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Agronomic and physiological aspects of nitrogen and water management for monocrop corn and corn competing with a ryegrass intercrop

by
Xiaomin Zhou

A thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfilment of the requirements
of the degree of Doctor of Philosophy

Department of Plant Science
McGill University
Montréal, Québec
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Dedication

To the memory of my mother, Jing Chen, and father, Zuogan Zhou.

Short Title

NITROGEN, WATER AVAILABILITY AND CORN PRODUCTION

Abstract

Ph.D. Xiaomin Zhou Plant Science

Concern about NO_3^--N leaching and groundwater pollution from monoculture corn (*Zea mays* L.) has prompted investigation of alternative production systems which reduce N leaching. Both intercrop systems and water table controls alone have been shown to increase nitrogen (N) uptake and reduce soil NO_3^--N accumulation in cropping systems. There is a need to maintain crop productivity while reducing the potential for soil NO_3^--N leaching into groundwater. However, there has been no information available regarding agronomic and physiological aspects of N and water management for monocrop corn and corn competing with annual Italian ryegrass (*Lolium multiflorum* Lam) in an intercrop system. A study was conducted in southwestern Québec during 1993 and 1994. Nitrogen and dry matter components in the plant-soil system were determined. Intercropped corn grain yield did not differ from monocropped corn under high N fertility. At harvest, the corn-annual ryegrass intercrop system increased total aboveground N uptake by 77.2 and 50.7 kg ha^{-1} when compared with the corn monocrop system in 1993 and 1994, respectively. The intercrop system reduced the amount of NO_3^--N in the top 1 m of soil by 47% (92.3 kg N ha^{-1}) at harvest in 1993. Water table controls had little effect on corn yield, N use efficiency and soil NO_3^--N accumulation over the two years of this study. Both plant establishment and weather conditions affected the ability of annual ryegrass to aid in the uptake of soil NO_3^--N. The reproductive development of water stressed plants after silking was limited more by overall plant changes due to water stress than assimilate supply. The delivery of C (sucrose) and N (^{15}N urea) into corn plants via stem-injection showed that externally supplied C changed both the source strength (photosynthetic inhibition) and sink strength (decreased total grain production), while distribution of ^{15}N was affected by proximity of sinks to the point injection and the strength of sinks. Injection of salicylic acid (SA) plus sucrose increased corn photosynthetic activity and productivity.

Résumé

Ph. D. Xiaomin Zhou Plant Science

Les problèmes de lessivage des NO_3-N et de la pollution de la nappe phréatique causés par la monoculture de maïs (*Zea mays* L.) ont poussé vers la recherche d'alternatives dans les systèmes de production. On a démontré que des cultures intercalaires et le contrôle du niveau de l'eau dans le sol augmentent l'assimilation azotée et réduisent l'accumulation des NO_3-N dans le sol. On a besoin de maintenir la productivité des pratiques culturales et de réduire le lessivage des NO_3-N vers les eaux souterraines. Cependant, il y a peu d'information sur les aspects physiologiques et agronomiques de la relation entre l'N et l'eau dans une monoculture de maïs et dans le cas d'une culture de maïs avec du ray-grass italien (*Lolium multiflorum* Lam). Une étude a été faite dans le sud-ouest du Québec durant les années 1993 et 1994. On a étudié l'N et les composantes de la matière sèche dans le système sol-plante. Dans le cas d'abondance de la fumure azotée, le rendement du maïs en monoculture et en polyculture était le même. A la récolte de 1993 et de 1994, l'assimilation azotée de la polyculture était plus élevée que celle de la monoculture de 77,2 et 50,7 kg ha^{-1} respectivement. A la récolte de 1993, le niveau des NO_3-N dans le premier mètre de sol dans le système intercalaire était réduit de 47% (92,3 kg N ha^{-1}). Le contrôle du niveau de l'eau dans le sol a eu peu d'effet sur le rendement du maïs, l'efficacité d'utilisation de l'N et l'accumulation des NO_3-N dans le sol durant les deux années. L'établissement des plantes et les conditions météorologiques ont affecté la capacité du ray-grass d'aider dans l'assimilation des NO_3-N du sol. Le développement reproducteur des plantes après l'apparition des soies était plus limité par les changements subis par les plantes dûs à la sécheresse et non aux éléments nutritifs. L'injection de C (sucrose) et d'N (^{15}N-urée) dans les tiges des plantes a démontré que le C de source externe a causé une diminution photosynthétique et une diminution dans la production des graines et que la distribution de l' ^{15}N était affectée par la proximité du point d'injection au réservoir et par la puissance de ce dernier. L'injection d'acide

salicylique et de sucrose a amélioré l'activité photosynthétique et la productivité du maïs.

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Contributions of co-authors to manuscripts for publication

This thesis has been written in the form of manuscripts to be submitted to scientific journals. This format has been approved as outlined in the "Guidelines Concerning Thesis Preparation". The following text, from "Guidelines Concerning Thesis Preparation" by the Faculty of Graduate Studies and Research, "must be cited in full in the introductory section of any thesis to which it applies":

" ***2/Manuscript and Authorship:*** *"Candidates have the option, subject to the approval of their Department, of including, as part of their thesis, copies of the text of paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), provided that theses copies are bound as an integral part of thesis. If this option is chosen, connecting texts, providing logical bridges between the different papers, are mandatory. The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation" and should be in a literary form that is more than a mere collection of manuscripts published or to be published.* ***The thesis must include, as separate chapters or sections:*** *(1) a Table of Contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rational and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and /or summary. Additional material (procedural and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail (eg. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis. In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; supervisors must attest to the accuracy of such claims at the Ph.D. Oral Defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored papers".*

This thesis contains nine manuscripts corresponding to chapters 3-11, all of

them are drawn from the manuscripts for publication. The manuscripts from which chapters 3, 9, 10 and 11 were taken were co-authored by myself, Dr. D.L. Smith, Dr. A.F. MacKenzie, and Dr. C.A. Madramootoo. My supervisor, Professor D.L. Smith provided supervisory guidance and funds from the onset of this study, arranged technical assistance during field and laboratory operation, offered many valuable suggestions and reviewed all of manuscripts before submission for publication. Dr. A.F. MacKenzie, Professor within the Department of Natural Resource Science of McGill University provided all field management, soil sampling and analysis materials, and also reviewed the manuscripts. Dr. C.A. Madramootoo, Professor within the Department of Agricultural and Biosystems Engineering provided funds from the onset of this study, weather data, assistance in the field work, and reviewed the manuscripts.

The manuscripts from which chapters 4 and 5 are drawn were co-authored by myself, Dr. A.F. MacKenzie, Dr. C.A. Madramootoo, J.W. Kaluli, and Dr. D.L. Smith. Dr. J.W. Kaluli, who was a former Ph.D student under Dr. C.A. Madramootoo's supervision, helped in harvest and soil sampling, and reviewed this manuscript before submission for publication. The contribution of Dr. Smith, Dr. A.F. MacKenzie and Dr. C.A. Madramootoo to this manuscript was as described previously.

The manuscript from which chapter 6 is drawn was co-authored by myself, S. Leibovitch, Dr. A.F. MacKenzie, Dr. C.A. Madramootoo, Dr. P. Dutilleul and Dr D.L. Smith. Mr. S. Leibovitch, is the technician in Dr. D.L. Smith's laboratory. He was involved in establishment of the ^{15}N microplots, helped with harvest and analysis of ^{15}N materials, and reviewed this manuscript before submission for publication. Dr. Dutilleul, is statistician within the Department of Plant Science, helped to analyze the data with Repeated methods, and reviewed this manuscript before submission for publication. The contributions of Dr. D.L. Smith, Dr. A.F. MacKenzie and Dr. C.A. Madramootoo to this manuscript were as described previously.

The manuscript from which chapter 7 is drawn was co-authored by myself and my supervisor, Dr. D.L. Smith. The contribution of Dr. Smith to this manuscript was

as described previously.

The manuscript from which chapter 8 is drawn was co-authored by myself, Dr. A.F. MacKenzie, Dr. C.A. Madramootoo, Dr. P. Dutilleul and Dr. D.L. Smith. The contributions of Dr. C.A. Madramootoo, Dr. A.F. MacKenize, Dr. P. Dutilleul and Dr. D.L. Smith to this manuscript were as described previously.

Chapter 1

General Introduction

Québec is the second largest corn production area in Canada. Corn has been widely used for grain and forage in this area (Bureau de la Statistique du Québec, 1994). Corn monoculture is a common management practice in Québec. Intensive corn production has resulted in loss of soil organic matter (Martel and MacKenzie, 1980) and increased potential for groundwater pollution (Liang and MacKenzie, 1991).

High corn grain yields can be achieved when there are sufficient supplies of nutrients, water and light. However, the improper use of nutrients and water can reduce grain yield or cause environmentally adverse effects. Over-fertilization with N was practised in the past and was even recommended as an insurance against N deficiency, but this may decrease the efficiency of N use and cause groundwater pollution (Keeney, 1986a). Corn generally takes up only about 50% of the applied N fertilizer (Bartolomew, 1965). Thus, large quantities of applied N may remain in the soil profile after corn harvest (Bock, 1984) due to high rates of N fertilizer applied to corn production and low fertilizer N recovery. There is potential to lose this N through leaching, denitrification, volatilization or immobilization in the soil during both the growing season and the non-growing season.

The most effective way of reducing NO_3^--N leaching from soil is to reduce the N application rate. However, reductions in the levels of applied N can result in corn yield reductions. Therefore, the best way to reduce leaching losses while maintaining yield is to improve N use efficiency. Studies on increased N use efficiency using intercropping systems, water table management, catch crops or reduced N application rates have been suggested (Juergens-Gschwind, 1989; Ferguson *et al.*, 1991; Madramootoo *et al.*, 1993; McCracken *et al.*, 1994). Efficient use of N in cropping systems is important for agronomic, economic or environmental reasons. A given N management system may provide highly efficient use of N from one perspective but be relatively inefficient from another. Unfortunately, there is no information available from an integrated study investigating the combination of intercropping and water table

management on N use efficiency. Information on N use efficiency in such a cropping system is essential for developing management practices to minimize environmental pollution without reducing corn yield.

The use of high N fertilizer levels increases grain yield but also causes groundwater pollution. The lack of sufficient water supply during corn development can lead to considerable yield reductions due to water stress. Water deficiency during the reproductive stage, especially during silking and early kernel growth, has been extensively reported to decrease corn yield due to abnormal kernel development (Schussler and West gate, 1991). The probable reason for the grain yield reductions have been suggested to be inhibition of photosynthesis by low leaf water potential (West gate and Boyer, 1985), and/or related low accumulation of photosynthetic reserves in the corn stem at anthesis (Boyle *et al.*, 1991b). It has been hypothesized that the supply assimilate at, or after anthesis could compensate yield loss due to water stress. However, the traditional supply methods (via roots or leaves) of materials may not be appropriate for some organic substances (e.g. sugar) or some expensive nutrients (e.g. isotope labelled materials). The development of a technique for suppling substances in physiological and biochemical studies will make it possible to more clearly examine the roles of these substances play in plant physiological process. This thesis emphasizes corn production and N use efficiency in the soil-plant system for corn intercropped with annual ryegrass under water table management and high N fertility. Changes in physiological activity and productivity of corn plants when injected with sucrose, plant growth regulators and ^{15}N enriched urea were also investigated as ways to explain possible aspects of the field data. Chapter 3 describes how corn development was affected by establishment of underseed annual ryegrass and water table controls during the corn growing season under climate and soil conditions of southwestern Québec; chapter 4 characterizes the effectiveness of a corn-annual ryegrass intercrop system and water table managements on final corn yield, N uptake, and fertilizer N recovery; chapter 5 illustrates the effects of the utilization of an corn-ryegrass intercrop system and water table management on NO_3^--N content in the soil

profile in the spring and fall; chapter 6 characterizes the use of confined ^{15}N microplots to minimize the cost of ^{15}N for this type of study; chapter 7 illustrates the development and use of a new stem injection technique for corn plants; chapter 8 characterizes the externally supplied sucrose on dry matter partitioning, photosynthetic activity and productivity of corn; chapter 9 characterizes the interactions between the supply of sucrose via stem injection and water availability on photosynthesis and kernel development by corn under controlled environmental conditions; chapter 10 describes changes in photosynthetic activity and productivity of corn due to addition, via stem injection, of combinations of plant growth regulators, including those generally thought to be involved in regulating water stress responses, and sucrose; finally, chapter 11 illustrates the distribution of N added via an artificial source relative to the various N sinks.

Chapter 2

Literature Review

2. Corn production, N fertilizer and environmental pollution

2.1. Corn production and utilization

Corn (*Zea mays* L.) is originally from Mexico and central America, and, since its domestication, has grown in importance such that now it is the second most widely produced cereal crop in the world. According to FAO reports (FAO, 1986), over 449 million metric tons of corn were harvested worldwide from 133 million ha, making corn second only to wheat among the world's cereal crops.

World corn production has increased since 1930, with a dramatic increase occurring in recent years. This increase has been due both to an increase in land area used in corn production and to increased yield per unit of land area (Benson and Pearce, 1987). Developed market economies account for 30% of the global corn area but provide 50% of total production, due to average yields that are three times higher than the world average.

At present, North America produces over 50% of the world's corn (Benson and Pearce, 1987). Canadian production makes up about 1% of the global planted corn area. The largest Canadian production areas are Ontario and Québec, where the crop is grown extensively for grain and fodder. In 1993, approximate by 295,000 hectare of grain corn and 30,500 hectares of fodder corn were grown in Québec, which was 29 and 17% of total grain and forage corn production areas in Canada (Bureau de la Statistique du Québec, 1994).

Corn is used in many ways: as human food, a feed grain, a fodder crop, and for hundreds of industrial purposes. Corn can supply abundant energy in the form of carbohydrates. Mangelsdorf (1967) tested the approximate starch concentration of cereal grains and found that corn grain contains more starch than that of wheat, barley and oat, but less than that of rice. The starchy endosperm of corn grain is an excellent source of carbohydrate and, as such, can provide energy for livestock and human

beings.

2.2. Corn yield, N fertilizer and N use efficiency

In this century, increases in crop yields have mainly been due to better genotypes and tremendous increases in fertilizer use. Nitrogen, an essential major plant nutrient, represents the mineral fertilizer most applied to agricultural lands. Nitrogen fertilization has been a major factor in increasing the world crop production (Peterson and Frye, 1989). There was a rapid increase in the use of N fertilizer during the period 1960 to 1970 (Thomposon, 1986). In Québec, the amount of N fertilizer sold in 1980 was 54,930 metric tonnes (AFEQ, 1995). By 1992, the sale of N fertilizer had increased more than 71% to 93,328 metric tonnes (AFEQ, 1995). Research in Québec has shown that the increase in corn grain yield was closely related to increased fertilizer N use (Tomar *et al.*, 1988). Cromwell *et al.* (1983) showed that production of corn per hectare increases quadratically with increasing N fertilization levels.

Broadbent and Carlton (1971) found that the recovery of N fertilizer by plants ranged from 35 to 71% and that between 18 to 41% of applied N fertilizer remained in the soil. Later, Bock (1984) reviewed relevant literature and concluded that N recovery from fertilizer ranged from 30 to 70% during the year of application. In Québec, fertilizer N-use efficiency by corn has been reported to be as low as 9% at high N rate (400 kg N ha^{-1}), and as high as 58% at the recommended N application rate (180 kg N ha^{-1}) (Liang and MacKenzie, 1994a). The fertilizer N not recovered by the crop and leached below the root zone is of environmental concern, as increasing NO_3^- concentrations in groundwater are often attributed to leached N fertilizer (Angle *et al.*, 1993).

2.3. Nitrogen fertilizer and environmental pollution

Since most crops take up N more than any other nutrient, the amount of applied N fertilizer generally far exceeds that of other nutrients (Hargett and Berry, 1987). Farmers apply excessive quantities of inorganic fertilizer N in an attempt to ensure maximum yields. Every year in Canada, over $100 million of N fertilizer is applied, but 20 to 60 percent of this fertilizer may be lost from soils through leaching or

denitrification. The large inputs of fertilizer N and intensive crop production for corn has caused soil degradation and affected groundwater quality (Forster *et al.*, 1986; Keeney, 1986b; Blackmer, 1987). Some researchers have estimated that 30% of the N fertilizer applied in Québec is leached out of agricultural soils and into waterways and groundwater due to autumn rainfall and surface runoff (Neilson and MacKenzie, 1977; Miller and MacKenzie, 1978).

Many studies have shown a direct relationship between NO^-_3-N concentration in groundwater and N fertilization rates. Pratt (1984) reviewed a number of studies in which high nitrate concentrations occurred in groundwater beneath irrigated agricultural sites in California, and found that there was a close relationship between the amount of nitrate leached and rates of fertilizer N use. Chaney (1990) showed that nitrate contents in soil increased linearly when the N applied exceeded 160 kg ha^{-1} for winter wheat.

Research over the last decade has clearly shown that agriculture has become the most extensive source of nitrate delivered to ground and surface waters (Pratt, 1984; Harrllberg, 1987a,b). In some areas, N pollution in ground water has reached alarming levels. For instance, Madramootoo (1992) measured nitrate concentrations as high as 40 mg L^{-1} in a subsurface drainage system from a tile drained potato field in Québec; this far exceeds the U.S. Environmental Protection Agency safe drinking water standard (10 mg L^{-1}). Concern arises when nitrate accumulates in ground water because when ingested in sufficient amounts by humans and animals, there are potential adverse health effects. These health effects are reported to include methaemoglobinaemia and elevated risk of cancer (Comly, 1945; Addiscott *et al.*, 1991).

Fertilizer N has become a focus of attention because of concerns with groundwater contamination by NO^-_3-N, as well as NO^-_3-N losses into the environment are typically coupled with its intensive and extensive utilization.

3. Nitrogen losses from soil

Fertilizer N losses from agricultural systems have been measured at 20 to 80%

of amounts applied (Henzell, 1972; Power, 1980). Plants consume only 40-50% of the added fertilizer N, and the other approximately 50% is lost from agricultural soils. Low recovery of fertilizer N by crops not only reduces fertilizer N profitability, but also contributes to groundwater and surface water pollution. Nitrogen loss from soil can occur in several ways: 1) leaching losses; 2) denitrification and gaseous N loss; 3) through tile drainage systems; 4) wind and water erosion of soil.

3.1. Leaching losses

Nitrate is the most important source contributing to groundwater contamination from agricultural system (Stanley *et al.*, 1990; Ferguson *et al.*, 1991a). The availability and use of commercial N fertilizers and expansion of agricultural land have increased the potential for nitrate leaching. Legg and Meisinger (1982) pointed out that nitrate leaching losses are the most significant way N is lost from soil. An average 25 to 50% of the N applied in most cropping lands is lost through leaching (McNeal and Pratt, 1978). Olson *et al.* (1970) observed that at an annual application rate of 112 kg N ha^{-1}, about 46% of N was recovered by corn, and 26% retained in soil as mineral N within a depth of 120 cm. When 336 kg N ha^{-1} was applied, the crop removed only 19% of the N, and 69% of the N remained in mineral form in the soil. It is clear that NO^-_3-N can easily accumulate in the soil when N supply exceeds uptake. This excessive NO_3-N is easily leached out of the soil.

3.2. Denitrification and gaseous N losses

Denitrification and gaseous losses are other ways that N can leave the soil. Denitrification can cause substantial losses of soil N with possible consequences in depletion of the ozone layer (Crutzen, 1981). Ryden (1983) reported that denitrification losses can increase as fertilizer N rates increase because of the greater availability of NO^-_3. Mosier *et al.* (1986) found that denitrification losses for corn in Colorado amounted to 2.5% of the 200 kg ^{15}N ha^{-1} fertilizer applied. Lindau *et al.* (1988) demonstrated that denitrification may increase due to flooded conditions where soil is waterlogged for long periods and no plants are growing. Except under special conditions, denitrification losses seem to be smaller than previously thought. In

Québec, Liang and MacKenzie (1994b) found that denitrification loss in two typical soils was relatively small when compared with leaching over the non-growing season.

3.3. Tile drainage

Logan *et al.* (1980) found that annual NO^-_3-N losses in tile effluent from extensive corn production land were generally < 30 kg ha^{-1}, but increased with N fertilizer application above crop needs. Nitrate-N concentrations were above 10 mg L^{-1} even when 20 kg of fertilizer N ha^{-1} or less were applied annually to continuous corn.

3.4. Wind and water erosion of soil

Legg and Meisinger (1982) estimated that 4.5 million metric tonnes of U.S cropland N are lost annually to wind and water movement. Twenty percent was attributed to wind. This soil can contain significant amounts of N.

4. Possible ways to reduce soil N losses due to leaching

Reduction of N leaching from agricultural land may be achieved in several ways: increased N uptake by crops, incorporation of high C/N crop residues, or raising the level of drainage and introducing forage grass species into the cropping system to improve soil quality.

4.1. Increased N use efficiency

Nitrogen fertilizer accounts for approximately 33% of the 'energy subsidy' required for growing corn (Pimental *et al.*, 1973), but only 40-70% or less of applied N is recovered as plant N (MacLean, 1977). Thus, increasing N uptake by the crop, reducing the potential for leaching, would lead to more efficient use of fertilizer N and minimize the potential for NO^-_3-N contamination of groundwater.

4.1.1. Crop N uptake

Increasing the uptake rate by plants will aid in improving the efficiency of fertilizer N utilization (Olson and Kurtz, 1982), and it is a recommended practice to reduce nitrate leaching (Keeney, 1986a). In general, the absorption of nitrate by plant roots is slow during the early seedling stage and increases during the period of major growth; the uptake rate becomes slow again during the reproductive phase (Hanway, 1960). Polisetty and Hageman (1981) observed a decline in NO^-_3 uptake by corn with age.

Hanway (1960) reported that corn typically accumulates much of its N in the period beginning about one month after emergence.

4.1.2. Nitrogen fertilizer management

Nitrate-N is the predominant form of N available to corn in most soils. McCracken *et al.* (1989) reported that soil NO^-_3-N concentration was highly correlated with corn yield and N uptake. A three-year experiment conducted by Jokela and Randall (1989) showed that corn grain yield, total dry matter yield and plant N uptake were all increased by high N inputs. Using ^{15}N Broadbent and Carlto (1978) found that maximum N uptake by corn occurred at fertilizer levels which produced maximum yield, while MacLean (1977) pointed out that low rates of fertilizer were less effective in increasing N uptake by corn.

4.1.2.1. Use of ^{15}N in the plant-soil system

The use of fertilizer labeled with the stable isotope ^{15}N has become increasingly important in field studies where direct measurement of fertilizer delivered N in plants and soil is required. Compared with non-tracer studies, determinations of labeled fertilizer N can be more accurate (Hauck and Bremner, 1976), and treatment effects can be detected with greater sensitivity (Russelle *et al.*, 1981). Moreover, N in the crop derived from fertilizer can be distinguished from soil-derived N, allowing fertilizer N efficiency to be calculated without regard to residual fertilizer N in the soil.

One major disadvantage of using ^{15}N is the high cost of field plot size research. One means of reducing ^{15}N cost is to apply labeled N to small areas (microplots), within the larger field plot. Plant and soil samples taken from the microplot provide information on plant uptake and soil recovery of fertilizer N.

Studies to determine suitable microplot dimensions have been conducted for both confined and unconfined microplots. Jokela and Randall (1987) evaluated the maximum acceptable corn plant sampling area in a 1.52 by 2.29 m unconfined microplot with uniform N fertilizer application and a 76 cm row spacing. They found that this microplot size provided reliable fertilizer N recovery data for corn. Stumpe *et al.* (1989) suggested that plants which were at least 50 cm from the border of a ^{15}N

microplot provided reliable data without concern as to the method of N fertilizer application. These studies on unconfined microplots confirmed that they provided reliable N recovery data for N uptake studies, but the high cost of ^{15}N required for larger application areas still needed to be addressed. The reason for this is that unconfined microplots still require an application of labeled N fertilizer over a much larger region surrounding the smaller central portion where samples may be reliably taken. On the other hand, confined microplots use barriers placed in the soil to delineate them (Malhi and Nyborg, 1983; Power and Legg, 1984) and eliminate problems with lateral movement of labeled N (Ma *et al.*, 1995). The major advantage of using confined ^{15}N microplots in field research is that they can prevent diffusion and mass flow of ^{15}N from inside to outside of the microplot area and the similar movement of unlabeled N from outside to inside (Carter *et al.*, 1967; Malhi and Nyborg, 1983; Power and Legg, 1984). Thus, there was potential for the use of confined ^{15}N microplots to reduce the high cost of ^{15}N in fertilizer N research.

4.1.3. Intercropping

A biological advantage to intercropping may result from complementary use of growth resources. Component crops may differ in their use of growth resources over time and space. When they are grown together they make more efficient use of light, water and nutrients than when grown separately. It is also frequently stated that intercropping can be an efficient method of weed control (Moss and Hartwing, 1980) and reduction of pests and disease (Martin *et al.*, 1989).

4.1.3.1. Corn yield under intercropping

Crop rotation has helped to maintain or even improve soil quality while increasing corn yield (Ketcheson and Webber, 1978; Bolton *et al.*, 1982). Intercropping corn with legumes is a possible alternative to crop rotation (Tomar and MacKenzie, 1988). Some field work has demonstrated the effectiveness of intercropping corn with legumes; intercrops resulted in greater productivity of land than monoculture production (Nair *et al.*, 1979; Allen and Obura, 1983; Ebelhar *et al.*, 1984; Martin *et al.*, 1990; Weil and Mcfadden, 1991).

An eight year field study of corn intercropped with rye, ryegrass or fescue demonstrated that intercropped corn yields were excellent and showed little tendency to decline when sufficient N was supplied regardless of the intercropping practice (Triplett, 1962). Scott *et al.* (1987) showed no corn yield reduction under corn intercropped with legumes. Searle *et al.* (1981) also observed that corn grain yield was not affected by legume intercrops, but N uptake by the following crop (wheat) was improved by intercropping. Francis and Stern (1986) observed that the N concentration in corn grain was not affected by intercropping, and total N uptake at the final harvest by corn increased significantly with increased N fertilizer application.

4.1.3.2. The potential use of intercropping to reduce nitrate leaching

Intercropping is a possible method to reduce leaching, as it may reduce the movement of NO^-_3-N into deeper soil layers. Nitrate leaching was reduced by the intercrops of corn with black gram (*Vigna mungo* (L.) Hepper) or mung bean (*Vigna radiata* (L.) R. Wiliczek var. vadiate), compared with corn alone, especially when soil fertility was adequate (Singh and Sekhon, 1978). Compared with monocropped corn and sugarcane (*Saccharm officinarum* L.), corn-pigeon pea and sugarcane-black gram intercrops reduced leaching loss (Yadav, 1982). Similar results were also found in Québec (MacKenzie, personal communication). In a number of countries, intercropping has been shown to reduce NO^-_3-N leaching by 40-95% (Juergens-Gschwind, 1989).

Increased crop dry matter production may enhance organic crop residual returns to soil, therefore, increasing soil quality (Liang and MacKenzie, 1992). Thus, the greater dry matter returned to the soil by intercropping than by monocropping will likely improve soil quality. Some researchers have found that when intercrop was practised on the same land for several years, the yield of subsequent crops was increased (Agboola and Fayemi, 1971), the same effect was recently demonstrated when intercropped corn was grown for only one year (Nair *et al.*, 1979).

4.1.3.3. Forage grass species

MacLean (1977) conducted a 3-year study on the movement of NO^-_3-N in different

cropping systems, and found that forage grass species were more effective than corn in the uptake of soil N. He observed that forage grass species took up 64-90% of the N applied at the optimum rate (112 kg ha^{-1}), with an average recovery of 85%. This recovery rate is greater than the rate usually reported for corn and implies that forage grass species can be used to reduce the movement of soil N into deeper soil.

Annual ryegrass has been used for soil stabilization (Schery, 1961; Musser *et al.*, 1969). It is extremely vigorous and emerges rapidly (Blaser *et al.*, 1956). In the seedling stage, ryegrass has a high shoot growth rate and competes very effectively for light (Appleby *et al.*, 1976). Steenvoorden (1989) reported that Italian ryegrass, winter wheat and rape proved to be very effective crops for reducing nitrate leaching. Muller (1989), using rape, rye and radish as catch crops, found that there was a positive effect of catch crop in decreasing soil mineral N leaching. Claude (1990) concluded that the ability of ryegrass to absorb and recycle NO_3^--N can be exploited in corn production to decrease soil NO_3^--N and reduce leaching of soil NO_3^--N. In addition, Scott *et al.* (1987) reported that annual ryegrass was most effective crops in terms of ground cover and dry matter production. Keeney (1982) pointed out that "a more effective cropping sequence for immobilizing residual N might be one which utilized an early season, rapid growing forage grass or small grain".

Since soil degradation resulting from losses of soil organic matter with intensive corn production has been recognized (Martel and MacKenzie, 1980), increased aboveground dry matter production may enhance organic crop residual returns to soil, thus reducing soil degradation (Liang and MacKenzie, 1992). Thus, there is potential to use annual ryegrass to increase nitrate uptake and reduce the available soil N content after harvest, as well as improve soil quality (Groffman *et al.*, 1987). Maintaining good soil quality will reduce the requirement for fertilizer N and maintain crop yield, which, in turn, could lessen the potential problems of excess N (Keeney, 1982).

4.1.4. Water table managements

At present, approximately 75,000 ha of crop land have been drained in Québec. Most of this cropland is used for extensive grain and silage corn, and small grain cereal

cultivation. Madramootoo *et al.* (1993) addressed several factors regarding the need for drainage in Québec crop production areas to maximize yield: 1) excess of precipitation over evapotranspiration; 2) most soils have low permeabililites: 3) the topography is relatively flat with low hydraulic gradients to river and water resources. Thus, many fields in Québec requires subsurface drainage to remove excess soil water in the spring and fall and during parts of the summer when rainfall is in excess of evapotranspiration. Conversely, there is sometimes a shortage of water for plant growth when dry conditions occur during the corn growing season. The presence of a shallow water table may reduce the need for supplementary irrigation (Chaudhary *et al.*, 1975; Follett *et al.*, 1974), it is also likely to cause poor soil aeration and restrict rooting volume (Williamson, 1964). Relatively deeper water tables have been shown to decrease crop growth due to decreased water availability (Chaudhary *et al.*, 1975; Follett *et al.*, 1974). Therefore, controlled drainage systems in Québec can be used not only to remove excess soil water which delays planting or harvest, but also for supplemental irrigation during water stress (Memon, 1985).

4.1.4.1. Water table controls to reduce nitrate leaching

There is also potential to use water table control as a management practice to reduce nitrate pollution from agricultural land. Nitrate N is the main form of soil N lost through leaching; its movement is highly correlated with water movement (Vinten and Smith, 1993). Higher soil NO^-_3-N concentrations and larger amounts of soil water movement promote NO^-_3-N leaching (Legg and Meisinger, 1982). Baker *et al.* (1975) and Miller (1979) reported that tile-drained fields can discharge considerable amounts of NO^-_3-N, especially when the N fertilizer rates are above the recommended rate for optimum crop yields.

Skaggs *et al.* (1994) and Tan *et al.* (1993) have reported reductions in nitrate loading of up to 50% with water table control. Research by Evans *et al.* (1989) has shown that controlled water table depths reduced N and P losses from a field by 47 and 44%, respectively, compared with conventional drainage. A water table depth as shallow as 0.4 m was found to reduce overall NO_3^--N levels in the soil profile by up to

50%, and to increase soybean yield by approximately 20%, compared with conventional, free-outlet drainage (Madramootoo *et al.*, 1993). Kalita and Kanwar (1992) reported that, under controlled water table depths, a water table depth from 0.6 to 1 m increased corn yield and soil NO_3^--N levels.

Denitrification is desirable when it occurs below the root zone because it reduces nitrate leaching to groundwater (Meek *et al.*, 1970). Raising the water table by controlled drainage may enhanced denitrification and increase N uptake (Steenvoorden, 1989), at the same time high soil moisture can slow the nitrification process, hence considerably reducing the NO_3^--N in the drainage water (Meek *et al.*, 1970). This occurs as denitrification usually happens when oxygen status is low and there is a readily available energy source (Meek *et al.*, 1970), conditions promoted through increasing the water content of the soil and the addition of high C/N crop residues.

Chaudhary *et al.* (1975) concluded that corn yield response to water table depths varied with rainfall during the growing season. They found that the deeper the water table the greater the corn grain yield under wet conditions while the grain yield was increased by shallow water tables under dry conditions. Therefore, there is need not only for determining the optimum water table levels for maximum crop yield, but also for maximum denitrification and reduction of nitrate pollution.

4.1.4.2. Water stress and reproductive failure

Availability of water is one of the most common limitations to plant growth. Water deficits during silking and early kernel growth decrease kernel number per ear, thus reducing sink size and yield potential (Westgate *et al.*, 1986). Loss of kernels can result from abnormal floral development, kernel abortion and developmental failure after fertilization. A lack of assimilate reserves is often found at anthesis (Westgate and Boyer, 1985; Schussler and Westgate, 1991) under water deficit.

4.1.4.2.1. Sucrose

Sucrose is the most important form of assimilate translocated by most plants (Setter and Meller, 1984). The lack of assimilate supply during flowering and early

kernel growth has been suggested as a major factor responsible for reproductive failure under water stress (Boyle *et al.* 1991b; Schussler and Westgate, 1991; Zinselmeier *et al.* 1995). Boyle *et al.* (1991b) used a stem infusion method to supply sucrose (150 g L^{-1}), dissolved in a tissue culture medium, to water stressed corn plants. They found that the grain yield of water-stressed corn plants infused with sucrose plus tissue culture medium was similar to the yield of well-watered control plants. In addition, water stressed plants with infused water or no-infusion showed nearly complete reproductive failure. Their results suggested that assimilate supply at flowering may regulate kernel set in water deficient plants.

4.1.4.2.2. Plant growth regulators

The idea of using plant hormones to improve crop growth or eliminate environmental stress is not new. In the 1930's, auxins were first discovered and shown to be natural plant hormones. Later, with recognition of gibberellic acids (GAs) in the early 1950's, and cytokinin and ethylene in the early 1960's, more growth regulators have been identified, produced and commercially used in horticultural and agronomic crop production (Arteca, 1996). The following discussion focuses on several synthetic plant growth regulators which have been tested and utilized in agricultural production.

Abscisic acid (ABA) has been demonstrated to act as a water stress signal in the resistance of plants to environmental stresses (Markhart *et al.*, 1979; Davies and Jones, 1991). It is known to cause stomatal closure (Raschke and Hendrick, 1985), which reduces water loss and photosynthesis, thereby acting as a mechanism to allow plants to survive periods of water deficit stress (Davies and Jones, 1991). In addition, foliar application of ABA has been shown to decrease photosynthetic rates and ribulose bisphosphate carboxylase/oxygenase (Rubisco) levels (Fisher *et al.*, 1985; Davies and Jones, 1991; Makeev *et al.*, 1992) and has a potential role in photosynthate partitioning and seed filling (Makeev *et al.*, 1992).

Brenner (1987) suggested that IAA is a promoter of stomatal opening, Rubisco activity, sucrose phosphate synthase activity, phloem loading, phloem transport, and the overall activity of sinks. It has also been reported that IAA alters the pattern of

seed weight between and within the spikes of wheat plants (Rademacher and Graebe, 1984; Bangerth *et al.*, 1985).

Salicylic acid (SA) is thought by some to be a new class of plant growth substance (Raskin, 1992). It has effects on many physiological processes in plants at low concentrations. Salicylic acid regulates some aspects of disease resistance and thermogenesis in plants. However, there have been no studies on the effects of salicylic acid on photosynthesis. It has been shown to stimulate flowering. Kling and Meyer (1983) reported that SA increased pod number and yield in mung bean. Jain and Srivastava (1981) found that SA increased the *in vivo* activity of nitrate reductase in corn seedlings. Rai *et al.* (1986) showed that SA greatly promoted stomatal opening by excised leaves of *Commelina communis* L. plants.

Ethephon is a synthetic growth regulator that releases ethylene slowly inside plant tissues through a pH-dependent reaction (Warner and Leopold, 1969). Ethephon has been shown to retard stem elongation leading to shorter plants that are less likely to lodge (Caldwell *et al.*, 1988). In addition to reducing lodging, ethephon applied to cereals may affect yield either positively or negatively (Moes and Stobbe, 1991). Ethephon application was shown to increase protein concentration of the grain and to reduce lodging of corn plants under high density conditions (Norberg *et al.*, 1988). Increased seed protein concentrations were also found in barley (*Hordeum vulgare* L.) (Ma *et al.*, 1994b; Foroutan-pour *et al.*, 1995) when ethephon was added via a peduncle perfusion technique.

4.1.4.2.3. Techniques for continuous supply of metabolism effectors

Traditional methods of supplying nutrient compounds to corn plants are through roots and leaves (Rendig and Crawford, 1985; Tomar *et al.*, 1988). However, in biochemical pathway or physiological studies, the small amounts supplied and the short durations of supply may be inappropriate.

Stem infusion can supply small quantities of some substances to different plants without the sometimes erratic uptake experienced with spraying, leaf abrasion or soil application (Brown and Neish, 1954; Grabau *et al.*, 1986; Schon and Blevins, 1987;

Boyle *et al.*, 1991a; Ma and Smith, 1992). In addition, stem infusion has proved to be less destructive and more effective than leaf feeding for supplying nutrients directly to corn plants for brief periods (Boyle *et al.*, 1991).

Infusion/perfusion techniques have also been developed for longer term delivery of materials into plant stem cavities. Ma and Smith (1992) delivered a steady supply of 30 mM N into the hollow stems of intact barley (*Hordeum vulgare* L.) plants for a period of 3 to 4 weeks (anthesis to physiological maturity). At present, the major obstacle to long term stem infusion of corn is the limited volume of solution that can be delivered and the duration of injection. An infusion technique developed by Boyle *et al.* (1991a) allowed delivery of large solution volumes, but only for a few days at a single infusion site. Corn stem infusion has been tested both under greenhouse conditions (Boyle *et al.*, 1991a,b) and field conditions (Ma *et al.*, 1994a), but the infusion uptake rate was limited to a short period irrespective of number of infusion sites.

5. Hypotheses

From the literature it is clear that over fertilization with N in corn production can cause groundwater pollution while a lack of water supply reduces corn grain yield. However, improving the agronomic efficiencies of N fertilizer or water use are complex matters.

There has been no information available on the effects of intercrop systems and water table management together as a management strategy to enhance N fertilizer use efficiency while maintaining corn yield. To do this, work is needed to identify and select alternative cropping system management practices that reduce leaching losses of N during both the corn growing season and the non-growing season. It is hypothesized in this study that the combination of an intercrop system and water table management, under sufficient N supply, will effectively increase N uptake by plants, thus reducing potential NO_3-N leaching after corn harvest, without reducing corn grain yield. In addition, the production of intercrops that are not harvested, but are returned to the soil (green manure) will result in addition of residues which decompose and mineralize, releasing their N into the soil. The specific hypotheses related to the use of an intercrop system and water table management, and crop ecophysiological aspects of corn plants growing under intercrop conditions are as follows:

1) Corn grain yield will not be affected by the presence of an annual ryegrass intercrop component when produced with high levels of fertilizer N.

2) Increased N uptake by a corn-annual ryegrass intercrop will reduce the soil NO_3^--N content, and thus, reduce the potential for nitrate pollution under soil and climatic conditions of southwestern Québec.

3) Greater dry matter accumulation in an intercrop system will increase the amount of crop residues returned to the soil.

4) Water table controls will increase corn grain yield, N uptake, and fertilizer N use efficiency while reducing soil NO_3^--N levels associated with corn production systems.

5) Confined microplots can reduce the microplot dimensions required to

produce reliable ^{15}N data in corn monocrop and intercrop (with annual ryegrass) systems.

6) A pressurized stem-injection system can continuously deliver concentrated nutrients into corn plants for periods of weeks to months.

7) The photosynthesis and productivity levels of corn plants will be altered by exogenously supplied sucrose via stem injection.

8) The reproductive failure of water stressed corn plants can be prevented by supplying assimilate (sucrose) post anthesis via a stem-injection technique.

9) Injection of plant growth regulators, with or without sucrose, will alter the growth and photosynthetic activities of corn plants.

10) The distribution of N added through an artificial source will be affected by both the proximity of plant N sinks to the source (site of injection) and the strength of various sinks.

6. Objectives

1) To compare dry matter production and distribution, and N allocation within monocropped and intercropped corn plants produced with or without subirrigation during corn development.

2) To evaluate the uptake of fertilizer and soil N by corn affected by the presence of an annual ryegrass intercrop component and water table controls.

3) To measure NO^-_3-N concentrations in soil solutions through the soil profile in order to evaluate the effectiveness of a corn-ryegrass intercropping system and water table management for reducing nitrate leaching to groundwater.

4) To use ^{15}N-enrichment to determine the fate of fertilizer N applied to the plant-soil system.

5) To contrast the level of % ^{15}N atom enrichment at different positions within confined microplots in order to assess the microplot dimensions required to produce reliable ^{15}N study for corn production systems.

6) To develop a stem injection technique which can deliver constant, high volumes of concentrated sucrose and other solutions into corn stems for a period of

weeks to months.

7) To determine whether assimilate supply (sucrose) by stem injection during active grain filling, can prevent the reproductive failure associated with water stress in corn plants.

8) To assess the effects of continuous plant growth regulator supply (via a stem injection), with or without sucrose, during the grain filling period on the growth and productivity of corn plants.

9) To determine distribution of ^{15}N, supplied via stem injection, in corn plants relative to the various N sinks.

Preface to Chapter 3

Chapter 3 is a manuscript prepared by myself, my supervisor Dr. D.L. Smith, Dr. A.F. MacKenzie and Dr. C.A. Madramootoo for publication in the *Agronomy Journal* in 1997. The format has been changed to be consistent within this thesis. All literature cited in this chapter are listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

In this chapter, we address the pattern and dynamics of dry matter and N accumulation in monocrop corn and a corn-annual ryegrass intercrop systems, as affected by water table management.

Chapter 3

Biomass production and N uptake as affected by monocrop and intercrop (ryegrass) corn systems and water table management

3.1. Abstract

Improving N uptake by crops reduces the quantity of N remaining in the soil profile at the end of the growing season and minimizes potential N losses, due to leaching, after crop harvest. Both intercropping and water table control (WTC) can help to minimize loss of N from the soil. Applied N fertilizer rate, plant growth stage, type of crop and WTC all affect N uptake. No previous study has reported the combined effect of cropping system (intercrop versus monocrop) and WTC on dry matter (DM) and N accumulation, and DM distribution and N allocation of corn (*Zea mays* L.) during crop development. A two-year study was conducted to assess DM production and N uptake during the development of monocrop corn and corn intercropped with annual ryegrass (*Lolium multiflorum* Lam.) on a fine sandy loam (fine, silty, mixed, nonacid, frigid Typic Humaquept) soil. Each of these corn production systems was produced under three drainage practices, with an annual application of 270 kg N ha^{-1}. The three drainage practices were free outlet conventional drainage, and subirrigation with the water tables maintained at 0.70 or 0.80 m from the soil surface. Zero N (control) and conventional N rate (180 kg N ha^{-1}) treatments were included for monocropped corn with conventional drainage. Plant tissues (corn or intercrop) of all treatments were sampled at four different corn growth stages (six leaf, tasseling, mid-grain filling and after physiological maturity). Dry matter production and N uptake were determined for each stage. DM production and N uptake of intercropped corn were lower than, or not different from the monocropped corn depending on growth stage, in both years. The total N uptake and DM production of the intercrop system (corn plus ryegrass plus weeds) was greater than that of the monocrop system. Application of 270 kg N ha^{-1} resulted in 20% less N recovery than 180 kg N ha^{-1} under monocrop corn production with free drainage. Both applied N

rates and the use of the corn-ryegrass intercrop system altered the allocation of DM and N among different parts of the corn plants. The ryegress and weeds were not able to take up enough of the extra N to completely negate an increased risk of nitrate leaching after the high N rate was applied for two consecutively years. Water table depth had less effect on DM production and N uptake than cropping system. This study showed that a corn-annual ryegrass system increased N uptake from the soil, thereby minimizing the potential for ground water pollution. However, the effect of environmental conditions on annual ryegrass growth was an important factor in N uptake by the intercropping system.

3.2. Introduction

Corn production in eastern Canada involves the addition of N, as chemical fertilizer and/or manure. Fertilizer N use efficiency by corn in Québec has been reported to be as low as 9% at high N application rates, and as high as 58% at recommended N rates (Liang and MacKenzie, 1994a). Therefore, large amounts of applied N fertilizer may not be recovered by the crop at harvest, and potentially contributing to groundwater pollution due to leaching of NO_3^--N. Efficient use of N fertilizer by plants plays an important role in establishing fertilizer N use efficiency and establishing levels of NO_3^--N leaching from soil.

There is evidence that the uptake of N by plants can be influenced by the applied N fertilizer rate, plant growth stage, cropping system and water table control (WTC). Increasing total N uptake and DM production by corn due to high N inputs has been documented extensively (Jokela and Randall, 1989; McCracken *et al.*, 1989). Results to this effect were reported for grain corn production by Liang and MacKenzie (1994a) in eastern Canada. Using ^{15}N, Broadbent and Carlton (1978) showed that maximum proportional N uptake by corn occurred at N fertilizer levels which produced maximum yield. Goh and Haynes (1986) pointed out that in high fertility soil, excess N can decrease crop yields.

Absorption of NO_3^--N by corn is generally slow during the early seedling stages and increases exponentially during the period of rapid growth; the uptake rate slows

again during the reproductive phase (Hanway, 1960). Hanway (1960) reported that corn typically accumulated much of its N one month after emergence.

Annual ryegrass (*Lolium multiflorum* Lam.) has a high shoot growth rate, competes very effectively for light (Appleby *et al.*, 1976) with winter wheat, and has been used as a catch crop to utilize residual soil N after crop harvest (Meisinger *et al.*, 1991; Shipley *et al.*, 1992). Keeney (1982) pointed out that "a more effective cropping sequence for immobilizing residual N might be one which utilized an early season, rapidly growing grass or cereal". Intercropping systems can make more efficient use of light, water and nutrients than crops grown separately. Thus, it is possible to increase N uptake by corn intercropped with annual ryegrass, thereby reducing potential NO_3-N leaching losses.

Water table control (WTC) has been recommended as a best management practice to reduce NO_3^--N pollution and increase crop yield in agricultural systems (Kalita and Kanwar, 1993; Madramootoo *et al.*, 1993). Maintaining a desirable water table depth (WTD) should aid in plant N uptake and increase crop yields (Madramootoo *et al.*, 1993). Very shallow water tables have been reported to reduce N uptake and retard plant growth due to decreased oxygen supply to the roots (Williamson, 1964). Kalita and Kanwar (1993) found that a WTD at 0.2-0.3 m decreased corn grain yields whereas WTD from 0.6 to 0.9 m increased yields. Chaudhary *et al.* (1975) concluded that variations in corn response to WTD were attributable to the variation in rainfall during growing seasons.

Increased inputs of plant residues may be expected to enhance N conservation and improve soil structure. The growing of intercrops that are not harvested but are returned to the soil results in addition of residue which is decomposed and mineralized, releasing N into the soil (Juergens-Gschwind, 1989). In addition, the incorporation of large amounts of organic C into soil may minimize residual NO_3^--N leaching from fertilizer applications through immobilization of inorganic N in soil (Barthdomew, 1965; Keeney, 1982). Keeney (1982) pointed out that the residue of high-yielding corn may account for as much as 5,000-10,000 kg ha^{-1} of dry matter with a C/N ratio of

40:1 to 60:1. This high C/N ratio provided a favourable condition for immobilization of N. The presence of ample C substrate can also cause rapid O_2 consumption and possible depletion in the soil microenviroment; thus it indirectly enhances the potential for denitrification (Firestone, 1982).

Increasing N uptake by plants necessarily improves the efficiency of fertilizer N utilization (Olson and Kurtz, 1982), and it is a recommended practice to reduce NO_3^--N leaching (Keeney, 1986a). To our knowledge, there has been no previous study of seasonal DM production and distribution, and N uptake and allocation by monocrop and intercrop (with annual ryegrass) corn under various levels of WTC. Knowledge of the extent to which intercrops respond to, and compete for individual resources would aid in improving the management of corn-annual ryegrass intercrop systems. A better understanding of crop response to WTC is necessary for planning drainage and irrigation to optimize corn production and reduce NO_3^--N leaching. We hypothesized that corn intercropped with annual ryegrass and with controlled WTDs would have increased N uptake during the development of the cropping system, thus reducing residual N in the soil after corn harvest. In addition, it has previously been demonstrated that the presence of an under-seeded intercrop component may not lead to lower yields of the main crops when competition for water and nutrients is minimal (Searle *et al.*, 1981). However, this phenomenon has not been previously investigated over the course of crop development. Therefore, our objectives during the development of the crop system were to: 1) evaluate the combined effects of cropping system and WTD on DM production and distribution, and N uptake and allocation by the plants in the cropping system; 2) compare the effect of N rates on the development and N uptake of monocrop corn plants with free drainage, in order to determine the effects of intercrop competition for N.

3.3. Materials and Methods

3.3.1 Field conditions

The experiment was conducted in the 1993 and 1994 growing seasons on a 4.2 ha field in southwestern Québec, Canada. The top 50 cm soil layer was a well-drained

Soulanges sandy loam, below which was a deeper clay layer. The soil was classified as a nonacid, frigid Typic Humaquept. The soil characteristics were as follows: pH 6.6; organic C 50 g kg^{-1}; total N 4.5 Mg ha^{-1}; 48% sand, 26% silt and 23% clay. Precipitation data were collected from a weather station located 500 m from the experimental site. The monthly average precipitation and 20-year average at the experimental site are presented in Fig 3.1.

The treatments were factorial combinations of two cropping systems (monocropped corn and corn intercropped with annual Italian ryegrass) and three drainage practices (water table controls at 0.70 and 0.80 m from the soil surface, and conventional free outlet drainage where the drain tiles were at 1.0 m below the soil surface). Due to deep seepage and evaporation, the subirrigated WTDs were maintained, on average, at 0.70 and 0.80 m for designed WTDs of 0.50 and 0.75 m, respectively (Kaluli, 1996), in both years. Details of the research facility structure and instrumentation of water tables are reported in Tait *et al.* (1995). The resulting six treatment combinations were fertilized each year with 270 kg N ha^{-1}, the N rate resulting in maximum corn production in this area (Liang and MacKenzie, 1994a). In order to determine the specific effects of N fertilization on corn growth without subirrigation, we included two additional treatments in each block. These were the fertilizer application rate recommended for corn in this area (180 kg N ha^{-1}) (CPVQ, 1992) and zero fertilizer N, with monocrop corn and free drainage. These three treatments were included to provide a calibration, at least for free drainage, whereby the effects of intercrop competition for N could be assessed. Therefore, a total of eight treatments were arranged in a randomized complete block design with three blocks. Details of the eight treatments are given in Table 3.1. All treatments were repeated at the same plots for two years.

Each plot was 75 m x 15 m with a corn row spacing of 0.75 m. Corn (Pioneer 3921) was sown on May 27 in 1993 and May 31 in 1994, resulting in populations of 63,000 and 71,000 plants per ha^{-1}, respectively. The difference in corn plant density in the two years was due to wet conditions at seeding time in 1993, leading to low

germination as a result of soil compaction. Annual Italian ryegrass (*Lolium multiflorum* Lam. *cv.* Barmultra) was sown between corn rows in the intercropping plots using a forage planter (Brillion Model SS60-01, Brillion Iron Works Inc. Brillion, U.S.A) at a rate of 28 kg ha^{-1} on June 6 in 1993 and June 9, 1994. At planting, the 0 N fertilizer plots received 52 kg P as 0-46-0 (N-P-K) and all other plots received 47 kg N ha^{-1} as 18-46-0 (N-P-K). Two weeks after planting, 133 kg N ha^{-1} and 223 kg N ha^{-1} as NH_4NO_3 (34-0-0) were applied to the 180 and 270 kg N ha^{-1} fertilizer treatments, respectively.

Herbicide (atrazine: 2-chloro-4-ethylamino-6-isopropylamino-1,3,5 triazine) was applied to the monocrop plots at a rate of 1.52 kg a.i. ha^{-1} in both years. In 1994, 1.1 kg a.i. ha^{-1} Basagran (bentazon) [(3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide)] were applied to the intercropped plots to control broad-leaf weeds. In 1993, ragweed (*Ambrosia artemisiifolia* L.) constituted the major weed in intercropped plots while barnyard grass (*Echinochloa crusgalli* L.) dominated in 1994. All plant residues including ryegrass and weed biomass in the intercropping system were incorporated into the soil shortly after corn harvest.

3.3.2. Plant sampling and analysis

Corn plant samples were taken at four times: 1) at the six leaf stage (July 6 in 1993 and July 7 in 1994), 2) at tasseling (July 28 in 1993 and August 2 in 1994), 3) at mid grain-filling (August 28 in 1993 and August 22 in 1994) and 4) after physiological maturity (October 12 and 13 in 1993 and 1994, respectively). At the first three sampling dates, four randomly selected corn samples per plot were collected in 1993. In 1994, this was increased to 5 randomly selected plants per plot. At the final harvest, 30 randomly selected corn plants were sampled in each plot in both years. Harvested plant samples were separated into leaves, stalks and grain (when present). At the first sampling time (six leaf stage) in 1993, we did not separate the leaves and stalks. Plant samples were dried to a constant weight in a forced air dryer for determination of DM production. Dried plant tissues were sub-sampled and ground using a Wiley mill (A.H. Thomas Co., Philadelphia, PA, U.S.A.) fitted with a 2 mm screen, for subsequent

total N analysis.

Ryegrass and weeds were harvested at the same time as corn at all four sampling times. In 1993, ryegrass and weeds in each intercropping treatment were collected from two random quadrats of 0.75 m X 0.5 m at the first three sampling times, and the same three sampling areas were used at final harvest. In 1994, three 0.75 m X 0.5 m areas of samples were collected at each of four sampling times. The ryegrass and weeds were separated, dried, subsampled and ground as described above for DM production and total N analysis.

Nitrogen concentration in the plants was determined by Kjeldahl digestion (Tecator, Kjeltec 1030 autoanalyer, Sweden). Nitrogen uptake by corn (leaves, stalks and grain), ryegrass, and weed plants were calculated by multiplying the N concentration (separately for each plant tissue in the case of corn) with its corresponding DM weight at each sampling time.

Differences in DM production and N uptake among cropping systems, WTC and applied N levels were tested using ANOVA with a model appropriate to a randomized complete block design (SAS Inc., 1985). The effects of cropping system and WTC were tested without including the treatments in which N was applied at 0 and 180 kg N ha^{-1}. The effects of applied N levels were tested with free drainage, monocropped corn treatments receiving 0, 180 and 270 kg N ha^{-1}.

3.4. Results and Discussion

Precipitation levels varied between the two growing seasons (Fig 3.1). The 1993 growing season was relatively wet with accumulated rainfall between May and October being 30 mm above normal. By contrast, precipitation in the 1994 growing season (May to October) was 98 mm below normal.

3.4.1. *Response to cropping system and WTC*

3.4.1.1. DM accumulation and N uptake

Intercropping in 1993 greatly increased total aboveground DM production, leading to a higher N uptake for the intercropping than the monocropping system at all stages, while in 1994 this difference only occurred at the six leaf and at final harvest

stages (Fig 3.2c, d, g and h). By final harvest, intercropping increased the mean total DM produced by 3.1 Mg ha^{-1} (19%) and 2.7 Mg ha^{-1} (15%) in 1993 and 1994, respectively, compared to the monocropping system. Use of the intercropping system increased N uptake by 77.2 kg N ha^{-1} (27% of the total N uptake was by the ryegrass plus weeds) in 1993 and 50.7 kg N ha^{-1} (21% of total N uptake was by the ryegrass and weeds) in 1994 by final harvest, when compared with the monocropping system. These observations demonstrated that the increased intercrop biomass production not only increased overall N uptake, but also increased organic plant residual returns to the soil, thus improving soil quality (Liang and MacKenzie, 1992). These will also be a long term residual benefit in the term of increasing mineralized N in this cropping system. Yadav (1981) reported that sugarcane yield following corn intercropped with legume pigeon pea was increased by 40% over that obtained following corn alone. This occurred even though corn yields were not influenced by the intercrop.

The lower N uptake by ryegrass in 1994 than 1993 was consistent with DM production (Fig 3.4). The dry conditions in 1994 did not affect corn and weed growth, but did reduce that of ryegrass, relative to 1993. There was 79% less ryegrass DM production at final harvest in 1994 than 1993, indicating that under the conditions of our experiment, ryegrass was more sensitive to soil moisture than corn (Fig 3.4). Because corn plants are taller than ryegrass, and therefore compete more effectively for light, the development, including root development, of the ryegrass was probably restricted under intercrop conditions. Under these conditions, corn would have been the stronger competitor for soil water. Thus, when soil moisture becomes limiting, corn plants are able to capture more water for plant development. In addition, more vigorous growth of weeds relative to ryegrass at the six leaf stage (998.6 kg ha^{-1} of weeds vs 13.9 kg ha^{-1} of ryegrass) (Fig 3.4) in 1994 may also have depressed the development of ryegrass due to weed competition for nutrients and soil moisture. Weeds comprised a large proportion of harvested ground cover biomass in 1994, emphasizing the importance of weed control in intercropping systems. The results of this study also showed that ryegrass utilized more N than weeds when their DM

production was similar, suggesting that ryegrass would be a particularly good species for conservation of N, if well established in the absence of weeds (Fig 3.4).

During this study, the effects of WTC were less pronounced than those of cropping system. The lack of WTC effect in both years, was probably due to the small differences in WTD during this study. On the other hand, Kalita and Kanwar (1993) showed corn yield improvements with WTD as deep as 0.9 m.

The general pattern of DM accumulation by whole corn plants was consistent with other previously reported research on monocropped corn (Hanway, 1960). Dry matter production and N uptake by corn at any sampling time was unaffected by either cropping system or WTC over the two years (Fig 3.2a, b, e and f). The general lack of intercropping effect on corn DM and N uptake over the two years could have been due to: 1) the combination of abundant N (270 kg N ha^{-1}) and sufficient soil water (subirrigation) such that the only real competition between intercropped corn and the mixture of ryegrass and weeds was for light, which the much taller corn was likely most effective at capturing; 2) lack of strong competition among the intercrop components (corn, ryegrass and weeds) due to the establishment of ryegrass later than corn, possibly coupled with reduced growth of ryegrass in 1994. However, one exception to this lack of effect was noted: there was 7% less DM production in intercropped corn than monocropped corn plants at the mid-grain filling stage in 1993 (Fig 3.2a). We assumed that the reduction in DM production of intercropped corn at that time was the result of interspecific N competition among corn, ryegrass and weed components between the tasseling and mid-grain filling stages of corn development (Fig 3.3g, h and Fig 3.4). During this period, the lower N content in both leaves and stalks of intercropped corn indicated that there was greater degree of competition for N from ryegrass and weeds than at other samplings. However, this lower DM production by intercropped corn at the mid-grain filling in 1993 compared with monocropped corn did not result in decreased final corn N uptake.

Averaged over the two years, the maximum N accumulation (235 kg ha^{-1}) by corn in this study was reached at mid-grain filling (Fig 3.2g, h). Wetselarr and

Farquhar (1980) indicated that the total amount of N in aboveground biomass (grain plus stover) of annual grain crops reaches a maximum before maturity, often followed by a subsequent decline. Substantial post-anthesis N losses have been reported for wheat, ranging from 15 to 80 kg N ha^{-1} (Daigger *et al.*, 1976; Papakosta and Gagianas, 1991). Francis *et al.* (1993) found that 7 to 34 kg N ha^{-1} of labelled N was lost from aboveground corn plant tissues when the plants were fertilized with 50 to 300 kg N ha^{-1}. It has been suggested that this loss of N from the aboveground biomass was due to the N volatilization from foliage, particularly at high N fertilizer levels; this decline occurs largely after the reproductive stage (Terman and Allen, 1974; Crasswell and Martin, 1975; Stutte and Weiland, 1978). Assessing volatilization N losses by corn plants may be important in understanding N efficiency in cropping systems.

3.4.1.2. DM distribution and N allocation within corn plants

The allocation of DM and N among different parts of the corn plant was not the same in monocropped and intercropped corn for the two years of this study (Fig 3.3). The effects of cropping system on these variables occurred more frequently at tasseling and mid-grain filling, and were more pronounced in 1993 than in 1994. Intercropping corn with ryegrass did not alter the general pattern of corn DM accumulation and N allocation with time (Fig 3.3). In 1994, the effect of cropping system was more pronounced for DM production and N in leaves than in stalks.

At tasseling in 1993, the lower N content in both leaves and stalks in intercropped corn did not result in lower DM production, indicating that intercropped corn was more efficient in utilizing N. At mid-grain filling in 1993, DM production in corn leaves and stalks of the intercropped corn were both lower than those of monocropped corn, resulting in a reduction in total corn DM production (Fig 3.2a). Nitrogen contents in leaves or stalks of monocropped corn were also higher than those of intercropped corn at this time. This result indicated that the ryegrass and weeds were more competitive for resources (especially N) at this stage. The competition for N at tasseling was also evident for corn intercropped with cowpea (Francis and Stern, 1986). It seems probable that the peak demand on the environment for growth-limiting

factors (e.g N) by intercrop ryegrass plus weeds occurred at the same time as peak demand was reached by corn; the latter being the dominant component of the intercrop system.

At harvest, corn grain yield was lower in 1993 than 1994, probably due to the lower population in 1993. The yield of intercropped corn was not affected by cropping system or WTC although DM accumulation in corn leaves and stalks was reduced by the presence of ryegrass and weeds at some growth stages in both years. Competition among corn, ryegrass and weeds was apparently not sufficient to cause a reduction in grain yield. It is probable that the abundant supplies of N and water alleviated or at least minimized interspecific competition among plants. Similar effects on corn yield were reported for corn intercropped with soybean or peanuts (Searle *et al.*, 1981).

3.4.2. Response to N

3.4.2.1. DM accumulation and N uptake

Differences in DM production and N accumulation between applied N treatments and the 0 N treatment did not occur at the earliest measured growth stage (the six leaf stage), indicating that there was a minimal N requirement for corn growth at this stage (Fig 3.5). The DM production and N uptake by monocropped corn at the 0 N treatments through all corn growth stages suggested a greater degrees of N stress for 0 N plots in 1994 than in 1993. This was probably responsible for the reduction of in corn DM production in these plots in 1994 relative to 1993 (Fig 3.5).

Dry matter production and N uptake were much greater for treatments which received N fertilizer than for the 0 N treatment at tasseling, indicating that N was limiting to plant growth for the 0 N treatment. There were no differences in DM production or N uptake for the applied N rates of 180 and 270 kg N ha^{-1} at any sampling date in either year. Therefore, if the applied N exceeds the recommended N rate of 180 kg N ha^{-1} for monocropped corn, it will result in a lower N recovery, which may lead to increased N accumulation in the soil profile after corn harvest. In terms of N use efficiency (yield divided by the amount of applied N), when 180 kg N ha^{-1} were applied, 67% was recovered in the monocrop corn at harvest while an application of

270 kg N ha^{-1} resulted in recovery of only 48%, averaged over the two years of this study. The highest N rate (270 kg ha^{-1}) was used, as it has been shown to produce maxim grain yields (Liang and MacKenzie, 1994a), and would minimize competition for N between the ryegrass component and the corn, allowing the system to maintain corn grain yield levels. However, the continuous application of high N rates for corn production can lead to the accumulation more N in the soil profile after crop harvest. In the fall of 1993, FIN_{270} accumulated 28% less N in the top 1 m of the soil profile than FMN_{180} (the recommend N rate treatment) (97.5 kg N ha^{-1} vs 134.9 kg N ha^{-1}). By the following fall, FIN_{270} accumulated over 50% more N in the top 1 m of the soil profile than FMN_{180}. Theses results suggest that use of a corn-ryegrass intercrop reduces soil N accumulation after crop harvest, but not sufficiently to overcome the increased accumulation of soil NO_3^--N due to consecutive applications of N levels well above those recommended in the second season.

3.4.2.2. DM distribution and N allocation within corn plants

The applied N levels greatly affected allocation of plant N and DM to each plant part (Fig 3.6), but did not change the pattern of DM production and N accumulation in plant parts with time. Dry matter distribution or N uptake in the different tissues of monocropped corn did not differ between 180 kg N ha^{-1} and 270 kg N ha^{-1} while the 0 N treatment accumulated less DM and N, than treatments receiving N fertilizer.

Nitrogen in corn leaves declined rapidly after tasseling, and leaf weight showed a similar trend. Conversely, both DM production and N accumulation in corn stalks decreased after tasseling while the grain weight increased linearly from mid-grain filling to harvest time, indicating that corn stalks may play an important role in supplying assimilates to grain. The net accumulation of sugars by the corn stems occurs over a short period of 2-3 weeks and the amount of sugar accumulated is substantial, sometimes equivalent to 12 to 25% of final grain dry matter (Daynard *et al.*, 1969).

3.5. Conclusions

This study showed that an intercrop of corn and ryegrass increased N uptake

from soil. We have shown that during corn development, aboveground DM production and N uptake in the intercropping system was greater than in the corn monocropping system due to a greater DM production per unit land area by the intercrop. The cropping system altered the pattern of DM distribution and N allocation within corn plant parts. Competition among corn, ryegrass and weeds at some growth stages resulted in no reduction of corn grain yield at harvest due to intercropping. Intercropping can provide more crop residues into the soil than corn monocropping. Eventual decomposition of the greater crop residues from an intercropping system will result in the release of mineralized N, benefiting both crops and soil in the long term. Production of corn intercropped with annual ryegrass reduces soil N accumulation after crop harvest. However, intercropping does not completely mitigate the increased soil NO_3^--N levels resulting from the consecutive application of high N fertilizer levels substantially above the recommended values. Water table controls had no effect on DM production and N allocation. Addition of N above the recommended rate led to low proportional N recovery. Finally, we showed that the establishment of ryegrass greatly affected N uptake by the intercropping system and that besides N levels, environmental factors (such as precipitation) can limit DM production and N uptake by ryegrass.

Table 3.1 Description of experimental treatments (1993-1994).

Treatments	Water table depth	cropping systems	N levels
FMN_0	free drainage	monocropping	0
FMN_{180}	free drainage	monocropping	180
FMN_{270}	free drainage	monocropping	270
FIN_{270}	free drainage	intercropping	270
$S_{70}MN_{270}$	70 cm	monocropping	270
$S_{70}IN_{270}$	70 cm	intercropping	270
$S_{80}MN_{270}$	80 cm	monocropping	270
$S_{80}IN_{270}$	80 cm	intercropping	270

Fig 3.1. Monthly precipitation during 1993-1994 and 20-year average.

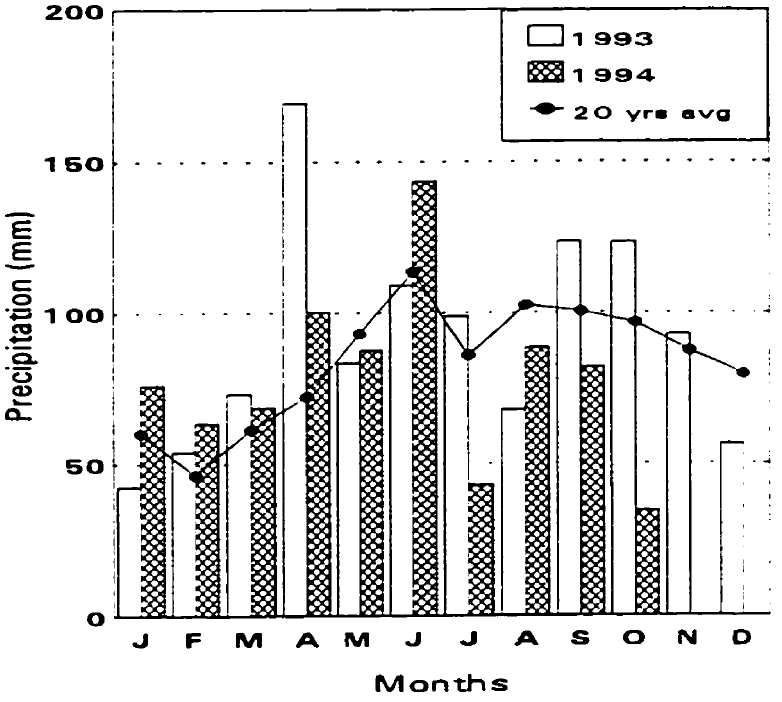

200
150
100
50
0
Precipitation (mm)
1993
1994
20 yrs avg
J F M A M J J A S O N D
Months

Fig 3.2. The effect of cropping system on dry matter production and N uptake at each sampling time (the same letters indicate that no difference within the sampling time due to the effect of cropping system was found at the 0.05 probability level; ns = no significant difference).

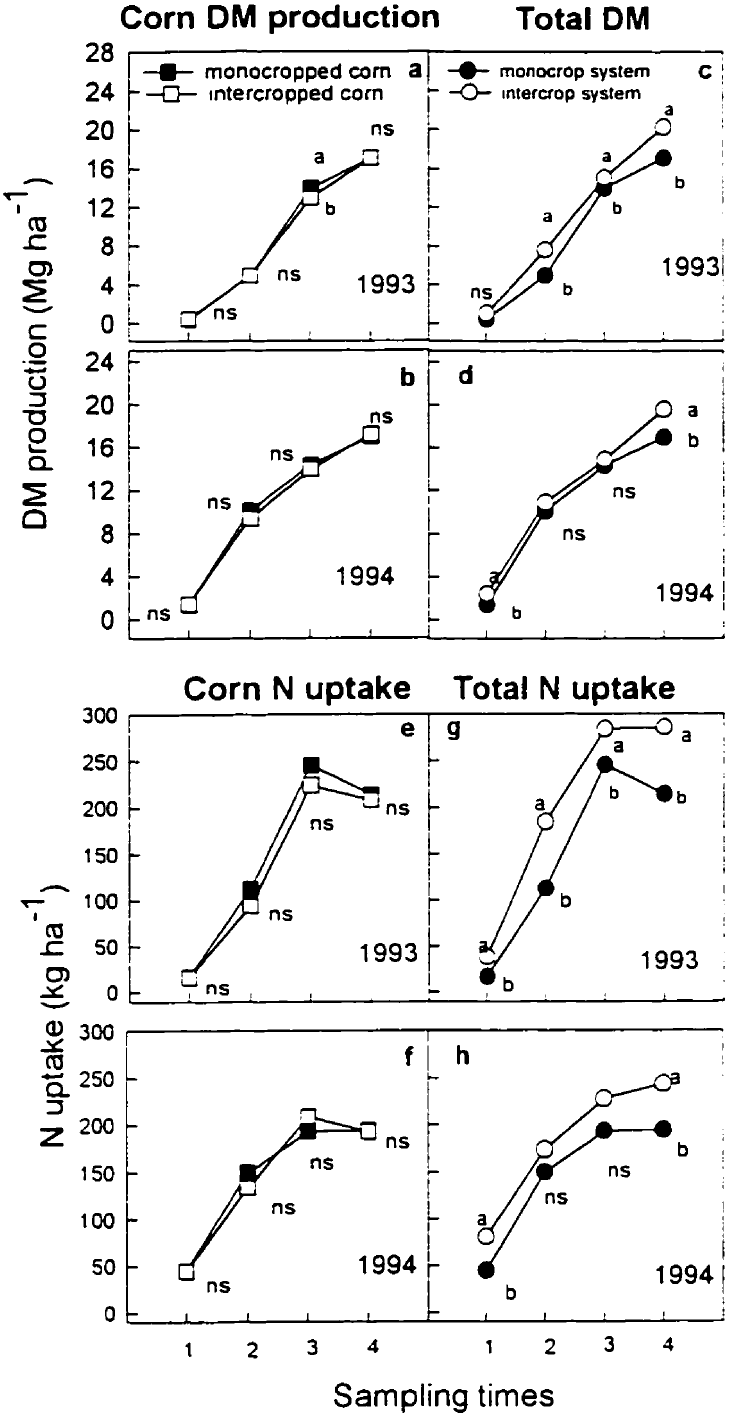

Corn DM production
Total DM
monocropped corn
intercropped corn
monocrop system
intercrop system
a
b
c
d
1993
1994
DM production (Mg ha-1)
Corn N uptake
Total N uptake
e
f
g
h
N uptake (kg ha-1)
Sampling times
ns

Fig 3.3. The effect of cropping system on dry matter distribution and N allocation at each sampling time (the same letter indicates a lack of difference within sampling times due to cropping system at a 0.05 probability level; ns = no significant difference).

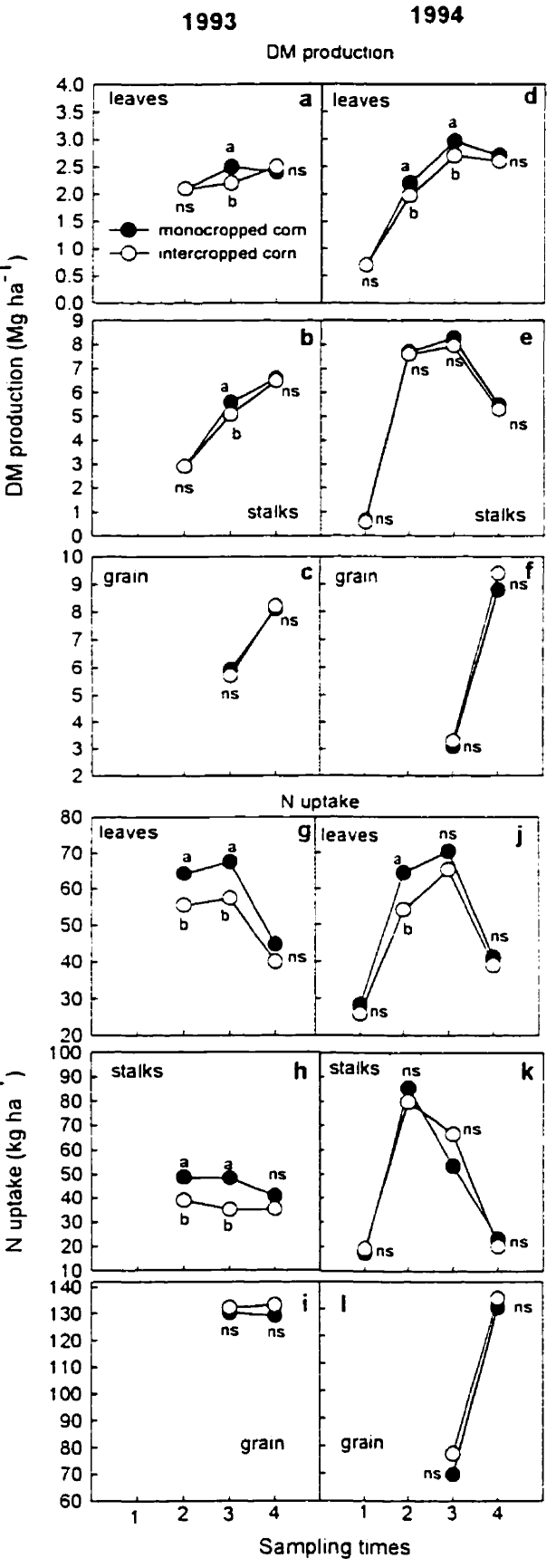

1993
1994
DM production
leaves
a
leaves
d
monocropped corn
intercropped corn
b
stalks
e
stalks
grain
c
grain
f
N uptake
leaves
g
leaves
j
stalks
h
stalks
k
grain
i
grain
l
DM production (Mg ha-1)
N uptake (kg ha-1)
Sampling times

Fig 3.4 Dry matter production and N uptake by ryegrass and weeds at each sampling time in the 1993-1994 growing seasons.

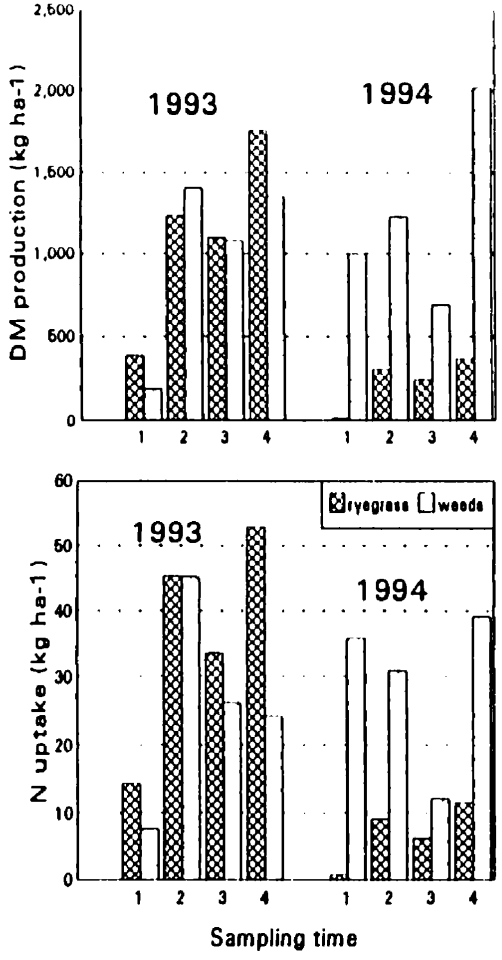

DM production (kg ha-1)
2,500
2,000
1,500
1,000
500
0
1993
1994
1 2 3 4 1 2 3 4
N uptake (kg ha-1)
60
50
40
30
20
10
0
ryegrass weeds
1993
1994
1 2 3 4 1 2 3 4
Sampling time

Fig 3.5. The effect of applied N rates on DM production and N uptake at each sampling time (the same letter indicates that no difference within the sampling time due to applied N rates was found at the 0.05 probability level; ns = no significant difference).

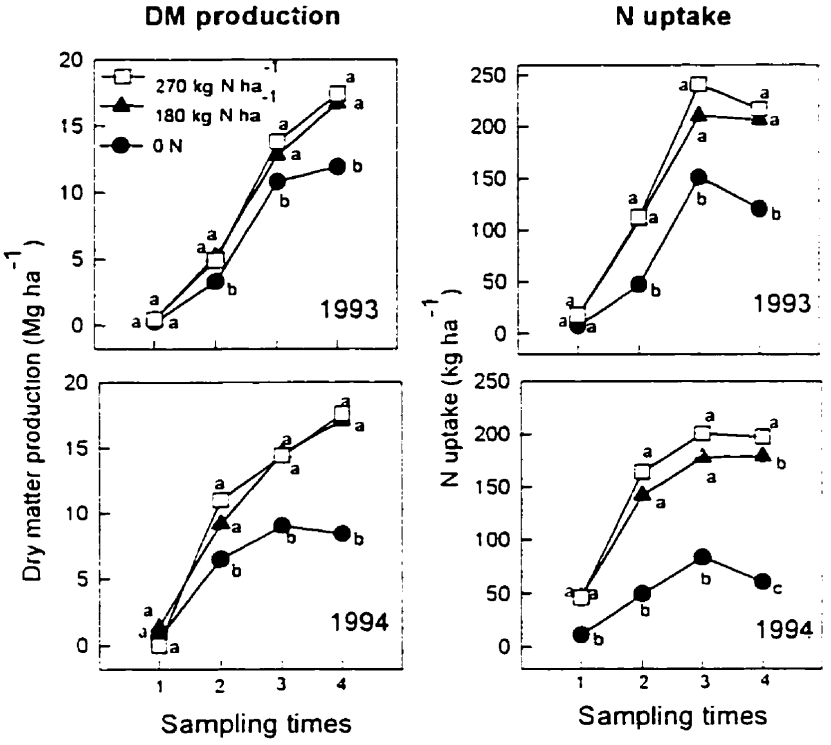
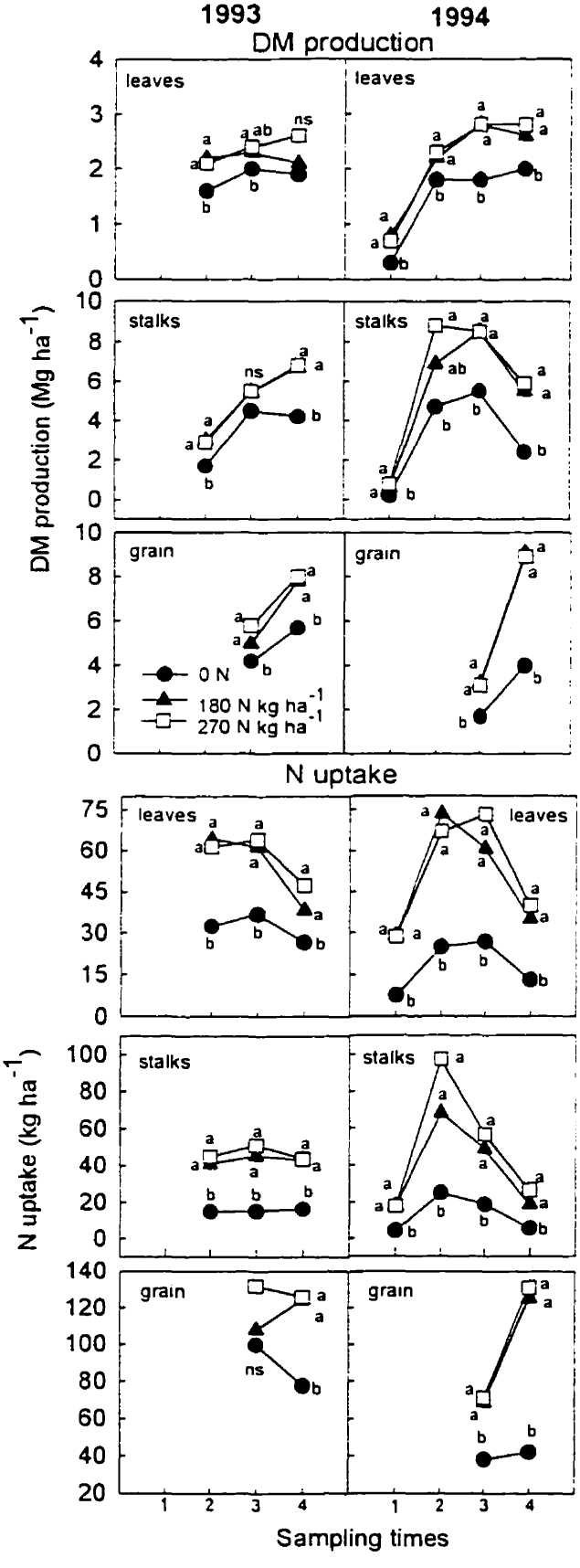

DM production
N uptake
270 kg N ha-1
180 kg N ha-1
0 N
Dry matter production (Mg ha-1)
N uptake (kg ha-1)
1993
1994
Sampling times
Sampling times

Fig 3.6. The effect of applied N rates on dry matter distribution and N allocation at each sampling time (the same letter indicates that no difference within the sampling time due to cropping system was found at the 0.05 probability level; ns = not significant difference).


1993
1994
DM production
leaves
stalks
grain
0 N
180 N kg ha-1
270 N kg ha-1
N uptake
DM production (Mg ha-1)
N uptake (kg ha-1)
Sampling times

Preface to Chapter 4

Chapter 4 is comprised of materials contained in a manuscript by myself, my supervisor Dr. D.L Smith and three other co-authors, Dr. C.A. Madramootoo, Dr. A.F. MacKenzie, and Dr. J.W. Kaluli, submitted to the *Journal of Environmental Quality* for publication in 1997. The format has been changed to be consistent with this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

Following discussion of corn growth patterns and dynamics in monocrop and an intercrop systems with ryegrass, as affected by water table managements in chapter 3, final corn grain yield, ground cover production, N uptake and fertilizer N use efficiency, as affected by the combination of the cropping system and controlled drainage, are investigated in this chapter.

Chapter 4

Water Table Control and Intercropping to Minimize Soil NO_3-N Accumulation and Leaching Under Corn I. Corn Yield, Dry matter Production, N Uptake, and N Use Efficiency

4.1. Abstract

Concern about NO_3^- leaching and groundwater pollution from corn monoculture has prompted investigation of alternative production systems which reduce leaching. It is hypothesized that both intercropping and water table control could increase N uptake after corn harvest. This study compared corn (*Zea mays* L.)-annual Italian ryegrass (*Lolium multiflorum* Lam) intercrop and corn monocrop systems under three controlled drainage levels (free drainage, and water table depths via subirrigation maintained at 0.70 or 0.80 m from the soil surface). Corn yield, aboveground dry matter production, and soil N utilization were assessed over two years on fine, silty, mixed, nonacid, frigid Typic Humaquept sandy loam soil. Corn grain yield and N uptake increased linearly with applied N in 1993, and quadratically in 1994. Corn grain yields were not affected by the corn-annual ryegrass intercrop with various water table depths in either year. The intercropping system at harvest increased total aboveground N uptake by 77.2 and 50.7 kg ha^{-1} when compared with monocropping in 1993 and 1994, respectively. Fertilizer nitrogen recovery (FNR) was higher in the monocropped corn grain and stover than the intercropped corn. Water table depths had less effect on grain yield and N uptake than cropping system. Precipitation during the growing seasons may also have been a limitation to N conservation by annual ryegrass. This study indicated that a corn-annual ryegrass intercrop system is an effective practice for increasing soil N uptake without reduction of corn grain yield when adequate N and moisture are present.

4.2. Introduction

There is growing concern that leaching of NO_3^--N from soil used for monoculture corn production constitutes a major source of NO_3^--N pollution of groundwater (Neilson and MacKenzie, 1977; Martel and MacKenzie, 1980; Liang *et*

al., 1991). It has been estimated that 30% of the N fertilizer applied in Québec is lost, and may make its way to groundwater and waterways via leaching and surface runoff (Neilson and MacKenzie, 1977). In Québec, Madramootoo *et al.* (1992) measured NO_3^--N concentrations of 45 mg L^{-1} in tile-drained potato fields. The U.S. Environmental Protection Agency safe drinking water limit is 10 mg L^{-1} NO_3^--N. The adverse health and environmental impacts of NO_3^--N contaminated groundwater make it imperative to determine NO_3^--N leaching losses from cropland, and to investigate crop production practices which could reduce leaching.

The objectives of producing crops in intercropping systems range from its higher land productivity (Ebelhar *et al.*, 1984; Martin *et al.*, 1990; Weil and Mcfadden, 1990), to providing green manure or cover crop biomass for soil enrichment and conservation. More recently in North America, intercropping cereals (mainly corn and sorghum) with legumes has been considered as a possible way to maintain high productivity while improving soil fertility and structure (Allen and Obura, 1983; Ebelhar *et al.*, 1984). In Europe, intercropping has also been shown to reduce NO_3^--N leaching by increasing plant N uptake (Juergens-Gschwind, 1989). Increasing N uptake by plants will aid in improving the efficiency of fertilizer N utilization (Olson and Kurtz, 1982).

Grass species have been shown to be very effective in reducing NO_3^--N leaching (MacLean, 1977; Steenvoorden, 1989). Annual Italian ryegrass (*Lolium multiflorum* Lam), with its high dry matter production and extensive root system, can increase soil organic matter, improve soil structure, reduce soil erosion, and decrease the loss of NO_3^--N through leaching, by absorbing soil NO_3^--N and incorporating it into organic matter (Schery, 1961; Musser and Pekins, 1969; Kunelius and Veinot, 1984; Bergstrom, 1986; Groffman *et al.*, 1987). The ability of ryegrass to absorb and recycle NO_3^--N can be exploited in corn production to decrease soil NO_3^--N and reduce leaching of soil NO_3^--N (Claude, 1990).

Kurtz *et al.* (1952) suggested that competition in corn-intercrop systems is essentially competition for N and water, and high corn yields could be achieved

regardless of the extensive growth of intercrop components if sufficient amounts of these two resources were available. Researchers have found that corn yield did not decrease when corn was intercropped with legumes or grass species such as rye and ryegrass (Chang and Shibles, 1985; Scott *et al.*, 1987). In an experiment with sweet corn, intercropped with white clover (*Trifolium repens* L.), ladino clover (*T. repens* L. forma *lodigense* Hort. ex Gams.) and alfalfa (*Medicago sativa* L.) seeded at corn planting time or 4 weeks later, intercropped corn yields were comparable to monocropped corn (Vrabel, 1981). However, Nordquist and Wicks (1974) reported that corn dry matter was reduced by up to 47% and yield reductions up to 31% when alfalfa was interseeded at corn planting. Allen and Obura (1983) suggested that the competition for N was responsible for lower corn yields within an intercropping system.

Water table control has been recommended as a management practice to reduce NO_3^--N pollution from agricultural land and increase crop yield (Kalita and Kanwar, 1993; Madramootoo *et al.*, 1993). Research by Evans *et al.* (1989) has shown that controlled water table depths reduced N and P losses from a field by 47 and 44%, respectively, compared with conventional drainage. Studies by Meek *et al.* (1970) reported reductions of soil NO_3^--N of up to 50% through water table control, due to denitrification. A water table depth as shallow as 0.4 m was found to reduce overall NO_3^--N levels in the soil profile by up to 50%, and to increase soybean yield by approximately 20%, compared with conventional, free-outlet drainage (Madramootoo *et al.*, 1993). Kalita and Kanwar (1992) reported that, under controlled water table depths, a water table depth from 0.6 to 1 m increased corn yield and soil NO_3^--N levels. On the other hand, Chaudhary *et al.* (1975) concluded that corn response to water table depths varied with rainfall during the growing season. They found that the deeper the water table the greater the corn grain yield under wet conditions while grain yield was increased by shallow water tables under dry conditions.

The most effective way of reducing NO_3^--N leaching from soil is to reduce the N application rate. However, reductions of applied N can result in corn yield

reductions. Therefore, the best way to reduce leaching losses is to improve N use efficiency. A study by Miller and MacKenzie (1978) found that between 30 to 60% of soil applied fertilizer N was recovered under Québec conditions. The determination of an optimum N rate for maximum corn production and increased ground cover production by intercropping will increase N use efficiency and minimize potential leaching losses.

No previously reported work has evaluated the combination of both intercropping and water table controls as a method of reducing NO_3^--N in the soil profile without decreasing corn yield. In this study, the term intercropping refers to the practice of seeding annual Italian ryegrass between corn rows and plowing the corn stover and ryegrass residues into the soil after corn harvest. Our objectives in this work were to: i) compare corn yield, uptake of soil N and N use efficiency as affected by an annual Italian ryegrass intercrop component and controlled water table depths (via subirrigation) under conditions of sufficient N supply; and ii) assess monocropped corn yield and N uptake response to N fertilizer levels under soil and climatic conditions for southwestern Québec.

4.3. Materials and Methods

4.3.1. Field conditions

An experiment was conducted during the 1993 and 1994 growing seasons, in Soulanges County, Québec, Canada. The field had been used for corn production for two years prior to the experiment. The soil was a Soulanges very fine sandy loam (fine, silty, mixed, nonacid, frigid Typic Humaquept). This soil type extends to about 0.5 m, and is underlain by a layer of clay loam and clay. The field slope was approximately 0.5%.

Treatments included two cropping systems (monocrop corn and a corn-annual Italian ryegrass intercrop), and three water table controls (conventional, free-outlet subsurface drainage and two water table levels with average water tables depths of 0.70 and 0.80 m below the soil surface). The average water table depth of the free drainage treatments over the two growing seasons of this study was 1.0 m. The controlled water

table depths had originally been set for 50 and 75 cm, but due to deep seepage, the water table depths could be maintained only at 70 and 80 cm, respectively. The factorial combination of cropping system and water table depth resulted in six treatment combinations which were fertilized annually with 270 kg N ha^{-1}, an maximum N rate (Liang and MacKenzie, 1994a) in this area. Each block had two additional monocrop corn plots with conventional free-outlet drainage. Of these two plots, one received no N fertilizer and the other received 180 kg N ha^{-1}. The design of this experiment was a randomized complete block with three blocks (see Table 3.1 in chapter 3).

Each plot had a surface area of 1125 m^2 (15 by 75 m) and contained a total of 20 corn rows, with 0.75 m between adjacent rows. Corn hybrid Pioneer 3921 was planted in the last week of May 1993 and 1994. The population densities were 63,000 and 71,000 plants ha^{-1} in 1993 and 1994, respectively. The lower corn density in 1993 was caused by soil compaction due to the late arrival of spring planting conditions, which resulted in seeding into very wet soil. One week after corn planting, annual Italian ryegrass (*Lolium multiflorum* Lam. *cv.* Barmultra) was sown between corn rows of intercrop treatment plots at approximately 28 kg ha^{-1} using a forage seeder (Brillion model SS60-01, Brillion Iron Works Inc, Brillion, U.S.A).

In both years, monocrop corn plots were treated with 4.0 L ha^{-1} atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5 triazine). Herbicide was not applied to intercropped treatments in 1993. In 1994, however, 2.25 L Basagran (bentazon) ha^{-1} (3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) was applied to the intercrop treatment in order to control broad-leaf weeds. Ragweed (*Ambrosia artemisiifolia* L.) was the dominant weed in the intercropping plots in 1993, while barnyard grass (*Echinochloa crusgalli* L.) was the major weed in 1994.

Precipitation data were collected at a weather station 500 m away from the experimental site. During the 1993 growing season (May to October), precipitation was 30 mm above the 20-year average. By comparison precipitation, the 1994 growing season was 98 mm below the 20-year average (see Fig 3.1 in chapter 3).

4.3.2. Water table control and fertilizer application

Two buildings (each 5 m by 5 m) were constructed at the site for water table control, to monitor drainage water flow, and to collect drainage water samples. A subsurface drainage lateral (76 mm i.d.) was centrally located with 0.3% slope along the length of each plot at a depth of 1 m, in the same direction as the natural drainage. The downstream end of each drainage lateral was connected to a 5 m long non-perforated PVC pipe with a 51 mm diameter. These non-perforated PVC pipes then entered the instrument buildings. Each plot was separated by a double-thickness, 6-mil polyethylene plastic sheet buried vertically below the soil surface to a depth of 1.5 m. The purpose of this plastic sheet was to reduce intra-plot seepage. A complete description of the entire water management research facility can be found in Tait *et al.* (1995).

In both years, 141 kg K ha^{-1} as K_2O were applied prior to planting in all plots. At planting, the 0 N fertilizer plots received 52 kg P ha^{-1} as 0-46-0 (N-P-K) while all other plots received 47 kg N ha^{-1} and 52 kg P ha^{-1} as 18-46-0 (N-P-K). In order to establish the 180 and 270 kg N ha^{-1} N rates, 133 kg N ha^{-1} and 223 kg N ha^{-1} as 34-0-0 (NH_4NO_3) were applied to the 180 and 270 kg N ha^{-1} fertilizer plots, respectively, two weeks after planting.

4.3.4. Plant sampling and analyses

Corn was harvested in the second week of October in both years. Ten corn plants were randomly chosen at three subsampling sites within each plot, and hand-harvested. The remaining corn plants in each plot were harvested for grain with a combine. Corn stover (leaves plus stalks) was plowed (moldboard plow, incorporation to 20 cm) into the field shortly after harvest. Corn plant subsamples harvested from each plot were used to determine grain yield and dry matter production. The harvested plants were separated into leaves, stalks and ears, then weighed, chopped, subsampled, air dried, and finally weighed again. Final grain yields in both years were expressed at 0% moisture. Dried composite subsamples of grain, leaf and stalk samples were ground with a Wiley mill (A.H. Thomas Co., Philadelphia, PA, U.S.A) to pass through a 2 mm sieve for plant tissue and grain N determinations.

In order to allow estimation of total above-ground N uptake, three areas (0.75m by 0.5 m) of intercrop ryegrass and weeds were harvested in the intercropped plots at the same time as the corn was harvested in both years. The ryegrass and weeds were separated and dried to a constant weight in a forced-air dryer, subsampled and ground as described above for estimation of dry matter production and total N content.

Total plant N was determined by Kjeldahl analysis (Tecator, Kjeltec 1030 auto analyzer, Höganäs, Sweden). Plant tissue samples (approximately 0.5 g) were digested for 90 min at 420 °C in 12 mL of concentrated H_2SO_4 containing 6.8 g catalyst (3.5 g K_2SO_4 per 3.5 mg Se). Digested samples were then distilled and titrated. Nitrogen uptake levels by corn, ryegrass and weeds were determined from the total N concentration of plant tissues and dry weights at the end of each season.

In order to detect the fate of applied fertilizer N, ^{15}N microplots were established in the plots with N fertilized at 270 kg N ha^{-1} in two blocks in both years. Before N fertilizer application in 1993, two locations on opposite sides of each plot, were chosen randomly from five middle rows for establishment of ^{15}N microplots for the 1993 and 1994 growing seasons. In both years, a 2.25 m by 3 m unfertilized area was marked to prevent any unlabeled N fertilizer application to the areas which were later used for ^{15}N microplots. After corn emerged, a 1.15 m by 1 m area within the remarked area was selected as the ^{15}N microplot. In order to delimit the microplot, a solid plastic border was hammered into the soil along both sides and both ends of each microplot to a depth of 20 cm. Disturbance of the soil within the microplots was minimal. A solution of $^{15}NH_4{}^{15}NO_3$ (5% atom enrichment) was sprayed evenly on the microplot surface areas on June 17, 1993 and June 16, 1994. In order to move ^{15}N fertilizer below the soil surface, an additional 2 L of water was added immediately after the ^{15}N solution. Corn in the microplots was harvested at the same time as the main plots were harvested, as three separate sampling groups: group 1 was formed by the plants at 60 cm from both ends of the microplots; group 2 was formed from two plants 40 cm from the ends of the microplot; and group 3 was formed by the two plants, 20 cm from the ends the of microplot. All harvested plant

samples were separated into leaves, stalks, and grain. Harvested samples were dried to a constant weight. Total N was determined as described above. The titration solutions from the Kjeldahl analysis were used for ^{15}N analysis (Martin *et al.*, 1991). The % ^{15}N atom enrichment in the plant samples was determined by emission spectroscopy using a JASCO N-150 ^{15}N analyzer (JASCO, LTD, Tokyo, Japan). Fertilizer nitrogen recovery (FNR) was calculated by the following equation:

$$\% \text{ N recovered} = p(c-b)/f(a-b) \times 100$$

where p is the total N in corn grain, or corn stover, or ryegrass, or weeds, f is the amount of N fertilizer applied, and a, b, and c, are the atom % ^{15}N concentrations in the fertilizer, unlabeled control plants, and labeled plants, respectively.

We found that ^{15}N enrichment of plant tissue was not affected by plant position in the microplot, and we used the % ^{15}N enrichment from the plants at the central position to calculate the N use efficiency. In order to relate the final plant growth values to the whole field, the total N values of plant tissue from outside of the microplots were used to calculate N use efficiency.

Three sampling groups of intercrop ryegrass (including weeds) were harvested in the same manner as the corn plants within the microplots. In order to obtain reliable ^{15}N recovery (Jokela and Randall, 1987), the intercrops sampled were at least 0.38 m from the end of the microplots. The sampled intercrops were separated into ryegrass and weeds, dried, weighed and ground. Total N and ^{15}N were determined as described above for corn plants.

Data were analyzed using analysis of variance with the SAS PROC GLM (General Linear Models) procedure (SAS, 1985) and single degree of freedom contrasts. Orthogonal polynomial trend comparisons were applied only for FMN_0, FMN_{180} and FMN_{270} (see Table 3.1 in chapter 3 for treatment descriptions) for the effect of fertilizer N rates. To test for cropping system and water table effects, orthogonal contrasts were employed without treatments FMN_0 and FMN_{180}. Since there was no cropping system by water table depths interaction, we do not indicate the significance level of this interaction in the tables.

4.4. Results and Discussion

4.4.1. Grain yield and total aboveground dry matter production

A linear yield response to soil added N was obtained in 1993, while for 1994 this relationship was best described by a quadratic function. Corn grain yields ranged from 5.7 to 8.5 Mg ha^{-1} in 1993 and from 4.0 to 9.9 Mg ha^{-1} in 1994 (Table 4.1). In 1994, a period of dry weather occurred in July (43 mm less precipitation than the 20 year average) (see Fig 3.1 in chapter 3). This dry period was coincident with the tasselling stage of field-grown corn in that year. Thus, the quadratic grain yield response of 1994 may have been the result of moisture stress rather than a fully exploited yield potential at the highest N rate. The N fertilizer rate for maximum grain corn yield in 1994 was calculated as 215 kg N ha^{-1}.

Grain yield and dry matter production by the 0 N control treatment in 1994 were 1.7 and 3.4 Mg ha^{-1}, respectively, less than in 1993. In the past, corn leaf N concentration has been used to help explain corn responses to fertilizer practices and management (Jones *et al.*, 1967; Voss *et al.*, 1970). We observed that corn leaf N concentration in the 0 N treatment in 1994 was approximately half of that of 1993 (6.7 vs 12.9 g kg^{-1}). In addition, nitrogen deficiency symptoms were more apparent during the early growth stages of corn plants in the 0 N plots in 1994 than in 1993. The lower tissue N concentrations in 1994 indicated a greater degree of N limitation stress for the plants of the 0 N plots in 1994 than in 1993. This may explain the decrease in corn yield and dry matter production in the 0 N plots in 1994 relative to 1993.

Corn grain yields were not affected by the presence of ryegrass in either year (Table 4.1). The grain yield was similar to the 3 year mean yield of monocrop corn in Québec (Liang and MacKenzie, 1994a). Similar results have been noted previously in intercropped with legumes (Kurtz *et al.*, 1952; Searle *et al.*, 1981). Studies by Scott *et al.* (1987) found that corn yield was not affected by the presence of underseeded intercrop components if the latter was planted when the corn was 0.15 to 0.30 m high. In our experiment, the ryegrass was planted one week later than the corn. The ryegrass started to geminate when the corn plants were about 0.30 m tall in both years.

Indigenous weeds had not fully established by that time. Therefore, the reduction in competition from the ryegrass or weeds during the early growth stages and sufficient N (270 kg N ha^{-1} applied) to minimize any competition between the corn, ryegrass or weeds for this soil nutrient, was probably responsible for this lack of reduction in corn grain yield in both years.

Nitrogen fertilization caused a linear increase in the stover dry matter biomass in both years. Intercropping had no affect on this variable. Shallow water table depths reduced stover dry matter biomass by 10% when compared with conventional drainage in 1994 ($P=0.06$). Lower stover dry matter yields were also observed in 1994 than in 1993. This suggests that the summer moisture stress observed in 1994, although not severe, limited corn stover yield.

Total biomass production by ryegrass in this study (0.3-1.9 Mg ha^{-1}) was similar to that reported by other intercrop researchers in this region (eg. Hope-Simpson, 1992). Greater total intercrop biomass was measured in 1993 than in 1994. This was primarily due to the greater biomass of ryegrass in 1993. The ryegrass did not develop well early in the 1993 season. However, one month before corn harvest, the ryegrass had achieved good establishment due to the senescence of weeds. The poor establishment of ryegrass in 1994 was probably due to its poor germination as result of wet soil conditions at planting (June) (see Fig 3.1 in chapter 3), stronger competition due to an earlier establishment of weeds relative to ryegrass, and drought stress starting in July. In this study, weeds comprised a large proportion of harvested ground cover biomass, emphasizing the importance of weed control in intercrop systems.

Total above ground dry matter production ranged from 11.8 to 21.1 Mg ha^{-1} in 1993 and 8.4 to 20.7 Mg ha^{-1} in 1994 (Table 4.1). Above ground biomass of monocropped corn under free drainage response to N was similar to that of its grain yield. Intercropping increased the mean total dry matter produced by 3.1 Mg ha^{-1} and 2.7 Mg ha^{-1} in 1993 and 1994, respectively, compared with the monocrop system.

In both years, water table depths did not affect corn grain yield, intercrop biomass, or total dry matter production. This lack of effect may have been due to the

small differences in water table depths during this study.

For treatments that received N fertilizer, grain yields were 14% higher in 1994, than in 1993 (Table 4.1). The differences between the two years may have been due to better corn emergence, resulting in greater grain yield in 1994, than in 1993. Below normal precipitation during the 1994 growing season resulted in high grain yield but low intercrop dry matter production, in particular a lower ryegrass biomass. This suggested that ryegrass growth may exhibit a greater response to soil moisture than corn.

4.4.2. Nitrogen uptake

In general, treatment effects on N accumulation in monocrop corn, intercropped corn and total aboveground cover (ryegrass plus weeds) were similar to those observed for corn grain, stover, intercrop biomass or total dry matter biomass (corn plus ryegrass plus weeds) (Table 4.2). A linear response for grain, stover and N uptake of monocropped corn to added N in 1993 suggested that N uptake was limited by fertilizer N up to 270 kg N ha^{-1}. In 1994, the quadratic response of these variables (except stover) to N fertilizer addition indicated that dry conditions that year limited grain yield potential at the high N rate.

Grain N ranged from 77.6 to 138.6 kg ha^{-1} in 1993 and 42.1 to 144.0 kg ha^{-1} in 1994. Neither water table depth nor cropping system affected corn grain N uptake. The same lack of effect of water table depth on monocropped grain N uptake was also noted by Chaudhary *et al.* (1975). The intercropping system resulted in less N (15%) in corn stover than in the monocropping system ($P=0.06$) in 1993. In contrast, corn grain N uptake was higher in the intercropping system than the monocropping system. This suggests that assimilated N was more efficiently translocated to the corn grain from the vegetative plant parts under competition pressure from other plant species.

The higher ryegrass biomass (1.4 Mg ha^{-1}) in 1993 than 1994 resulted in 41.3 kg N ha^{-1} more N accumulation in the ryegrass in 1993 than 1994. This suggests that the establishment of ryegrass after weed senescence approximately one month before harvest greatly increased N utilization by ryegrass. The lower N uptake by ryegrass in

1994 than 1993 was consistent with dry matter production. These observations demonstrated that increased intercrop growth increased N conservation.

In 1993, ryegrass and weeds comprised 19% and 8%, respectively, of the total aboveground N uptake in the intercropping treatments. Weeds dominated the ryegrass in 1994, and comprised 85% on a dry weight basis of the total weed-ryegrass mixture. At harvest, intercropping systems had taken up 77.2 and 50.7 kg N ha^{-1} more, than the monocrop systems in 1993 and 1994, respectively. These results show that the intercropping of corn with annual ryegrass is an effective practice to remove N that would otherwise be left in the soil after corn is harvested.

4.4.3. Nitrogen use efficiency

Cropping system affected the fertilizer N recovery (FNR) of corn grain and stover. On average, monocrop corn recovered 29.1 and 19.7% of applied fertilizer N in grain and stover, respectively (Table 4.3). Intercropping effects on FNR varied between the two years. In 1993, monocrop corn recovery values were 40 and 30% greater than that of intercrop corn grain and stover utilization, respectively, while in 1994 monocrop corn recovered 60 and 43% more, than intercrop corn for grain and stover, respectively. The difference in FNR between the cropping systems was expected since other plant species (ryegrass plus weeds) competed for the same nutrients in the intercropping system. The between year variations in the recovery of applied N fertilizer in the cropping systems were probably the result of soil moisture stress (see Fig 3.1 in chapter 3) and relatively higher accumulated residual soil N during the 1994 growing season. This indicated that environmental factors (eg. soil moisture) or N in the soil can be limiting to FNR.

Total aboveground FNR values were not affected by cropping system. This indicated that the corn-annual ryegrass intercrop, and monocrop corn had similar abilities to absorb the applied N fertilizer. The FNR values for cropping system did not follow the total N uptake of the plants. This result indicated that the increased total N uptake by the intercropping system was due to its high efficiency in absorbing both applied N fertilizer and mineralized organic soil N. Free drainage resulted in 16%

higher FNR values for stover than water table controls in 1993. This result may have been due to the higher denitrification in the water table control treatments than that of free drainage.

4.5. Conclusions

Intercropping corn with annual ryegrass is an effective method for conserving soil N under conditions of high fertilizer N application. Intercropping caused no reduction in corn grain yield if adequate nutrients and soil water were supplied. Later establishment of annual ryegrass (one month before corn harvest) not only reduced interspecific plant competition for available nutrients and soil moisture in the early season, but also allowed recovery of residual soil NO_3^--N late in the growing season. Fertilizer N recovery (FNR) was increased in the monocrop corn relative to intercrop corn. Intercropping had a substantially higher N use efficiency not only in applied N fertilizer but also the N realised from the soil by mineralization. Environmental factors such as soil moisture can limit the performance of the intercropping system. Water table depth had less effect on the development of corn and N uptake from the soil.

Table 4.1. Water table depths, cropping system and N fertilizer rates combination effects on dry matter production in 1993 and 1994.

	1993					1994				
Treatments	Stover	Grain	Ryegrass	Weeds	Total†	Stover	Grain	Ryegrass	Weeds	Total
	Mg ha^{-1}									
FMN_0	6.2	5.7	-	-	11.8	4.3	4.0	-	-	8.4
FMN_{180}	8.9	7.8	-	-	16.7	8.1	9.1	-	-	17.1
FMN_{270}	9.4	8.0	-	-	17.4	8.6	8.9	-	-	17.6
FIN_{270}	8.2	8.0	1.8	1.6	19.6	8.6	9.9	0.4	1.9	20.7
$S_{70}MN_{270}$	8.8	8.1	-	-	16.9	8.1	9.1	-	-	17.3
$S_{70}IN_{270}$	8.9	8.2	1.7	1.3	20.1	7.4	8.7	0.3	2.3	18.6
$S_{80}MN_{270}$	8.9	8.1	-	-	17.0	7.6	8.5	-	-	16.0
$S_{80}IN_{270}$	9.6	8.5	1.9	1.2	21.1	7.7	9.6	0.5	1.9	19.6
Contrast‡										
N rates	L	L	-	-	L	L	Q	-	-	Q
Cropping Systems										
Intercropping vs monocropping										
	NS	NS	-	-	*	NS	NS	-	-	*
Water Table Depths										
	NS	NS	NS	NS	NS	0.06	NS	NS	NS	NS

† total dry matter in the monocrop system=corn grain+stover, in the intercrop system= corn (grain+stover) + ryegrass + weeds; ‡ Orthogonal polynomial contrast significant at $P < 0.05$ (*), or not significant at 0.1 level (NS); L = linear contrast significant, Q = quadratic contrasts significant.

Table 4.2. Water table depths, cropping system, and N fertilizer rate combination effects on crop N uptake in 1993 and 1994.

	1993					1994				
Treatments	Stover	Grain	Ryegrass	Weeds	Total†	Stover	Grain	Ryegrass	Weeds	Total
	kg ha^{-1}									
FMN_0	42.7	77.6	-	-	120.3	18.4	42.1	-	-	60.6
FMN_{180}	82.1	124.4	-	-	206.5	53.7	125.5	-	-	179.2
FMN_{270}	90.9	125.8	-	-	216.7	66.3	131.1	-	-	197.4
FIN_{270}	69.4	131.8	56.0	27.9	285.0	66.0	144.0	10.5	35.5	255.9
$S_{70}MN_{270}$	79.4	131.0	-	-	210.5	61.0	135.7	-	-	196.8
$S_{70}IN_{270}$	73.2	129.1	53.2	23.2	278.6	55.1	123.0	8.5	46.1	232.7
$S_{80}MN_{270}$	85.8	130.7	-	-	216.5	65.2	124.8	-	-	189.6
$S_{80}IN_{270}$	83.4	138.6	49.6	21.7	293.4	55.8	134.6	15.7	35.9	242.3
Contrast‡										
N rates										
	L	L	-	-	L	L	Q	-	-	Q
Cropping Systems										
Intercropping vs monocropping										
	0.06	NS	-	-	*	NS	NS	-	-	*
Water Table Depths										
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† total nitrogen uptake in the monocrop system=corn grain+stover, in the intercrop system= corn (grain+stover) + ryegrass + weeds; ‡ Orthogonal polynomial contrast significant at $P < 0.05$ (*), or not significant at 0.1 level (NS); L = linear contrast significant, Q = quadratic contrasts significant.

Table 4.3. Water table depths, cropping system and N fertilizer rate combination effects on fertilizer nitrogen recovery (FNR) in 1993 and 1994.

Treatments	1993				1994			
	Stover	Grain	intercrop†	Total††	Stover	Grain	intercrop	Total
	%							
FMN_{270}	21.3	26.8	-	48.1	10.5	21.8	-	32.3
FIN_{270}	17.8	26.9	20.7	65.4	8.3	15.1	7.5	30.8
$S_{70}MN_{270}$	17.8	30.6	-	48.4	11.2	27.2	-	38.4
$S_{70}IN_{270}$	14.3	17.7	18.4	50.4	8.8	16.0	9.0	33.8
$S_{80}MN_{270}$	19.9	29.9	-	49.9	12.8	23.4	-	27.6
$S_{80}IN_{270}$	13.5	17.8	13.9	45.2	6.9	13.8	6.9	27.6
Contrast‡								
Cropping Systems								
Intercropping vs monocropping								
	*	0.06	-	NS	*	*	-	NS
Water Table Depths								
Free drainage vs water table controls								
	*	NS	NS	NS	NS	NS	NS	NS

† intercrop = ryegrass + weeds; †† total FNR = monocrop system (corn grain + stover) plus intercrop system (corn grain + stover + ryegrass + weeds); ‡ Orthogonal polynomial contrast significant at $P < 0.05$ (*), or not significant at 0.1 level (NS).

Preface to Chapter 5

Chapter 5 is comprised of materials contained in a manuscript by myself, my supervisor Dr. D.L. Smith and three other co-authors, Dr. A.F. MacKenzie, Dr. C.A. Madramootoo, and Dr. J.W Kaluli, submitted to the *Journal of Environmental Quality* for publication in 1997. The format has been changed to be consistent within this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

Following demonstration of the effects of cropping systems and controlled drainage on corn grain yield, aboveground dry matter production, N uptake and fertilizer N use efficiency in chapter 4, the soil N accumulation and distribution in the top 1 m of the soil profile in the spring and fall of this two-year study, as affected by the cropping system and controlled drainage, are addressed in this chapter.

Chapter 5

Water Table Control and Intercropping to Minimize Soil NO_3^--N Accumulation and Leaching Under Corn. II. Soil NO_3^--N Distribution and Accumulation

5.1. Abstract

Soil NO_3^--N that remains in the soil after crop harvest or produced as a result of mineralization is susceptible to leaching during the late fall and the following early spring, and therefore is a potential groundwater contaminant. A two-year study was conducted to assess soil NO_3^--N distribution and accumulation in the top 1 m of the soil profile of a Soulanges fine sandy loam soil (fine, silty, mixed nonacid, frigid Typic Humaquept). The experiment tested two factors, cropping system [monocrop corn (*Zea mays* L.), and corn intercropped with annual Italian ryegrass (*Lolium multiflorum* Lam.)] and three water table controls (free drainage, or controlled drainage via subirrigation to establish water table depths at 70 and 80 cm from the soil surface) combined in a factorial design. The resulting six treatments were fertilized annually with 270 kg N ha^{-1}. The effects of adding N fertilizer on NO_3^--N in the soil profile were also investigated under monocrop corn with free drainage. Soil cores of 1 m in depth were collected in spring and fall of 1993 and 1994. Intercropping reduced the amount of NO_3^--N in the top 1 m of the soil profile by 47% (92.3 kg N ha^{-1}) at harvest time, in 1993. Water table depth had no effect on total soil NO_3^--N accumulation in the top 1 m soil profile in the fall of 1993 and 1994. Free drainage enhanced NO_3^--N movement deeper into the soil and leaching losses over the winter season. Accumulation of NO_3^--N increased as fertilizer N levels increased in the monocrop system with free drainage, especially when N rates exceeded 180 kg N ha^{-1}. Leaching of soil NO_3^--N was detected in deeper soils with the highest N rate, and conventional free drainage. Above normal precipitation during the 1993-1994 winter season may have promoted leaching.

5.2. Introduction

The potential for NO_3^--N pollution of groundwater increases significantly when N is applied in excess of crop requirements for corn (Roth and Fox, 1990) and other

field crops (eg. Ayoub *et al.*, 1995). Nitrogen remaining in the soil profile following corn harvest is susceptible to leaching during late fall and the following spring (Chichester, 1977; Liang and MacKenzie, 1991). Nitrogen fertilizer rate, cropping system, water movement, and soil sampling time (Jokela and Randall, 1989; Macdonald *et al.*, 1989; Rochester *et al.*, 1991; Liang and MacKenzie, 1994b) have been reported to directly affect the amount of NO_3^--N accumulation in the soil profile. A study by Liang and MacKenzie (1994b) found that a large amount of N fertilizer applied at rates higher than 170 kg N ha^{-1} remained in the root zone as NO_3^--N at the time of corn harvest, but little NO_3^--N was detected in the soil profile after three years of corn production at 170 or 400 kg N ha^{-1}. They concluded that leaching played a very important role in soil NO_3^--N loss from corn production systems. Liang and MacKenzie (1994b) also reported that NO_3^--N changes in the soil profile over winter were greater with increased precipitation during the winter and with high NO_3^--N in the soil the previous fall.

Grass species have been found to be very effective in conserving fall N when used as winter cover crops (Groffman *et al.*, 1987; Meisinger *et al.*, 1991; Shipley *et al.*, 1992). Scott *et al.* (1987) demonstrated that annual ryegrass can be successfully used as an intercrop in the continuous production of corn in the northeastern United States. They reported that annual ryegrass produced 56% ground cover if interseeded when corn was 0.30 m high. The high dry matter production by annual ryegrass in an intercrop system aids in the utilization of soil N during the corn growing season (see chapter 4). Bergstrom (1986) found that annual ryegrass interseeded with barley (*Hordeum vulgare* L.) reduced with mineral N content in the top 1 m of the soil profile by 23 kg N ha^{-1} compared to plots without an intercrop.

The potential use of water table control to reduce soil NO_3^--N has been documented by other researchers (Evans *et al.*, 1989; Steenovoorden, 1989; Kalita and Kanwar, 1993; Madramootoo *et al.*, 1993). Chaudhary *et al.* (1975) suggested that a water table depth of 0.60 to 0.90 m was desirable for corn growth. However, shallow water table depth (0.2-0.3 m) reduced corn growth due to poor soil aeration and

restricted rooting volumes (Kalita and Kanwar, 1993). Denitrification is desirable when it occurs below the root zone because it reduces NO_3^--N leaching to ground water (Meek *et al.*, 1970). Raising the water table by controlled drainage may increase both denitrification and crop N uptake (Steevnoorden, 1989). At the same time high soil moisture can slow the nitrification process, hence reducing soil NO_3^--N in drainage water (Meek *et al.*, 1970).

There is no available information regarding the combination of corn intercropped with annual ryegrass and controlled water tables on soil NO_3^--N distribution and accumulation after corn harvest, and during the non-growing (over winter) season. Therefore, the objectives of this study were i) to evaluate the effect of a corn-annual Italian ryegrass intercrop system with water table controls via subirrigation on NO_3^--N accumulation and distribution in the top 1 m of the soil profile, and ii) to determine the effect of N rates on soil NO_3^--N accumulation and leaching losses of monocrop corn plots under free drainage, supplied with three levels of N fertilizer.

5.3. Materials and Methods

The instrumented field research facility was constructed on a 4.2 ha field in Soulanges County, Québec (Tait *et al.*, 1995). The soil type is a Soulanges fine sandy loam (fine, silty, mixed nonacid, frigid Typic Humaquept). The surface soil characteristics are given in (Table 5.1). The study was conducted during the 1993 and 1994 seasons. Two cropping systems [monocrop corn (*Zea mays* L.) and corn intercropped with annual Italian ryegrass (*Lolium multiflorum* Lam. *cv.* Barmultra)] and three water table controls (conventional, free outlet drainage, or water table depths maintained at 70 and 80 cm below the soil surface via subirrigation) were combined in a factorial fashion. Plots of the resulting six treatments were fertilized annually with 270 kg N ha^{-1}. In this study, water table via subirrigation could be maintained only at 70 and 80 cm for expected water table depths of 50 and 75 cm, respectively. The water table depth for conventional, free drainage was measured at 1 m in both years. In addition, to assess the effect of fertilizer N rate, one free drainage monocropped

corn plot was not fertilized in each block and another received 180 kg N ha^{-1}. Therefore, a total of eight treatments was used in this study. The treatments were arranged in a randomized complete block design with three blocks.

5.3.1. Soil Sampling and Analyses

Soils were sampled to a depth of 1 m, prior to addition of N fertilizer in the spring, and shortly after harvesting in the fall of 1993 and 1994. Three sub-soil samples were taken randomly within each plot using hydraulically-inserted aluminum sampling tubes. Each soil column was cut into four sections representing depths of 0-20, 20-50, 50-75, and 75-100 cm. Representative soil samples were taken from each section. The three sub-soil samples from the same depths were mixed thoroughly, and stored in a cold room prior to extraction for NO_3^--N and NH_4^+-N.

Nitrate-N and NH_4^+-N were extracted using a 1 M KCl solution. Approximately 15 g of fresh soil sample were shaken in 100 mL of 1 M KCl solution for about 60 minutes. The resulting suspensions were filtered and the filtrates analyzed using an autoanalyzer (Lachat Instruments, Quik Chem @AE, Milwaukee, WI, USA). Moisture contents were also determined for each depth and plot. Soil NO_3^--N and NH_4^+-N accumulation in kg ha^{-1} were calculated for each sample, using soil total NO_3^--N and NH_4^+-N concentrations, and soil bulk densities (Table 5.1) from representative soil profile samples. The differences in NO_3^--N values between spring 1994 and fall 1993 were considered as changes in NO_3^--N over winter.

5.3.2. Statistical Analysis of Results

Results were analyzed by analysis of variance using the SAS PROC GLM (General Linear Model) procedure (SAS, 1985) and single degree of freedom contrasts. Orthogonal contrasts were used to test the effects of the cropping system and the water table control at the applied N rate of 270 kg ha^{-1}. Monocropped corn under conventional drainage at the applied N rate of 0, 180 and 270 N kg ha^{-1} were used to detect the effects of fertilizer N rate. The response of soil NO_3^--N at the different sampling times to applied N fertilizer rates were subjected to a regression analysis.

5.4. Results and Discussion

Averaged over the two years, the exchangeable NH_4^+-N was less than 10% of total inorganic N at every soil depth in the soil profile. Therefore, exchangeable NH_4^+-N is not discussed in this paper.

5.4.1. Effects of cropping system and water table control

Intercropping decreased total soil N accumulation in the top 1 m of the soil profile in the fall of 1993, while there was no effect of cropping system in the fall of 1994 (Table 5.2 and Fig 5.1). The total amount of NO_3^--N in the top 1 m of the soil profile in the fall of 1993 was reduced by 47% due to intercropping. This meant an average reduction of total 92.3 kg N ha^{-1} in the top of 1 m the soil profile after corn was harvested over all water table depths. The reduction in soil NO_3^--N levels after harvest due to intercropping indicates that increased N uptake by intercropping resulted in less residual soil N (see chapter 4). Poor early ryegrass establishment and drier weather conditions in 1994 (than in 1993) resulted in lower ryegrass biomass accumulation in that year. Therefore, the ability of the ryegrass to aid in the uptake of NO_3^--N from soil in 1994 was limited by both plant establishment and weather conditions.

The effects of cropping system and water table controls on soil NO_3^--N distributions in each of the two years varied. Averaged over water table depths, decreases in N residues due to intercropping ranged from 8 to 36.2 kg N ha^{-1} depending on sampling depths in the fall of 1993 (Fig 5.1). However, use of monocropping in the fall of 1993 resulted in a substantial accumulation of soil NO_3^--N under the deeper water table (80 cm) in the 20-50 cm soil layer after corn harvest, while intercropping resulted in the least NO_3^--N accumulation. This could have led to the interaction between cropping system and water table controls ($P < 0.1$) (Table 5.2). In the fall of 1994, treatments with water table control accumulated an average of 8.5 kg N ha^{-1} less soil NO_3^--N than the free drainage treatments at a soil depth of 50-75 cm ($P < 0.1$). In the fall of 1994, monocrop corn with the water table depth of 70 cm led to the least soil NO_3^--N accumulation, while intercropping under free drainage resulted in the most

accumulation in the 20-50 cm soil layer. This caused an interaction of cropping system and water table controls (Table 5.2 and Fig 5.1).

Changes in soil NO_3^--N concentration between the fall of 1993 and the spring of 1994 were varied. In general, total soil NO_3^--N in the top 1 m of the soil profile in the spring of 1994 was remarkably lower than NO_3^--N levels measured in the previous fall (Fig 5.1). The losses ranged from 17 to 99 kg N ha^{-1}. Reductions in NO_3^--N accumulation could have been a result of leaching losses and/or denitrification since precipitation was high (606 mm) between fall crop harvest and the following spring soil sampling. Liang and MacKenzie (1994b) concluded that changes in soil NO_3^--N (0-80 cm) over winter were a function of fall NO_3^--N levels and winter precipitation. Their study showed 40 kg N ha^{-1} to be the threshold level for NO_3^--N loss when winter precipitation was 432 mm. In our study, the estimated changes in NO_3^--N over winter were much higher than the threshold level estimated by Liang and MacKenzie (1994b) (data not shown). This suggests that leaching losses over winter may be responsible for the observed reductions in soil NO_3^--N by the following spring. Most intercropping treatments showed soil NO_3^--N reductions by the following spring, compared with the previous fall. The above normal precipitation or immobilization may have been responsible for the losses.

Compared with the water table control treatments, the free drainage treatments in the spring of 1994 had 10.7 kg N ha^{-1} less NO_3^--N accumulation in the 20 to 50 cm soil layer. The 40% increase in NO_3^--N accumulation in deeper soil layers under free drainage indicated that increasing water table depth enhanced NO_3^--N movement deeper into the soil and early spring leaching losses. Kaluli (1996) also found that the free drainage treatments had the highest NO_3^--N concentrations in drainage water. Intercropping with conventional, free-outlet drainage resulted in the least soil NO_3^--N carryover in the spring of 1994, while monocropping increased the soil NO_3^--N content in the below 50 cm soil layer. This caused a cropping system by water table depth interaction (Table 5.2). The lower NO_3^--N carryover under intercropped corn with free drainage were probably due to immobilization or residual effect of intercropping from

the previous fall.

5.4.2. Effect of applied N fertilizer rates

During the two year study period, addition of N fertilizer linearly increased the amount of NO_3^--N at each depth for monocropping corn with conventional free-outlet drainage, except in the 75 cm to 1 m soil layer in the fall 1993, the 20 to 50 cm soil layer in the spring of 1994, and 20 to 50 cm soil layer in the fall of 1994 (Table 5.2 and Fig 5.2). This N rate effect in the fall of 1994 was only significant at 0.1 level. These results suggest that conventional monocrop corn production with free drainage and added N fertilizer rates above the 180 kg N ha^{-1} resulted in high soil N residues in the fall. The potential for high soil N residues present in the fall to leach out of the root zone is high during the non-growing season, if precipitation (between crop harvest and the following spring) was above average (Liang and MacKenzie, 1994b).

Over the two years, most NO_3^--N in the fall accumulated between 0 and 50 cm for both applied N rates (Fig 5.2). This N accounted for 73% of total NO_3^--N accumulation in the top 1 m of the soil profile in 1993 and 47% in the fall of 1994. Compared with the spring of 1993, the increases in NO_3^--N in the deeper portion of the soil profile in the fall of 1993 for plots where N fertilizer had been applied, showed that leaching had occurred during the growing season. In the fall of 1994, soil NO_3^--N levels in the top 1 m of the soil profile varied from 3.6 to 43.7 kg N ha^{-1}, but the values were lower in the spring of 1994. Leaching losses during the 1994 growing season were less pronounced due to the relatively dry conditions (see Fig 3.1 in chapter 3). The lower NO_3^--N value in the fall of 1994 could have been due to plant N uptake, denitrification or immobilization.

Total NO_3^--N accumulations in the top 1 m of the soil profile, averaged over all N treatments, were 79.3, 126.0, 102.0 and 61.0 kg N ha^{-1} for the spring of 1993, fall of 1993, spring of 1994 and fall of 1994, respectively. The following linear regression equations describe the total NO_3^--N accumulation in the top 1 m of the soil profile as the function of applied N fertilizer rates, for the three sampling times: $Y_1=63.3 + 0.42N_1$ ($R^2=0.66^{**}$); $Y_2=46.5 + 0.37N_1$ ($R^2=0.45^{*}$); $Y_3=28.6 + 0.22N_2$ ($R^2=0.41$,

$P < 0.1$). Where, N_1 represents fertilizer N rates in spring of 1993, and N_2 the N fertilizer rates in spring of 1994. Y_1, Y_2 and Y_3 represent the total amount of soil NO_3^--N in the top 1 m of the soil profile in fall of 1993, the spring of 1994 and the fall of 1994, respectively. Based on the regression models, total soil NO_3^--N accumulation decreased with time. The increased NH_4-N in the spring and fall of 1994 could explain the low NO_3^--N in the soil profile (data not shown). In fall of 1994, the correlation between applied N and soil NO_3^--N was weaker than in the fall of 1993. Weather conditions were drier in 1994 than 1993, and this may account for the lower correlation observed in 1994.

Residual fertilizer N in the fall of 1993 had no effect on NO_3^--N content in the top soil layer (0-20 cm) in the spring of 1994 (Table 5.2). Residual N in the top 1 m of the soil profile in the spring of 1994 was 20% less than the residual N in the previous fall. The decrease in NO_3^--N at a soil depth of 50 cm and the increase in NO_3^--N at the 75 cm soil depth in the spring of 1994 indicated downward movement of soil NO_3^--N. We found that the greatest NO_3^--N loss occurred when the highest N rate was applied. Liang and MacKenzie (1994) found high potential for over winter NO_3^--N leaching when soil NO_3^--N residues were high the previous fall. Leaching of soil NO_3^--N during the winter could have accounted for the observed decrease in soil NO_3^--N. In the fall of 1994, soil N was less in plots that received 180 kg N ha^{-1} than those that received 270 kg N ha^{-1}, probably as the result of lower N inputs. Based on this observation the recommended N rate (180 kg N ha^{-1}) may have minimized ground water contamination through leaching for monocropped corn under conventional drainage.

Liang and MacKenzie (1994b) found that soil NO_3^--N accumulated between 0 to 60 cm in the soil profile when N fertilizer was applied at levels above the recommended for the area: 170 kg N ha^{-1}. We found that large amounts of N had accumulated in the top layer (0-20 cm) (Fig 5.2) in the fall, especially when applied N exceeded the crop demand. Similar results have been reported in previous work by Liang and MacKenzie (1994b). The NO_3^--N accumulation in the top 1 m of the soil

profile was highly correlated with NO_3^--N in the 0-20 cm soil layer ($R^2_{fall\ 1993}$=0.91**, $R^2_{fall\ 1994}$=0.97**). The mean soil NO_3^--N level across all N treatments was 33 and 45% of the total NO_3^--N in the top 1 m of the soil in the autumn of 1993 and 1994, respectively. These results show that surface NO_3^--N accumulation may be a good indicator of total N accumulation in the top 1 m of the soil profile. It therefore appears that NO_3^--N leaching in the following spring can be minimized by decreasing NO_3^--N levels in the top 0-20 cm of the soil profile in the previous fall.

5.5. Conclusions

In this study, we found that intercropping corn with annual ryegrass was an effective method for conserving residual soil N under conditions of excessive fertilizer N application, especially when environmental conditions were favourable to ryegrass development. Water table control had no effect on total soil NO_3^--N accumulation in the top 1 m of the soil profile in the fall of 1993 or 1994. Free drainage enhanced NO_3^--N movement deeper into the soil, and as a result enhanced leaching losses over the winter season. Conventional corn production (monocrop and free drainage) with the addition of N fertilizer in excess of the recommended N rate contributed greatly to leaching losses in the following spring. Top soil (0-20 cm) NO_3^--N level could be used as an indicator for total NO_3^--N accumulation and in estimating the potential for NO_3^--N leaching. We also found that leaching losses were high if winter precipitation levels were high and free drainage was used.

Table 5.1. Soil characteristics of the experimental field.

Total N, Mg ha^{-1}	4.5
Organic matter	50 g kg^{-1}
pH	6.6
Saturated hydraulic conductivity, m day^{-1}	0.1-11
Bulk density (g/cm^3)	
0-0.25 (cm)	1.6
0.25-0.50 (cm)	1.6
0.50-1.00 (cm)	1.5

Table 5.2 . Analysis of variance of soil NO_3-N in the top 1 m of the soil profile during the 1993-1994 seasons.

	1993					1994									
	fal					spring					fall				
	0-20	20-50	50-75	75-100	Total	0-20	20-50	50-75	75-100	Total	0-20	20-50	50-75	75-100	Total
	cm														
N rate															
linear	*	**	*	NS	**	NS	*	*	**	0.1	0.1	NS	0.1	0.1	0.1
Contrast															
Cropping systems															
intercropping vs															
monocropping	0.1	*	*	0.1	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Water table depth (WTD)															
Free drainage															
vs WTD	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	0.1	0.1	NS	NS
S_{70} vs S_{80}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Intercrop vs															
monocrop															
X Free drainage															
vs WTD	NS	NS	NS	NS	NS	NS	NS	NS	**	0.1	NS	*	NS	NS	NS
Intercrop vs															
monocrop X															
S_{70} vs S_{80}	NS	0.1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

L = linear contrast significant, Q = quadratic contrasts significant. **, and * represent significant differences at the 0.01 and 0.05 probability levels, respectively. NS = not significantly different at the 0.1 probability level. S_{70} and S_{80} represent water table depths maintained at 70 and 80 cm from the soil surface, respectively. represent NO_3-N accumulation in top 1 m of the soil profile.

Fig 5.1. Effect of cropping system and water table depth on distribution of soil NO_3^--N in the top 1 m of the soil profile.

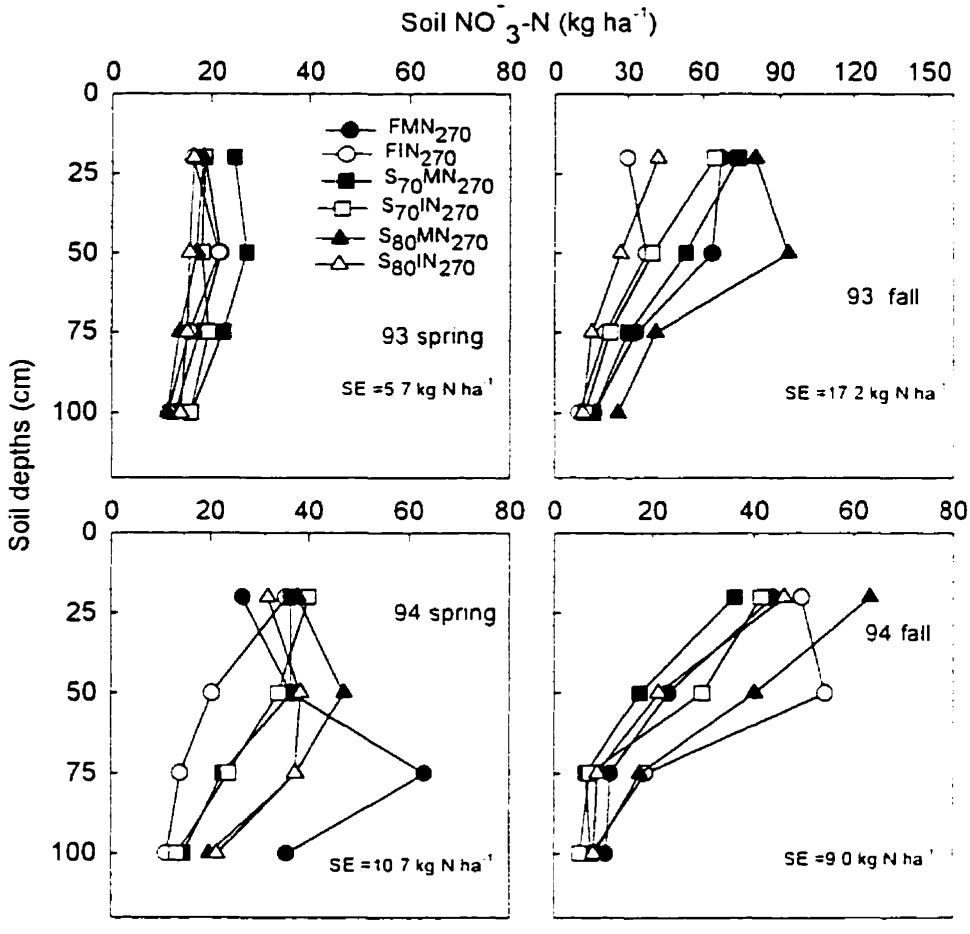
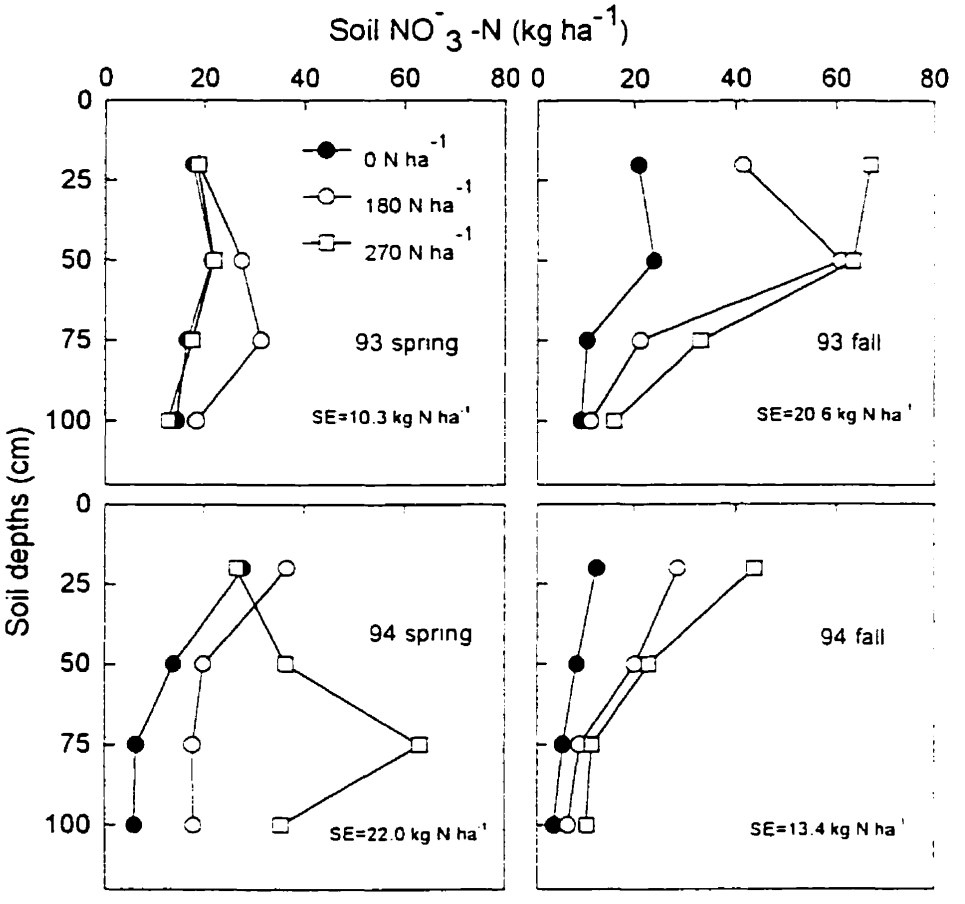

Soil NO^-_3-N (kg ha^{-1})
Soil depths (cm)
0
20
40
60
80
30
90
120
150
25
50
75
100
FMN_{270}
FIN_{270}
$S_{70}MN_{270}$
$S_{70}IN_{270}$
$S_{80}MN_{270}$
$S_{80}IN_{270}$
93 spring
SE =5 7 kg N ha
93 fall
SE =17 2 kg N ha
94 spring
SE =10 7 kg N ha
94 fall
SE =9 0 kg N ha

Fig 5.2. Effect of three applied N rates on distribution of soil NO_3^--N in the top 1 m of the soil profile.


Soil NO^-_3-N (kg ha^{-1})
0
20
40
60
80
Soil depths (cm)
0
25
50
75
100
0 N ha^{-1}
180 N ha^{-1}
270 N ha^{-1}
93 spring
SE=10.3 kg N ha^{-1}
93 fall
SE=20.6 kg N ha^{-1}
94 spring
SE=22.0 kg N ha^{-1}
94 fall
SE=13.4 kg N ha^{-1}

Preface to Chapter 6

Chapter 6 is comprised of materials contained in a manuscript by myself, my supervisor Dr. D.L Smith and four other co-authors, Mr. S. Leibovitch, Dr. A.F. MacKenzie, Dr. C.A. Madramootoo and Dr. P. Dutilleul, submitted to the *Agronomy Journal* for publication in 1997. The format has been changed to be consistent within this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

The previous three chapters addressed how crop N uptake, fertilizer N use efficiency and soil profile N contents responded to cropping systems and controlled drainage. Chapter 6 deals with reducing the cost of enriched ^{15}N used in field studies through the use of confined microplots.

Chapter 6

Variability in ^{15}N Uptake of Corn Plants in Confined Microplots Under A Controlled Drainage and Intercropping Management System

6.1. Abstract

Increasing concerns about groundwater pollution caused by excessive N fertilizer application in agricultural systems has led to an emphasis on increasing fertilizer N use efficiency. The ^{15}N tracer technique is a useful way to detect the fate of applied fertilizer N. However, the high cost of labeled N fertilizer places practical limitations on such work. The objective of this study was to assess microplot dimensions required to produce reliable ^{15}N data under a regime of monocropped corn (*Zeas mays* L.) and corn intercropped with annual Italian ryegrass (*Lolium multiflorum* Lam.) and water table controls via subirrigation. An experiment was conducted for two years on a fine, silty, mixed, nonacid and frigid Typic Humaquept soil. Labelled N fertilizer was applied in the microplots at 270 kg N ha^{-1}, the same rate as unlabeled N fertilizer was applied in the remainder of the plots. Corn plants inside the confined microplots were sampled at 0.2, 0.4 and 0.6 m from the end borders of microplots. Monocropping increased % ^{15}N enrichment in corn leaves, stalks and total dry matter in 1993 relative to intercropped corn, while no cropping system effect was found in 1994. Water table depths did not affect plant N fertilizer accumulation in either year. Plants within microplots were similar in size to plants fertilized with unlabeled N fertilizer outside the microplots. There was no difference in % ^{15}N enrichment for plants harvested at three sampling positions. These results suggest that a confined microplot with an area as small as 0.4 m by 1.15 m could provide a reliable measure of fertilizer N recovery for corn plants.

6.2. Introduction

The use of isotopically labeled N to trace the fate of applied N fertilizer has become increasingly important in field studies. The extent to which excessive N fertilizer application causes groundwater pollution in agricultural systems is of particular interest. The stable isotope ^{15}N provides a safe and effective tool for

quantifying fertilizer N in N-uptake studies. Nitrogen-15 can be used to distinguish between fertilizer N and indigenous N, providing a meaningful way to measure both the total amount of fertilizer N utilized by the crop and fertilizer N loss during crop production, a major concern to both environmental and agronomic researchers (Khanif *et al.*, 1984; Reddy and Reddy, 1993).

Because of the high cost of labeled N fertilizer, it is desirable to reduce the amount of labeled N fertilizer required for field studies. Studies to determine suitable microplot dimensions have been conducted for both confined and unconfined microplots. Studies examining unconfined microplots are primarily concerned with microplot dimensions and microplot sampling to reduce ^{15}N dilution. Olson (1980) demonstrated that accurate values for plant N uptake could be obtained by sampling the three center row plants of 2.13 m by 2.14 m in unconfined corn microplots. Jokela and Randall (1987) evaluated the maximum acceptable corn plant sampling area in a 1.52 m by 2.29 m unconfined microplot with uniform N fertilizer application and 0.76 m row spacing. They found that reasonable ^{15}N recovery from microplots could be obtained if plants were sampled from the center row at least 0.38 m from the edge of the microplots. Sanchez *et al.* (1987) banded N fertilizer on the soil surface in the spring and fall, and modeled the lateral movement of N at various positions in or near the microplot. They proposed that a 2 m by 2 m unconfined microplot provided a reliable measure of ^{15}N recovery. Stumpe *et al.* (1989) suggested that plants which were at least 0.50 m from the border of a ^{15}N microplot provided reliable data without concern as to the method of N fertilizer application. These studies on unconfined microplots confirmed that they provided reliable N recovery data for N uptake studies. However, unconfined microplots still required an application of labeled N fertilizer in a much larger region surrounding the smaller central portion where samples may be reliably taken.

Confined microplots use barriers placed in the soil to delineate them (Malhi and Nyborg, 1983; Power and Legg, 1984) and eliminate problems with lateral movement of labeled N (Ma *et al.*, 1995). The major advantage in using confined ^{15}N

microplots in field research is that they can prevent diffusion and mass flow of ^{15}N from inside to outside of the microplot area and the similar movement of unlabeled N from outside to inside (Carter *et al.*, 1967; Malhi and Nyborg, 1983; Power and Legg, 1984).

The objective of the present study was to assess microplot dimensions required to produce reliable ^{15}N data under corn monocropping and intercropping (with annual ryegrass) systems with water tables controlled via subirrigation used to contrast the level of % ^{15}N atom enrichment in three sampling groups of corn plants within confined microplots.

6.3. Materials and Methods

As the objectives of this study relate to N uptake by corn, we will not discuss the ryegrass component of the intercrop system in this paper.

6.3.1. Field methods

The study was conducted during the 1993 and 1994 growing seasons in Soulanges County, Québec, Canada. The field had been used for corn production for at least two years prior to the experiment. The soil was a Soulanges very fine sandy loam (fine, silty, mixed, nonacid, frigid Typic Humaquept). The field slope was approximately 0.5%. The surface soil characteristics were pH 6.6, soil total N 4.5 Mg ha^{-1}, and organic matter 50 g kg^{-1}.

The study reported here is part of a larger experiment consisting of two cropping systems (monocrop corn and a corn-annual ryegrass intercrop), and three water table controls via subirrigation (conventional, free-outlet subsurface drainage and two controlled drainage systems with water tables at 0.70 and 0.80 m below the soil surface). A complete description of the water table research facility can be found in Tait *et al.* (1995). The design of this experiment was organized in a randomized complete block with three blocks.

Each treatment plot was 75 m by 15 m. In both years, 141 kg K ha^{-1} as K_2O was applied prior to planting. In order to apply N fertilizer at an annual rate of 270 kg N ha^{-1}, each plot received 47 kg N ha^{-1}, 52 kg P ha^{-1} as 18-46-0 (N-P-K) at planting

and 223 kg N ha^{-1} as NH_4NO_3 two weeks after planting. Corn hybrid Pioneer 3921 was planted in the last week of May 1993 and 1994. In both years, annual Italian ryegrass (*Lolium multiflorum* Lam. *CV.* Barmaltra) was seeded one week after the corn in intercropping treatments.

In both years, monocrop corn plots were treated with 4.0 L ha^{-1} atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5 triazine). Herbicide was not applied to intercropped treatments in 1993. In 1994, however, 2.25 L Basagran (bentazon) ha^{-1} (3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) was applied to control broad leaf weeds in the field. Ragweed (*Ambrosia artemisiifolia* L.) was the most frequent weed in the intercropping plots in 1993, while barnyard grass (*Echinochloa crusgalli* L.) was the major weed in 1994.

Precipitation data were collected from a nearby weather station (0.5 km from the experimental site). The monthly precipitation for 1993, 1994, and 20-year average can be found in Fig 3.1 (see chapter 3).

In order to trace the fate of applied fertilizer N, ^{15}N microplots were established in all plots of two blocks in both years. Before the fertilizer N application in 1993, two locations on opposite sides of each plot were chosen randomly in five middle of rows for establishment of ^{15}N microplots for the 1993 and 1994 growing seasons (Fig 6.1. A). At each location a 2.25 m by 3 m area was marked before N fertilizer was applied. These unfertilized areas were used to prevent any unlabeled N fertilizer from being applied to the areas which were later used for ^{15}N microplots. After placement of microplots, the rest of the surrounding area was evenly fertilized with NH_4NO_3 (34-0-0) by hand at the same rate as main plots. The areas marked (2.25 m by 3 m) in 1993 for placement of the 1994 ^{15}N microplots were remarked in 1994 to avoid application of any unlabeled N fertilizer in 1994. After the corn emerged, a 1.15 m by 1 m area within the remarked area was selected for the ^{15}N microplot. The microplot area was chosen based on evenness of corn emergence. A rigid plastic barrier, 20 cm in height, was placed into the soil around the perimeter of the microplots by using a square edged spade to cut a slot in the soil and then hammering the plastic barrier into the slot. The

plastic barriers extended 1 cm above the soil surface. Disturbance of the soil within the microplots was minimal. A solution of $^{15}NH_4^{15}NO_3$ at 5 % ^{15}N excess was applied in the microplots at the same rate of N fertilization (270 kg N ha^{-1}) as was received by the plot in which they were located on 17 June 1993 and 16 June 1994. The solution containing 5 % ^{15}N excess was sprayed to the microplot surface areas using a watering can with a sprinkler head. In order to move N fertilizer below the soil surface and evenly distribute the ^{15}N throughout the microplot, an additional 2 L of water were added immediately after the ^{15}N solution.

6.3.2. Plant sampling and analyses

At the same time as the main plots were harvested (the second week of October in both years), five plants within each microplot were harvested. These were retained in three separate groups based on their positions within the microplots. Two corn plants 20 cm from each end of the microplot formed one group (group 3). Two corn plants adjacent to the plants at the end of the microplot (i.e. 40 cm from the end of the microplot) formed group 2. Finally, the plant 60 cm from either end of the microplot, the center plants, constituted group 1. Tillering of some plants within the microplots occurred in 1994, probably due to the higher temperatures just after planting and wet conditions in June (see Fig 3.1 in chapter 3) of that year. The details of sampling positions are presented in Fig 6.1 (B).

Harvested corn plants were cut at ground level and divided into ears (grain), leaves, and stalks, weighed, air dried (70 °C) to a constant weight, weighed again and ground with a Wiley mill fitted with a 1 mm screen. Total N was determined using Kjeldahl analysis (Tecator, Kjeltec System 20, Box 70. S-26301 Höganäs, Sweden). Plant tissue samples (approximately 0.5 g) were digested at 420 °C in 12 mL of concentrated H_2SO_4 containing 6.8 g of catalyst (3.5 g K_2SO_4 per 3.5 mg Se) for 90 min. Digested samples were then distilled and titrated with 0.05 M HCl for total N determination. The titrated solutions were subsequently acidified and used for ^{15}N analysis. To avoid sample cross contamination during the steam-distillation process, the distillation unit was flushed with distilled water for three minutes between samples.

A volume of Kjeldahl distillate solution calculated to contain approximately 7 μg of N was placed into a 15 cm length glass tube (6 mm, o.d.). All samples were oven dried at 90 °C. A catalyst (CuO) and a drying agent (CaO) were added to the glass tubes and the tubes were sealed under vacuum, and baked in an oven at 500 C for 4 to 6 hours, to oxidize the NH_3 to N_2 gas. The % ^{15}N atom enrichment in the samples were determined by emission spectroscopy using a JASCO N-150 ^{15}N analyzer (JASCO, LTD, Tokyo, Japan). The details of the sample drying process and the determination of % ^{15}N atom enrichment followed standard methods (Preston *et al.*, 1981; Martin *et al.*, 1991).

6.3.3. Statistical analyses

For a given variable, the three resulting values (one for each position) constituted repeated measures for each microplot. Repeated measures arise whenever the same characteristic or variable of interest is measured on more than one occasion, in time or in space, on the same observational unit, in this case, the microplot; thus they extend the case of paired observations. As a result, two classical assumptions underlying ANOVA (Analysis of Variance) are likely to be violated by repeated measures data; the independence of adjacent samples and homoscedasticity (homogeneity of variance) (Sokal and Rohlf, 1995). MANOVA, a multivariate version of ANOVA, and a modified univariate version based on a modified *F* test were developed to overcome this type of problem (Littel, 1989; Crowder and Hand, 1990) and applied here.

We used the Greenhouse and Geisser (1959) correction factor in a modified ANOVA (as recommended for cases like ours; Crowder and Hand, 1990). The repeated measures ANOVA was performed using the GLM procedure of SAS and included the statements MANOVA and REPEATED (SAS institute Inc., 1985).

We discuss the main effect of cropping system and water table depth over sampling positions using the P-values from standard ANOVA. In the statistical analysis for sampling position, we found that the two procedures, the multivariate analysis based on Wilks' Lambda, and the modified univariate procedure using the

Greenhouse-Geisser adjustment, agreed and concluded that effects of sampling position and its interactions with cropping system and water table controls on dry matter production and % ^{15}N atom enrichment in the microplots were not significant. Therefore, we examined the effects of sampling positions and its interaction with cropping system and water table controls using the probability value adjusted by the Greenhouse-Geisser epsilon.

6.4. Results and Discussion

6.4.1. Dry matter production

Grain yield was not affected by cropping system in either year (Table 6.1). Compared with intercropping, monocropping resulted in an increase in leaf, stalk and total dry matter production of 22, 41, and 30%, respectively, in 1993 within microplots (Fig 6.2), while in 1994 no effect of cropping system was found (Table 6.1 and Fig 6.2). The reduction of total dry matter production in 1993 for the intercropped corn can be attributed to the decreased leaf and stalk biomass. In July 1994, rainfall was 43 mm below normal (see Fig 3.1 in chapter 3) which could be responsible for the 34% reduction of plant dry matter production within microplots compared with the 1993 growing season. In both years, there were no water table depth or water table depth by cropping system interaction effects on grain yield or dry matter production in the microplots.

Sampling positions (adjusted Greenhouse-Geisser probability in Table 6.2) did not affect corn grain yield, stalk mass, or total dry matter production in either year. Leaf dry matter production was influenced by sampling position in 1993, but there was not enough difference to affect the total aboveground dry matter production of corn plants within the sampling positions (Fig 6.2). These results indicate that whole plant growth parameters were similar at different sampling positions within the confined microplots. Average dry matter production values for plants within the microplots were 280.4 and 209.7 g plant^{-1} for 1993 and 1994, respectively, while the average dry matter production for plants outside the microplots were 224.8 and 267.3 g plant^{-1} in 1993 and 1994, respectively. For each year, the within microplot and outside-

microplot values were not statistically different from each other. These results supported the view that plant growth in confined microplots was similar to plant growth in the rest of the plot.

6.4.2. Atom % ^{15}N enrichment

Averaged across sampling positions and treatment factors, the % ^{15}N enrichments for grain, leaf, and stalk tissues were 2.81, 3.08, and 2.92, respectively in 1993; in 1994, the same parameters were 2.21, 2.59, and 2.30, respectively (Fig 6.3). Cropping system affected ^{15}N accumulation in the grain, stalks and whole plants in both years, while no effect on the % ^{15}N atom enrichment occurred for leaf tissue. Compared with intercropped corn, the % ^{15}N atom enrichment in monocropped corn was consistently higher for both years (Table 6.2 and Fig 6.3), regardless of plant parts or sampling position. The % ^{15}N enrichment in monocropped were 19 and 34% higher than intercropped corn over two years. This higher ^{15}N accumulation in the monocropped corn plants was expected as there were no other plants to compete for N fertilizer in the monocrop system. During the two years of this study, no effect of the water table depths was observed on the % ^{15}N atom enrichment for all plant parts or total dry matter accumulation (Table 6.2). The lack of effect of water table controls may have been due to the small difference in water table depths.

In both years, sampling position (adjusted Greenhouse-Geisser values) had no effect on the atom % ^{15}N enrichment of corn grain, leaf and stalk samples (Table 6.2 and Fig 6.3). This suggested that the ^{15}N content of the plants at different sampling positions in the microplots was not affected by the unlabeled N from outside the microplots, probably because the barriers prevented the lateral exchange of ^{15}N labeled fertilizer inside the microplot and unlabeled N fertilizer outside the microplot. This result is consistent with the findings of Ma *et al.* (1995), who suggested that their 0.25 m deep frame (microplot plastic boundary) was efficient in preventing lateral movement of ^{15}N in the upper root zone when they tested a single ^{15}N labeled plant in a 0.76 m by 0.23 m microplot. Since we found that the % ^{15}N atom enrichment of the total plant sample was similar at the three sampling positions (Fig 6.3), we concluded

that the microplot area of corn for ^{15}N work could be reduced to 1.15 m by 0.4 m for corn plants grown in rows 0.76 m apart. We did not observe any difference in % ^{15}N atom enrichment of plants growing near the edge of the confined microplot border and those growing in the center of the microplot (Fig 6.3). Follett *et al.* (1991) found that the highest enrichment often occurred for the central plants within unconfined microplots. In addition, the lowest enrichment was found near the edge of unconfined microplots by Stumpe *et al.* (1989). Sanchez *et al.* (1987) studied lateral movement of labeled N fertilizer in unconfined microplots, and found that the isotopic composition of corn grain declined rapidly near the edge of the microplot. The even distribution of % ^{15}N atom enrichment at the three sampling positions measured in our study may have been due to: i) the establishment of a plastic border around the microplot which would have greatly reduced the possibility for the lateral exchange of labeled and un labeled N between the soil outside and inside of microplots; and ii) careful and even application of ^{15}N solution in the microplots, which should minimize the microplot size.

We observed consistently greater data variability in 1994 than in 1993 (data not shown). Furthermore, % ^{15}N atom enrichment values were lower in 1994 than in 1993 (Fig 6.3). A possible cause of this lower ^{15}N enrichment in 1994 was the gradual accumulation of free soil NO_3^--N, largely as $^{14}NO_3$, due to changes in N fertilization practices. Prior to the establishment of the experimental site, the field was used for commercial corn production and received N fertilizer at approximately 180 kg N ha^{-1} $year^{-1}$. Starting in the spring of 1993, we added 270 kg N ha^{-1} $year^{-1}$, as per Liang and MacKenzie (1994a). We would expect, and our data also demonstrated, that the application of 270 kg N ha^{-1} resulted in fall soil residual NO_3^--N values at a depth of 0 to 50 cm higher than 180 kg N ha^{-1}. The fall 1993 values were 130.4 versus 102.4 kg N ha^{-1} in the spring 1993. Compared with spring soil NO_3^--N in 1993, the soil NO_3-N in spring 1994 was approximately twice as high (36.4 vs 62.9 kg N ha^{-1}) due to the cumulative N level resulting from soil residual N plus applied fertilizer N.

6.5. Conclusions

This study demonstrated the advantages of small confined microplots for ^{15}N

tracer studies under field conditions. A single plant in the center of a sampling area 1.15 m by 0.4 m ^{15}N microplot can supply a reliable estimation of ^{15}N recovery by corn plants. The % ^{15}N enrichment and plant growth were greatly influenced by the cropping system and environmental conditions.

Table 6.1. Probability values of analysis of variance and modified univariate of ^{15}N corn plant dry matter production within ^{15}N microplots under cropping system, water table controls and sampling ^{15}N plant positions.

Year	Sources	Grain		Leaves		Stalks		Total	
		ANOVA† Pr > F	adjusted‡ G-G	ANOVA Pr > F	adjusted G-G	ANOVA Pr > F	adjusted G-G	ANOVA Pr > F	adjusted G-G
1993	Cropping systems (C)	0.14	-	0.03	-	0.01	-	0.02	-
	Water table controls (W)	0.47	-	0.8	-	0.47	-	0.59	-
	C X W	0.72	-	0.91	-	0.75	-	0.98	-
	Sampling positions (P)	-	0.34	-	0.03	-	0.84	-	0.83
	C X P	-	0.54	-	0.46	-	0.67	-	0.98
	W X P	-	0.41	-	0.59	-	0.82	-	0.70
	CX W X P	-	0.63	-	0.50	-	0.64	-	0.70
1994	Cropping systems (C)	0.77	-	0.96	-	0.36	-	0.95	-
	Water table controls (W)	0.37	-	0.34	-	0.05	-	0.21	-
	C X W	0.28	-	0.99	-	0.66	-	0.38	-
	Sampling positions (P)	-	0.67	-	0.95	-	0.60	-	0.98
	C X P	-	0.58	-	0.46	-	0.88	-	0.61
	W X P	-	0.73	-	0.97	-	0.75	-	0.91
	CX W X P	-	0.63	-	0.95	-	0.59	-	0.82

† Probability of the analysis of variance. ‡ Probability value adjusted by the Greenhouse-Geisser epsilon.

Table 6.2. Probability values of analysis of variance and modified univariate of % ^{15}N atom enrichment of corn plants under cropping system, water table controls and sampling plant positions.

Year	Sources	Grain		Leaves		Stalks		Total	
		ANOVA† Pr > F	adjusted‡ G-G	ANOVA Pr > F	adjusted G-G	ANOVA Pr > F	adjusted G-G	ANOVA Pr > F	adjusted G-G
1993	cropping systems (C)	0.01	-	0.21	-	0.02	-	0.02	-
	Water table controls (W)	0.35	-	0.73	-	0.15	-	0.37	-
	C X W	0.26	-	0.3	-	0.31	-	0.25	-
	Sampling positions (P)	-	0.5	-	0.76	-	0.70	-	0.89
	C X P	-	0.35	-	0.28	-	0.50	-	0.83
	W X P	-	0.79	-	0.58	-	0.13	-	0.24
	CX W X P	-	0.48	-	0.19	-	0.56	-	0.31
1994	Cropping systems (C)	0.02	-	0.14	-	0.04	-	0.05	-
	Water table controls (W)	0.60	-	0.37	-	0.46	-	0.46	-
	C X W	0.64	-	0.61	-	0.57	-	0.63	-
	Sampling positions (P)	-	0.67	-	0.32	-	0.75	-	0.64
	C X P	-	0.19	-	0.32	-	0.93	-	0.40
	W X P	-	0.34	-	0.47	-	0.38	-	0.31
	CX W X P	-	0.34	-	0.29	-	0.53	-	0.24

† Probability of the analysis of variance. ‡ Probability value adjusted by the Greenhouse-Geisser epsilon.

Fig 6.1. Field layout: the position of enriched ^{15}N microplots within the large plot (A) and the location of plants sampled at various positions in the ^{15}N microplots (B).{ * indicates the sampled corn plants, 1 stands for sampling group (plant sampled in the center of the ^{15}N microplot, 0.6 m away from the end of the ^{15}N microplot); 2 indicates sampling group 2 (plants sampled about 0.4 m away from the end of the ^{15}N microplot; 3 indicates sampling group 3 (plants sampled about 0.2 m away from the end of the ^{15}N microplot)}.

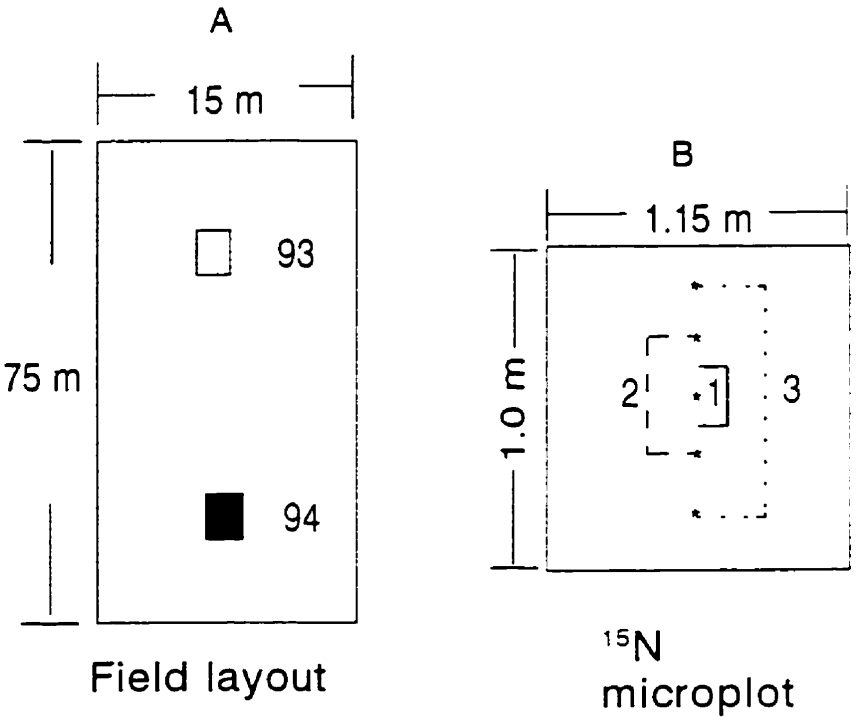

A
15 m
93
75 m
94
Field layout
B
1.15 m
1.0 m
2
1
3
^{15}N
microplot

Fig 6.2. The effects of cropping system and sampling positions on dry matter production of corn plants in the ^{15}N microplots. (Vertical bars represent standard errors of the mean with N=6; AVG represents the mean over the three sampling positions).

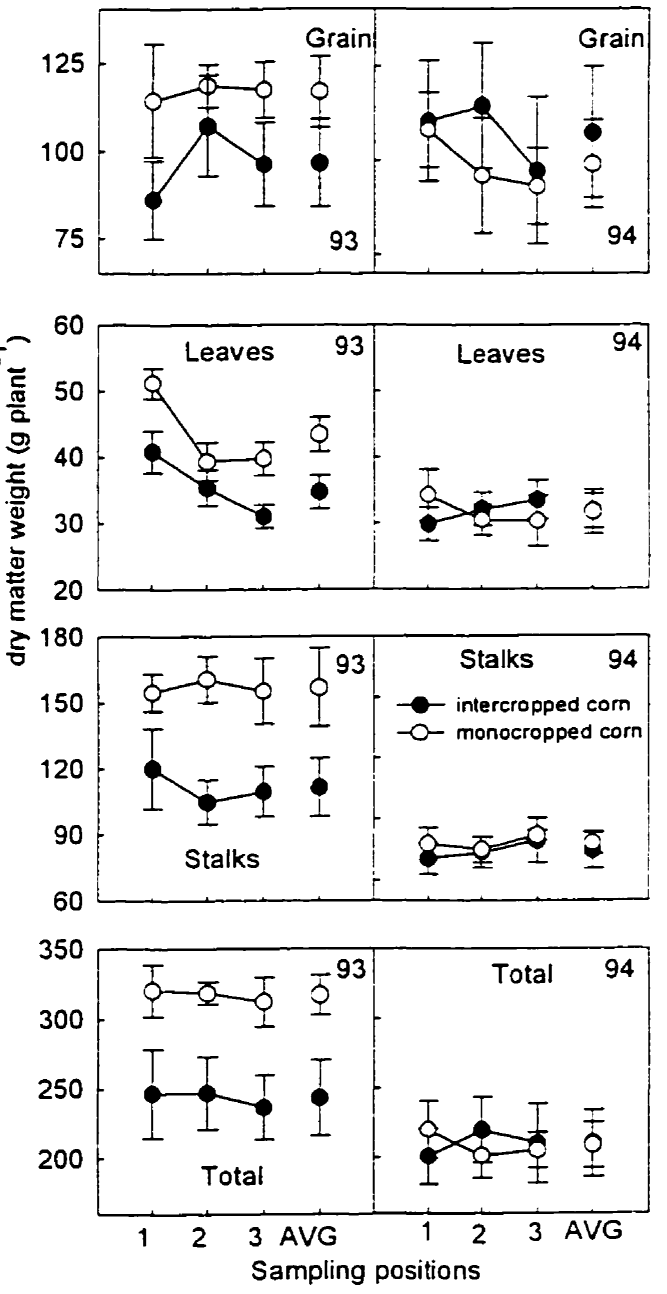
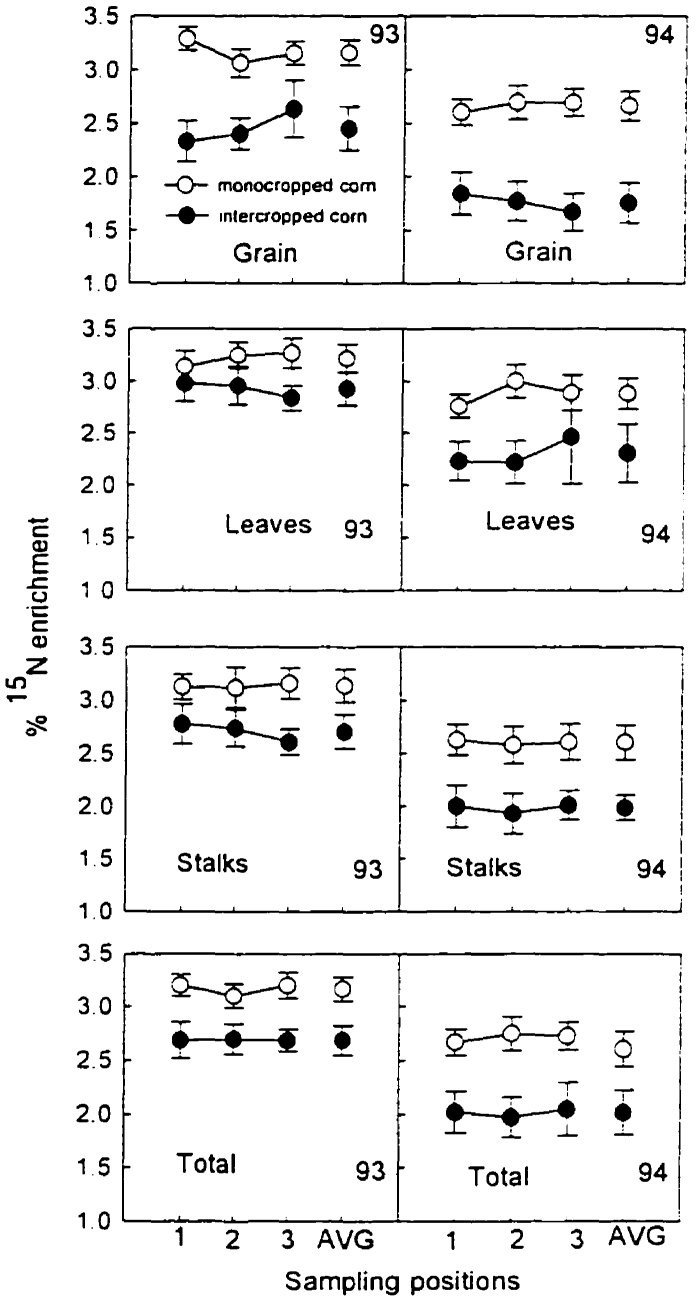

Grain
93
Grain
94
125
100
75
Leaves
93
Leaves
94
60
50
40
30
20
dry matter weight (g plant -1)
Stalks
93
Stalks
94
intercropped corn
monocropped corn
180
150
120
90
60
Total
93
Total
94
350
300
250
200
1 2 3 AVG
1 2 3 AVG
Sampling positions

Fig 6.3. The effects of cropping system and sampling positions on % ^{15}N atom enrichment of corn plants in the ^{15}N microplots. (Vertical bars represent standard errors of the mean with N=6; AVG represents the means over the three sampling positions).


3.5
3.0
2.5
2.0
1.5
1.0
93
94
monocropped corn
intercropped corn
Grain
Grain
Leaves
93
Leaves
94
Stalks
93
Stalks
94
Total
93
Total
94
% 15N enrichment
1
2
3
AVG
Sampling positions

Preface to Chapter 7

Chapter 7 is comprised of materials contained in a manuscript by myself and my supervisor Dr. D.L. Smith published in the *Crop Science* Journal in 1996. The format has been changed to be consistent within this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

In the last four chapters, we described crop dry matter accumulation and partitioning and N utilization in corn intercrop and monocrop systems as affected by water table management. We found that the monocrop corn grain yield response to applied N rates may have been the result of moisture stress rather than a fully exploited yield potential at the highest N rate. In addition, the competition for water in the intercrop system could become more severe when drier weather occurred. Thus, it is desirable to study aspects of corn plant physiology, potentially affected by water stress and N nutrition. To do this, we described and tested a technique for delivery of potential metabolic effectors into corn plants. The development of this system is the subject of this chapter.

Chapter 7

A New Technique for Continuous Injection into Stems of Field-Grown Corn Plants

7.1. Abstract

Chronic addition of nutrients, metabolites, growth regulators, or toxins to intact plants is a useful way to study numerous aspects of plant physiology. However, continuous delivery of large amounts of test solutions into plant tissues has been difficult. Stem infusion methods have proven less destructive and more effective than other methods, such as leaf and root feeding, for supplying nutrients and other materials to developing plants. Infusion into solid-stemmed plants has been limited by its short delivery period and the damage that it causes at the delivery site. A field experiment was conducted to evaluate a new technique for supplying sucrose solution or water to corn (*Zea mays* L.) stems. This injection technique delivered pressurized solutions or water through syringe needles sealed to the stem with latex. The pressure was applied to the syringe plunger with ceramic construction bricks. Solutions containing sucrose at 0, 150, and 300 g L^{-1} were injected over a 32 day injection period. The average solution uptake rate was 5.1 mL day^{-1} $plant^{-1}$. Distilled water was more easily delivered than sucrose solutions. No difference in uptake rates were observed between 150 and 300 g L^{-1} sucrose solutions. The tested injection system involves a simple, efficient and inexpensive method that is easily used in the field or greenhouse.

7.2. Introduction

The traditional methods of supplying nutrient compounds to corn plants are through roots and leaves (Rendig and Crawford, 1985; Tomar *et al.*, 1988). However, in biochemical pathway or physiological studies, the small amounts supplied and the short durations of supply may be inappropriate. Previously reported methods for stem infusion can supply small quantities of these substances to plants without the sometimes erratic uptake experienced with spraying, leaf abrasion or soil application.

Stem infusion has proved to be less destructive and more effective than leaf

feeding for supplying nutrients directly to crop plants for brief periods. Brown and Neish (1954) successfully injected radioactive compounds into the hollow internodes of wheat (*Triticum aestivum* L.). Grabau *et al.* (1986) used a novel infusion technique to introduce the amino acid methionine into intact soybean (*Glycine max* (L.) Merr.) stems during seed development. This increased the methionine content in the resulting seeds. In soybean, infusion of a small amount of boron into the stems resulted in an 84.8% increase in the number of lateral pods and a 17.6% increase in seed weight (Schon and Blevins, 1987). Infusion/perfusion techniques have also been developed for longer term delivery of materials into plant stem cavities. Using a stem perfusion technique to deliver nitrogen into the hollow stems of intact barley (*Hordeum vulgare* L.) plants, Ma and Smith (1992) were able to increase the nitrogen concentration in barley grains by up to 40% and total grain N per spike by 25%, relative to non-perfused or distilled water-perfused controls. This perfusion technique delivered a steady supply of 30 mM N into the peduncle cavity over a period of 3 to 4 weeks (anthesis to physiological maturity).

At present, the major obstacle to long term stem infusion of corn is the limited volume of solution that can be delivered and the duration of infusion. Boyle *et al.* (1991a) developed an infusion technique to deliver agar-free kernel culture medium into artificial cavities made by removing tissue from the stems of corn plants. This method allowed delivery of large solution volumes, but only for a few days at a single infusion site. They concluded that the daily rate for infusion into corn plants was limited by the composition of the fluid infused, the number of infusion sites, and the duration of infusion at a given site. In their study, the average infused volume per corn plant at a single infusion site was 85 mL over a five day period. The highest uptake volume occurred on the first day, followed by a much lower uptake on the second day, and almost no uptake by the fifth day. By using sequential infusion sites, Boyle *et al.* (1991a) increased the total cumulative volumes of infusion, but still the duration of infusion for each infusion site was limited to five days and, since this technique involves removing several mL of tissue for each injection site, there would have been

substantial stem damage over time. Ma *et al.* (1994a) modified Boyle's infusion method, but again found that the greatest rate of infusion was during the first 24 hours, after which it declined sharply, reaching zero by 48 to 72 h. In our own preliminary testing (data not shown), we found that the method of Boyle *et al.* (1991a) generally caused the death of plant tissue at the infusion sites. Tissue death and wound response at the site of tissue removal are probably the causes of limited solution uptake after the first few days (Ma *et al.*, 1994a). Corn stem infusion has been tested both under greenhouse conditions (Boyle *et al.*, 1991a) and field conditions (Ma *et al.*, 1994a), but the infusion uptake rate was limited to a short period irrespective of number of infusion sites.

The development of a stem infusion method causing minimal tissue damage and suitable for longer periods of study would be beneficial for crop physiological research. We chose sucrose as the infusion solution, as it is the most important form of assimilate translocated throughout most plants (Setter and Meller, 1984). Therefore, this study was designed to develop a stem injection technique for corn plants which could continuously supply sucrose and water over a period of weeks to months under field conditions.

7.3. Materials and Methods

7.3.1. Field Conditions

The experimental site was located at the Horticulture Research Station of the Macdonald Campus of McGill University, Québec in the summer of 1993. The experimental plots were established on St. Bernard (fine loamy, mixed, nonacid, frigid, typic Eutrochrept) soil. During the previous year the field was used for vegetable and sweet corn production. Each plot was 4.5 m x 2 m with six rows of corn plants. Corn hybrid Pioneer 3921 was hand seeded at 60000 plants ha^{-1} in rows 0.75 m apart and with 0.20 m between plants within rows. Corn was sown on 22 May and harvested on 4 October, 1993. Before planting, the plots were fertilized with 40 kg ha^{-1} $N\text{-}P_2O_5\text{-}K_2O$ (5-20-20), according to a soil test. The plants received only rainwater during the growing season. Weeding was done by hand and no herbicides were applied during the

growing season.

Since N fertilizer status can affect overall plant growth and thus the injection uptake rate, fertilizer N level was included as an experimental factor. Ammonium nitrate was applied at 0 or 270 kg N ha^{-1} at seeding. The experiment was arranged as a split-plot factorial with three replicates. Soil N fertility rate was the main-plot factor, and injection concentration of sucrose the sub-plot factor. Three sucrose concentrations (0, 150 and 300 g L^{-1}) were injected into corn plants starting on 18 August, 1993. Distilled water-injected and non-injected plants were included as controls.

7.3.2. Stem Injection Methodology

7.3.2.1. The Injection System:

A 1.2-m length of flexible plastic tubing (i.d. 0.8, o.d. 2.4 mm) (Tygon, Norton Co., Akron, OH, USA) was attached to a 21-gauge standard disposable hypodermic needle (Becton Dickinson, Rutherford, NJ, U.S.A) with the plastic portion removed. Silicon sealant (GE Silicones Canada, Pickering, ON, Canada) was used to seal the tubing to the needle. An 18-gauge hypodermic needle was inserted into the other end of this tubing and masking tape was wrapped around the connection site to form a cup, which was filled with latex (Vultex, General Latex Canada, Candiac, QC, Canada) to seal this needle to the plastic tubing. The latex was allowed to dry completely (about 5 days) before this needle-tubing system was applied to corn plants in the field.

To supply a consistent volume of injected solution, several bricks (approximately 2.7 kg each) were used to produce pressure to force the solution into the plant stem. The bricks used were standard (22.5 X 8.5 X 7 cm) ceramic three hole construction type. Two 2.4-metre (2 cm o.d.) steel pipes were used to support the bricks. Plywood sheets were cut to the same surface dimensions as the bricks, and three holes were drilled in each piece of plywood. The two outside holes in the plywood were large enough to pass the pipe through and the middle hole was slightly larger than the outside diameter of a 60-mL syringe barrel. Two pipes were hammered into the field beside each corn plant to be injected. The distance between the pipes was

such that they fit into the two outside holes of the bricks. A cut plywood sheet was put over the pipes and supported at the desired height by hose clamps placed on the pipes. This arrangement is illustrated in Fig 7.1.

All injected solutions were autoclaved for thirty minutes and placed in sterile syringes before they were taken to the field. A 750 ml L^{-1} ethanol solution was sprayed on the injection site to surface sterilize the stem around it before the needle was inserted.

7.3.2.2. Injection:

Two rows of corn plants in each plot were randomly chosen for injection treatments. Two injection points were prepared: one on the internode below the ear (first injection internode) and one on the internode above the ear (second injection internode) so that the syringe reservoir could be switched to the alternate injection point in case of leakage or blockage. During the experiment, most injections were conducted entirely on the first injection internode. The 21-gauge needle connected to the end of the needle-tubing system described above was inserted downward into a corn internode at approximately a 60-degree angle to the stem and to a depth of approximately 1.5 cm. The site of injection was approximately 5 cm below the ear node. To protect the injection site from damage by wind or leakage, and to seal the needle to the stem, masking tape was wrapped around the needle such that it formed a cup against the stem. This cup was filled with latex, which was allowed to dry for several days. The open upper plastic portions of the 18 gauge needles were covered by the masking until ready for use. At 15 days after silking (early grain filling), a syringe was filled with 60 mL of sucrose solution or distilled water and placed through the middle hole of the plywood. The syringe was then connected to the 18-gauge needle of the needle-tubing system. Bricks were placed on the pipe supports with their weight resting upon a small piece of plywood which was placed on top of each syringe plunger. Given the weight per brick (2.7 kg) and diameter of the opening of the 21 gauge needle (0.5mm), the calculated pressure of the solution above it entered the plant would have been 1644 M Pa per brick. When injection into a plant was started, bricks were added until the

solution's meniscus in the tubing reached the injection site. At the beginning of injection, each treatment required the use of 1 to 3 bricks in order for the solution's meniscus to reach the injection site. After a few days, if there was no apparent flow of solution, more bricks were added. The total number of bricks applied ranged from 3 to 12 (approximately 33 kg). Given that the internal diameter of the 21-gauge needle inserted into corn stem was 0.5 mm, and the flow rate (several ml day^{-1}) was low enough to make frictional effects negligible, the pressure would have been approximately 1644 M Pa at the site of injection.

After injection was initiated, the system was examined daily to make sure that no bubbles appeared in the tubing. Whenever leakage occurred, the injection site was immediately moved to the adjacent injection internode, which was either above or below the initial injection internode, but not at the same internode. This precaution was taken because we observed that the pressure added to the injected internode will cause fluid to drip out of the tube connected to the back up injection site if it is placed on the same internode. A minimum of six plants was used for each treatment. When the solution in the syringe was exhausted, the syringe was replaced by a new syringe filled with the same solution. In order to limit air entry into the injection tube, the luer-lock of the new syringe was filled with liquid by slightly depressing the syringe plunger after the syringe was placed on the plywood support. The decrease in the syringe barrel volume was recorded daily to monitor solution uptake by the plants. Injection continued for 32 days, which extended to a few days beyond plant physiological maturity.

7.3.3. Data Analysis

The data were analyzed with the PROC GLM procedure of the SAS package (SAS Institute, 1985), according to Steel and Torrie (1980). Differences between individual means were tested with a GLM analysis protected Least Significant Difference (LSD) test. Due to the lack of any detectable fertilizer effect on the injection rate, the data analysis was based on the variable means pooled over fertilizer N rates.

7.4. Results and Discussion

7.4.1. Rate of injection

In general, this experiment showed that injection under continuous pressure was minimally destructive, and could deliver concentrated sucrose or water solutions into corn stems for long periods under field conditions. The average cumulative injection volume over all treatments was 163 mL (5.1 mL day^{-1}) (Fig 7.2). We did not observe tissue necrosis at the injection site during this work.

The injection period lasted 32 days (Fig 7.2). Uptake effectively ceased a few days before the end of the injection period, even though additional weight (bricks) had been added in an attempt to maintain the injection rate. This cessation of uptake was abrupt (Fig 7.2) and occurred a few days before the plants reached physiological maturity, as determined by the black layer method (Daynard and Duncan, 1969). The solution uptake for plants injected with sucrose treatments declined to 0 mL about 4 days earlier than that of plants which received only distilled water. As the cessation of uptake coincided with physiological maturity, it may have been caused by the decreasing plant metabolism at this stage, or rapid production of senescence related tissue, such as the callus like deposition in abscision layers, leading to final complete blockage of the injection sites.

The daily per plant injection rates were quite varied during the 32 day injection period. The variability in injection rate for any treatment during the first fifteen days of the injection period was less than that of the last fifteen-days. The variability in daily rate was greater for distilled water than the sucrose solutions during the last 15 days. The most rapid uptake of the 300 g L^{-1} sucrose solution occurred on the 19th day while for the distilled water treatment peaks occurred on the 17th, 25th, and 29th days. These injection peaks were due to one or two individual plants which had brief periods of abnormally high uptake. We did not observe any leakage at those times.

The studies of Boyle *et al.* (1991a) and Ma *et al.* (1994a) showed a rapid decline in infused volume during the first five days. We observed a similar pattern for our 300 g L^{-1} sucrose treatment. This was overcome by additional of more bricks on

day five. For the distilled water and the 150 g L^{-1} sucrose treatments solution uptake varied gradually, declining or increasing from day to day. By the fifth day, uptake volumes for the 150 g L^{-1} sucrose solutions had reached a minimum, although uptake had not stopped completely while the uptake of distilled water solution reached a maximum on the fifth day of injection. The declining sucrose solution uptake may indicate that injection with sucrose solutions stimulated callus or tyloses as part of a wound response production and that some of the injected carbohydrate was used to produce these structures. Using ^{14}C infused sucrose into sorghum (*Sorghum bicolor* (L.) Moench) by the method of Boyle *et al.* (1991a), Tarpley *et al.* (1994) suggested that some infused ^{14}C near the infusion point could be incorporated into wound response structures as insoluble compounds such as cellulose or lignin. Boyle *et al.* (1991a) and Ma *et al.* (1994a) reported that the infusion rate for their artificial cavity system reached zero 48 to 72 hours after infusion began. Ma *et al.* (1994a) concluded that cessation of solution intake was probably due to the plants' response to wounding. Previously reported results for other infusion methods (Boyle *et al.*, 1991a; Ma *et al.*, 1994a) and the results reported here may indicate that by the 5th day the plant had made a reasonably complete reaction to the injection wound. After the 5th day, the uptake volume of our two sucrose solutions was increased due to the addition of more bricks, which increased the pressure on the injected solutions. After this the rate of solution uptake remained reasonably constant, except for occasional brief surges in individual plants.

Converting the injection volume into grams of added sucrose, average total sucrose uptake values were 17.7 and 40.9 g for the 150 and 300 g L^{-1} solutions, respectively. The method of Boyle *et al.* (1991b), infusion of approximately 3 g sucrose into corn stems as a 150 g L^{-1} solution, at a single infusion site, and by using five infusion sites the authors were able to infused approximately 15 g sucrose per plant. Ma *et al.* (1994a) also infused 13.5 g and 21.6 g sucrose per corn plant for the 150 and 300 g L^{-1} solutions using a modification of Boyle's method (Boyle *et al.*, 1991a). In an experiment conducted by us with the same hybrid and at the same time

and near the experiment reported here, corn plants were sampled for total dry matter at the early grain filling stage and at maturity, which corresponded to the beginning and end of the injection period in the experiment reported here. In that experiment the dry matter gained during the time equivalent to the injection period was 140 g plant^{-1}. Based on that weight gain we injected sucrose, in this study, equivalent to approximately 30% of the dry matter gain during the injection period, which corresponded to the period of most active grain filling.

Transpiration rate was measured with a Li-Cor 6200 Portable Photosynthesis Measurement Systems (Lincoln, NE, U.S.A) between 10:00 and 16:00h and at 24 and 48 hours after injection commenced and once a week thereafter. Transpiration rate was not affected by the solution injected and showed no relationship to the amount of solution taken up (data not shown). This agrees with the observations of Foroutan-pour *et al.* (1995) who found that the uptake rate of stem-perfused barley plants was very similar under field conditions to rates of uptake reported by Ma and Smith (1992) under greenhouse conditions, in spite of the greater vapour pressure deficits and light intensities likely to have been present under field conditions.

7.4.2. Effect of injection concentrations

Solution concentrations affected the injection rates and total volumes. Plants injected with sucrose took up an average of 4 mL day^{-1}, while those injected with distilled water took up 7.2 mL day^{-1}. The average total volume of injected distilled water was approximately 100 mL greater than that of the sucrose treatments. Ma *et al.* (1994a) reported 30 mL more distilled water than sucrose solution taken up per plant by corn. These results differed from those of Boyle *et al.* (1991a), who found distilled water and culture medium to be taken up in similar volumes by corn plants. Variability during the course of the injection period was also higher for the distilled water treatment than for the sucrose solutions (Fig 7.2). This is in agreement with the findings of Boyle *et al.* (1991a). The total injected volumes were not different between the 150 and 300 g L^{-1} sucrose concentrations (Fig 7.3). A similar result was also noted by Ma *et al.* (1994a), who used a modification of the infusion technique of Boyle *et al.*

(1991a).

The continuous addition of bricks provided additional pressure which acted to largely overcome developing blockages. When injection was initiated, we observed that the number of bricks required to bring the meniscus to the injection point did not differ among concentration treatments. After three or four days, the injection fluid of all treatments showed a pattern of gradually decreasing uptake, which was reversed by the addition of more bricks on day 5. In most cases, the maintenance of reasonable flow rates required more bricks, usually two or three more, for the sucrose containing solutions than distilled water. There are two factors that could have contributed to the higher pressure requirement for the sucrose solutions: i) plant response to wounding may have occurred more rapidly when sucrose was injected as some of the injected sucrose could have been used to facilitate the production of callus or tyloses since sucrose is the main compound providing sugars for construction of the plant cell wall (Ma *et al.*, 1994a), ii) the osmotic potential of the sucrose solutions is higher than that of distilled water and some additional pressure will be required to overcome this osmotic potential. Foroutan-pour *et al.* (1995) used a peduncle perfusion technique to infuse distilled water, ethephon (15 μM) and nitrogen (15 and 30 mM) into barley plants grown under field conditions. They observed that the slowest uptake rate occurred for the plants perfused with the 30 mM nitrogen solution, while plants perfused with distilled water had the most rapid solution uptake rate. They suggested that the cause of the different solution uptake rates was differences in the osmotic potentials.

In general, continuous injection of sucrose can be accomplished under field conditions by employing this method. This is a simple, efficient and inexpensive method that is easily used under field conditions. The rate of injection depends on the concentration of the injected solution and the pressure applied to those solutions.

Fig 7.1. System for continuous pressure injection of corn stems.

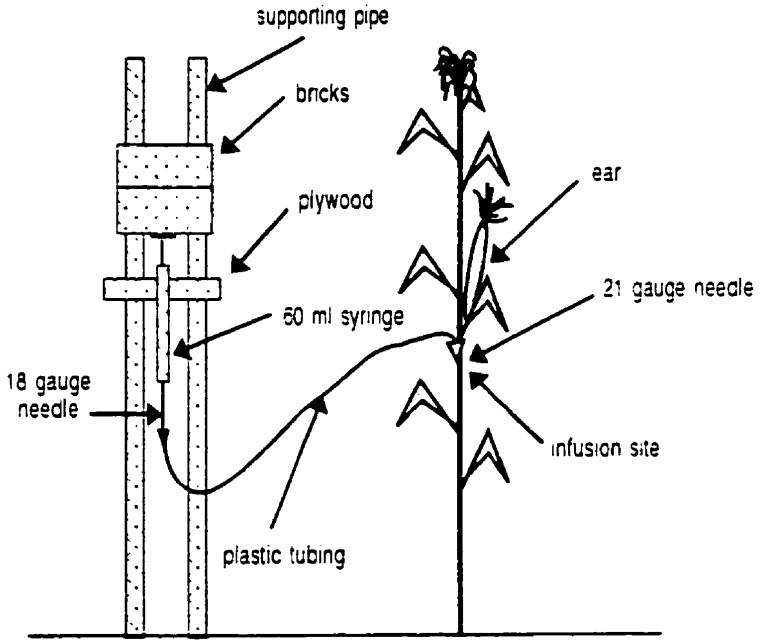

supporting pipe
bricks
plywood
60 ml syringe
18 gauge needle
plastic tubing
ear
21 gauge needle
infusion site

Fig 7.2. Average daily uptake of injected solutions during a 32 day period.

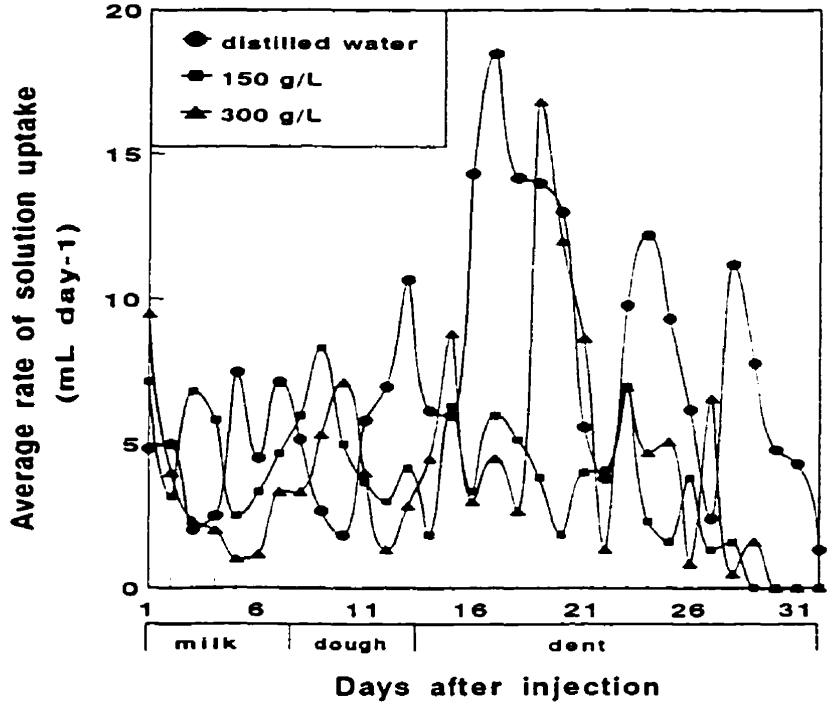
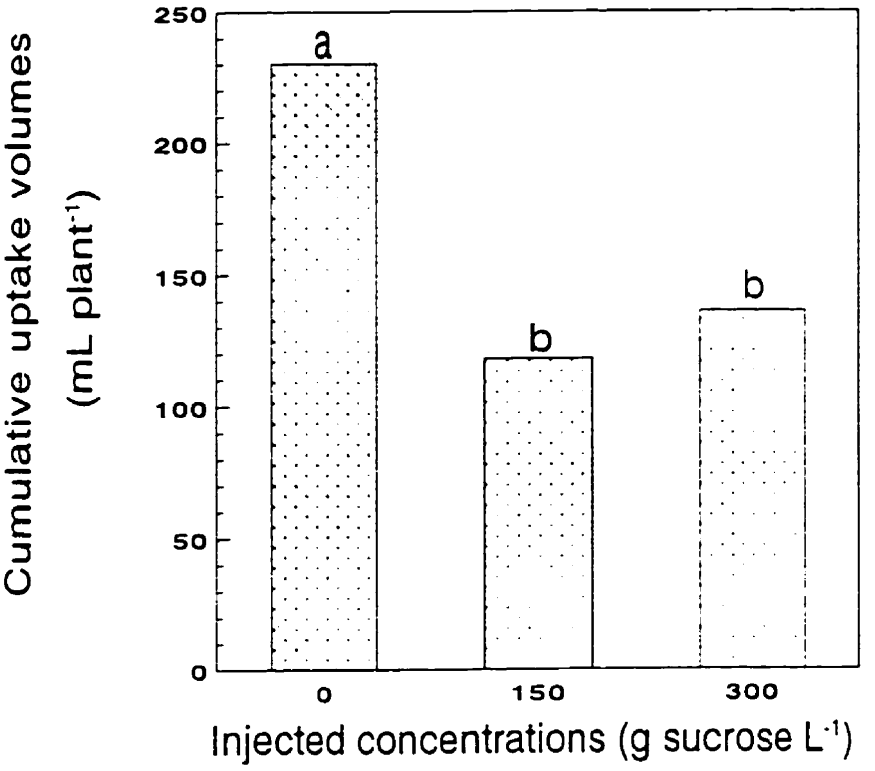

distilled water
150 g/L
300 g/L
Average rate of solution uptake
(mL day-1)
20
15
10
5
0
1
6
11
16
21
26
31
milk
dough
dent
Days after injection

Fig 7.3. Effects of solution concentrations on accumulative injected volumes over a 32 day injection period.


Cumulative uptake volumes
(mL plant-1)
250
200
150
100
50
0
a
b
b
0
150
300
Injected concentrations (g sucrose L-1)

Preface to Chapter 8

Chapter 8 is comprised of materials contained in a manuscript by myself, my supervisor, Dr. D.L. Smith and other two co-authors, Dr. A.F. MacKenzie, and Dr. C.A. Madramootoo, submitted to the *Journal of Agronomy and Crop Science* for publication in 1997. The format has been changed to be consistent within this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

In the chapter 7, we discussed solution uptake volumes as affected by the concentrations of sucrose solutions added with a stem injection system. The photosynthesis and productivity of the corn plants as affected by exogenous supply sucrose via a stem injection system, are the subject of in this chapter.

Chapter 8

Effects of Stem-Injected Sucrose on Grain Production, Dry Matter Distribution, and Chlorophyll Fluorescence of Field-Grown Corn Plants

8.1. Abstract

A field experiment was conducted to evaluate the effect of long term exogenous sucrose supply on aspects of corn plant physiology and development during the grain filling period. Concentrated sucrose solution was supplied to corn (*Zea mays* L.) stems by an injection technique. This injection technique delivered pressurized solutions through syringe needles sealed to the stem with latex. The pressure was applied to the syringe plunger with ceramic construction bricks. Solutions containing sucrose at 0, 150, and 300 g L^{-1} were injected over a 32-day period encompassing the duration of the active grain filling period. The primary ears of plants injected with sucrose produced approximately 55% more kernels and 37% more grain weight than those injected with distilled water. The injected internode was also considerably heavier (49%) for plants receiving sucrose than plants receiving distilled water. For all measured variables, plants injected with distilled water were either not different from or had larger values than the non-injected controls. After one week, sucrose injection caused photosynthetic inhibition (as measured by chlorophyll fluorescence) in the leaf just above the ear and the ear leaf, and this was more severe for plants receiving 300 g sucrose L^{-1} than for those receiving 150 g sucrose L^{-1}. For sucrose injected plants, the increased size of the primary ear was concomitant with a large decrease in grain production by the secondary ear and an overall decrease in per plant grain production. These results suggest that the mechanisms for signalling between sinks (the primary and secondary ears) and the primary sink and the source (leaves) are different.

8.2. Introduction

Sucrose, the major form of assimilate translocated in corn (*Zea mays*. L) (Prioul *et al.*, 1990), is produced in the leaves (Prioul *et al.*, 1990) and translocated to grain during the grain filling period. In the absence of environmental stress, pathological conditions, or nutrient deficiency, cereal yield may be limited by the

plant's ability to match assimilatory potential with sink capacity for optimal dry matter accumulation in grain (Bingham, 1966). The factors having the largest effects on corn yield are the number of kernels per plant, i.e. the number of available sinks, and the rate and the duration of grain filling. Salvador (1984) found that the final number of corn kernels carried to physiological maturity is set two to three weeks after silking. Limitation of carbohydrate supply during ear development can lead to reduced kernel set.

Previous stem infusion techniques have provided an effective way to supply nutrients, growth regulators or small amounts of ^{14}C sucrose directly to corn plants for brief periods (Boyle *et al.*, 1991a; 1991b; Ma *et al.*, 1994b; Tarpley *et al.*, 1994; Zinselmeier *et al.*, 1995). In corn plants the major impediment to corn stem infusion work is the short duration of infusion. The stem infusion technique developed by Boyle et al. (1991a) allowed delivery of large solution volumes, but for only a few days, regardless of how many infusion sites were made. Ma *et al.* (1994a), using a modification of the infusion method of Boyle *et al.* (1991a), also found that infusion uptake by corn plants ceased after 48 to 72 hours for any single infusion site. They concluded that infusion stopped due to a plant wounding response. Although multiple infusion sites increased uptake volumes per plant when the method of Boyle *et al.* (1991a) was used, the large number of infusion sites seems likely to cause a serious wounding response by infused corn plants, as each infusion site resulted in the removal of several mLs of stem tissue. To achieve minimal plant tissue damage during corn stem infusion, Zhou and Smith (1996) developed a pressurized stem injection system for field-grown corn plants. This technique both minimizes destructive damage at the injection site and makes for longer injection periods (weeks) possible.

The present study was conducted to evaluate the effect of a long term supplementation of sucrose supply to corn plants, in the form of continuously injected sucrose, on aspects of plant development; and to determine whether supplying additional sucrose from early grain filling to physiological maturity would positively

affect grain production and alter aspects of corn plant physiology.

8.3. Materials and Methods

8.3.1. Field conditions

The experiment was conducted on St. Bernard soil (fine loamy, mixed, nonacid, frigid, typic Eutrochrept) at the Horticulture Research Centre of the Macdonald Campus of McGill University in 1993. Corn hybrid Pioneer 3921 was hand seeded on 22 May. Rows were 4.5 m long, and were spaced 75 cm apart. Plants were approximately 20 cm apart within rows. This resulted in a final stand density of 60,000 plants ha^{-1}.

During the previous year, the field had been used for vegetable and sweet corn production. Before planting, plots were fertilized with 40 kg ha^{-1} N-P_2O_5-K_2O (5-20-20) according to a soil test. Plants received only rainwater during the growing season. Weeding was done by hand and no herbicides were applied.

Treatments consisted of three concentrations of injected sucrose: 0 (distilled water), 150 and 300 g sucrose L^{-1}. Data were also collected on non-injected plants. The distilled- water injected plants provided controls for the effects of sucrose injection, while non-injected plants acted as overall controls for the injection process. We initiated the stem injection treatments at two weeks after silking (early grain filling). Silking was defined as the time when 80% of all plants had ears with exposed silks.

Since it seemed possible that soil N fertility could affect sink development, and therefore the injection uptake rate, N fertilizer levels were also included as an experimental factor. Ammonium nitrate was applied at 0 or 270 kg N ha^{-1} at seeding. The experiment was arranged as a split-plot factorial with three blocks. Soil N fertility rate was the main-plot factor, and injection treatments the sub-plot factor.

Chlorophyll fluorescence indirectly measures photosynthesis efficiency (Krause and Weis, 1984). When a leaf has been in the dark for a few minutes and then is illuminated, fluorescence rises quickly to an initial level (F_0). Due to the rapid reduction of electron accepting Q_A (quinone-type acceptor) molecules, fluorescence increases from F_0 to a maximum (F_m), and the difference between F_0 and F_m is termed

variable fluorescence (F_v). The variable fluorescence is highly sensitive to changes in the ultrastructure of membranes and rates of electron transfer. Therefore, the potential yield of the photochemical reactions can be expressed as F_v/F_m (Krause and Weis, 1988), with a high F_v/F_m indicating high photosynthesis efficiency. In order to evaluate the effect of a long term injection technique on the physiological aspects of corn plants during grain filling, chlorophyll fluorescence was measured (CF-1000 Chlorophyll Fluorescence System, P.K. Morgan Instruments, Inc., Andover, MA, USA) on the leaves of injected and non-injected plants. Chlorophyll fluorescence measurements took place between 10:00 and 16:00h and were taken the first day after injection commenced and at weekly intervals thereafter. Measurements were taken on the primary ear leaf, the leaf just above the primary ear (ear+1 leaf), and the leaf just below the primary ear (ear-1 leaf). Cuvettes were placed on the middle of the leaves for 10 min before measurement. Chlorophyll fluorescence measurements on ear+1 leaves were stopped at the second week after commencement of injection due to the pre-mature senescence of these leaves (see Results and Discussion section below).

Corn plants were harvested on 4 October 1993. Harvested injected corn plants and non-injected control plants were separated into ears (primary ear and secondary ear), injected internodes (first and second injected internodes, see Stem Injection section below), ear leaf, ear+1 leaf, ear-1 leaf, and the stalk (including leaf sheaths and the tassel). These plant parts were dried to a constant weight at 70°C. The number and weight of seeds per ear were measured and recorded.

8.3.2. Stem injection

Zhou and Smith (1996) described the stem injection method in detail. Briefly, a flexible plastic tubing (i.d. 0.8, o.d 2.4 mm) was connected to a 21-gauge standard disposable hypodermic needle with the plastic portion removed. The needle was sealed to the tubing with silicon sealant (GE Silicones Canada, Pickering, ON, Canada). A 18-gauge hypodermic needle was inserted into the other end of this tubing and masking tape was wrapped around the connection site to form a cup, which was filled with latex to seal the needle to the plastic tubing.

Several ceramic three-hole construction bricks (approximately 2.7 kg each) were used to produce pressure to force the solution into the plant stem. Two steel pipes (2 cm, o.d.) vertically positioned with an attached horizontally positioned plywood sheet were used to support the bricks. The plywood was fixed at the desired height by hose clamps placed on the pipes. Prior to the commencement of injection, 60-mL sterile syringes (Becton Dickinson Inc, NJ, U.S.A.) were filled with each test solution and placed through the middle hole of the plywood. The syringe was then connected to the 18-gauge needle of the needle-tubing system. The 21-gauge needle connected to the needle-tubing system described above was inserted downwards into a corn internode (approximately 1.5 cm deep). Two injection points were established on each selected plant: one on the internode below the ear (first injection internode) and one on the internode above the ear (second injection internode) so that the syringe reservoir could be switched to the second injection point in case of leakage or blockage of the first injection internode. Most injections were conducted entirely at the first injection internode. The connection sites between injection point and corn stem were warped to form a cup shape, and filled with latex (Vultex, General Latex Canada, Candiac, Québec, Canada). The latex was allowed to dry for several days before injection was started. At two weeks after silking (early grain filling), the test solutions were supplied through the 21-gauge needle from the syringe reservoir. The two outside holes of the bricks were matched to the pipes, and the weight of the bricks rested on a small piece of square wood placed on the top of the syringe plunger. The bricks provided approximately 1644 M Pa per brick of constant pressure on the solution being fed into the plants.

Injection treatments were randomly assigned to 2-row plots in each main plot of each block and were applied to one plant selected at random within each sub-plot. A minimum of six plants were used for each treatment. The decrease in the syringe barrel volume was recorded daily to monitor solution uptake by the plants. All injected treatments were applied from 15 days after silking to a few days beyond physiological maturity, which was defined as the appearance of the black layer (Daynard and Duncan, 1969).

In order to prevent contamination, sterile test solutions were used; after every feeding the unused solutions were stored at +4°C and were discarded if any contamination was detected. When the solution in a syringe was exhausted, the syringe was replaced by a new sterilized syringe filled with the new solution.

8.3.3. Data analysis

The dry matter distribution and yield data were analyzed with the GLM procedure of the SAS package (SAS Inc., 1985), following Steel and Torrie (1980). The F-ratio test for main treatment effects was redirected in accordance with the split-plot experimental design (Steel and Torrie, 1980, p.380). Differences between treatment means were associated with a protected Least Squares Difference (LSD) test. In general, differences are reported at the $P < 0.05$ level.

An analysis of variance incorporating data from the three leaf positions (ear leaf, ear-1 leaf and ear+1 leaf) and the first two weeks after injection was performed on the data of chlorophyll fluorescence measurement. The ANOVA procedure was modified in order to take into account the potential heterogeneity of the variances and lack of independence, over space (from position to position) and over time (from week to week), of the spatio-temporal repeated measurements of chlorophyll fluorescence made repeatedly on the same corn plants (Crowder and Hand, 1990; Dutilleul, 1996). The model used for the modified univariate analysis of variance was derived by adding the position and time effects plus all the interactions to the terms of the usual ANOVA model. That analysis of variance was carried out using the REPEATED statement of the GLM procedure (SAS Inc., 1985)

We did not find any effect of N application on the uptake volumes of injected solutions, total plant dry weight, grain yield, and yield components (see next section) of injected or non-injected corn plants in this study. The lack of effect of applied N rates may have been due to the relatively high nitrogen levels contained in the soils at the site, which had previously been used for horticultural research. We found that the total N accumulated in the non-injected plants was equivalent to 145 and 123 kg N ha^{-1} for the 270 and 0 N kg ha^{-1} treatments, respectively; these values were not different ($P \geq 0.05$). Therefore, no main effect of the N fertilizer rates nor any interaction

between the N fertilizer rates and injection concentrations are shown; the data were pooled over the N fertilizer rates for statistical analysis. Similarly, the effects of the N fertilizer rate were not significant for changes in chlorophyll fluorescence of ear leaves, ear-1 leaves and ear+1 leaves during the first two weeks of injection, so chlorophyll fluorescence data were also pooled over N fertilizer rates before performing the repeated measures ANOVA. The homogeneity of the variance among N fertilizer rates was assessed prior pooling using Bartlett's test (Steel and Torrie, 1980, p.471).

8.4. Results and Discussion

8.4.1. Dry matter distribution

The injection period lasted 32 days (Zhou and Smith, 1996). The average solution uptake rate was 4-7 mL day^{-1} over all injection treatments (data not shown). Plants injected with 150 g sucrose L^{-1} received a total of 19 g of sucrose while those injected with 300 g sucrose L^{-1} received 38 g. The latter value was equal to about 30% of the total plant weight gain during the period of active grain fill.

Sucrose injection increased the dry weight of the injected internodes by 2.4 g (49%), compared with the distilled water injected treatment (Table 8.1). This increase in injected internode dry weight may indicate a sink limitation in the developing primary ear, leading to stalk accumulation of sucrose (Hume and Campbell, 1972) or a response of the plants to wounding, resulting in production of insoluble compounds such as cellulose or lignin near the site where additional sucrose was supplied (Tarpley *et al.*, 1994). Injection of the 300 g sucrose L^{-1} solution decreased ear+1 leaf dry weight more than the other treatments (Table 8.1). Injected sucrose caused premature leaf senescence near the primary ear (Fig 8.1) (see discussion in the section on Chlorophyll Fluorescence and Leaf Colour below), especially for ear+1 leaves. This was probably the reason for the decrease in the ear+1 leaf dry weight.

There were no differences among injection treatments for the second injected internode, total stalk, ear leaf, ear-1 leaf, and total plant dry weights (Table 8.1). Surprisingly, plants which received the distilled water treatment had the highest

average leaf dry matter weight when compared with other injection treatments. The cause of this higher leaf weight for plants injected with water is uncertain, but may be related the apparent inhibition of photosynthetic activity in leaves near the injection site (see Results and Discussion below). In general, there were no differences for dry matter distributions and total dry matter weight between injected distilled water plants and non-injected plants, except for total leaf weight.

8.4.2. Grain production

Significant injection effects on final grain weight per plant for injection treatments were attributed to the development of the secondary ear. The highest grain yield was for the plants injected with distilled water, which was 31% higher on a per plant basis, than the other injection treatments. This was mainly due to the well developed secondary ears of these corn plants, relative to other injection treatments.

Plants injected with 150 and 300 g sucrose L^{-1} developed only the primary ear (Table 8.2). The grain weight in primary ears of plants injected with sucrose was 37% greater than that of plants injected with distilled water. No differences in primary ear grain weight occurred between the two sucrose injection concentrations, or between non-injected and distilled water injected plants. The number of seeds in the primary ear was increased by approximately 55% due to the sucrose injection treatment when compared with distilled water control. Ma *et al.* (1994a) reported that infusion of sucrose at 150 g L^{-1} increased kernel set in one of two hybrids tested.

At the beginning of rapid kernel growth, corn stems accumulate carbohydrate (Hume and Campbell, 1972). Partitioning of ^{14}C photoassimilate revealed that the stem is a major sink at this stage (Alofe and Schrader, 1975). The net accumulation of sugars by corn stems occurs over a period of 2-3 weeks and the amount of sugar accumulated is substantial, sometimes equivalent to 12 to 25% of final grain dry matter (Daynard *et al.*, 1969). Therefore, the stem represents a large temporary sink which can compete with other sinks for available photosynthate. In this experiment, although depression of secondary ear growth occurred for the sucrose treatments, the primary ear grain weights of sucrose injected plants were greater than those of plants injected with distilled water, probably due to direct provision of assimilate through the

stems (i.e. injected sucrose) leading to increased the sink strength for primary ear, and increased dominance of the primary ear over secondary ear. Both plants injected with distilled water plants and non-injected plants produced secondary ears. However, the secondary ear development of the non-injected plants was smaller than that of distilled water injected plants, leading to lower total grain yield. The greater development of secondary ears in the plants injected with water, relative to plants injected with sucrose or non-injected plants, may have been related to altered assimilate flow and water relationships among tissue proximate to the injection site.

Zinselmeier *et al.* (1995) infused corn plants with a complete tissue culture medium containing 150 g sucrose L^{-1} and plant growth regulators (2,4-D and kinetin) through an artificial cavity made in the stems of field-grown corn plants. They did not find any effect of this test medium on grain development. The grain weight per plant of plants infused with tissue culture medium, in the Zinselmeier *et al.* (1995) study, was similar to that of plants injected with sucrose at 150 g L^{-1} in our study. In their study (Zinselmeier *et al.*, 1995), the secondary ears were fully developed for all treatments (injected with tissue culture medium or water) while secondary ears were fully developed only for distilled water injected treatments in our work. Several factors may have contributed to the differences between our findings and those of Zinselmeier *et al.* (1995): the length of the infusion periods was different; 32 days for our work vs 5 days for Zinselmeier *et al.* (1995); the injected solutions used in our work did not contain plant growth regulators or other constituents of tissue culture medium as was the case with the work of Zinselmeier *et al.* (1995); the hybrids used in the two studies were different; the environmental conditions were different at the two locations; and the sucrose supply techniques used in the two studies differed.

8.4.3. Chlorophyll fluorescence and leaf colour

Across all leaf positions and times, there was no simple effect of sucrose concentration on chlorophyll fluorescence. However, there was an interaction between leaf position and sucrose solution concentration, leaf position and injection time, and injection time and injection concentrations (Table 8.3). The effect of

sucrose solution concentration was more apparent for both ear leaves and ear+1 leaves (Fig 8.1). No changes were found in F_v/F_m values on ear-1 leaves during injection (Fig 8.1). The F_v/F_m ratio of ear leaves of plants injected with 300 g sucrose L^{-1} declined during the first week of injection and then recovered to values similar to those of other plants injected with lower sucrose concentrations, during the second week of injection. By one week after the beginning of injection, the F_v/F_m ratio in the ear+1 leaves for sucrose injected plants was lower than that of the control plants (Fig 8.1). By day 10 after the commencement of injection, some ear+1 leaves of the plants injected with sucrose (especially the 300 g sucrose L^{-1} treatment) began to show signs of carbon-phosphorus imbalance as indicated by leaf redening. Leaf redening was apparently due to anthocyanin accumulation and the loss of leaf chlorophyll. In addition, a decrease in the ratio of chlorophyll fluorescence (F_v/F_m) in ear+1 leaves further indicated depressed photosynthesis by ear+1 leaves for plants injected with 300 g sucrose L^{-1}. By five to six days after the red pigmentation appeared, we were unable to detect any chlorophyll fluorescence, demonstrating the absence of chlorophyll. This indicated premature senescence of these leaves when internodes nearest the primary ear were injected with sucrose.

The long term effect of pressurized sucrose injection on leaf physiology is unknown. Tarpley *et al.* (1994) found that some of the ^{14}C sucrose infused via a pulse application into sorghum (*Sorghum bicolor* (L.) Moench) moved upwards through the xylem and into leaves. In our case, the needles were inserted approximately 1.5 cm into the stem, which means most uptake of added sucrose would have been by pith parenchyma cells. It seems unlikely that these cells would have transported sucrose to xylem cells. However, we do not know that this sort of transport did not occur and we do not know if it would have contributed to leaf redening if it did occur. It may be that sink limitation resulted in restricted sucrose export from leaves near the injection site, which caused the premature ear+1 leaf senescence in sucrose-injected plants. Prioul *et al.* (1992) found that ear excision (sink removal) led to an accumulation of anthocyanin in the leaves of corn plants and caused an accumulation of carbohydrate in source leaves, which depressed

photosynthesis and induced senescence.

In this experiment, we found that injection with sucrose decreased total plant dry weight as compared with distilled water injected control plants. This may have been due to: 1) an overall shift in the pattern of dry matter partitioning between primary and secondary ears with increased dominance of the former over the latter, 2) the effects of osmotic stress on carbon dioxide fixation in plants injected with concentrated sucrose solutions, or 3) corn plants may have a built-in regulation of growth rate which acted to compensate, and perhaps over compensate, for the extra reduced carbon availability. It is clear from the data that the first of these played a strong role.

There were two possible reasons for causing the difference in grain weight between primary ear and secondary ear of injection of sucrose plants: 1) injection of sucrose into the stem adjacent to the developing primary ear caused the secondary ear to perceive a large developing sink, inhibiting the development of the secondary ear, while causing the leaves normally supplying the primary ear to behave as though the sink strength had been reduced to zero (Prioul *et al.*, 1992), as if the primary ear had been removed; 2) it could be due to some unknown artifact from the pressure damage or the osmotic potential of sucrose. Collectively, these observations suggest that the mechanism for signalling between the primary and secondary sinks, and between the primary sink and the source are different.

8.5. Conclusion

The long term constant pressure injection of solutions containing concentrations of sucrose, especially 300 g sucrose L^{-1}, led to earlier senescence of the ear+1 leaves, and caused a decrease in the F_v/F_m chlorophyll fluorescence ratio. The injected internodes of plants receiving sucrose solutions weighed more than those of the distilled water injected controls. These internodes appear to have acted as a sink for a portion of the injected carbohydrate. Injection of sucrose increased the number of seeds produced in the primary ear and the weight of the primary ear, but inhibited the development of the secondary ear. The greater development of secondary ears in the plants injected with distilled water may have been related to

altered assimilate flow and water relationships among tissue proximate to the injection site. This work constitutes the first use of the stem injection technique to examine the effects of chronic sucrose addition to the development and physiology of corn plants undergoing active grain filling.

Table 8.1. Corn dry matter distribution (g plant^{-1}) means receiving the sucrose injection treatments.

Injection concentrations (g sucrose L^{-1})	Leaves	Total stalks	First injection internode	Second injection internode	Ear+1 leaves	Ear leaves	Ear-1 leaves	Total dry matter
non-injection	34.5 b†	70.6 a	4.4 b	-	3.4 a	3.3 a	3.7 a	272.8 a
0	43.3 a	75.1 a	5.0 b	5.6 a	3.2 a	3.5 a	3.4 a	305.4 a
150	33.5 b	83.5 a	7.1 a	6.9 a	2.7 ab	3.3 a	3.6 a	259.5 a
300	31.1 b	76.1 a	7.8 a	7.0 a	2.4 b	3.2 a	3.4 a	244.8 a

† Within columns, means followed by the same letter are not significant different at the 0.05 significance level by a protected Least Significant Difference test.

Table 8.2. Corn yield and yield components of corn plants means receiving sucrose injection treatments.

Injection	Primary ear		Secondary ear		Total	
concentration (g sucrose L^{-1})	grain number	grain weight (g ear^{-1})	grain number	grain weight (g ear^{-1})	grain number	grain weight (g ear^{-1})
non-injection	331 b†	100 b	176 b	28 b	507 b	128 b
0	323 b	90 b	323 a	75 a	648 a	165 a
150	492 a	125 a	--	--	492 b	125 b
300	509 a	122 a	--	--	509 a	122 b

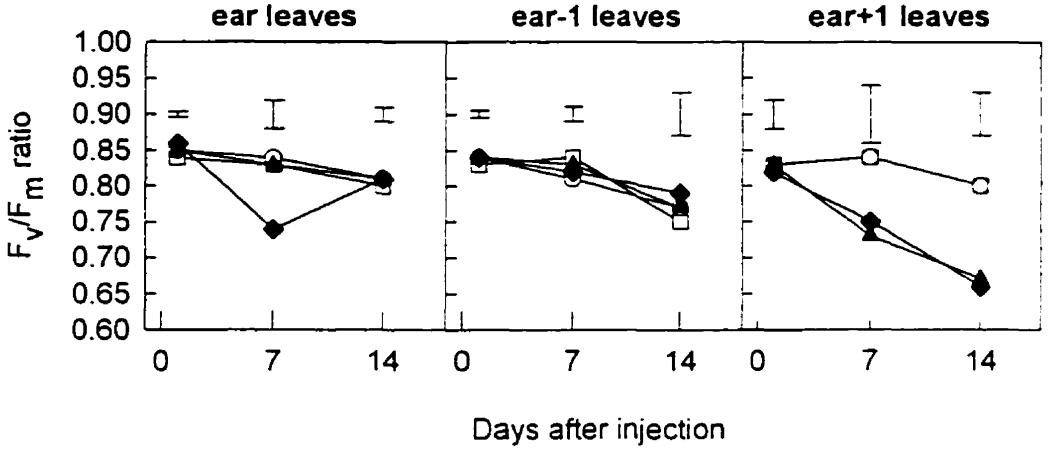
† Within columns, means followed by the same letter are not significantly different at the 0.05 level by a protected Least significant Difference test.

Table 8.3. Analysis of variance (ANOVA) and modified ANOVA on chlorophyll fluorescence (F_v/F_m) of ear leaves, ear-1 leaves and ear+1 leaves during the first two-weeks of the injection period.

Sources	ANOVA (Pr≥ F)	Modified ANOVA
Conc (C)†	NS	NA
Positions (P)‡	*	*
C X P	*	*
Time (T)	*	*
T X C	*	*
P X T	*	*
P X T X C	*	NS

NA= not applicable; † presented injected concentration; ‡ presented leaf positions [leaves just below ear (ear leaves), leaves just below ear leaves (ear-1 leaves) and leaves just above ear (ear+1 leaves)]; ANOVA=univariate analysis of variance; modified ANOVA= the probability of significance Pr ≥ F is adjusted by using the Greenhouse-Geisser estimate of Box's epsilon correction factor; * significantly different at 0.05 level; NS=not significant.

Fig 8.1. The effects of injected sucrose concentration on chlorophyll fluorescence (F_v/F_m) of corn plants [non-injection (□); injected with 0 g sucrose L^{-1} (○); injected with 150 g sucrose L^{-1} (▲); injected with 300 g sucrose L^{-1} (◆); vertical bars indicate the standard error of treatment means].


ear leaves
ear-1 leaves
ear+1 leaves
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
F_v/F_m ratio
0
7
14
Days after injection

Preface to Chapter 9

Chapter 9 is comprised of material from a manuscript prepared by myself, my supervisor, Dr. D.L. Smith and other three co-authors, Dr. C.A. Madramootoo, Dr. A.F. MacKenzie and Dr. P. Dutilleul for publication in the *Journal of Agronomy and Crop Science* in 1997. The format has been changed to be consistent within this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

In the last chapter, we demonstrated that the exogenous supply of sucrose affected the photosynthetic activity and dry weight accumulation of corn plants. This chapter deals with corn yield components and photosynthesis as affected by combinations of water availability (via a controlled water table) and sucrose (via corn stem injection) under controlled environment conditions.

Chapter 9

Sucrose Supplementation (Stem Injection) of Water Stressed Corn

9.1. Abstract

Water stress during silking or early kernel development decreases the number of kernels set by corn (*Zea mays* L.) plants. Previous work has suggested that lack of assimilate supply due to water stress at silking was a major factor in the resulting reproductive failure. A greenhouse experiment was conducted to test the hypothesis that sucrose supplementation of water stressed corn plants can prevent decreased kernel set. Sucrose was injected into corn stems at three concentrations [0 (distilled water), 150 and 300 g L^{-1}] for 30 days starting at silking. Water availability was controlled by either maintaining a water table at 50 cm from the soil surface (well watered) or by withholding water starting one week before silking (water stress) until the fifth day after silking. The photosynthesis rate of water stressed plants was 25% that of well-watered plants on the first day of silking. On average, the daily injection rate for distilled water was 1 mL higher than that of the sucrose treatments over a 30 day injection period. No difference in daily uptake rate was observed between the 150 and the 300 g sucrose L^{-1} treatments. For over water availability treatments, approximately 17 g sucrose were injected into corn plants during the 30 day injection period. Corn plants receiving sufficient water supply produced bigger ears, with more seeds and greater 100-seed weight values, leading to higher total plant dry matter accumulation than water stressed plants. Injecting of 300 g sucrose L^{-1} increased the weight of the injected internodes by 28%, compared with distilled water injection. The highest grain yield was for the plants injected with 150 g sucrose L^{-1}, but only under sufficient water supply. Plants injected with 300 g sucrose L^{-1} produced the least grain regardless of moisture availability. Thus, the exogenous sucrose supplementation influenced kernel set only under conditions of sufficient soil water supply. These results indicate that plant reproductive development after silking was limited more by water availability than assimilate supply, suggesting that some overall plant response to water stress, perhaps mediated by hormonal signalling, was more important than carbohydrate supply.

9.2. Introduction

Water stress during the reproductive phase, and especially during silking and early kernel growth, reduces the number of kernels per ear in corn (Tollenaar and Daynard, 1978; Herrero and Johnson, 1981; Frey, 1981). This reduces both potential sink size and final grain yield (Schussler and Westgate, 1991). Lack of assimilate supply during flowering and early kernel growth has been suggested as a major factor responsible for reproductive failure under water stress (Boyle *et al.*, 1991b; Schussler and Westgate, 1991; Zinselmeier *et al.*, 1995). This overall shortage of assimilates in corn is probably caused by the inhibition of photosynthesis by low leaf water potential (Westgate and Boyer, 1985; Schussler and Westgate, 1991) and related reductions in accumulation of photosynthate reserves in the corn stem at anthesis (Boyle *et al.*, 1991b). On the other hand, Schussler and Westgate (1994) found that C and N reserves in the corn stalk are not sufficient to offset the assimilate deficit caused by water stress. They concluded that a high level of assimilate reserves alone was not sufficient to overcome the lack of readily available photosynthate at low water potential.

Photosynthesis is the ultimate physiological limitation to crop grain production (Zelitch, 1982). The reduction of photosynthesis by water deficit stress has been well documented (Boyer and McPherson, 1975; Boyer, 1976). Photosynthesis is reduced by the lowered availability of water, both as a result of stomatal closure and as a result of effects at the chloroplast level (Boyer, 1976). Boyer and McPherson (1975) showed that photosynthesis is a major factor controlling grain yield under water deficit stress.

Stem infusion is one of the most useful methods for delivering nutrients to plants (Grabau *et al.*, 1986; Boyle *et al.*, 1991a; Ma and Smith, 1992; Ma *et al.*, 1994; Zhou and Smith, 1996). Boyle *et al.* (1991b) used a stem infusion method to supply sucrose (150 g L^{-1}), dissolved in a complete liquid tissue culture medium containing plant growth regulators (2,4-D and kinetin), to water stressed corn plants. Final grain yield of some plants infused with tissue culture medium approached the

yield of well watered control plants. In addition, water stressed plants showed nearly complete reproductive failure, whether they were infused with a similar volume of water, or not, suggesting that assimilate supply at flowering may regulate kernel set in water deficient plants. In order to assess whether the plant growth regulators 2,4-D and kinetin were the media components responsible for restoration of grain yield in water stressed corn plants, they also tested the effect of the components in the medium by testing treatments with and without the plant growth regulators. Their results demonstrated that the plant growth regulators in the infused complete media were not the components responsible for preventing reproductive failure under water stress. However, they did suggest that some component of the media, besides plant growth regulators, played a role in overcoming drought stress effects.

The purpose of the present study was to examine the effects of soil water and assimilate supply (sucrose only), using the stem injection method of Zhou and Smith (1996), on corn growth during active grain filling. We chose sucrose as the supplemented assimilate since it is the carbohydrate most transported in corn plants (Prioul *et al.*, 1990).

9.3. Materials and Methods

The experiment was conducted in a greenhouse at the Macdonald Campus of McGill University, Ste Anne de Bellevue, Québec, Canada in the spring of 1994. Two water availability treatments (water table maintained at 50 cm from the soil surface, and water stress) and three sucrose injection concentrations [0 (distilled water), 150 and 300 g sucrose L^{-1}] were organized in a 2 X 3 factorial arranged within each of three randomized complete blocks.

9.3.1. Plant culture

Corn (*Zea mays* L.) hybrid Pioneer 3921, was planted in 68.2 L white plastic pails (57 cm in height) filled with 100 kg of 2:1 (v/v) sand/soil mixture on March 24, 1994. The experiment was conducted at 30/20°C day/night temperature, 85/45% relative humidity. High pressure sodium lamps (St Thomas de Joliette, Québec, Canada) provided a 14.5 h photoperiod.

At planting, 5 seeds were sown in each pail. The seedlings were thinned to

two plants per pail one week after emergence. Two weeks after emergence, the plants were thinned again to one plant per pail. All plastic pails were watered to field capacity daily until one week before silking when water availability treatments were begun. Starting at emergence, fertilizer (20-20-20) $N\text{-}P_2O_5\text{-}K_2O$ solution (3 g L^{-1}) was applied twice a week at the rate of 2 L per pail. The nutrient solution concentration was increased to 6 g L^{-1} at three weeks after emergence to provide additional nutrients for stem elongation and early reproductive development. The fertilizer application stopped after four weeks when the water availability treatments started. To simulate field conditions, the pails were arranged 30 cm apart, giving a plant density equivalent to approximately 75,000 plants ha^{-1}.

9.3.2. Water stress and water table treatments

In order to create a simulated controlled water table, four holes were drilled into the sides of each pail near the bottom to provide drainage for each treatment. When water table control commenced, the holes were blocked with rubber stoppers (0.65 cm, o.d.). An 80 cm length of PVC pipe (5.5 cm, o.d.) was used to control water table with four holes (1 cm, o.d.) drilled 5 cm above the bottom opening of the pipe. The pipe was positioned vertically near one side of each pail and buried in the soil mixture to a depth of 55 cm. To obtain a constant water table at 50 cm from the soil surface, a rubber stopper (6.5 cm, o.d at the top, 5.5 cm, o.d at the bottom and 2.6 cm in height) was tightly inserted into the top of the PVC pipe; a hole just large enough to fit the neck of a 1 L bottle had been drilled through the stopper. When the water table treatment started, the stopper was sealed tightly onto the top of the PVC pipe was attached to 1 L reservoir filled with water. Water from the bottle reservoir was delivered to the soil. When the water level in the pail dropped below 50 cm below the soil surface, water flowed out of the bottle (water reservoir) and raised the water table back to 50 cm from the soil surface.

To achieve water stress conditions at silking, water was withheld for approximately one week before the silking stage (R1) (Ritchie *et al.*, 1986). By R1 stage, the leaves of plants receiving the water stress treatment were wilting and curling, and the photosynthetic rates of these plants had already been reduced to 25%

of the 50 cm water-table treatment values (Table 9.2). This water stressed treatment was rehydrated at day five after silking (R1) stage. During this interruption, 2 L H_2O were added to water stress plants for a four-day period. The water was added as it was evident that without it the drought stressed plants would have died before the end of the experiment. Water stress symptoms developed again within one week of withholding water for the second time, but it was felt that the plants would probably survive to the end of experiment, which they did. Both water stress and water table control treatments were continued until the late dent stage (R5) (Ritchie *et al.*, 1986).

As the time between tasselling and silking increased for drought stressed plants, so pollen was collected in a paper bag from plants receiving the water table treatments and used to hand pollinate the water stressed plants during the early morning on the first, second and third days after silk emergence.

9.3.3. Stem injection

The details of this injection technique have described in a previous paper (Zhou and Smith, 1996). Briefly, flexible plastic tubing (i.d. 0.8, o.d 2.4 mm) (Tygon, Norton Co., Akron, OH, USA), an 18 gauge standard disposable hypodermic needle (Becton Dickinson, Rutherford, NJ, USA) and a similar 21 gauge needle with the plastic portion removed formed the delivery system while several standard (22.5 X 8.5 X 7 cm) ceramic three-hole construction bricks (approximately 2.7 kg each) and two 2.4 metre (1.25 cm i.d.) supporting steel pipes provided the pressure for injection. The injection end of the 18 gauge needle was inserted into the top of the tubing. Masking tape was wrapped around the connection site between the 18 gauge needle and the tubing to form a cup shape, which was filled with latex (Vultex, General Latex Canada, Candiac, Québec, Canada) and allowed to dry. The top of the 21 gauge needle was inserted into the bottom of the tubing and sealed there with white silicon sealant (GE Silicones Canada, Pickering, Ontario, Canada). The tubing-needle system was applied when the silicon and the latex completely dried. Before injection commenced, the 21 gauge needle indicated above was inserted downward into the internode below the ear, approximately 5 cm below the node which gave rise to the ear shank. Masking tape was applied to the corn stem to form a cup around

this injection site and the cup was filled with latex. Two steel pipes with a attached piece of plywood at a desirable hight were fixed to the edge of a nearby greenhouse bench in the greenhouse prior to injection. The plywood provide a platform to hold a test-solution filled 60 mL syringe, which was connected to the 18 gauge needle at top of the tubing-needle system. A number of bricks rested on the syringe plunger to produce a constant pressure to force tested solutions into the corn stems for a period of weeks.

Injection was initiated at the R1 developmental stage and continued to late in the R5 stage (Ritchie *et al.*, 1986), a period 30 days. By day 30 after the commencement of injection, 57% of the leaves of the water stressed plants (including the ear leaf) had died due to extreme water stress. The solution volume in the syringe was observed and recorded daily; the decrease in solution volumes indicated uptake by the injected plants.

9.3.4. Measurements and analyses

Forty-eight hours after the water and injection treatments were started, net photosynthesis was measured on the ear leaf with a Li-Cor 6200 portable photosynthesis system (Li-Cor Inc., Lincoln, NE) between 10:00 and 12:00. These measurements were then conducted at one week intervals for the next three weeks. Due to technical problems, we were not able to record data for stomatal conductance for the first measurement (48 hours after the imposition of the treatments).

Corn plants were harvested on July 7 by cutting at the soil level. Harvested plants were separated into leaves (including the leaf sheath), injected internodes, stalks, non-seed reproductive structures (husk, shank and tassel) and ears, then dried at 70 °C to a constant weight. The dry weight, grain weight, width and length of the ear, kernel rows per ear, number of kernels per row, and 100-seed weight of each plant were measured. Any developing secondary ears were removed as soon as they were noted, so that all data on grain yield and yield components would relate to primary ears only.

Statistical analyses were conducted with the PROC GLM procedure of the SAS Package (SAS Institute Inc., 1985), according to Steel and Torrie (1980).

Comparisons between means were made with a GLM analysis protected LSD test. Generally, differences reported in this paper were at the $P \leq 0.05$ level. However, some differences between the 0.05 and 0.1 levels are discussed; in these cases the P values are given in the text and in the tables.

9.4. Results and Discussion

9.4.1. Injection uptake rates

The injection period lasted for 30 days. The overall average daily injection rate was 3.1 mL day^{-1} (Table 9.1). The uptake rate for water stressed plants was not different among injection concentrations. Under sufficient moisture conditions, the uptake rate day^{-1} was highest for the distilled water treatment. These results agreed with Zhou and Smith (1996). In total, plants took up approximately 27 mL less of the 300 g sucrose L^{-1} solution than the distilled water over water availability treatments during the injection period. Interestingly, under water stress conditions, 30 mL more of the injected 300 g sucrose L^{-1} solution per day was taken up than of the 150 g sucrose L^{-1} solution. During the 30 day injection period, over water availability treatments, approximately 11.2 and 22.4 g sucrose were injected for the 150 and 300 g sucrose L^{-1} solutions, respectively.

In this study, we found that the 300 g sucrose L^{-1} solution occasionally resulted in flow of stalk solution back into the syringe reservoir, even through the solution in the reservoir was under pressure. Ma *et al.* (1994a) made a similar observation under field conditions, although in an unpressurized system. The 150 g sucrose L^{-1} was a better concentration for use under greenhouse conditions. Similar results have been observed under field conditions (Ma *et al.*, 1994a).

9.4.2. Leaf photosynthesis and stomatal conductance

In general, photosynthesis declined as the plants developed (Table 9.2). Treatment differences were present 2 days after silking, by which time the photosynthetic rate of water stressed plants was approximately 25% that of the well watered plants. The water stressed plants were given 2 L of water per day for four successive days, starting five days after silking after which the full water stress regime was reimposed (see Materials and Methods). As a result, measurements of

the photosynthetic rates taken one week after silking were not different between water availability treatments. The photosynthetic rates of the water stressed plants then continued to be lower than those of the well watered plants until the onset of senescence.

The effects of injected solution concentrations on leaf photosynthesis were observed at 48 hours and two weeks after the commencement of injection ($P=0.06$). This result was different from the finding of Ma *et al.* (1994a) who, using the modified method of Boyle *et al.* (1991a), observed no infused sucrose concentration effects on photosynthetic rates. By 48 hours after the onset of injection, the water stressed plants injected with sucrose had near zero photosynthetic rates. This inhibition of photosynthesis for the plants injected with sucrose may have been due to accelerated accumulation of assimilates (either free sugars or starch), especially under water stress. This end-product inhibition of photosynthesis has been observed perviously (Gifford and Evans, 1981). Recent investigations at the molecular level have shown that inhibition of photosynthetic gene expression by metabolic factors is related to high tissue carbohydrate content, which forms a mechanism for 'sink regulation' of photosynthesis (Sonnewald and Willmitzer, 1992; Krapp *et al.*, 1993; Van Oosten *et al.*, 1994). While such an inhibition was indicated by a reduced photosynthetic rate at 48 hours after the start of injection ($P \leq 0.06$), the measurements taken at one and two weeks ($P \leq 0.06$) after the start of injection showed elevated rates of photosynthesis for plants receiving sucrose. No previous work has investigated the effect of long-term injection of sucrose into corn stems on leaf photosynthesis, and the reasons for the increased photosynthetic rates due to injected sucrose are not understood.

By the third week of injection, there was an interaction between water availability and sucrose injection concentration ($P=0.05$). Injection of the 300 g sucrose L^{-1} solution strongly inhibited photosynthesis under water deficient conditions while the same concentration resulted in the highest photosynthetic rate under sufficient water supply. Under water deficit stress conditions, the injected 300 g sucrose L^{-1} solution more strongly inhibited photosynthesis than the 150 g sucrose L^{-1}

solution. Water stress may have reduced sink capacity very early on, at a time when photosynthetic rates of the 150 g sucrose L^{-1} plants were also affected. This inhibition of net photosynthesis at the highest sucrose concentration may have been caused by osmotic imbalance or a metabolic reaction to accumulated solutes (mainly sucrose) in the stem of water stressed plants, leading to decreased water potential pressure on the cell membranes, causing inhibition of photosynthetic electron transport (Keck and Boyer, 1974).

When plants are exposed to water stress, stomatal closure is one of the earliest and most sensitive responses (Hsiao, 1973). Changes in stomatal conductance coincided with changes in photosynthetic rate during the period when the water availability treatments were imposed (Table 9.3). One week after silking, stomatal conductance of the ear leaves was not different among any of the treatments. This was probably because the plants of the water stressed treatment were receiving water at this time, in order to prevent mortality (see Materials and Methods). However, the water stress led to reductions in stomatal conductance for the last two weeks. The concentration of sucrose injected did not cause any detectable changes in stomatal conductance at the first two measurements (one week and two weeks after injection). Although by the third week of injection, water stressed plants receiving sucrose showed inhibition of stomatal conductance when compared with well watered plants (Table 9.3). The stomatal conductance of water stressed plants injected with 300 g sucrose L^{-1} was not different from that of distilled water injected plants. However, for the well watered plants injection with 300 g sucrose L^{-1} reduced average stomatal conductance below that of 150 g sucrose L^{-1} injected plants.

9.4.3. Dry matter partitioning, yield components and grain yield

There was no effects of water availability treatments or sucrose injection concentrations on total stalk weight, leaf number and leaf weight (Table 9.1). Averaged over injection concentrations, total dry matter production was reduced 20% as a result of the water stress treatments. This reduced accumulation of total dry matter production confirmed the observed inhibition of photosynthesis under water deficit stress. A similar result was also reported by Schussler and Westgate (1991).

Under these controlled environment conditions, we also found no effect of injected sucrose concentrations on total stalk weight and total dry matter production, which was consistent with our findings under field conditions (Zhou and Smith, unpublished data).

Injected sucrose increased the weight of the injected internodes (Table 9.1) ($P \leq 0.07$). Injection of 300 g sucrose L^{-1} caused a 3.2 g (28%) increase in the weight of the injected internodes over that of distilled water injection. This agrees with our previous observations for field grown plants (Zhou and Smith, unpublished data). The increase in the weight of the injected internodes due to injection of 300 g sucrose L^{-1} could have had several causes. First, sink limitation may have resulted in extensive stalk storage of the injected materials. Second, the continuous injection of concentrated sucrose solutions with their high osmotic potentials may have caused cell membrane damage, which resulted in restriction of injected solution transport leading to accumulation of solute at the injection internodes. Third, the production of callus or tyloses due to wounding caused by needle injection, could also have increased internode weight.

Production of the non-seed reproductive structures (husk, shank, and tassel) by corn plants was affected by the water availability and injected sucrose concentrations, but only at $P=0.05$ and 0.07, respectively. Averaged over the injection concentrations, water availability increased dry weight of the non-seed reproductive structures by 7.8 g. The injection of 300 g sucrose L^{-1} increased non-seed reproductive structure weight by 4.5 g $plant^{-1}$, compared with distilled water and 150 g sucrose L^{-1} treatments, averaged over the water availability treatments.

The water availability treatment produced more and larger seeds, leading to greater grain weight per plant when compared with the water stressed plants (Table 9.4). Water availability increased the number of kernels per row by 15% and the 100 seed weight by 15%, when compared to water stress conditions. The reduced kernel set under water stress treatments was probably due to developmental failure within the ovary (Bassetti and Westgate, 1993).

There were interactions between water availability treatment and sucrose

injection treatment for ear length ($P \leq 0.05$) and grain yield ($P \leq 0.06$). Under water stress, plants injected with sucrose at 150 and 300 g L^{-1} had ear lengths that were 4.7 cm (23%) and 5.6 cm (28%) shorter, respectively, than those of the corresponding well watered treatments. Water stressed plants injected with distilled water had ear lengths similar to those of distilled water injected well watered plants (Table 9.4).

The highest grain yield was observed for the plants injected with 150 g sucrose L^{-1} under sufficient water supply. The plants injected with 300 g sucrose L^{-1} produced the lowest grain yields under water stress conditions. However, under water stress, both sucrose injection treatments produced lower yields than distilled water injection, and also the plants injected with 300 g sucrose L^{-1} produced 40.2 g (34%) less grain weight than the same injection concentration with sufficient water supply. Data presented here indicated that the 300 g sucrose L^{-1} solution was probably excessively concentrated, perhaps causing osmotic stress, or other disruptions. Other possible causes of the reduction of grain yield due to injection of the 300 g sucrose L^{-1} solution are: i) the inhibition of assimilate translocation capacity, as evidenced by the greater dry matter accumulation in the injection internodes, ii) cell membrane damage due to more solute accumulation as sugar concentration increased under water deficit (Premachandra *et al.*, 1992), iii) under water stress, the earlier ear leaf senescence and greater accumulation of dry matter in non-seed reproductive structures and injection internodes due to injection of 300 g sucrose L^{-1} may also have been factors responsible for the reduction in grain yield.

Some of the effects of sucrose injection observed under controlled environment conditions, differed from our previous findings under field conditions (Zhou and Smith, unpublished data). Injection of sucrose into corn stems under field conditions increased primary ear grain yield, but inhibited the development of the secondary ear, resulting in an overall yield reduction. However, for the greenhouse study, we attempted to avoid this complication by removing the secondary ears from all plants, hence, we artificially removed this alternate sink. In addition, disruption of sucrose translocation and sink relationships were obvious under field conditions, as evidenced by rapid senescence of the ear leaf and ear+1 leaf, with an accompanying

accumulation of red pigments for plants injected with sucrose, by two weeks after injection treatments were imposed. In the work reported here, we found that sucrose injection decreased grain production, although only under conditions of water stress. Although the field grown plants showed no visible signs of water stress, it is certain that these plants experienced lower average water availability than the 50 cm water table treatment used here, both because soil water was probably less available, at least for some time during the injection period in the field, and because the transpiration is certain to have been higher in the field (i.e. higher radiation and wind levels, and lower relative humilities than in the greenhouse). Under greenhouse conditions, the ear leaf of water stressed plants showed the greatest reductions in photosynthetic rates relative to other treatments by three weeks after the injection treatments were imposed. This reduction of photosynthesis indicated early ear leaf senescence for water stressed plants. However, in this work, the pre-senescent ear leaf did not show a red colour as was the case under the field conditions. This may have been due to lower light intensities and/ or better phosphorous nutrition in the greenhouse. Greenhouse light levels were about half of field values and this was found to be the case in our situation (e.g 474.2 μmol PAR m^{-2} s^{-1} vs 925.4 μmol PAR m^{-2} s^{-1} under clear skies). In addition, light levels in April and May, when most of the corn growth occurred in the greenhouse would have been well below those occurring in June and July when rapid growth occurs under field conditions.

Zinselmeier *et al.* (1995) reported that the exogenous supply of a solution containing only sucrose and water to water stressed corn plants maintained the grain development of these plants. A careful comparison of our results with the results of Zinselmeier *et al.* (1995), showed that the grain yield of water stressed plants receiving sucrose (150 g L^{-1}) was similar to their work and in our work (89.6 vs 74 g per ear). We injected approximately half the mass of sucrose infused by Zinselmeier *et al.* (1995) and our entire injection occurred at one injection site over a relatively long period of time [32 days for ours' work vs 5 days for Zinselmeier *et al.* (1995)'s work]. However, in our study, we did not observe positive effects of sucrose addition under water stress conditions, when compared to plants with injected water

only. The main cause of this difference may have been due to greater grain yield for the water injected plants in our work than their that of Zinselmeier *et al.* (1995) (108.5 vs 4 g per ear). Based on the photosynthetic rates of water stressed plants in our work and that of Zinselmeier *et al.* (1995), the level of water stress applied by Zinselmeier *et al.* (1995) was lower than the level applied by us (8 μ mol m^{-2} s^{-1} vs 3 μ mol m^{-2} s^{-1}) on the first day of water stress was imposed, and the rehydration of plants from their study appeared to result in faster recovery of photosynthetic rate than ours. This indicated that our plants were more stressed than those of Zinselmeier *et al.* (1995). It seems probable that the main cause for the different conclusion reached in our work and that of Zinselmeier *et al.* (1995) was a difference in the physiological ability of the two hybrids to tolerate water stress, which resulted in the lack of a positive effect of sucrose injection on grain yield. The hybrid used in our work is reported to be stress-tolerant.

9.5. Conclusion

Supply of sufficient water increased the number seeds per row and seed size, leading to greater yield per plant. Injected sucrose concentrations did not change yield components in water stressed plants. Injection of 300 g sucrose L^{-1} decreased grain yield, especially under water stress conditions. With sufficient water supply, injection of the 150 g sucrose L^{-1} increased grain yield. The 150 g sucrose L^{-1} solution was the more appropriate concentration for use under greenhouse conditions when supplying assimilate as sucrose into corn stems. These results indicated that exogenous sucrose supplementation influenced kernel set only under conditions of sufficient soil water supply. The observation that sucrose injection (150 g sucrose L^{-1}) increased grain production when the plants had sufficient water, but not when water stressed, indicated that plant reproductive development after silking was limited more by water availability than by the supply of assimilates, indicating that some overall plant response to water stress, perhaps mediated by hormonal signalling, was more important than carbohydrate supply. Different hybrids may respond differently to the injection sucrose.

Table 9.1. The effects of water availability and injected sucrose concentrations on injection daily solution uptake and partial corn dry matter distributions. **, NS indicates differences at the 0.05 probability level, and not significant at $P < 0.1$, respectively, P values between 0.1 and 0.05 are given.

Factors		uptake rate	non-seed reproductive structures	injection internode	stalks	ear leaf	leaves	leaf	total
		(mL day^{-1})	(g plant^{-1})	(g)	(g plant^{-1})	(g plant^{-1})	(g plant^{-1})	numbers	(g plant^{-1})
Water Availability	Infused Concentrations								
Water stress	distilled water	3.2	22.1	7.2	87.7	4.5	38.3	14.0	268.2
	150 g sucrose L^{-1}	2.4	23.6	8.9	94.7	4.3	34.5	13.0	255.6
	300 g sucrose L^{-1}	3.4	28.8	11.6	120.7	4.5	38.9	13.0	282.9
Water	distilled water	4.1	33.0	8.9	135.5	4.2	38.8	14.0	319.4
sufficiency	150 g sucrose L^{-1}	2.6	33.1	8.8	121.9	4.5	37.5	14.0	346.4
	300 g sucrose L^{-1}	2.1	36.1	10.8	131.8	4.3	38.2	14.0	339.6
$LSD_{0.05}$		1.0	7.7	3.2	43.1	0.6	5.6	1.8	67.3
Water Availability (W)		NS	0.05	NS	NS	NS	NS	NS	**
Concentrations (C)		*	0.07	0.07	NS	NS	NS	NS	NS
W X C		NS	NS	NS	NS	0.06	NS	NS	NS

Table 9.2. The effects of water availability and injected sucrose concentrations on corn leaf photosynthesis. † stands for 48 hours, one week, two weeks and three weeks after injection was imposed. **, NS indicates differences at the 0.05 probability level, and not significant at P< 0.1, respectively. P values between 0.1 and 0.05 are given.

Factors		48 hrs†	1 wk	2 wks	3 wks
		------ μ mol m^{-2} s^{-1} -------			
Water Availability	Infused Concentrations				
Water stress	distilled water	11.7	8.7	4.4	3.0
	150 g sucrose L^{-1}	2.4	9.9	5.3	3.3
	300 g sucrose L^{-1}	2.7	11.4	8.7	1.3
Water sufficiency	distilled water	24.8	6.8	5.0	5.3
	150 g sucrose L^{-1}	20.6	14.7	9.3	4.0
	300 g sucrose L^{-1}	22.7	11.6	10.9	5.8
$LSD_{0.05}$		8.0	7.8	4.8	2.2
Water Availability (W)		**	NS	0.09	**
Concentrations (C)		0.06	NS	0.06	NS
W X C		NS	NS	NS	0.05

Table 9.3. The effects of water availability and injected sucrose concentrations on stomatal conductance of corn leaves. † stands for 48 hours, one week, two weeks and three weeks after injection was imposed. **, NS indicates differences at the 0.05 probability level, and not significant at $P < 0.05$, respectively.

Factors			48 hrs†	1 wk	2 wks	3 wks
			m mol m^{-2} s^{-1}			
Water Availability	Infused Concentrations					
Water stress	distilled water	--		44.2	18.2	9.7
	150 g sucrose L^{-1}	--		24.7	18.9	6.4
	300 g sucrose L^{-1}	--		27.9	18.5	4.1
Water sufficiency	distilled water	--		32.8	32.7	23.7
	150 g sucrose L^{-1}	--		37.9	32.5	26.8
	300 g sucrose L^{-1}	--		39.2	30.0	16.5
$LSD_{0.05}$		--		46.2	16.4	9.3
Water availability (W)		--		NS	**	**
Concentrations (C)		--		NS	NS	**
W X C		--		NS	NS	NS

Table 9.4. The effects of water availability and injected sucrose concentrations on corn grain yield and yield components. **, NS indicates differences at the 0.05 probability level and not significant at P< 0.1, respectively, P values between 0.1 and 0.05 are given.

Factors		# rows	width	length	# seed DW	grain	100 seed
		(ear^{-1})	(cm ear^{-1})	(cm ear^{-1})	(row^{-1})	(g ear^{-1})	(g)
Water Availability	Infused Concentrations						
Water stress	distilled water	13.0	14.2	18.1	33.7	108.5	25.6
	150 g sucrose L^{-1}	13.0	13.8	16.1	31.3	89.6	25.2
	300 g sucrose L^{-1}	12.0	12.8	14.7	28.7	78.3	24.5
Water	distilled water	12.0	14.1	19.2	37.0	98.9	29.4
sufficiency	150 g sucrose L^{-1}	13.0	18.2	20.8	39.0	140.7	28.5
	300 g sucrose L^{-1}	11.0	13.8	20.3	34.0	118.5	30.9
$LSD_{0.05}$		3.9	5.8	2.6	11.0	52.7	3.7
Water Availability (W)		NS	NS	*	*	*	*
Concentrations (C)		NS	NS	NS	NS	NS	NS
W X C		NS	NS	0.05	NS	0.06	NS

Preface to Chapter 10

Chapter 10 is comprised of material from a manuscript prepared by myself, my supervisor Dr. D.L. Smith and two other co-authors, Dr. A.F. MacKenzie and Dr. C.A. Madramootoo for publication in the Journal of *Physiologia Plantarum* in 1996. The format has been changed to be consistent within this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

In chapters 9, it was demonstrated that plant reproductive development after silking was limited more by water availability than assimilate supply. As mentioned in the literature review, some plant growth regulators might increase grain weight, or induce stomatal closure, or stimulate photosynthetic rates. Therefore, we assessed the effects of continuous plant growth regulator supply (via a stem injection system), with or without sucrose, during the grain filling period on the growth and productivity of field grown corn plants in this chapter.

Chapter 10

Effects of Stem-Injected Plant Growth Regulators, with or without Sucrose, on Grain Production, Biomass, and Photosynthetic Activity of Field-Grown Corn plants

10.1. Abstract

Application of plant growth regulators (PGRs) or sucrose to cereal crop plants at anthesis has been demonstrated to affect plant photosynthetic activities and productivity. ABA causes stomatal closure, and photosynthesis restriction during drought. IAA stimulates many aspects of plant development, while little is known about SA in this regard. Sucrose has potential to mitigate inhibition of photosynthesis in water stressed corn plants. However, conventional foliar application or tissue culture with PGRs and other organic materials have limitations. No previous study has examined the effects of long term continuous addition of PGRs, with or without sucrose to field grown corn plants. A field study was conducted to supply PGRs and sucrose to corn plants (*Zea mays* L.) through a stem-injection technique. Our objective was to determine the effects of a continuous supply of PGRs, with or without sucrose, during the grain filling period on corn plant growth and productivity under field conditions. Our injection technique delivered pressurized solutions through syringe needles sealed to the stem with latex. Pressure was applied to the syringe plunger with ceramic construction bricks. Four PGRs (IAA, ABA, ethephon, salicylic acid) plus a distilled water control and two levels of sucrose (150 g L^{-1} and a distilled water control) were injected into corn plants for forty-two consecutive days during the grain filling period. Both ethephon and ABA are known to inhibit aspects of plant development. The plants injected with solutions not containing sucrose took up forty-two mL more than those of injected with sucrose containing solutions. Corn plants injected with salicylic acid (SA) produced 9% more grain yield than plants injected with no PGRs. The combination of SA and sucrose increased the photosynthetic rate by 42% when compared with distilled water. Injection of ethephon resulted in an 11% reduction in grain yield. Neither IAA nor ABA altered plant photosynthesis or productivity. Sucrose injection increased the dry weight of

injected internodes and stover, and induced partial stomatal closure, although without any measurable effect on net photosynthetic rate. This study showed that stem injection makes possible the study of changes in plant physiology during grain-filling due to the effects of PGRs and metabolites administered continuously over a protracted period of time. It also demonstrated a previously undocumented stimulation of plant photosynthesis and grain yield by SA.

10.2. Introduction

Plant growth regulators (PGRs) have been widely used in crop production. The influences of PGR application on crop growth and productivity are varied. Norberg *et al.* (1988) reported that ethephon application under high density conditions decreased corn (*Zea mays* L.) grain yield, and reduced plant height and lodging when applied at tasseling or post-tasseling. In the same study, ethephon application was shown to increase the protein concentration of the grain. Increased seed protein concentrations were also found in barley (*Hordeum vulgare* L.) (Ma *et al.*, 1994b; Foroutan-pour *et al.*, 1996) when ethephon was added via a peduncle perfusion technique.

ABA regulates transpiration by inducing stomatal closure (Raschke, 1975) which can reduce plant water loss (Davies and Jones, 1991), but also results in decreased CO_2 uptake. ABA can act to close stomata during the periods of drought (Davies and Mansfield, 1983). Foroutan-pour *et al.* (1994) found that peduncle perfused ABA decreased the total seed weight per spike and seed protein of barley plants. Boyle *et al.* (1991a) infused ABA into corn plants and suggested that ABA could be used to temporarily induce stomatal closure, thus reducing photosynthesis. Thus ABA may reduce stomatal aperture and photosynthesis, as occurs under dry conditions, and sucrose addition may overcome the resulting reductions in yield.

IAA acts as a promoter of stomatal opening and stimulates photosynthetic rates (Davies and Mansfield, 1987; Guinn *et al.*, 1993). It also has been reported that IAA alters the pattern of weight per seed between and within the spikes of wheat plants (Rademacher and Graebe, 1984; Bangerth *et al.*, 1985).

Salicylic acid (SA) is a member of an diverse group of plant phenolics, and has recently been reported to act as an endogenous signal for systemic-acquired resistance to infection by plant pathogens (Raskin, 1992). Patterson (1981) found that phenolic acids at a concentration of 1 mM reduced growth and physiological processes, including photosynthesis, of soybean [*Glycine max* (L.) Merr.]. Shettel and Balke (1983) reported that salicylic acid sprayed on the soil surface or plant foliage reduced shoot dry weight accumulation by several crops (including corn) and weed species. It has been suggested such a reduction could have been due to interference with membrane ion transport in roots (Glass, 1973; Harper and Balke, 1981). On the other hand, SA was reported to increase pod number and yield in mung bean (*Vigna radiata* L. Wiliczek) (Kling and Meyer, 1983). Jain and Srivastava (1981) found that SA increased the *in vivo* activity of nitrate reductase in corn seedlings. Rai *et al.* (1986) showed that SA greatly promoted stomatal opening by excised leaves of *Commelina communis* L. plants.

Sucrose is the main form of assimilate transported in corn (Prioul and Schwebel-Daugue, 1992) and is translocated to seeds during the grain filling period (Daie, 1985). Ma *et al.* (1994a) reported that stem infusion with sucrose after silking increased kernel set in one of two corn hybrids tested. Milthorpe and Moorby (1969) indicated that the application of IAA increased the movement of soluble carbohydrates, nitrogen substances and ions into the treated regions over a period of several days. Furthermore, they concluded that the direct effect of IAA on transport mechanisms and those arising from enhanced growth could not be distinguished. Hew *et al.* (1967) demonstrated that the export of photosynthate from the primary leaves was increased when IAA was applied to decapitated soybean plants. They proposed that IAA influences sucrose translocation in the soybean stem. Aspirin, a trade name for acetylsalicylic acid, plus sucrose were reported to enhance flower opening in orchid species (Hew, 1987). Thus, it is possible that interactions between PGRs and sucrose could alter plant development.

Stem infusion or injection techniques have been used successfully to supply

nitrogen, plant growth regulators or sucrose to different plants (Boyle *et al.*, 1991a,b; Ma *et al.*, 1992; Foroutan-pour *et al.*, 1995; Zhou and Smith, 1996). No previous study has documented the effects of long term injection of PGRs alone or combination with sucrose on corn plant growth during the grain filling period. Thus, our objective was to determine the effects of continuous PGR supply (via a stem injection system), with or without sucrose, during the grain filling period on the growth and productivity of field grown corn plants.

10.3. Materials and Methods

The experiment was conducted during the 1994 growing season at the Horticulture Research Centre of the Plant Science Department, McGill University, Ste-Anne-de-Bellevue, Québec, Canada. Prior to this experiment, the experimental field was used for grain corn production. The experimental plots were established on St. Bernard loam (fine loamy, mixed, nonacid, frigid, Typic Eutrochrept) soil. The plots were fertilized based on the results of a soil test. Each plot received 25 kg N ha^{-1} as 5-20-20 (N-P-K) at planting. An additional 50 kg N ha^{-1} as NH_4NO_3 were applied at the four leaf stage. Each plot was 4.5 m x 4 m. Seed of corn hybrid Pioneer 3921 was planted in six rows with a row spacing of 0.75 cm, giving a final plant density of 66,666 plants ha^{-1}. Seeding was conducted on May 29 and the plants were harvested on October 22, 1994. The corn plants received only rainwater and no herbicides were applied. Hand weeding was applied when necessary. The total monthly precipitation, and temperatures during the 1994 growing season and 30 years average are given in Table 10.1.

Four PGRs (IAA at 1.5 mg L^{-1}, ABA 1 mg L^{-1}, ethephon 15 μM, salicylic acid at 1 mM) plus a distilled water control, and two levels of sucrose (150 g L^{-1} and a distilled water control) were combined in a 5 by 2 factorial randomized complete block design with three blocks. Injection treatments were randomly assigned to plants in the two middle rows of each plot and each was applied to one plant within these two rows. All test solutions were prepared in a sterile environment and stored in a refrigerator until use. In order to prevent the photooxidization of IAA, the IAA solution was stored in a brown bottle and the injection apparatus for IAA was covered

by aluminium foil when in use in the field.

A full description of the stem injection system can be found in Zhou and Smith (1996). Briefly, a flexible plastic tubing (i.d. 0.8 mm, o.d. 2.4 mm) (Tygon, Norton Co., Akron, OH, USA), a 21 gauge and an 18 gauge standard disposable hypodermic needle (Becton Dickinson, Rutherford, NJ, USA) were used to construct the delivery system. The injection end of 18 gauge needle was inserted into the top of the tubing. Masking tape was wrapped around the connection site between the 18 gauge needle and the tubing to form a cup shape which was filled with latex (Vultex, General Latex Canada, Candiac, Québec, Canada). The top end of the 21 gauge needle, with the plastic portion removed, was inserted into the bottom of the tubing and sealed with white silicon sealant (GE Silicones Canada, Pickering, Ontario, Canada). The tubing-needle system was applied in the field and allowed to stand until the silicon and the latex were completely dried (approximately five days). Before the injection commenced, 21 gauge needle described above was inserted downward into the internode below the ear, 5 cm below the node which gave rise to the ear shank. In order to prevent leakage and damage by wind, masking tape was applied to corn stem to form a cup around the injection site; this was filled with latex, which was allowed to dry for several days. The other end of tubing-needle system was taped to stem above the injection point during the time the later was drying. The upper plastic portion of the 18 gauge needles were covered by masking tape until the injection started. Several standard (22.5 X 8.5 X 7 cm) ceramic three-hole construction bricks (approximately 2.7 kg each) provided a pressure source and two 2.4 metre (1.25 cm o.d.) steel pipes provided support for the bricks. These two steel pipes were hammered into the soil beside the test plants prior to injection, and a piece of plywood fixed at the desired height provided a platform to hold a test-solution filled 60 mL syringe, which connect to the top of the 18 gauge of the tube-needle system, with the bricks resting on the syringe plunger. The bricks provided a constant pressure to force the tested solutions into the corn stems over a six week period.

Injection was initiated one week after silking (August 3, 1994) and continued for forty-two days. The solution volume in the syringe was observed and recorded

daily; the decrease in solution volumes indicated uptake by the injected plants. Two or three of the stem injection attempts showed signs of leakage or blockage. This problem was overcome by establishing an additional injection site on the internode above the ear. If leakage or blockage of the first injection setup was detected, the experimental treatments were immediately switched to the second injection site.

Photosynthesis was measured on ear leaves at 24 hours and one week after injection. At harvest, the number of rows per ear, the number of seeds per row, ear size (width and length), stover (leaves plus stalks) dry weight, injected internode dry weight, and grain yield were measured. All plant tissues were dried to a constant weight then weighed. Grain was sub-sampled and ground with a Wiley mill (A.H. Thomas Co., Philadelphia, PA, U.S.A) to pass through a 2 mm sieve for N determinations by the Kjeldahl method (Bradstreet, 1965). Ground tissue samples were digested with H_2SO_4 and Kjeltab catalyst, distilled (Tecator, Höganäs, Sweden), and titrated with 0.05 M HCl.

The data were analyzed with the GLM procedure of the SAS package (SAS, 1985) according to Steel and Torrie (1980). When the GLM analysis indicated their presence, differences between means were determined by a protected least significant difference (LSD) test at the 0.05 level. In general, differences are reported at the $P \leq 0.05$ level. However, some differences at 0.1 level are discussed; in these cases the P values are given in the text. Because injected distilled water and non-injected plants were not different for all measured variables, the distilled water injected plants were used as the controls in this study.

10.4. Results and Discussion

10.4.1. Uptake volumes

The injection period lasted forty-two days. The uptake volumes of plants injected without sucrose were 42 mL more than those injected with sucrose (Table 10.3). A similar result was reported previously by Zhou and Smith (1996). Overall, injected plants took up an average of 290 mL of tested solution, which was equivalent to 7 mL per plant per day. The injected volumes were greater than in our previous study (Zhou and Smith, 1996). Two factors may have contributed to this difference:

i) the relatively dry and warmer than normal weather during the 1994 growing season probably enhanced the uptake volumes due to elevated evapotranspiration rates (Table 10.1); ii) we started the injection one week after silking in this study while in the previous study injection was begun 15 days after silking. We noted in the previous study that the cessation of uptake coincided with physiological maturity, and suggested that this may have been caused by decreasing plant metabolism at this stage, or rapid production of senescence-related tissues, such as callus-like deposition in abscision layers, leading to final complete blockage of the injection sites (Zhou and Smith, 1996). This also appears to have been the case in the current study. The total volumes of solution injected were not significant as a covariable for grain yield and grain N concentration in this experiment.

10.4.2. Photosynthesis

Plant growth regulators can affect photosynthetic CO_2 uptake either by regulating stomatal aperture, by affecting photosynthetic activity at the chloroplast level or both. There were no differences among treatments for photosynthetic rates twenty-four hours after injection (data not shown). Net photosynthesis, transpiration, intercellular CO_2 concentration and stomatal conductance were affected by both PGRs and sucrose by one week after the commencement of injection (Table 10.2). In most cases, plants injected with PGRs alone had higher photosynthetic rates than plants injected with PGRs in combination with sucrose. The exception was the combination of sucrose and SA, which stimulated stomatal opening and photosynthesis relative to control plants or plants injected with sucrose alone. Injection of SA alone stimulated stomatal opening and increased transpiration one week after the onset of injection in comparison with distilled water or sucrose injected alone. Rai *et al.* (1986) reported that SA reversed ABA-induced stomatal closure. We measured an increased intercellular CO_2 concentration for most treatments that injected with PGR and sucrose together. However, the combination of SA and sucrose had the reverse effect. The increased photosynthetic rate with low intercellular CO_2 indicated that this combination may have caused the plants to utilize the available CO_2 inside the leaf more effectively. Conversely, injection of the combination of ABA and sucrose

caused the lowest net photosynthesis, but the highest intercellular CO_2 concentration, suggesting a decreased CO_2 uptake rate inside the leaf. Neither IAA nor ABA alone affected photosynthetic activity during the period of photosynthesis measurement when compared with distilled water treatment. The lack of effects of ABA or IAA on photosynthesis have been reported for other species. Arteca and Tsai (1988) found that the short-term pulses of ABA (via root application) to tomato transplants could reduce photosynthesis and transpiration for four days, but by the sixth day there was no difference between ABA treated plants and untreated controls. The study by Robinson *et al.* (1978) showed no effect of IAA on the rate of CO_2 fixation by isolated chloroplasts.

Sucrose injection induced stomatal closure and decreased transpiration rates relative to distilled water injection. Increased assimilate in the chloroplast, resulting from the exogenous supply of sucrose, probably explains the stomatal closure.

In this study we observed that the ear leaves of some plants injected with combinations of PGRs and sucrose became red one week after injection. However, there were no leaf colour changes for the plants injected with sucrose alone or with only distilled water. In our previous study, in 1993, we found a similar change of leaf colour occurred on ear leaves or the leaves just above the ears for plants injected with sucrose alone. The leaf colour change was more apparent on plants injected with a higher sucrose concentration (300 g sucrose L^{-1}) than those injected with a lower sucrose concentration (150 g sucrose L^{-1}). The reddening colour on these leaves was associated with early leaf senescence. Several factors may contribute to the much reduced leaf reddening in our current study: 1) the only sucrose concentration used in our current study was 150 g sucrose L^{-1}, while the most obvious reddening in the previous study was associated with injection of 300 g sucrose L^{-1}; 2) the long term injection of combination of PGRs and sucrose may change metabolism in the plants; and 3) the warmer and drier than normal weather in 1994 probably caused some water stress or heat stress on the plants growing in that year (Table 10.1); 4) the difference in the duration of injection (42 days in this study vs 32 days in the previous study).

10.4.3. Dry weight, yield components and grain N concentration

Since there was no interaction between PGRs and sucrose, only the main effects of PGR and sucrose concentrations are presented.

The injection of ethephon and SA altered total dry weight per plant. Compared with the treatments injected with no PGRs (distilled water and sucrose), injection with ethephon decreased total dry weight by 13% while SA increased it by 9% (Table 10.3). There were no effects of injection with PGRs on injection internode or stover weight.

Sucrose injection increased stover production by 13%, relative to the treatments without injected sucrose. Injected sucrose increased the dry weight of the injected internodes by an average of 1.2 g per plant, compared to the treatments without injected sucrose. A similar increase in the dry weight of the injected internodes was found in our previous study (Zhou and Smith, 1996). This increased dry weight for injected internodes may be due to a sink limitation in the developing ear, leading to stalk accumulation of sucrose (Hume and Campbell, 1972), and/or a wounding response, which causes insoluble compounds such as cellulose or lignin to accumulate at the injection site (Tarpley *et al.*, 1994).

Injection with PGR solutions only affected ear size (width and length) at the 0.1 level of probability. There was no effect on kernel number per row due to injection of PGRs (Table 10.3). The peduncle perfusion of ethephon did not alter kernel numbers in barley plants (Foroutan-pour *et al.*, 1995). Sucrose injection caused a small increase in kernel numbers per row ($P \leq 0.1$). Since kernel number is set before tasseling while injection started one week after silking, the lack of difference in kernel numbers might have been expected. However, there was the possibility of reduced kernel abortion due to the applied treatments, but this did not occur in our study.

In the absence of significant lodging, Langan and Oplinger (1987) reported there were reductions in corn yield, yield per ear and kernel weight due to ethephon application. In our study, plants injected with ethephon produced the lowest average kernel weight, which was 13% lower than plants injected with no PGRs. The 1994

growing season was warmer and drier than normal (Table 10.1). Ethephon has been suggested to aggravate stress conditions leading to grain yield reductions (Ma *et al.*, 1994b). Reduction in grain yield due to injection with ethephon may be related to a reduction in weight per kernel (Langan and Oplinger, 1987). In contrast, Foroutan-pour *et al.* (1995) found that application of ethephon through a peduncle perfusion method did not effect kernel weight or grain yield of barley plants.

Plants injected with SA produced 9% more grain than sucrose and distilled water treatments, respectively. Since the corn population did not vary in this study and each plant had only one ear, the increase in grain yield due to injection of SA had to have been the result of either an increase in the number of the kernels per row or on the size of kernels produced. However, there was no statistically detectable increase in either of these two variables. The number of kernels per ear (based on the number of kernels per row) did show a numerical increase, which the yield increase suggests may have been real, although below the level of statistical detection. The weight per kernel values for SA injected plants and those not injected with SA were almost identical. Singh and Kaur (1980) found that pod number and yield in mung bean were increased by application of SA.

Neither PGRs nor sucrose changed the grain nitrogen concentration of injected plants. Injection with ethephon did not altered grain nitrogen concentration, which confirms the results of Norberg *et al.* (1988). A similar finding was reported for wheat (*Triticum aestivum* L.) by Mohamed *et al.* (1990). However, Ma *et al.* (1994b) and Foroutan-pour *et al.* (1995) showed that the application of ethephon by peduncle perfusion increased the grain protein concentration of barley while injection of ABA decreased grain protein concentration, compared to distilled water controls.

We did not observe any effects on dry weight, grain yield and grain N concentration due to injection of IAA or ABA. Bousquet *et al.* (1990) did not find any relationship between ABA content and grain weight in wheat, while Foroutan-pour *et al.* (1994) demonstrated that infused ABA decreased total seed weight and grain N concentration in barley. Bangerth *et al.* (1985) found a positive correlation

between IAA concentration and dry weight accumulation rate in wheat grain. A study with hydroponically grown tomato plants showed that root application of IAA had either no effect on photosynthetic rate, and total plant dry weight, or an inhibitory effect was observed (Dong and Arteca, 1982). The lack of consistent results among previous research reports and the lack of previous work on long term injection of IAA or ABA on grain productivity and grain N concentration indicated a need for further study.

10.5. Conclusions

Our data suggested that stem injection makes possible the study of changes in plant physiology during grain-filling due to the addition of PGRs and sucrose. For grain yield, SA had an effect by itself, but here was no effect of sucrose, and there was no interaction with sucrose. However, for photosynthesis, there was a significant interaction, the injection of the combination SA and sucrose was higher than either alone. Environmental stresses may have aggravated the effects of ethephon, leading to a grain yield reduction. The injection of IAA or ABA did not alter injected plant photosynthetic activity and productivity. This study provided evidence that injection of some PGRs, with or without sucrose can alter photosynthetic rate and grain yield.

Table 10.1. Monthly mean precipitation and temperature for 1994 growing season and 30 years average.

	Precipitation					Temperature				
	June	July	August	September	October	June	July	August	September	October
			(mm)					(°C)		
1994	143.8	50.6	84.6	67.2	19.8	19.1	21.8	18.2	14.8	9.4
Average†	82.5	85.6	100.3	86.5	75.4	17.9	20.8	19.4	14.6	8.3

†30-year average.

Table 10.2. The interaction Means ear leaf net photosynthesis, transpiration, intercellular CO_2 concentration for injected corn plants one week after injection.

Treatments	net photosynthesis rate (μmol cm^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	Intercellular CO_2 concentration (ppm)	Stomatal conductance (mmol m^{-2} s^{-1})
Ethephon +Sucrose	6.5	1.4	212.4	57.9
Ethephon	23.4	2.8	112.2	126.9
IAA+Sucrose	6.3	2.1	274.3	83.1
IAA	12.6	2.6	216.6	120.0
ABA+Sucrose	1.7	1.8	357.5	66.1
ABA	7.9	2.1	258.0	86.7
Salicylic acid +Sucrose	24.7	3.0	102.6	125.0
Salicylic acid	15.1	3.4	226.3	156.6
Sucrose	7.8	2.4	256.9	99.3
Distilled water	14.4	2.4	158.9	97.4
LSD_a†	6.2	0.7	56.9	38.5
LSD_b	3.9	0.4	35.9	24.3
LSD_c	8.8	-	80.4	-
Significance of Factors				
PGR§	**	*	**	*
Sucrose	*	*	*	*
PGR X Sucrose	*	NS	**	NS

**, * different at 0.01 and 0.05 probability levels, respectively; NS= not significant between 0.1 and 0.05 probability level. † LSD_a is for comparison within plant growth regulators; LSD_b is for comparison of means of sucrose and without sucrose; LSD_c is for comparison of means for the interaction between plant growth regulators and sucrose. § plant growth regulators.

Table 10.3. The effects of injected plant growth regulators (PGR) and sucrose on uptake volumes, dry matter distributions and yield components.

Treatments	Uptake volumes (ml)	Ear widths (cm)	Ear length (cm)	Kernels row^{-1}	Injection internode weight	Stover weight	Weight kernel^{-1}	Grain weight	Total[¶] weight	N (%)
					g plant^{-1}					
Ethephon	299	13.8	17.7	39	5.8	86.7	0.29	113.0	228.6	1.65
ABA	294	14.3	18.1	39	7.6	99.4	0.29	132.4	271.6	1.75
IAA	296	14.4	17.9	38	6.8	96.4	0.29	133.6	268.7	1.65
Salicylic acid	278	14.3	18.8	40	7.6	104.0	0.31	145.2	291.1	1.65
without PGRs[§]	281	14.1	18.0	39	6.8	95.1	0.30	129.9	262.5	1.62
$LSD_{0.05}$	68	0.1	0.8	2	1.5	13.6	0.03	13.5	26.4	0.18
Sucrose	269	14.3	18.3	39	7.5	100.3	0.29	131.0	270.7	1.70
Without sucrose	311	14.0	17.9	38	6.3	92.3	0.29	129.6	258.5	1.64
$LSD_{0.05}$	20	0.3	0.5	2	0.9	8.6	0.02	8.5	16.4	0.11
PGR	NS	0.1	0.1	NS	NS	NS	*	**	*	NS
Sucrose	*	NS	NS	0.1	*	*	NS	NS	NS	NS
PGR*Sucrose	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

**, * different at 0.01 and 0.05 probability levels, respectively; NS= no significant between 0.1 and 0.05 probability level;§ plant growth regulators;¶ the total weight of stover, grain weight, husk and cobs.

Preface to Chapter 11

Chapter 11 is comprised of material from a manuscript prepared by myself, my supervisor Dr. D.L. Smith and two other co-authors, Dr. A.F. MacKenzie and Dr. C.A. Madramootoo for publication in the *Journal of Plant Nutrition* in 1997. The format has been changed to be consistent within this thesis. All literature cited in this chapter is listed in the reference section at the end of this thesis. Each table or figure is presented at the end of this chapter.

The previous four chapters demonstrated that injected sucrose, sucrose in combination with plant growth regulators, or plant growth regulators alone affected photosynthesis and the pattern of dry matter distribution within corn plants. Nitrogen, as the most important macronutrient in crop development, can alter N accumulation and distribution within corn plants. Thus, in this chapter we investigated the effects of sink proximity and strength on the distribution of N added via an artificial source.

Chapter 11

Distribution of ^{15}N-Labeled Urea Injected into Field-Grown Corn Plants

11.1. Abstract

Nitrogen assimilate supply to developing corn (*Zea mays* L.) ears plays a critical role in grain dry weight accumulation. The use of stem-perfused/injected ^{15}N labeled compounds to determine the effects of an artificial N source on the subsequent distribution of injected N and grain weight of field grown corn plants has not been reported previously. Our objective was to assess the distribution of N added via an artificial source. Three soil N fertilizer levels (0, 180 and 270 kg N ha^{-1}) and three N solutions (distilled water control and ^{15}N enriched urea at 15 and 30 mM N) were arranged in a split-plot design. Three N concentrations were injected using a pressurized stem injection technique. The injection started fifteen days after silking and continued until immediately prior to plant physiological maturity. The average uptake volume was 256 mL over the 30-day injection period. The N supplied via injection represented 1.5 to 3% of the total plant N. Neither soil applied N fertilizer nor injected N altered dry matter distribution among plant tissues. As the concentration of the injected solutions increased N concentrations increased in the grain and upper stalks, while % ^{15}N atom excess in ear+1 leaves and leaves increased as the N concentrations of the injected solutions increased. The relative degree of ^{15}N enrichment for each of the tissues measured was: injected internode > grain > upper stalks > leaves > lower stalks > cob > husk > ear+1 leaf > ear leaf. This study indicated that the exogenous N supplied via stem-injection, was incorporated into all the measured plant parts, although not uniformly. The distribution of the injected ^{15}N was affected both by the proximity of sinks to the point of injection and the strength of the various sinks. The lack of increase in grain N concentration as soil N fertility increased, combined with the increase in grain N concentration as the concentration of the injected N solutions increased demonstrated that the ability of the plant roots to take up N from the soil was more limiting to grain N accumulation than was the ability of grain to take up N from the rest of plant.

11.2. Introduction

Assimilate supply to the developing corn (*Zea mays* L.) ear is an important determinant of grain yield. Although carbohydrates are the major components of corn grain, the supply of N also plays an important role in grain dry weight accumulation (Below *et al.*, 1981; Reed *et al.*, 1988). Nitrogen fertilizer, applied to the soil or foliage, after anthesis has been shown to greatly increase the grain yield and the protein content of crop plants (Below *et al*, 1985; Bulman and Smith, 1993). Below *et al.* (1985) reported a higher net recovery of added N in corn when ^{15}N urea was foliar applied after anthesis than before anthesis.

Nitrogen partitioning is affected by both source capacity and sink capacity. When the grain sink was limiting (Uhart and Andrade, 1991; Reed *et al.*, 1988) greater leaf and stem N accumulations occurred, and conversely, greater vegetative N remobilization occurred when the source was limiting (Reed *et al.*, 1988). In addition, N assimilates from sources (such as leaves) was often translocated preferentially to nearest sink (grain) (Daie, 1985). During the grain filling period the grain itself is the strongest sink for both photosynthate and N (Pate, 1980).

Foliar and root applications of N have been used to overcome specific N deficiencies in horticultural or agronomic corps (Peterson and Frye, 1989). However, in biochemical pathway or physiological studies, foliar or root application of organic (such as sucrose) or expensive (such as isotope labeled materials) substances may be inappropriate. The stem infusion method was developed to supply small quantities of substances to plants, to study aspects of plant physiology without the erratic uptake experienced with leaf abrasion and spraying, or the plant regulation of uptake following soil application. Previous stem infusion techniques have provided effective ways to supply small amounts of sucrose directly to corn plants, but only for brief periods of time (Boyle *et al.*, 1991a; Boyle *et al.*, 1991b; Ma *et al.*, 1994a). In order to overcome the short period of stem perfusion, Zhou and Smith (1996) developed a pressurized stem injection system for field grown corn plants which involved a simple, efficient and inexpensive method that is easily used in the field or greenhouse.

Using a peduncle perfusion technique, Ma *et al.* (1994c) supplied 5% atom enriched ^{15}N into barley plants (*Hordeum vulgare* L.) after spike emergence. They found that perfusion supplied N increased grain N concentration and that the ^{15}N tracer was most concentrated in the chaff while the grain, flag leaf and peduncle had similar % atom ^{15}N excesses, demonstrating that peduncle-perfused N was transported and incorporated into all plant parts, but was preferentially incorporated into structures immediately adjacent to the grain.

The use of stem-perfused/injected labeled ^{15}N compounds to determine the effect of this artificial N source on the subsequent accumulation and distribution of dry matter and injected N in field grown corn plants has not been reported before. Nitrogen is a readily mobile macronutrient and is suitable for testing this technique. Thus, our objective in this study was to assess the distribution of N added via an artificial source relative to the various N sinks.

11.3. Materials and methods

The experiment was conducted at the Horticulture Research Station of the Macdonald Campus of McGill University, Québec in the summer of the 1993. The field was used for vegetable and sweet corn production in the year prior to this experiment. The soil type in the experimental plots was St. Bernard loam (fine loamy, mixed, nonacid, frigid, typic Eutrochrept) soil. Before planting 40 kg ha^{-1} N-P_2O_5-K_2O (5-20-20) was hand broadcast onto the plots, according to a soil test.

Each plot was 4.5 m x 2 m with six rows of corn plants. Corn hybrid Pioneer 3921 was sown on 22 May and harvested on 4 October, 1993. Corn seeds were planted in rows 0.75 m apart, with 0.20 m between plants within rows. This gave a corn population at 60,000 plants ha^{-1}. Weeding was done by hand when necessary.

Since N fertilizer status can affect overall plant growth and thus the injection fluid uptake rate, fertilizer N level was included as an experimental factor. Ammonium nitrate was applied at 0, 180, and 270 kg N ha^{-1} at seeding. The experiment was arranged as a split-plot factorial with three blocks. Soil N fertility rate was the main-plot factor, and injected N solutions (0, 15 and 30 mM N urea) was the sub-plot factor. Distilled water-injected and non-injected plants were included as

controls. The injected N source was 5% ^{15}N atom excess, double-labeled ^{15}N urea (ISOTEC Inc, Miamisburg, OH).

A stem injection system, as described by Zhou and Smith (1996), was started 15 days after silking and continued until a few days prior to plant physiological maturity, a period of 30 days. Briefly, a flexible plastic tubing (i.d. 0.8 mm, o.d. 2.4 mm) (Tygon, Norton Co., Akron, OH, USA), a 21 gauge with the plastic portion removed and an 18 gauge standard disposable hypodermic needle (Becton Dickinson, Rutherford, NJ, USA) were used to construct the delivery system. The injection end of the 21 gauge needle was inserted into the top of the tubing and sealed with silicon sealant (GE Silicones Canada, Pickering, Ontario, Canada). The 18 gauge needle was inserted into the bottom of the tubing. Masking tape was wrapped around the connection site between the 18 gauge needle and the tubing to form a cup shape which was filled with latex (Vultex, General Latex Canada, Candiac, Québec, Canada). The tubing-needle system was applied in the field until the silicon and the latex were completely dried (approximately five days). When the initiation of injection, the 21-gauge needle connected to the end of the needle-tubing system described above was inserted downward into corn internode (below ear internode). Several standard (22.5 X 8.5 X 7 cm) ceramic three-hole construction bricks (approximately 2.7 kg each) provided a pressure source and two 2.4 m (1.25 cm o.d.) steel pipes provided support for the bricks. These two steel pipes were hammered into the soil beside the test plants before injection, and a piece of plywood fixed at the desired height provided a platform to hold a 60 mL syringe which connected to the luer-lok of 18 gauge needle described above, with the bricks resting on the syringe plunger. The test solution was filled in 60 mL syringe before injection. The bricks provided a constant pressure to force the tested solutions into the corn stems. The reduced volumes in the syringe were recorded daily as the uptake volumes.

At harvest, each corn plant was cut and separated into ear leaf, leaf just above ear (ear+1 leaf), other leaves (not including ear leaves and ear+1 leaves), injected internode, upper stalks (above the injection internode, including leaf sheaths), lower stalks (below the injection internode, including leaf sheathes), grain, husk and cob.

All plant parts were dried to a constant weight at 90°C, and the dry weight of each plant part was measured.

All dried plant samples were ground with a Wiley mill fitted with a 1 mm screen. Total N was determined using Kjeldahl analysis (Tecator, Kjeltec System 20, Sweden). Plant tissue samples were digested in the concentrated H_2SO_4 plus 6.8 g of catalyst (3.5 g K_2SO_4 per 3.5 mg Se) for 90 min. Digested samples were then distilled and titrated with 0.05 M HCl for total N determination. The titration solutions were subsequently acidified and used for ^{15}N analysis. To avoid sample cross contamination during the steam-distillation process, the distillation unit was flushed with distilled water for three minutes between samples. A volume of Kjeldahl distillate solution calculated to contain approximately 7 μg of N was placed into a 15 cm length glass tube (6 mm, o.d.). All samples were oven dried at 90 °C. A catalyst (CuO) and a drying agent (CaO) were added to the glass tubes and the tubes were sealed under vacuum, and baked in an oven at 500°C for 4 to 6 hours, to oxidize the NH_3 to N_2 gas. The % ^{15}N atom enrichment in the samples was determined by emission spectroscopy using a JASCO N-150 ^{15}N analyzer (JASCO, LTD, Tokyo, Japan). The details of the sample drying process and the determination of % ^{15}N atom enrichment were as per standard methods (Preston *et al.*, 1981; Martin *et al.*, 1991). The % atom ^{15}N excess for each sample was calculated from the difference in the % atom ^{15}N enrichment of the injected plants and the level in the distilled water control plant in the same plot.

Statistical analyses were conducted with the PROC GLM procedure of the SAS Package (SAS Inc, 1985), according to Steel and Torrie (1980). Comparisons between means were made with a GLM analysis protected LSD test at the $P \leq 0.05$ level. There were no applied N fertilizer X injected concentration interactions or main effects of soil applied N levels for all measured variables, so main effect means of the injected urea ^{15}N levels were pooled over the three applied soil N levels. Since the measured variables were not different between the non-injected and distilled water injected plants, only data from the distilled water-injected controls are presented.

11.4. Results and discussions

11.4.1. Injection volumes and supplied N

There were no differences in injection volumes among the three injected N levels (Table 11.1). The average uptake volume by corn plants was 256 mL during the 30 day injection period. This result was different from that of Ma et al. (1994c) who found that distilled water was more easily delivered to barley plants than N solutions. We assumed that this lack of difference in injection volumes among solutions of different concentrations was due to the different supply methods (perfusion in their study versus pressurized injection in our study), and perhaps also to differences between the two crop species (primarily differences in plant size and stem structure). During the 30-day injection period, the amounts of stem-injected N were 52.7 and 120.3 mg N per plant for the 15 and 30 mM N solutions, respectively, which represented 1.5 and 3%, respectively, of total N in the plants (Table 11.1).

11.4.2. Dry matter production and N accumulation

Neither the applied soil N fertilizer nor the injected N caused any difference in dry matter accumulation or distribution within plants when compared with the appropriate controls (data not shown). The average total biomass of the corn plants was 279.8 g per plant. The lack of dry matter weight response to applied soil N fertilizer was probably due to a high soil N content in the experimental field, which was used for horticultural crop production prior to the experiment. In addition, corn grain N concentration in the past has been used to help explain corn responses to N fertilizer practices and management. The grain N concentration in the 0 N control treatment was not different from the plots which received N fertilizer (15.8 g kg^{-1} verse 16.9 g kg^{-1}, respectively). The lack of N fertilizer addition or N injection effect on corn plant growth indicated that our injected N would act as a new N source but that its presence would not alter the pattern of sink strength, by allowing additional growth due to alleviation of N limitation. This situation allows direct comparison between the various injection treatments.

Stem-injected N increased the N concentration in the injected internodes and grain by 100 and 15%, respectively, relative to the distilled water. The average N concentration for the grain was up to 2.3 g kg^{-1} higher than that of distilled water

injected control plants (Table 11.1). However, there was no difference in N accumulation and distribution within plants between the 15 and 30 mM injected N concentrations although the amount of N supplied by injection of the 15 mM N solution was half that supplied by the 30 mM N. The N concentrations of grain, ear leaf, ear+1, leaves and injected internode for all N treatments were higher than other vegetative tissues (upper stalks and lower stalks) and non-grain reproductive structures (cob and husk).

The observation that grain N concentration did not increase as the levels of added soil N increased from 0 to 270 kg ha^{-1} indicates that the ability of corn roots to take up N was saturated in this soil. However, the measured increase in grain N concentration as the N concentration of the injected solutions increased suggested that the ability of the grain to take up N was not saturated in this soil. This demonstrates that grain N accumulation was more limited by the ability of the plant roots to extract N from the soil than by the ability of the grain to extract N from other plant tissues. This was previously demonstrated for indoor grown barley plants (Foroutan-pour *et al.*, 1996).

11.4.3. Nitrogen-15 distribution among plant tissues

The % atom ^{15}N excesses were higher for all tissues of the ^{15}N injected N treatments than the distilled water injected controls, indicating that stem-injected ^{15}N was incorporated into all measured plant parts during the injection period (Table 11.2). The % atom ^{15}N excess values varied among plant parts, demonstrating that injected N was not uniformly incorporated within plants. Injection of 30 mM N resulted in % atom ^{15}N excess in ear+1 leaves and other leaves (not including ear leaves and ear+1 leaves) that were 0.04 and 0.08, respectively, greater than in the same structures which the plants were injected with 15 mM N.

The lowest % atom ^{15}N excess value occurred in ear leaves while the highest total N concentration (^{14}N plus ^{15}N) occurred in these leaves. For treatments injected with N, the % atom ^{15}N excess was highest in the injected internode (0.5% on average for all injected N treatments). The reminder of the plant tissues ranked for % atom ^{15}N excess as follows: grain > upper stalks > leaves > lower stalks > cob >

husk > ear+1 leaf > ear leaf. Thus, injected internodes, as a primary receiver of the exogenous N source, may have served as a storage organ for urea-derived N, which could be relayed to ear. The higher % atom ^{15}N excess in grain and upper stalks further supported the role of both grain and upper stalks as storage organs (sinks) for injected N. Since the leaves are the fundamental site for photosynthate production, a large amount of N also accumulated in the leaf tissues. The % atom ^{15}N excess N in the non-grain reproductive structures was much lower than in the grain. This is probably because the grain was the most active sink during the injection period (Daie, 1985). However, this finding differed from the work using barley (Ma *et al.*, 1994c) for which the highest N enrichment were found in the chaff.

The distribution of the injected N was apparently affected by both sink strength and sink proximity by the source. The injected internode was more ^{15}N enriched than the grain, although the filling grain was probably a much stronger sink (Pate, 1985). The grain, the cob, the husk and the ear leaf were all sinks of about equal proximity to the injection site, yet the strongest sink, the grain, was much more enriched in ^{15}N. The heavy enrichment of the upper stalks may represent N storage in that sink. The timing of sink demand would also have played a role in ^{15}N distribution. The bulk of the N in leaf and husk tissues occurs as ribulose bisphosphate carboxylase, PEP carboxylase and photosystem proteins, which would have been largely in place by the time injection was begun. However, at the onset of injection grain sink strength was just developing.

11.5. Conclusion

The exogenous N supplied via stem-injection, was incorporated into all measured plant parts, although not uniformly. The stem-injected N did not alter overall plant growth, but affected the % ^{15}N distribution among plant issues. Both sink strength and sink proximity play a role in the distribution of injected N.

Table 11.1. Effects of stem-injected N concentrations on the uptake volumes, amount of stem-supplied N and the concentration of N in corn plant tissues.

Injected N concentration	uptake volumes	injected N	injected internode	upper stalks	lower stalks	ear leaf	ear+1‡	leaves	Husk	cob	grain
	mL	mg					g kg^{-1}				
distilled water	227	NA	6.3 a†	4.7 b	5.3 a	16.5 a	17.4 a	14.4 a	6.3 a	3.7 a	14.9 b
15 mM N	256	53.7	12.1 a	7.7 a	6.5 a	18.4 a	18.6 a	15.7 a	4.9 a	5.0 a	17.0 a
30 mM N	286	120.3	13.6 a	7.7 a	6.4 a	19.1 a	16.2 a	15.0 a	4.1 a	5.6 a	17.4 a

NA, not applicable.† Variable means followed by the same letter were not significantly different from each other at $P \leq 0.05$ level by a GLM protected LSD test; ‡ Represents the leaf just above ear.

Table 11.2. Effects of stem-injected N concentrations on % atom ^{15}N excess in corn plant tissues at harvest.

Injected N concentrations	injected internode	upper stalks	lower stalks	ear leaf	ear+1‡	leaves	Husk	cob	grain
					%				
distilled water	0.0 b†	0.0 b	0.0 b	0.0 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
15 mM N	0.57 a	0.09 a	0.11 a	0.04 a	0.05 a	0.07 b	0.06 a	0.09 a	0.13 a
30 mM N	0.39 a	0.17 a	0.07 a	0.06 a	0.09 b	0.15 a	0.09 a	0.10 a	0.28 a

† Variable means followed by the same letter were not significantly different from each other at $P \leq 0.05$ level by a GLM protected LSD test; ‡ Represents the leaf just above ear.

Chapter 12

General Discussion

Management of both fertility and water management is important for efficient crop production. The availability of N in corn production systems plays a critical role in promoting higher grain yields and, also the risk of environmental pollution. When N was applied in excess of corn crop requirements, the potential for nitrate leaching from soil and subsequent contamination of groundwater increases. To minimize nitrate leaching, crop management practices which minimize the size of soil nitrate pools remaining in the soil after corn harvest should be employed.

More efficient use of resources is a major motivation for intercropping (Ofori and Stern, 1987) and this objective is achieved by managing the way component crops compete for available light, water and nutrients. Thus, intercropping has the potential to increase N use efficiency in crop production (Juergens-Gschwind, 1989). During the two years of our study, under the soil and climatic conditions of southwestern Quebec, the corn-forage grass (annual ryegrass) intercrop system demonstrated an ability to minimize the negative effects of high applied N rates (270 kg ha^{-1}) while maintaining corn grain yields (chapters 3, 4 and 5). The intercrop system produced more total aboveground dry matter than the monocrop corn system and reduced soil NO_3^--N in the top 1 m of the soil profile after corn harvest (chapter 5). This reduction was due to greater N accumulation by plants of the intercrop system (chapters 3 and 4) (Yadav, 1982). However, a large amount of soil NO_3^--N was present in the soil profile after corn harvest when applied N rate (270 kg N ha^{-1}) exceed the recommended rate for the area (chapter 5). In addition, in our study, the second successive year of the high N fertilizer application (270 kg ha^{-1}) resulted in the soil NO_3^--N pool in the intercrop system was as high as monocrop system with applied recommend N rate (chapter 5). Perhaps, this is due to soil NO_3^--N accumulation over time, even in intercrop system, or mineralization of corn, ryegrass, and weeds during the second season. The data of chapter 3 demonstrated that the high N application rate (270 kg ha^{-1}) did allow production of considerable ryegrass plus weed biomass, which would provided long term benefit by aiding in maintaining

soil organic matter levels. However, the ryegrass and weeds were not able to take up enough of this extra added N to completely negate the increased risk of nitrate leaching in the second season if high N rate consecutively applied (chapter 5). Therefore, the efficiency of the intercrop system for reducing soil NO_3^--N accumulation after crop harvest can be limited by the need to apply N in excess the recommend rates, in order to maintain corn yields.

When two crops were grown together, they always compete for light, nutrients and water during the growing season (Fukai and Trenbath, 1993). The results of chapters 3 and 4 indicated that competition for nutrients and water play important roles in determining corn grain and total intercrop productivity for a corn-annual ryegrass intercrop system; final grain yield, dry matter production and the N uptake ability of intercrop corn were not affected by the presence of annual ryegrass and weeds in either year of this study (chapters 3 and 4). The availability of adequate N (270 kg N ha^{-1}) and water (via subirrigation) to the intercrop system explains the success of the corn (chapters 3 and 4). Although intercropped corn had lower dry matter production and N contents of both leaves and stalks than monocrop corn at the mid-grain filling stage, the interspecific N competition among corn, ryegrass and weeds did not result in reduced final corn dry matter production or N uptake (chapters 3 and 4). All of these findings supported the postulate that sufficient N supply alleviates the negative effects of interspecific N competition in intercropping systems.

Resource availability to the dominated intercrop component (annual ryegrass) is greatly decreased by the dominant (corn) component. Hence, agronomic manipulation of the corn can strongly effect growth of the ryegrass. For example, there was 79% less ryegrass dry matter at the final harvest of 1994 than the final harvest of 1993, due to drier weather conditions of 1994. Under the conditions of our experiment, ryegrass was more sensitive to soil moisture deficit stress than corn (chapters 3 and 4). Because corn plants are taller than ryegrass, and therefore compete more effectively for light, the development, including root development, of the ryegrass was probably restricted under intercrop conditions. Thus, when soil moisture becomes limiting, corn plants are better able to capture water for plant

development than ryegrass. The comparable corn grain yields in 1994, a drier than normal year, and 1993, a year of approximately normal precipitation, further demonstrates that if growth of all component crops is equally limited by shortage of a particular resource, application of that resource normally favours the growth of the dominant crop, leading to greater suppression of the dominated crop (Cordero and McCollum, 1979).

Corn and annual ryegrass vary in their ability to absorb NO_3^--N from the soil profile in a corn-ryegrass intercrop system. The growth of the dominated component(s) intercrop (ryegrass) plays a key role in N use efficiency of the cropping system, even though the corn was the dominant N user. The establishment of and sequential N uptake by annual ryegrass greatly depended on the strength of N and water competition between corn and ryegrass plus weeds, and on environmental factors (such as precipitation). Poor early ryegrass establishment in 1994, relative to 1993, resulted in lower ryegrass biomass and N accumulation in 1994 than in 1993. In addition, more vigorous growth of weeds relative to ryegrass in 1994 may also have depressed the development of ryegrass due to weed competition for nutrients and soil moisture (chapters 3 and 4). Therefore, both plant establishment and weather conditions limited the ability of the ryegrass to utilize soil N (chapters 3, 4 and 5).

The greater amount of dry matter produced by the intercrop system not only increases overall N uptake from the soil and reduced nitrate leaching (Singh *et al.*, 1978; Yadav, 1982), but also has the potential to improve soil quality due to the additional crop residues added to the soil (Juergens-Gschwind, 1989). After corn harvest, approximately 3 Mg ha^{-1} more plant dry matter was incorporated into the soil for the intercrop than the monocrop system (chapter 4). Although the benefits from soil incorporated intercrop residues were not apparent after only two years in our study, improved soil fertility may be imparted over a longer time frame. Thus, we can speculate that a yield-enhancing benefit of this system may be present in the future.

Nitrogen losses from soil have received a great deal of research attention. However, aboveground N losses from crop tissues through volatilization of N

containing compounds should also be considered in efforts to increase N use efficiency in cropping systems. Over the two years of this study, we observed that the maximum N accumulations for both monocrop and intercrop corn were reached at mid-grain filling, rather than at the end of the growing season (chapter 3). It has been suggested the losses of N from the aboveground biomass are due to N volatilization from foliage, particularly at higher N fertilizer levels; this decline has been shown to occur largely after the reproductive stage (Terman and Allen, 1974; Crasswell and Martin, 1975; Stutte and Weiland, 1978). Thus, when assessing N losses from the plant-soil system, not only underground but, also volatile N losses from aboveground corn plant tissues should be investigated. Measurement and understanding of aboveground plant tissue loss of N are important in understanding N efficiency in cropping systems.

When we attempt to develop crop management strategies which benefit the environment while maintaining yield levels, the study methods used must be economical. Nitrogen-15, as a tracer, allows clear and accurate determination of the fate of the N fertilizer in agricultural systems (Khanif *et al.*, 1984; Reddy and Reddy, 1993). However, the cost of ^{15}N is high. This high cost places practical limitations on such research. The ^{15}N microplot investigation in our work proved that a small confined microplot, with area as small as 0.4 m by 1.15 m could provide a reliable measure of fertilizer N recovery by corn plants (chapter 6). In addition, ^{15}N data from this study indicated that corn, in intercrop or monocrop systems had similar abilities to absorb applied N fertilizer. The increased total N uptake by the intercrop system (chapters 3 and 4) was due to the additional uptake of both applied N fertilizer and mineralized organic soil N by the ryegrass-weed component (chapter 6).

Water table management had little effect on the development of corn and ryegrass, N use efficiency and total soil residual N accumulation in top 1 m of the soil profile, during the two years of our study (chapters 3, 4, 5 and 6). Although increased water table depth was shown to enhance soil NO_3^--N movement deeper into the soil and early spring leaching losses, water table control failed to increase N uptake and reduce soil nitrate content. In this study, water table control, via

subirrigation, could be only maintained at 70 and 80 cm, for expected water table depths of 50 and 75 cm, respectively, due to deep seepage. The lack of response to water table control effects may have been due to the small differences in water table depths.

When adequate N supply was available to the intercrop system, the interspecific competition for this resource is reduced (chapters 3 and 4); however, in the corn-annual ryegrass intercrop, competition for light was apparent, with corn, the much taller crop, effectively out competing the ryegrass. The potential for severe competition for water is increased by dry weather conditions. Water stress during corn development can lead to considerable yield reductions, especially when it occurs during grain-filling stage. In our greenhouse experiment, we tested the hypothesis that sucrose supplementation (via stem-injection) of water stressed and well-watered (maintain water table at 50 cm from the soil surface) corn plants can prevent decreased kernel set. The results of this experiment showed that exogenous sucrose influenced kernel set only under conditions of sufficient soil water supply, implying that plant reproductive development after silking was limited more by some over all change in plant physiology triggered by water stress, than assimilate supply (chapter 9). Water table control can remove excess water from a field, or can raise the water level to the crop root zone in order to overcome water stress. Aspects of water management and its effects on crop physiology need to be investigated.

Both plant growth regulators (Arteca, 1996) and the level reduced carbon available within plants (chapter 8) affect photosynthetic activity, and can alter the pattern of dry matter distribution (chapter 10). Salicylic acid (SA), a recently recognized plant growth regulator (Raskin, 1992) promoted stomatal opening by excised leaves of *Commelina communis* L. plants (Rai *et al.*, 1986). However, information regarding the direct effects of SA on plant photosynthesis is not available. When SA was supplied via stem-injection, we found that, in conjunction with sucrose, it stimulated stomatal opening and increased photosynthetic rate one week after the onset of injection, when compared with injection of distilled water or sucrose alone. Corn plants injected with SA produced 9% more grain than treatments injected with

distilled water or sucrose without SA. The application of other plant growth regulators via stem injection, affected plant growth and productivity (chapter 10) as described in previous work (Robinson *et al.*, 1978; Langan and Oplinger, 1987; Arteca and Tsai, 1988; Ma *et al.*, 1994b; Foroutan-pour *et al.*, 1995).

The roles of carbohydrate production and remobilization in kernel growth have been investigated (Tollenaar, 1977) while other researchers have implied that availability of N can be more limiting than that nonstructural carbohydrates for grain dry weight accumulation (Below, 1981). The delivery of C (sucrose) and N (^{15}N urea) into field-grown corn plants via stem-injection showed that externally supplied sucrose changed both of the source strength (photosynthetic inhibition) and sink strength (decreased total grain production) (chapter 8) while distribution of ^{15}N was affected by proximity of sinks to the point injection and the strength of sinks (chapter 11).

Chapter 13

General Conclusions

The following conclusions may be drawn based on the findings contained in this thesis:

1. Corn grain yields are not changed by either a corn-annual ryegrass intercrop system or controlled water table depth under conditions of high N fertility in spite of measurable effects related to N and water competition from ryegrass and weeds at some corn-growing stages.

2. A corn-annual ryegrass intercrop system increased total aboveground dry matter production, resulting in higher N uptake for intercrop than monocrop systems at all corn growing stages in 1993, while in 1994 this difference only occurred at the six leaf and at final harvest stages. By the end of the growing season, the intercropping system increased total aboveground N uptake by 77.2 and 50.7 kg N ha^{-1} when compared with a similarly fertilized monocrop system in 1993 and 1994, respectively.

3. The greater total aboveground biomass in corn-ryegrass intercrop increased organic plant residues returned to soil, thus potentially improving soil organic matter content and quality over the long term.

4. The total amount of NO_3^--N in the top 1 m of the soil profile was reduced by intercropping in the fall of 1993 when climatic conditions allow good development of intercrop biomass.

5. Nitrate-N level in the soil (0-20 cm) can be used as an indicator of total NO_3^--N accumulation in the top 1 m of the soil profile and in estimating the potential for NO_3^--N leaching.

6. Dry matter production and N uptake in different plant tissues of intercropped corn is only slightly affected by the other intercrop components when competition for N and water are minimized

7. Both plant establishment and weather conditions greatly affect the ability of intercropped ryegrass to take up soil N.

8. When the N application rate was high (eg. 270 kg ha^{-1}), intercropped

annual ryegrass and weeds were able to grow well and contribute organic matter to the soil, but they were unable to utilize enough of the extra added N to completely negate an increased risk of nitrate leaching.

9. Water table depth had less effect on corn grain yield, dry matter partitioning, N accumulation in aboveground biomass, and soil NO_3^--N content, than cropping system.

10. A confined microplot with an area as small as 0.4 m by 1.15 m can provide a reliable measure of fertilizer N recovery for corn plants in intercrop or monocrop systems.

11. Injection of sucrose causes photosynthetic inhibition (pre-mature senescence) in the leaf just above the ear and the ear leaf, and this is more severe for plants receiving higher sucrose concentrations.

12. Exogenous sucrose supplementation via stem injection influenced kernel set only under conditions of sufficient soil water supply.

13. The injection of a combination of SA and sucrose stimulated plant photosynthesis while SA increased grain yield of injected plants.

14. Both the proximity of sinks to the point of injection and the strength of the sinks affected the distribution of the ^{15}N supplied from a artificial source (injected).

Chapter 14

Acceptance or Rejection of Hypotheses

Hypothesis 1:

Corn grain yield will not be affected by the presence of an annual ryegrass intercrop component when produced with high levels of fertilizer N.

Results related to hypothesis 1:

In this study, intercropped corn grain yield was comparable to monocrop corn yield when annually applied 270 kg N ha^{-1} (chapter 3 and 4). Thus, we **accept hypothesis 1.**

Hypothesis 2:

Increased N uptake by a corn-annual ryegrass intercrop will reduce the soil NO_3^--N content, and thus, reduce the potential for nitrate pollution under the soil and climatic conditions of eastern Canada.

Results related to hypothesis 2:

At harvest, the intercrop systems had taken up 77.2 and 50.7 kg ha^{-1} more N, than the monocrop systems in 1993 and 1994, respectively (chapter 4). Intercropping decreased total soil N accumulation in the top 1 m of the soil profile in the fall of 1993 (chapter 5). The total amount of NO_3^--N in the top 1 m of the soil profile in fall 1993 was reduced by 47% due to intercropping (chapter 5). Thus, we **accept hypothesis 2.**

Hypothesis 3:

Greater dry matter accumulation in an intercrop system will increase the amount of crop residue returned into soil.

Results related to hypothesis 3:

After crop harvest, corn stover and all other intercrop components (ryegrass and weeds) were incorporated into soil. Corn intercropped with annual ryegrass led to incorporation 3.1 and 2.7 Mg ha^{-1} more crop residue in the fall of the 1993 and 1994, respectively, than the monocrop system (chapter 4). Thus, we **accept hypothesis 3.**

Hypothesis 4:

Water table controls will increase corn grain yield, N uptake, and fertilizer N use efficiency while reducing soil NO_3^--N levels associated with corn production systems.

Results related to hypothesis 4:

We did not find any effect of water table control on corn grain yield, aboveground dry mater production, N uptake, fertilizer N use efficiency, or soil nitrate-N content in the top 1 m of the soil profile (chapters 3, 4, 5 and 6). Therefore, we **reject hypothesis 4.**

Hypothesis 5:

Confined microplots can reduce the microplot dimensions required to produce reliable ^{15}N data in corn monocrop and intercrop (with annual ryegrass) systems.

Results related to hypothesis 5:

Confined microplots with area as small as 0.4 m by 1.15 m could provide a reliable measure of fertilizer N recovery by corn plants (chapter 5). Thus, we **accept hypothesis 5**.

Hypothesis 6:

A pressurized stem-injection system can continuously deliver concentrated nutrients into corn plants for periods of weeks to months.

Results related to hypothesis 6:

The results contained in chapter 7 demonstrated that concentrated sucrose could be injected into corn stems for a period of 32 days through constant pressure supplied from construction bricks. Therefore, we **accept hypothesis 6**.

Hypothesis 7:

The photosynthesis and productivity levels of corn plants will be altered by exogenous supply sucrose via stem injection.

Results related to hypothesis 7:

The results contained in chapter 8 showed that continuous injection of concentrated sucrose solutions caused earlier senescence of the ear+1 leaves, and caused a decrease in the F_v/F_m chlorophyll fluorescence ratio. The injected internodes of plants receiving sucrose solutions weighed more than those of the distilled water

injected controls. Injection of sucrose increased the number of seeds produced in the primary ear and the weight of the primary ear, but inhibited the development of the secondary ear. Thus, we **accept hypothesis 7.**

Hypothesis 8:

The reproductive failure of water stressed corn plants can be prevented by supplying assimilate (sucrose) post anthesis via a stem-injection technique.

Results related to hypothesis 8:

Chapter 9 demonstrated that sucrose injection had no effect on the grain yield components of water stressed plants. Supplementation with exogenous sucrose altered kernel set only under conditions of sufficient soil water supply. Thus, we **reject hypothesis 8.**

Hypothesis 9:

Injection of plant growth regulators, with or without sucrose, will alter the growth and photosynthetic activities of corn plants.

Results related to hypothesis 9:

Plants injected with SA produced 9% more grain yield than plants injected with distilled water and sucrose. When compared with injected distilled water injection, the combination of SA with sucrose increased the photosynthetic rate by 42%, while the injection of IAA or ABA did not alter plant photosynthesis or productivity. Injection of ethephon resulted in 10% less grain yield than distilled injection. Sucrose injection increased the dry weight of injected internodes and stover; it also induced partial stomatal closure, although without any apparent effect on net photosynthetic rate (chapter 10). Thus, we **accept hypothesis 9.**

Hypothesis 10:

The distribution of N added through an artificial source will be affected by both the proximity plant sinks to the source (site of injection) and the strength of various sinks.

Results relate to hypothesis 10:

The results contained chapter 11 demonstrated that the relative degree of enrichment for each of the tissues measured was: injected internode > grain > upper

stalks > leaves > lower stalks > cobs > husk > ear+1 leaf > ear leaf. Since the injected internode, the grain, the husks, the cob and the ear leaves were all sinks close to the point of injection, but the grain and upper stalks should have been the strongest sinks. Thus, we **accept hypothesis 10**.

Chapter 15

Contributions to Knowledge

The work presented in this thesis contained new information regarding the use of corn-forage grass intercrop systems in conjunction with water table management as a way to reduce nitrate-N losses into groundwater. For the first time, seasonal corn plant development and final yield under the combination of intercrop and water table management was detailed. In addition, a pressurized stem-injection technique, developed in this study, allowed the first study of aspects of corn physiology related to water and N availability. More specific contributions include the following:

1. The corn-annual ryegrass intercrop system is an effective practice for increasing soil N uptake, and reducing residual soil NO_3^--N after corn harvest.
2. When there is sufficient N and water, the grain yield of intercropped corn is not decreased due to minimized N and water competition among corn, annual ryegrass and weeds.
3. Dry weather conditions can greatly limit ground cover production and N uptake by ryegrass when it competes with corn for light, nutrient and water.
4. A high N application rate (270 kg ha^{-1}) allows production of considerable ryegrass plus weed biomass to aid in maintaining soil organic matter levels, but the ryegrass and weeds are not able to take up enough of this extra N to completely negate an increased risk of nitrate leaching.
5. Water table management has little effect on the grain yield, plant growth and residual soil NO_3-N content after crop harvest.
6. A single plant in the centre of a 1.15 m by 0.4 m ^{15}N microplot can supply a reliable estimation of ^{15}N recovery by corn plants in a corn-annual ryegrass intercrop or corn monocrop systems.
7. Externally supplied C (sucrose) restricts both source (pre-mature senescence of leaves) and sink strength, indicating that mechanisms for signalling between sinks (the primary and secondary ears) and the primary sink and the source (leaves) are different.
8. The distribution of the injected ^{15}N is affected by both the proximity of the sinks to

the point of injection and the strength of the sinks.

9. Injection of corn plants with the combination of SA and sucrose promotes photosynthesis while SA alone increase grain yield of injected plants.

10. Stem injection makes possible the study of changes in plant physiology during grain-filling due to addition of carbohydrate, N and plant growth regulators under both controlled environments and field conditions.

Chapter 16

Suggestions For Future Research

To expand on the work reported here, research should be continued to confirm or complete the following:

1. An nitrogen fertilizer rate between 180 and 270 should be included in a future study, since this may allow the production of corn and ryegrass together, without any reduction in corn yield, while allowing the ryegrass component to take up all of the extra N added to the soil.

2. Research should be considered to investigate whether annual ryegrass can be maintained after crop harvest and not incorporated into soil until the following spring.

3. Volatile N losses from corn plant tissues, from mid-grain filling to the final harvest, should be further examined.

4. Weed control in the corn-annual ryegrass intercrop system should be the subject of a future study.

5. The exudation of NO_3^--N from corn roots into the soil should be investigated, using ^{15}N injection into corn stalks.

6. The effects of an auxiliary reduced carbon sucrose on the dominance of the primary sink over the secondary sink needs further investigation.

7. The apparent inability of injected sucrose to overcome water deficit effects on corn grain filling should be investigated using a wide range of corn hybrids.

References:

Addiscott, T.M., A.P. Whitmore, and D.S. Powlson. 1991. Nitrate: perception of the problem. *In*. Framing, Fertilizers, and the Nitrate Problem. (Chapter 1) p.1-14. Rothamsted Experimental Station, Harperden, UK.

AFEQ. 1995. Fertilizer sales in Québec. 1995. Association des fabricants dengrais du Québec.

Agboola, A.A, and A.A. Fayemi. 1971. Preliminary trials on the intercropping of maize with different tropical legumes in western Nigeria. J. Agric. Sci.77:219-225.

Allen, J.R., and R.K. Obura. 1983. Yield of corn, cowpea, and soybean under different intercropping systems. Agron. J. 75:1005-1009.

Alofe, C.O., L. E. Shrader. 1975. Photosynthate translocation in tillered *Zea mays* following $^{14}CO_2$ assimilation. Can. J. Plant. Sci. 55:407-414.

Angle, J.S., C.M. Gross., R.L. Hill, and M.S. McIntosh. 1993. Soil nitrate concentrations under corn as affected by tillage, manure, and fertilizer applications. J. Environ. Qual. 22:141-147.

Appleby, A.P., P.D. Olson, and D.R. Colbert. 1976. Winter wheat yield reduction from interference by Italian ryegrass. Agron. J. 68:463-466.

Arteca, R.N. 1996. Plant growth substances: principles and applications. Historical aspects and fundamental terms and concepts. p.1-22. Chapman & Hall, New York.

Arteca, R.N., and D.S, Tsai. 1988. Effects of abscisic acid on photosynthesis. transpiration and growth of tomato plants. Crop Res. 27:91-96.

Ayoub, M., A.F. MacKenzie, and D.L. Smith. 1995. Evaluation of N fertilizer rate and timing and wheat cultivars on soil residual nitrate. J. Agron. Crop Sci. 174:1-11.

Baker, J.L., K.L. Campbell., H.P. Johnson, and J.J. Hanway. 1975. Nitrate, phosphorus, and sulphate in subsurface drainage water. J. Environ. Qual. 4:406-412.

Bangerth, F., W. Aufhammer, and O. Baum. 1985. IAA level and dry matter accumulation at different position within a wheat ear. Physiol. Plant. 63:121-125.

Barthdomew, W.V. 1965. Mineralization and immobilization of nitrogen in the decomposition of plant and animal residues. *In.* Soil Nitrogen. W.V. Barthdomew and F.E. Clark (eds.). Agronomy 10:285-306.

Bassetti, P., and M.E. Westgate. 1993. Water deficit affects receptivity of maize silks. Crop Sci. 33: 279-282.

Below, F.E., L.E. Christensen., A.J. Reed, and R.H. Hageman. 1981. Availability of reduced N and carbohydrate for ear development of maize. Plant Physiol. 68:1186-1190.

Below, F.E., R.J. Lambert, and R.H. Hageman. 1984. Foliar applications of nutrients on maize. I. Yield and N content of grain and stover. Agron. J. 76:773-777.

Below. F.E., S.J. Crafts-Brander, J.E. Harper, and R.H. Hageman. 1985. Uptake, distribution, and remobilization of ^{15}N-labeled urea applied to maize canopies. Agron. J. 77:412-415.

Benson, G.O., and R.B. Pearce. 1987. Corn perspective and culture. *In.* Corn (Chemistry and Technology). Watson and Ramastad (eds.). p.1-29.

Bergstrom. L. 1986. Distribution and temporal changes of mineral nitrogen in soil supporting annual and perennial crops. Swed. J. Agric. Res. 16:105-112.

Blackmer, A.M. 1987. Losses and transport of nitrogen from soils. *In.* Rural Groundwater Contamination. F.M.D'Itri and L.G. Wolfson (eds.). p.85-103. Lewis Publish Inc., Chelsea, MI.

Blaser, R.E., T. Taylor, W. Griffeth, and W. Skrdla. 1956. Seedling competition in establishing forage plants. Agron. J. 48:1-6.

Bock, B.R. 1984. Efficient use nitrogen in cropping system. *In.* Nitrogen in Crop Production. R.D. Hauk (ed.). American Society of Agronomy, Madison, WI.

Bolton, E.F., V.A. Dirks, and M.M. McDonnell. 1982. The effect of drainage, rotation and fertilizer on corn yield, plant height, leaf nutrient composition and physical properties of Brookston soil in southeastern Ontario. Can. J. Soil. Sci. 2:297-309.

Bousquet, J.F., G. Touraud, M.T. Piollat, U. Bosch, and M. Trottet. 1990. ABA accumulation in wheat heads inoculated with Septoria Nordorum in the filed conditions. J. Agron. Crop Sci. 165:297-300.

Boyer, J.S. 1976. Photosynthesis at low water potentials. Philos. Trans. R. Soc. London 273: 501-512.

Boyer, J.S., and H.G. McPherson. 1975. Physiology of water deficits in cereal crops. Adv. Agron. 27:1-23.

Boyle, M.G., J.S. Boyer, and P.W. Morgan. 1991a. Stem infusion of maize plants. Crop Sci. 31:1241-1245.

Boyle, M.G., J.S. Boyer, and P.W. Morgan. 1991b. Stem infusion of liquid culture medium prevents reproductive failure of maize at low water potential. Crop Sci. 31:1246-1252.

Bradstreet, R. B. 1965. The Kjeldahl Method for Organic Nitrogen. Academic Press, New York, NY.

Brenner, M.L. 1987. The role of hormones in photosynthate partitioning and seed filling. *In*. Plant Hormones and Their Role in Plant Growth and Development. P.J. Davies (ed.). p:474. Martinus Nijhoff Publishers, Boston.

Broadbent, F.E., and A.B. Carlton. 1971. Field trials with isotopically labelled nitrogen fertilizer. *In*. Nitrogen in the Environment. R.N. Donald and J.G. Macdonald (eds.). p.1-43. Academic Press, New York.

Brown, S.A., and A.C. Neish. 1954. The biosynthesis of cell wall carbohydrate: glucose-^{14}C as a cellulose precursor in wheat plants. Can. J. Biochem. Physiol. 32:170-177.

Bulman, P., and D.L. Smith. 1993. Grain protein response of spring barley to high rates and post-anthesis application of fertilizer nitrogen. Agron. J. 85:1109-1113.

Bureau de la statistique du Québec. 1994. Statistiques Agricoles. Grandes cultures: superficie. 2^{e} semestre. Ste-Foy, Québec, Canada. p:175.

Carter, J.N., O.L. Bennett, and R.W. Pearson. 1967. Recovery of fertilizer nitrogen under field conditions using nitrogen-15. Soil Sci. Soc. Am. Proc. 31:50-56.

Chaney, K. 1990. Effect of nitrogen fertilizer rate on soil nitrogen content after harvesting winter wheat. J. Agri. Sci. 114:171-176.

Chang, J.F., and R.M. Shibles. 1985. An analysis of competition between intercropped cowpea and maize. I. Soil N and P levels and their relationships with dry matter and seed productivity. Field Crop Res. 12:133-143.

Chaudhary, T.N., V.K. Bhatnagar, and S.S. Prihar. 1975. Corn yield and nutrient uptake as affected by water-table depth and soil submergence. Agron. J. 67:745-749.

Chichester, F.W. 1977. Effects of increased fertilizer rates on nitrogen content of runoff and percolate from monolith lysimeters. J. Environ. Qual. 6:211-217.

Claude, P.P. 1990. Effets des cultures intercalaires dans le maïs-grain, sur le rendement en grain, la qualité édaphique, et la teneur en azote inorganique des sols. Ph.D. dissertation, McGill University, Québec, Canada.

Conseil des Productions Végétales du Québec. 1992. Maïze Culture. Agdex 111/20.

Comly, H.H. 1945. Cyanosis in infants caused by nitrates in well-water. J. Ameri. Medical. Assoc. 129:112-116.

Cordero, A., and R.E. McCollum. 1979. Yield potential of interplanted annual food crops in Southeastern U.S. Agron. J. 71:834-842.

Crasswell, E.T., and A.E. Martin. 1975. Isotope studies on the nitrogen balance in a cracking clay. I. Recovery of added nitrogen from soil and wheat in the glass house and gas lysimeter. Aus. J. Soil Res. 13:43-52.

Cromwell, G.L., M.J. Bitzer, T.S. Stahly, and T.H. Johnson. 1983. Effects of soil nitrogen fertility on protein and lysine content and nutritional value of normal and opaque-2 corn (Chicks). J. Anim. Sci. Champaign: Am. Soc. Ani. Sci. 57:1345-1351.

Crowder, M.J., and D.J. Hand. 1990. Analysis of Repeated Measures. Chapman and Hall, London.

Crutzen, P.J. 1981. Atmospheric chemical processes of the oxides of nitrogen, including nitrous oxide. *In*. Denitrification, Nitrification, and Atmospheric Nitrous Oxide. C.C. Delwiche (ed.). p.17-44. John Willy & Sons, New York.

Daie, J. 1985. Carbohydrate partitioning and metabolism in crops. Hort. Rev. 7:69-108.

Daigger, L.A., D.H. Sander, and G.A. Peterson. 1976. Nitrogen content of winter wheat during growth and maturation. Agron. J. 68: 815-818.

Davies, W.J., and T.A. Mansfield. 1983. The role of abscisic acid in drought avoidance. *In*. Abscisic Acid. F.T. Addicott (ed.). p.327-268. Praeger Publ. New York.

Davies, W.J., and T.A. Mansfield. 1987. Auxin and stomata. *In*. Stomatal Function. Z.E. Farquha and L.R. Cowan (eds.). p.293-309. Stanford University Press. Stanford, CA.

Davis, W.J., and H.G. Jones. 1991. Abscisic Acid: Physiology and Biochemistry. Bios Scientific Publishers. Oxford.

Daynard, T.B., J.W. Tanner, and D.J. Hume. 1969. Contribution of stalk soluble carbohydrates to grain yield in corn (*Zea mays* L). Crop Sci. 9:831-834.

Daynard, T.B., and W.G. Duncan. 1969. The black layer and grain maturity in corn. Crop Sci. 9:473-476.

Dong, C.N., and R.N. Arteca. 1982. Changes in photosynthetic rates resulting from phytohormone treatments to the roots of tomato plants. Photosyn. Res. 3:45-52.

Dutilleul, P. 1996. Incorporating Scale in Study Design and Data Analysis. *In*. Scale Issue in Ecology. D.L. Peterson and V.T. Parker (eds.). Columbia University Press, New York: in Press.

Ebelhar, S.A., W.W. Frye, and R.L. Blevins. 1984. Nitrogen from legume cover crops for no tillage corn. Agron. J. 76:51-55.

Evans, R.O., J.W. Gilliam, and R.W. Skaggs. 1989. Managing water table management systems for water quality. ASAE paper #89-2129.

FAO, 1986. Production Yearbook, Vol.34. Rome, Italy.

Ferguson, R.B., C.A. Shapiro, G.W. Hergert, W.L. Kranz, N.L. Klocke, and D.H. Krull. 1991a. Nitrogen and irrigation management practices to minimize nitrate leaching from irrigated corn. J. Proc. Agric. 4:186-192.

Ferguson, R.B., J.S. Schepers, G.W. Hergert, and R.D. Lohry. 1991b. Corn uptake and soil accumulation of nitrogen: management and hybrid effects. Soil Sci. Soc. Am. J. 55:875-880.

Firestone, M.K. 1982. Biological Denitrification. *In*. Nitrogen in Agricultural Soils. F.J. Stevenson (ed.). Agronomy 22:651-688.

Fisher, E., M. Still, and K. Raschke. 1985. Effects of abscisic acid on photosynthesis in whole leaves: changes in CO_2 assimilation, levels of carbon reduction cycle intermediates and activity of ribulose-1,5-bisphosphate carboxylase. International Plant Growth Substance 12th conference.

Follett, R.F., E.J. Doering, G.A. Reichman, and L.C. Benz. 1974. Effect of irrigation and water-table depth on crop yields. Agron. J. 66:304-308.

Follett, R.F., L.K. Porter, and A.D. Halvorson. 1991. Border effects on nitrogen-15 fertilized winter wheat microplots grown in the great plains. Agron. J. 83:608-612.

Foroutan-pour, K. 1994. Aspects of barley post-anthesis nitrogen physiology. M.Sc. Thesis. McGill University, Canada.

Foroutan-pour, K., B.L. Ma, and D.L. Smith. 1995. Field evaluation of the peduncle perfusion technique. J. Plant Nutri. 18:1225-1236.

Forouton-pour, K., B.L. Ma, and D.L. Smith. 1996. Protein accumulation potential in barley seeds as affected by soil- and peduncle- applied N and peduncle-applied plant growth regulators. Physiol. Plant.: in press.

Forster, S.S.D., L.R. Bridge, A.K. Geake, A.R. Lawrence, and J.M. Parker. 1986. The groundwater nitrate problem: A summary of research on the impact of agricultural land-use practices on groundwater quality between 1976 and 1985. Hydrogel. Rep. Br. Geol. Surv., no.86/2. Br.Gelo.Surv., Oxfordshire, England.

Francis, O, and W.R. Stern. 1986. Maize/cowpea intercrop system: effect of nitrogen fertilizer on productivity and efficiency. Field Crops Res. 14:247-261.

Francis, D.D., J.S. Schepers, and M.F. Vigil. 1993. Post-anthesis nitrogen loss from corn. Agron. J. 85:659-663.

Frey, N.M. 1981. Dry matter accumulation in kernels of maize. Crop Sci. 21:118-222.

Fukai, S., and B.R. Trenbath. 1993. Processes determining intercrop productivity and yield of components crops. Field Crops Res. 34:247-271.

Gifford, R., and L.T. Evans. 1981. Photosynthesis, carbon partitioning, and yield. Ann. Rev. Plant Physiol. 32: 485-509.

Glass, A.D.M. 1973. Influence of phenolic acids on ion uptake: I. Inhibition of phosphate uptake. Plant Physiol. 51:1037-1041.

Goh, K.M., and R.T. Haynes. 1986. Nitrogen and agronomic practice. *In*. Mineral Nitrogen in the Plant-Soil System. R.J. Haynes, K.C. Cameron, K.M. Goh and R.R. Sherlock (eds.). p.379-468. Orlando, Fla. Acdemic Press.

Grabau, L.J., D.G. Blevins, and H.C. Minor. 1986. Stem infusions enhanced methionine content of soybeans. Plant Physiol. 82:1013-1018.

Greenhouse, S.W., and S. Geisser. 1959. On the methods in the analysis of profile data. Psychometrika 24:95-112.

Groffman, P.M., P.F. Hendrix, C.R. Han, and D.A. Crossley. 1987. Nutrient cycling processes in a southeastern agroecosystem with winter legumes. *In*. The role of Legumes in Conservation Tillage System. J.F. Power (ed). p. 7-8. Proc. Soil Cons. Soc. of America. Ankeny, Iowa.

Guinn, G., and D.L Brummett. 1993. Leaf age, decline in photosynthesis, and changes in abscisic acid, indole-3-acetic acid, and cytokinin in cotton leaves. Field Crop Res. 32:269-275.

Hallberg, G.R. 1987a. Nitrates in Ground Water Contamination. p.23-68. Lewis Publish Inc., Chelsea, Mich.

Hallberg, G.R. 1987b. The impacts of agricultural chemicals on ground water quality. Geo. J. 15:283-295.

Hanway, J.J. 1960. Growth and nutrient uptake by corn. Extension Service Pamphlet 277, Iowa State Univ. Extension, Ames.

Hargett, N.L., and J.T. Berry. 1987. Fertilizer summary data-1986. Bull.Y-197. Natl. Fertilizer Develd. Cntr., Tennessee Valley Authority, Muscle Shoals, Al. p.135.

Harper, J.R., and N.E. Balke. 1981. Characterization of the inhibition of K^+ absorption in oats roots by salicylic acid. Plant Physiol. 68:1349-1353.

Hauck, R.D., and J.M. Bremner. 1976. Use of tracers for soil and fertilizer nitrogen research. Adv. Agron. 26:219-266.

Henzell, E.F. 1972. Loss of nitrogen from nitrogen-fertilized pastures. J. Aus. Inst. Agric. Sci. 38:309-310.

Herrero, M.P., and R.R. Johnson. 1981. Drought stress and its effects on maize reproductive systems. Crop Sci. 21:105-110.

Hew, C.S., C.D. Nelson, and G. Krotkov. 1967. Hormonal control of translocation of photosynthetically assimilate ^{14}C in young soybean plants. Amer. J. Bot. 54:252-256.

Hew, C.S. 1987. The effects of 8-hydroxylquinoline sulphate, acetylsalicylic acid and sucrose on bud opening of *Oncidium* flowers. J. Hort. Sci. 62:75-78.

Hope-Simposon, M.E. 1992. An evaluation of corn yield, intercrop growth and soil nitrogen levels in silage and grain corn intercrop system. M.Sc. Thesis. McGill University, Canada.

Hsiao, T.C. 1973. Plant response to water stress. Ann. Rev. Plant. Physiol. 24: 519-570.

Hume, D.J., and D.K. Campbell. 1972. Accumulation and translocation of soluble solids in corn stalks. Can. J. Plant. Sci. 52:363-368.

Jain, A., and H.S. Sivastava. 1981. Effect of salicylic acid on nitrate reductase activity in maize seedlings. Physiol. Plant. 51:339-342.

Jokela, W.E, and G.W. Randall. 1987. A nitrogen-15 microplot design for measuring plant and soil recovery of fertilizer nitrogen applied to corn. Agron. J. 79:322-325.

Jokela, W.E., and G.W. Randall. 1989. Corn yield and residual soil nitrate as affected by time and rate of nitrogen application. Agron. J. 81:720-726.

Jones, J.B., Jr. 1967. Interpretation of plant analysis for several agronomic crops. p.49-58. *In.* Soil Testing and Plant Analysis. G.W. Hard (ed.). Part 2. SSSA Spec. Pub. No.2. Soil Sci. Soc Am., Madison, Wis.

Juergens-Gschwind, S. 1989. Ground water nitrates in others developed countries (Europe)- Relationships to land use patterns. *In.* Nitrogen Management and Groundwater Protection. p.75-138. R.F. Follett (ed.). Amsterdam.

Kalita, P.K., and R.S. Kanwar. 1992. Shallow water table effects on photosynthesis and corn yield. Trans. ASAE 35:97-104.

Kalita, P.K., and R.S. Kanwar. 1993. Effects of water table management practices on the transport of nitrate-N to the shallow groundwater. Trans. ASAE 36:413-422.

Kaluli, J.W. 1996. Water table management and cropping systems for sustainable corn production. Ph.D Thesis, McGill University, Canada.

Keck, R.W., and J.S. Boyer. 1974. Chloroplast response to low leaf water potentials. III. Differing inhibition of electro transport and photophosphorylation. Plant Physiol. 53:474-479.

Keeney, D.R. 1982. Nitrogen management for maximum efficiency and minimum pollution. *In*. Nitrogen in Agricultural Soils. F.J. Stevensoon (ed.). Agronomy 22:605-649.

Keeney, D.R. 1986a. Nitrate in groundwater-agricultural contribution and control. *In*. Agricultural Impacts on Ground Water. p.329-351. Nat'l. Water Well Assoc. Worthington, OH.

Keeney, D.R. 1986b. Sources of nitrate to ground water. C.R.C. Crit. Rev. Environ. Control 16:257-304.

Ketcheson, J.W., and L.R. Webber. 1978. Effect of soil erosion on yield of corn. Can. J. Soil Sci. 58:459-463.

Khanif, Y.M., O.V. Cleemput, and L. Baert. 1984. Field study of the fate of labeled fertilizer nitrate applied to barley and maize in sandy soils. Fert. Res. 5:289-294.

Kling, G.J., and M.M. Meyer. 1983. Effect of phenolic compounds and indoleacetic acid on adventitious root initiation in cuttings of *Phaseolus aureus, Acer saccharinum, and Acer griseum*. Horti. Sci. 18:352-354.

Krapp, A., B. Hofmann, C. Schafer, and M. Stitt. 1993. Regulation of expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis? J. Plant. 3: 817-828.

Krause, G.H., and E. Weis. 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosyn. Res. 5. 139-157.

Krause, G.H., and E. Weis. 1988. The photosynthetic apparatus and chlorophyll fluorescence: an introduction. *In*. Application of Chlorophyll Fluorescence. H. K. Lichtenthaler (ed.). p.3-11. Kluwer Academic Publishers.

Kunelius, H.T., and R.L. Veinot. 1984. Root mass of Italian and Westerworlds ryegrasses. Canadex 127.4. Agriculture Canada, Ottawa, ON.

Kurtz, T., S.W. Melsted, and R.H. Bray. 1952. The importance of nitrogen and water in reducing competition between intercrops and corn. Agron. J. 44:13-17.

Langan, T.D., and E.S. Oplinger. 1987. Growth and yield of ethephon treated maize. Agron. J. 79:130-134.

Legg, J.O., and J.J. Meisinger. 1982. Soil nitrogen budgets. *In*. Nitrogen in Agricultural Soils. F.J. Stevenson (ed.). p.503-566. Soil Science Society of America, Inc. Madison, Wisconsin U.S.A.

Liang, B.C., M. Remillard, and A.F. MacKenzie. 1991. Influence of fertilizer, irrigation, and non-growing season precipitation on soil nitrate-nitrogen under corn. J. Environ. Qual. 20:123-128.

Liang, B.C., and A.F. MacKenzie. 1992. Changes in soil organic carbon and nitrogen after six years of corn production. Soil. Sci. 153:307-313.

Liang, B.C., and A.F. MacKenzie. 1994a. Corn yield, nitrogen uptake and nitrogen use efficiency as influenced by nitrogen fertilization. Can. J. Soil Sci. 74:235-240.

Liang, B.C., and A.F. MacKenzie. 1994b. Changes of soil nitrate-nitrogen and denitrification as affected by nitrogen fertilizer on two Québec soils. J. Environ. Qual. 23:521-525.

Lindau, C.W., W. H. Patrick, R.D. Delaune, K.R. Reddy, and P.K. Bollich. 1988. Entrapment of nitrogen-15 dinitrogen during soil denitrification. Soil Sci. Soc. Am. J. 52:538-540.

Littel, R.C. 1989. Statistical analysis of experiments with repeated measurements. Hort. Sci. 24:37-40.

Logan, T.J., G.W. Randell, and D.R. Timmons. 1980. Nutrient content of title drainage from cropland in the north central region. Research Bulletin 1119. Ohio Agric. Res. Develop. Centre, Wooster, OH.

Ma, B.L., and D.L. Smith. 1992. New method for supplying substances to cereal inflorescence. Crop Sci. 32:191-194.

Ma, B.L., L.M. Dwyer, D.W. Stewart, C.J. Andrews, and M.Tollenaar. 1994a. Stem infusion of field-grown maize. Commun. Soil Sci. Plant Anal. 25:2005-2017.

Ma, B.L, S. Leibovitch, and D.L Smith. 1994b. Plant growth regulator effects on protein content and yield of spring barley and wheat. J. Agro. Crop. Sci. 172:9-18.

Ma, B.L., L.M. Dwyer, and D.L. Smith. 1994c. Evaluation of peduncle perfusion for in *Vivo* studies of carbon and nitrogen distribution in cereal crops. Crop Sci. 34:1584-1588.

Ma, B.L., L.M. Dwyer, B.C. Liang, and E. Gregorich. 1995a. Use of nitrogen-15 microplots for field studies of maize nitrogen-use efficiency. Commun. Soil. Sci. Plant. Anal. 26:1813-1822.

Macdonald, A.J., D.S. Powlson, P.R. Poulton., and D.S. Jenkinson. 1989. Unused fertilizer nitrogen in arable soils -Its contribution to nitrate. J. Sci. Agric. 46:407-419.

MacLean, A.J. 1977. Movement of nitrate nitrogen with different cropping systems in two soils. Can. J. Soil Sci :57-33.

Madramootoo, C.A., K. Wiyo, and P. Enright. 1992. Nutrient losses through tile drains from two potato fields. Appli. Eng. Agric. 8:639-646.

Madramootoo, C.A., G.T. Dodds, and A. Papadopoulos. 1993. Agronomic and environmental benefits of water-table management. J. Irrig. Drain Eng. ASCE 119:1052-1065.

Makeev, A.V., T.E. Krendeleva, and A.T. Mokronosov. 1992. Photosynthesis and abscisic acid. Soviet Plant Physiol. 39:118-126.

Malhi, S.S., and M. Nyborg. 1983. Field study of the fate of fall-applied ^{15}N-labeled fertilizers in three Alberta soils. Agron. J. 75:71-74.

Mangelsdorf, P.C., R.S. Macneish, and W.C. Galinat. 1967. Prehistoric wild and cultivated Maize. *In*. The Prehistory of the Tehuacan Valley. Vol.1. Environment and subsistence. D.S. Byers. (ed.). p.178-200. Univ. of Texas Press, Austin.

Markhart, A.H., E.L. Fiscur, A.W. Naylor, and P.J. Kramer. 1979. Effect of abscisic acid on root hydraulic conductivity. Plant Physiol. 64:611-614.

Martel, Y.A., and A.F. MacKenzie. 1980. Long-term effects of cultivation and land use on soil quality in Québec. Can. J. Soil Sci. 60:411-420.

Martin, R.C., J.T. Arnason, J.D.H. Lambert, P. Isabelle, H.D. Voldeng, and D.L. Smith. 1989. Reduction of European corn borer (*Lepidoptera: Pyralidae*) damage by intercropping corn with soybean. J. Econ. Entomol. 82:1455-1459.

Martin, R.C., H.D. Voldeng, and D.L. Smith. 1990. Intercropping corn and soybean for silage in a cool-temperate region: yield, protein and economic effects. Field Crops Res. 23:295-310.

Martin, R.C., H.D. Voldeng, and D.L. Smith. 1991. Nitrogen transfer from

nodulating soybean to maize or to nonodulating soybean in intercrops: the ^{15}N dilution methods. Plant and Soil. 132:53-63.

McCracken, D.V., S.J. Corak, M.S. Smith, W.W. Frye, and R.L. Blevins. 1989. Residual effects of nitrogen fertilization and winter cover cropping on nitrogen availability. Soil. Sci. Soc. Am. J. 53:1459-1464.

McCracken, D.V., M.S. Smith, J.H. Grove, C.T. MacKown, and R.L. Blevins. 1994. Nitrate leaching as influenced by cover cropping and nitrogen source. Soil. Sci. Soc. Am. J. 58:1476-1483.

McNeal, B.L., and P.F. Pratt. 1978. Leaching of nitrate from soils. *In.* Proc. Natl. Conf. on Management of Nitrogen in Irrigated Agric. Sacramento, Calif. Pratt (ed.). 15-18 May 1978. Univ. of California, Riverside.

Meek, B.B., L.B. Grass., L.S. Willardson, and A.J. MacKenzie. 1970. Nitrate transformations in a column with controlled water table. Soil. Sci. Soc. Am. Proc. 34:235-239.

Meisinger, J.J., W.L. Hargrove, R.B. Mikkelsen, J.R. Williams, and V.W. Benson. 1991. Effects of cover crop on groundwater quality. *In.* Cover crop for clean water. W.L. Hargrove (ed.). p.57-68. Proc Int. Conf., Jackson TN. 9-11 Apr. 1991. Soil and Water Conserv. Soc. Am., Ankeny, IA.

Memon, N.A. 1985. Experiments with subsurface irrigation. Ph.D. Thesis. McGill University, Canada.

Miller, P.L., and A.F. MacKenzie. 1978. Effects of manures, ammonium nitrate and S-coated urea on yield and uptake of N by corn and on subsequent inorganic N level in soil in southern Québec. Can. J. Soil. Sci. 58:153-158.

Miller, M.H. 1979. Contribution of nitrogen and phosphorus to subsurface drainage water from intensively cropped mineral and organic soils in Ontario. J. Environ. Qual. 8:42-48.

Milthorpe, F.L., and J. Moorby. 1969. Vascular transport and its significance in plant growth. Ann. Rev. Plant Physiol. 20:117-138.

Moes, J., and E.H. Stobbe. 1991. Barley treated with ethephon: I. Yield components and net grain yield. Agron. J. 83:86-90.

Mohamed, M.A., J.J. Steiner, S.D. Wright, M.S. Bhangoo, and D.E. Millhouse. 1990. Intensive crop management practices on wheat yield and quality. Agron. J. 82:701-706.

Mosier, A.R., W.D. Guenzi, and E.E. Schweizer. 1986. Soil losses of dinitrogen and nitrous oxide from irrigated crops in Northern Colorado. Soil. Sci. Soc. Am. J. 51:271-276.

Moss, P.A., and N.L. Hartwig. 1980. Competitive control of common lambsquarter in a corn-soybean intercrop. Proc. Northeast. Weed. Sci. Soc. 34:21-28.

Muller, J.C., D. Denys, G. Morlet, and A. Mariotti. 1989. Influence of catch crops on mineral nitrogen leaching and its subsequent plant use. *In.* Management Systems to Reduce Impact of Nitrates. J.C. Germon (ed.). p.85-98. Elsevier Applied Science, London and New York.

Musser, H.B., and A.T. Pekins. 1969. Guide to plant planting. *In.* Turfgrass Science. A.A. Hanson and F.J. Juska (eds.). p.474-490. Amer. Soc. Agron. Madison, Wis.

Nair, K.P.P., U.K. Patel, R.P. Singh, and M.K. Kaushik. 1979. Evaluation of legume intercropping in conservation of fertilizer nitrogen in maize culture. J. Agri. Sci. 93:189-194.

Neilson, G.N., and A.F. MacKenzie. 1977. Soluble and sediment nitrogen losses as related to land use and type of soil in eastern Canada. J. Environ. Qual. 6:318-321.

Norberg, O.S, S.C. Mason, and S.R. Lowry. 1988. Ethephon influence on harvestable yield, grain quality, and lodging of corn. Agron. J. 80:768-772.

Nordquist, P.T., and G.A. Wicks. 1974. Establishment methods for alfalfa in irrigated corn. Agron. J. 66:377-380.

Ofori, F., and W.R. Stern. 1987. Cereal-legume intercropping systems. Adv. Agron. 41:41-90.

Olson, R.J., R.F. Hensler, O.J. Attoe, S.A. Witzel, and L.A. Peterson. 1970. Fertilizer nitrogen and crop rotation in relation to movement of nitrate nitrogen through soil profiles. Soil. Sci. Soc. Am. Proc. 34:448-452.

Olson, R.A., and L.T. Kurtz. 1982. Crop nitrogen requirements, utilization and fertilization. *In.* Nitrogen in Agricultural Soils. F.J.Stevenson (ed.) Agronomy 22:567-604.

Olson, R.V. 1980. Plot size requirements for measuring residual fertilizer nitrogen and nitrogen uptake by corn. Soil. Sci. Soc Am. J. 44:428-429.

Papakosta, D.K., and A.A. Gsgianas. 1991. Nitrogen and dry matter accumulation, remobilization, and losses for Mediterranean wheat during grain filling. Agron. J. 83:864-870.

Pate, J.S. 1980. Transport and partitioning of nitrogenous solutes. Ann. Rev. Plant Physiol. 31:313-340.

Patterson, D.T. 1981. Effects of allelopathic chemicals on growth and physiological response of soybean (*Glycine max*). Weed. Sci. 29:53-59.

Peterson, G.A., and W.W. Frye. 1989. Fertilizer nitrogen management. *In*. Nitrogen Management and Ground Water Protection. R.F. Follett (ed.). Chapter 7. p.183-219. Developments in agricultural and amanged-forest ecology 21. Elsevier Science Publishing Company Inc., New York.

Pimental, D., L.E. Hurd, A.C. Bellott, M.J. Forster, I.N. Oka, O.D. Sholes, and R.T. Whitman. 1973. Food production and the energy crisis. Science. 182:443-449.

Power, J.F. 1980. Response of semiarid grassland sites to nitrogen fertilization. II. Fertilizer recovery. Soil. Sci. Soc. Am. J. 44:550-555.

Polisetty, R., and R.H. Hageman. 1981. Nitrate absorption by corn: independent of root nitrate reductase activity. Curr. Sci. 50:495-496.

Power, J.F., and J.O. Legg. 1984. Nitrogen-15 recovery for five years after application of ammonium nitrate to crested wheatgrass. Soil. Sci. Soc. Am. J. 48:322-326.

Pratt, P.F. 1984. Nitrogen use and nitrate leaching in irrigated agriculture. *In*. Nitrogen in Crop Production. Hauck. R.D (ed.). p.319-333. Am. Soc. Agron., Madison. WI.

Premachandra, G.S., H. Saneoka., K. Fujita, and S. Ogata. 1992. Osmotic adjustment and stomatal response to water deficits in maize. J. Exp. Bot. 43:1451-1456.

Preston, C.M., J.M. Preston, and E.G. Callway. 1981. Inexpensive ^{15}N analysis of agricultural samples by optical emission spectroscopy employing a simple, one-step Dumas sample preparation procedure. Can. J. Spectrosc. 26:239-244.

Prioul, J.L., and N. Schwebel-Dugue. 1992. Source-sink manipulations and carbohydrate metabolism in maize. Crop Sci 32:751-756.

Prioul, J.L., A. Reyss, and N. Schwebel-Dugue. 1990. Relationships between carbohydrate metabolism in ear and adjacent leaf during grain filling in maize genotypes. Plant Physiol. Biochem. 28:485-493.

Rademacher, W., and J.E. Graebe. 1984. Hormonal changes in developing kernels of two spring wheat varieties differing in storage capacity. Berichte Deutschen Botanischen Gesellschaft 97:167-181.

Rai, V.K, S.S. Sharma, and S. Sharma. 1986. Reversal of ABA-induced stomatal closure by phenolic compounds. J. Exp. Bot. 37:129-134.

Raschke, K., and R. Hendrick. 1985. Simultaneous and independent effects of abscisic acid on stomata and the photosynthetic apparatus in whole leaves. Planta. 163:105-118.

Raschke, K. 1975. Stomatal action. Ann. Rev. Plant. Physiol. 26:309-340.

Raskin, I. 1992. Role of salicylic acid in plants. Ann. Rev. Plant. Physiol. Plant. Mol.Biol. 43:439-463.

Reddy, G.B., and K.R. Reddy. 1993. Fate of nitrogen-15 enriched ammonium nitrate applied to corn. Soil. Sci. Soc. Am. J. 57:111-115.

Reed, A.J., G.W. Singletary, J.R. Schussler, D.R. Williamson, and A.L. Christy. 1988. Shading effects on dry matter and nitrogen partitioning, kernel number and yield of maize. Crop Sci. 28:819-825.

Rendig, V.V., and T.W. Crawford, Jr. 1985. Partitioning into maize grain N fractions of N absorbed through the roots before and after pollination. J. Sci. Food Agric. 36:645-650.

Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1986. How a corn plant develops. Rev. ed. Iowa Coop. Ext. Serv. Special. Report. Vol.48.

Robinson, S.P., J.T. Wiskich, and L.G. Paleg. 1978. Effects of indoleacetic acid on CO_2 fixation, electron transport and phosphorylation in isolated chloroplasts. Aus. J. Plant Physiol. 5:425-431.

Rochester, I.J., G.A. Cinstable, and D.A. Macleod. 1991. Mineral nitrogen dynamics in a fallow grey clay. J. Exper. Agric. 31:237-244.

Roth, G.W., and R.H. Fox. 1990. Soil nitrate accumulations following nitrogen-fertilized corn in Pennsylvania. J. Environ. Qual. 19:243-248.

Russelle, M.P., E.J. Deibert, R.D. Huack, M. Stevanovic, and R.A. Olson. 1981. Effects of water and nitrogen management on yield and ^{15}N-depleted fertilizer use efficiency of irrigated corn. Soil. Sci. Soc. Am. J. 45:553-558.

Ryden, J.C. 1983. Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. J. Soil. Sci. 34:355-365.

Salvador, R.J. 1984. Assimilate uptake by maize (*Zea mays*. L) kernels as affected by source-sink manipulation. M. Sc. Thesis. Iowa State University., Ames.

Sanchez, C.A., A.M. Blackmer, R. Horton, and D.R. Timmons. 1987. Assessment of errors associated with plot size and lateral movement on nitrogen-15 when studying fertilizer recovery under field conditions. Soil. Sci. 144:344-351.

SAS Institute. 1985. SAS user's guide. Statistics. Vers 5 ed. SAS Inst., Cary, NC.

Schery, R.W. 1961. Making and maintaining a lawn. Am. Hortic. Mag. 40:199-209.

Schon, M.K., and D.G. Blevins. 1987. Boron stem infusions stimulate soybean yield by increasing pods on lateral branches. Plant Physiol. 84:969-971.

Schussler, J.R., and M.S. Westgate. 1991. Maize kernel set at low water potential: Sensitivity to reduced assimilates during early kernel growth. Crop Sci. 31:1189-1195.

Schussler. J.R., and Westgate. M.E. 1994. Increasing assimilate reserve does not prevent kernel abortion at low water potential in maize. Crop Sci. 34: 1569-1576.

Scott, T.W., J.Mt. Pleasant, R.F. Bur, and D.J. Otis. 1987. Contributions of ground cover, dry matter, and nitrogen from intercrops and cover crops in a corn polyculture system. Agron. J. 79:792-798.

Searle, P.G.E., Y. Comudom., D.C. Shedden, and R.A. Nance. 1981. Effect of maize/legume intercropping systems and fertilizer nitrogen on crop yields and residual nitrogen. Field Crops Res. 4:133-145.

Setter, T.L., and V.H. Meller. 1984. Reserve carbohydrate in maize stem. {^{14}C (carbon isotope) glucose and {^{14}C} sucrose uptake characteristics (phloem import versus export from the stem). Plant Physiol. 75:617-622.

Shettel, N.L., and N.E. Balke. 1983. Plant growth response to serval allelopathic chemicals. Weed. Sci. 31:293-298.

Shipley, P.R , J.J. Meisinger, and A.M. Decker. 1992. Conserving residual corn fertilizer nitrogen with winter cover crops. Agron. J. 84:869-876.

Singh, B., D.S. Rana, and G.S. Sekhon. 1978. Some measures of reducing leaching loss of nitrates beyond potential rooting zone. IV. Intercropping. Plant. Soil. 49:633-639.

Singh, G., and M. Kaur. 1980. Effect of growth regulators on podding and yield of mung bean (*Vigna radiata* (L.) Wiliczek). Indian. J. Plant Physiol. 23:366-370.

Skaggs, R.M., M.A. Breve, and J.W. Gilliam. 1994. Hydrologic and water quality impacts of agricultural drainage. Critical Review in Environmental Science and Technology 24:1-32.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. Third edition. Freeman, New York.

Sonnewald, U., and L. Willmitzer. 1992. Molecular approaches to sink-source interactions. Plant Physiol. 99:1267-1270.

Stanley, C.D., R.E. Green, M.A. Khan, and L.T. Santo. 1990. Nitrogen fertilization rate and soil nitrate distribution for microirrigated sugarcane. Soil. Sci. Soc. Am. J. 54:217-222.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics: a biometrical approach. McGraw-Hill Co., New York.

Steenvoorden, J.H.A.M. 1989. Agricultural practices to reduce nitrogen losses via leaching and surface runoff. *In*. Management Systems to Reduce Impact of Nitrates. J.C. Germon (ed.). p.72-84. Commission of the European Communities, Elsevier Science Publishers Ltd., London, England.

Stumpe, J.M., P.L.G. Viek, S.K. Mughogho, and F. Ganry. 1989. Microplot size requirements for measuring balances of fertilizer nitrogen-15 applied to maize. Soil. Sci. Soc. Am. J. 53:797-800.

Stutte, C.A., and R.T. Weiland. 1978. Gaseous nitrogen loss and transpiration of several crop and weed species. Crop Sci. 18:887-889.

Tait, R.T., C.A. Madramootoo, and P. Enright. 1995. An instrumented, field-scale research facility for drainage and water quality studies. Computers Electron. Agric. 12:131-145.

Tan, C.S., C.F. Drury, J.D. Graynor, and T.W. Welacky. 1993. Integrated system to abate herbicide and nitrate contaminations of Great lakes. Water Sci. Tech. 28:497-507.

Tarpley, L., D.M. Vietor, and F.R. Miller. 1994. Internodal compartmentation of stem-infused ^{14}C sucrose in sweet and grain sorghum. Crop Sci. 43:1116-1120.

Terman, G.L., and S.E. Allen. 1974. Losses of nitrogen and mineral nutrients from corn grown in greenhouse pot experiments. Soil. Sci Soc. Am. Proc. 38:322-326.

Thomposon, L.M. 1986. Climatic change, weather variability, and corn production. Agron. J. 78:649-653.

Tollenaar. M. 1977. Source-sink relationship during reproductive development in maize. Maydica. 22:49-79.

Tollenaar. M., and T.B. Daynard. 1978. Effect of defoliation on kernel development in maize. Can. J. Plant. Sci. 58:207-212.

Tomar, J.S., A.F. MacKenzie, G.R. Mehuys, and I. Alli. 1988. Corn growth with foliar nitrogen, soil applied nitrogen, and legume intercrops. Agron. J. 80:802-807.

Triplett, G.B.Jr. 1962. Intercrops in corn and soybean cropping systems. Agron. J. 54:106-109.

Uhart, S.A., and F.H. Andrade. 1991. Source-sink relationship in maize grown in a cool temperate area. Agronomie. 11:863-875.

Van, O., J, D. Wilkins, and R.T. Besford. 1994. Regulation of the expression of photosynthetic nuclear genes by CO_2 is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high CO_2? Plant. Cell. Environ. 17:913-923.

Vinten, A.J.A., and K.A. Smith. 1993. Nitrogen cycling in agricultural soils. p:39-73. *In.* Nitrate: Precesses, Patterns and Managements. T.P. Burt, A.L. Heathwaite and S.T. Trudgill (eds.). John Willey and Sons. New York.

Voss, R.E., J.J. Hanway, and L.C. Dumenil. 1970. Relationship between grain yield and leaf N, P, and K concentrations for corn (*Zea mays* L.) and the factors that influence the relationship. Agron. J. 62:726-728.

Vrabel, T.E. 1981. The use of legumes as living mulch crops with sweet corn (*Zea*

mays L.)-feasibility studies. M.Sc. Thesis. Cornell Univ., Ithaca, NY.

Warner, H.L., and A.C. Lepold. 1969. Ethylene evolution from 2-chlorethyly phosphoric acid. Plant Physiol. 44:156-158.

Weil, R.R., and M.E. Mcfadden. 1990. Fertility and weed stress effects on performance of maize/soybean intercrop. Agron. J. 83:717-721.

Westagate, M.E., and J.S. Boyer. 1985. Carbohydrate reserves and reproductive development at low water potentials in maize. Crop Sci. 25:762-769.

Westgate, M.E., and J.S. Boyer. 1986. Reproduction at low silking and pollen water potentials in maize. Crop Sci. 26:951-956.

Wetselarr, R., and G.D. Farquhar. 1980. Nitrogen losses from tops of plants. Adv. Agron. 33:263-302.

Williamson, R.E. 1964. The effect of root aeration on plant growth. Soil. Sci. Soc. Am. Proc. 28:86-90.

Yadav, R.L. 1982. Intercropping pigeonpea to conserve nitrogen in maize and produce residual effects on sugarcane. Expl. Agric. 17:311-315.

Zelitch, I. 1982. The close relationship between net photosynthesis and crop yield. Bio. Sci. 32:796-802.

Zhou, X.M., and D.L Smith. 1996. A new technique for continuous injection into stems of field-grown corn plants. Crop Sci. 36:452-456.

Zinselmeier, C., M.J. Lauer, and J.S. Boyer. 1995. Reversing Drought-induced losses in grain yield: sucrose maintains embryo growth in maize. Crop Sci. 35:1390-1400.

IMAGE EVALUATION
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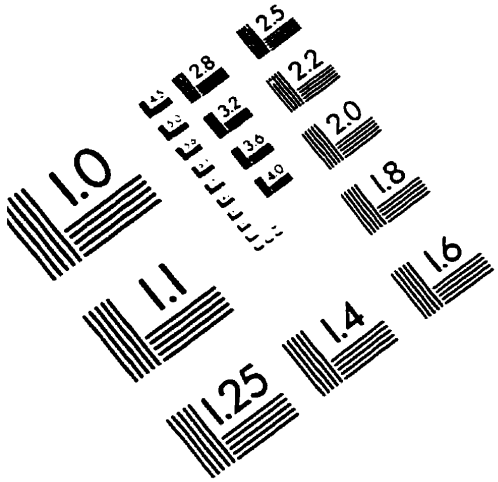

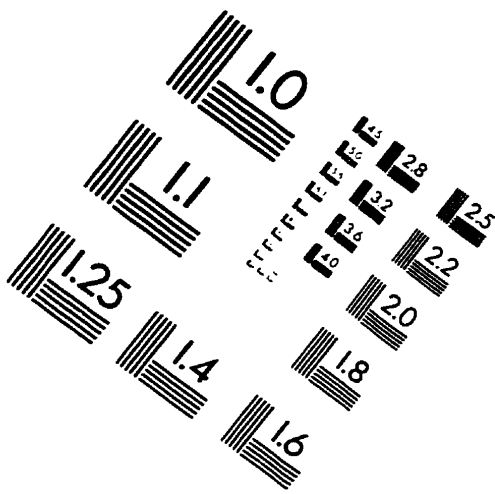

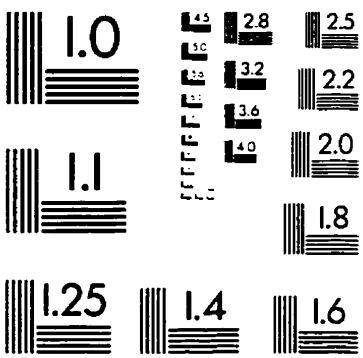

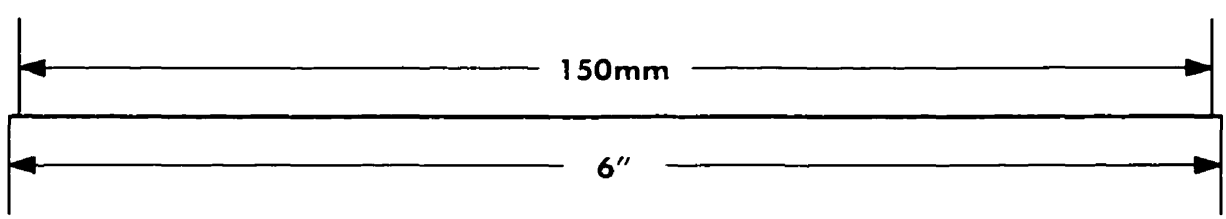

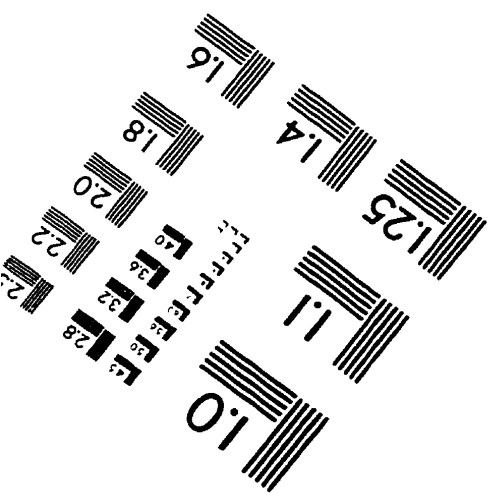

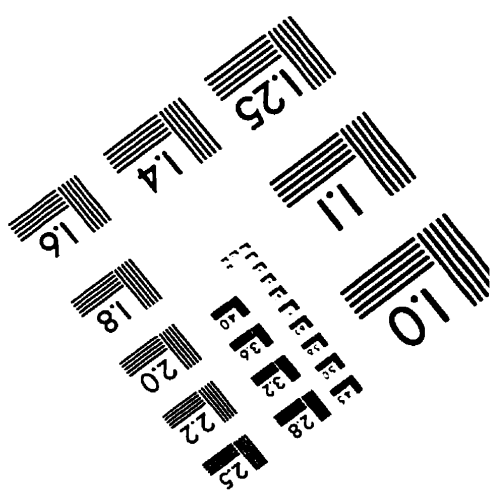

APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989