Swedish University of Agricultural Sciences

Master thesis in the Horticultural Science Programme
2008**:4 45 hp**
ISSN 1403-0993

Effect of irrigation and nitrogen treatments on yield, quality, plant nitrogen uptake and soil nitrogen status and the evaluation of sap test, SPAD chlorophyll meter and Dualex to monitor nitrogen status in broccoli

by

Rickard Mellgren

Department of Horticulture, SLU, Alnarp

Master thesis in biology, 45 hp.

Title: Effect of irrigation and nitrogen treatments on yield, quality, plant nitrogen uptake and soil nitrogen status and the evaluation of sap test, SPAD chlorophyll meter and Dualex to monitor nitrogen status in broccoli

Swedish title: Effekt av bevattning och kvävegödning på skörd, kvalitet, växtens kväveupptag och markens kväveinnehåll samt utvärdering av växtsaftprov, SPAD klorofyllmätare och Dualex för att bestämma kvävestatusen i broccoli

Author: Rickard Mellgren

Supervisor: Dr Nicolas Tremblay
Researcher in Crop Management and Fertilization
Agriculture and Agri-Food Canada

Examiner: Associate Professor Håkan Asp
Department of Horticulture
Swedish University of Agricultural Sciences

Title page photo by author:
Broccoli field during irrigation at L'Acadie research substation

Abstract

Broccoli (*Brassica oleracea* L. ssp. *italica*) is a crop benefiting greatly from nitrogen fertilization and the risk of leaching to groundwater is high. Nine N fertilizer treatments, from 0 to 225 kg N/ha and two irrigation treatments, irrigated and non-irrigated, were arranged in a split-plot design. Nitrogen had a curvilinear effect on marketable yield; an increase was seen up to an application of 165 kg N/ha where the response plateaued. Hollow stem was the only disorder related to N application noticed in this project. It was seen only in treatments receiving more than 165 kg N/ha and the number of heads affected was very low. Plant uptake of N was strongly affected by nitrogen application and a large effect from irrigation was observed at harvest. The difference could only be explained by leaching early in the season and denitrification during the season. The risk of leaching early in the season is critical when the amount of precipitation and/or irrigation is above field capacity. Sap test, SPAD chlorophyll meter and Dualex were compared as a way to monitor nitrogen status. Sap test was the method showing the largest effect among different N applications but also the largest variability. The SPAD chlorophyll meter was very sensitive to differences in nitrogen application, even though it had the narrowest span from high to low fertilized plants. The Dualex showed the best relationship with plant nitrogen uptake. It quickly showed differences among N application and a good correlation with yield. The use of a reference plot is recommended to decrease the influence of factors other than N affecting test results and to obtain relative values that can be used in different conditions.

Sammanfattning

Broccoli (*Brassica oleracea* L. ssp. *italica*) är en gröda som har ett stort utbyte av kvävegödning, så risken för läckage till grundvattnet är stort. Nio olika kvävegivor, mellan 0 och 225 kg N/ha och två bevattningsfrekvenser, bevattning och ej bevattning, arrangerades i ett split-plot utförande. Kvävet hade en kurvlinjär effekt på säljbar skörd; en ökning observerades upp till 165 kg N/ha, därefter planade den ut. Broccoliplantor med ihålig stam var den enda fysiologiska skadan relaterad till kväve som observerades i detta projekt. Det var endast i kvävegivor över 165 kg N/ha som ihålig stam observerades och antalet var väldigt lågt. Upptaget av N var starkt relaterat till kvävegivans storlek och mätningarna vid skörd visade att påverkan från bevattningen var stor. Skillnaden kunde endast förklaras med läckage tidigt på säsongen och denitrifikation

under säsongen. Risken för kväveläckage tidigt på säsongen är stor när nederbörden och/eller bevattningen är över jordens fältkapacitet. Växtsaftprovet var det test som visade störst skillnad mellan olika kvävegivor men också den största variationen mellan samma kvävegiva. SPAD klorofyllmätare var väldigt känslig för skillnader mellan kvävegivor även om den visade minst skillnad mellan hög och låg kvävegödning. Dualex hade bästa sambandet mellan växtens upptag av kväve och instrumentets resultat, den reagerade snabbt på kvävegödsling och hade en bra korrelation med skörden. Användandet av en referensruta rekommenderas för att minska påverkan från andra faktorer än kvävet på testresultatet och för att erhålla relativa värden som kan användas i olika förhållanden.

Acknowledgement

I would like to express my great gratitude to my supervisor Nicolas Tremblay and his team of experts in crop management and fertilization, Carl Belec, Edith Fallon, Elisabeth Fortier, Marcel Tétreault and Philippe Vigneault. The help and kindness from these people made my stay in Quebec so much easier and more fun and I see them as very dear friends. Thanks also to the summer students at both Saint Jean research station and L'Acadie research substation for their assistance. I would also like to thank my examiner Håkan Asp for his patience in dealing with questions regarding this thesis. Jan-Erik Englund was of great help in giving me feedback on the statistical part and that was very much appreciated. The financial contributions from the Faculty of Natural Resources and Agricultural Sciences and the foundation "Stiftelsen Oscar och Lili Lamms Minne" were of valuable assistance in this thesis.

Contents

List of tables

List of figures

General introduction

Nitrogen

Of all the major nutrients provided to agricultural crops, nitrogen is generally considered the most important for plant growth. It is a constituent of many components in plants, including all proteins, which build cell material and plant tissue and production of chlorophyll, making photosynthesis possible. Many enzymes need nitrogen for assimilation of nutrients and also the nucleic acids involved in reproduction of the genetic code, DNA and RNA, is dependent of nitrogen. (Teiz & Zeiger, 2004)

Nitrogen can be taken up by the plant as either nitrate NO_3^- or ammonium NH_4^+. Nitrate is the form most easily taken up by plant roots since it is soluble in water. In most agricultural soils, the amount of nitrate is significantly larger than the quantity of ammonium because of nitrification that converts ammonium to nitrate (Bartholomew & Clark, 1965).

Optimal nitrogen fertilization management

The amount of available nitrogen during the season is often not enough for high yields and good quality. The nitrogen in the soil at planting is affected by many factors, for example previous crop, fertilisation history, precipitation since the last crop, soil humus content, soil texture and temperature (Tremblay et al. 2001). Much of the nitrogen left in the soil the previous year can be lost through leaching. Often the root system of the seedlings or transplants is too limited to reach nitrogen from lower soil layers. Nitrogen fertilizer is therefore added to the field. Chemical fertilizers can be either organic (like manure) or inorganic with different composition of ammonium, nitrate, urea, etc. Since broccoli requires large quantities of nitrogen to produce high yields and the timing of nitrogen uptake is crucial for optimum growth, inorganic fertilizers are often used. They are easier to apply and the grower knows exactly the amount of nitrogen applied and approximately when it is available to the crop.

Basing nitrogen fertilization solely on crop yield response constitutes an environmental risk, since maximum yields are often obtained with high amounts of nitrogen corresponding to low recovery rates (Zebarth et al., 1995). Nitrogen should be added to the crop when the need is the largest. With only one application at transplanting, the grower is tempted to apply a high N rate to avoid deficiencies under favourable growth conditions. With this approach the risk of leaching is high, but the use of a split application can satisfy plant need and avoid environmental hazard. In Quebec the recommendation for broccoli is a split application of 85 kg N/ha at transplanting and another 50 kg N/ha, 4 to 5 weeks later (CRAAQ 2003).

Several authors have shown the importance of timing the application of nitrogen. Nkoa et al. (2003) showed in an experiment in hydroponics that the nitrogen uptake of broccoli is bell shaped. It increased to reach a maximum at 30 days after transplantation and then decreased to harvest time, 60 days after transplantation. They showed that both higher and lower contents of nitrogen in the water, compared to optimum, reduced growth rate. This was especially observed on young broccoli plants, 15 days after transplantation, with excessive plant nitrogen concentration. When comparing the optimum concentration of 100 mg N l^{-1} to 175 mg N l^{-1} they observed a 50 percent reduction in growth. The study also showed that the broccoli plants utilized the nitrogen best during the first 30 days of growth. During this time a concentration of 100 mg l^{-1} gave optimal growth rates, while after the 30 days a concentration of 175 mg l^{-1} was optimal. Even though the root system is small during the first half of the growth period, 62 percent of the nitrogen required to reach maturity was taken up during this time (54 percent between 15 and 30 days). Studies made by Bowen et al. (1999), Vågen (2003), Shelp and Liu (1991) and Magnifico et al., (1979) also showed the small nitrogen uptake during the first weeks after transplantation and a more rapid uptake around midseason. As compared to Nkoa et al. (2003), they showed a high nitrogen uptake for a longer time, until just a few weeks before harvest with the maximum during head formation.

Trials made by Riley and Vågen (2003) showed no effect of increasing nitrogen fertilizer from 150 to 250 kg per ha in broccoli. When plant nitrogen status was high the relation between crop yield and plant nitrogen status was low. They also showed that split application of nitrogen gave significantly higher yields as compared to a single application. However, Everaarts and de Willigen (1999a) showed that split application had no or a negative effect on yield. Babik and Elkner (2002) showed that split application was preferable when low pre-plant rates were used (100 kg N per ha) together with irrigation. At higher pre-plant rates (300 and 500 kg N per ha) or

when irrigation was not used, no increase in yield was observed. Bracey et al. (1995), showed no significant response on yield to either pre-plant or sidedress nitrogen rates, even though the amount differed from 179 to 348 kg N per ha.
Babik and Elkner (2002) showed that for every increase of nitrogen application (100, 200, 400 and 600 kg N/ha), yield increased significantly. Kowalenko and Hall (1987), showed that broccoli yield increased with nitrogen applications of up to 250 kg per ha. Broccoli yield increased curvilinearly with increasing rates of nitrogen fertilizer and maximum yields were obtained at rates between 436 and 558 kg per ha (Zebarth et al., 1995). Kahn et al. (1991), showed no significant effect on marketable head weight in three out of four experiments when using nitrogen rates between 112 and 224 kg/ha. The nitrogen concentration in heads and above-ground residues increased significantly with increasing amount of nitrogen fertilizer but the timing of application had little effect. Recoveries of around 50 percent of the applied nitrogen were recorded (Riley and Vågen, 2003).

When deciding the amount of nitrogen fertilizer to apply, the grower needs to consider the rate of mineralization during the season. Mineralization of organic matter and residues is more rapid in light-textured soils than heavier soils (De Neve & Hofman, 1998). Mineralization is strongly affected by the carbon : nitrogen ratio. The higher the ratio, the lower the rate of mineralization. Temperature and moisture play an important role in the microbial activity responsible for the mineralization. With lower temperatures and limited moisture the mineralization rates will be slower (Neeteson & Carton, 2001). A mineralization rate of around 5 kg N/ha per week has been estimated in earlier studies (Tremblay et al., 2001; Fink & Scharpf, 2000). This would mean that mineralization add around 50 kg N/ha during a broccoli season (10 weeks).

Nitrogen fertilizer is usually banded on vegetable crops, because of the distance between the rows, to minimize leaching and get an effective uptake. Everaarts and De Willigen (1999a), showed that there was a significant response in yield both to method of application and amount of nitrogen applied. Band placed nitrogen was more effective than broadcast. Their study showed that the nitrogen required in the soil, applied and mineralized during the growing season, for optimum yield was 270 kg N per ha for banded fertilizer. For broadcast application 275 kg N per ha were required, however with lower yield compared to banded. Bracy et al. (1995), on the other hand showed no difference in yield between fertilizer applied to the surface or banded. Tremblay et al. (2001) concluded that nitrogen uptake for an average yield is around 260 kg N per ha. Eveerarts and de Willigen (1999b) reported a maximum nitrogen uptake of 300 kg N per ha and that split application did not influence nitrogen uptake.

Most studies have shown that the proportion of nitrogen taken up by broccoli decreased with increasing amount of fertilizer (Riley & Vågen, 2003; Eveerarts & de Willigen, 1999b; Zebarth et al., 1995; Letey et al., 1983). One study has shown the opposite (Kowalenko & Hall, 1987). Fink and Scharpf (2000) and Greenwood and Draycott (1988), both showed that the recovery rate of fertilizer nitrogen and soil mineral nitrogen was not affected by the amount of nitrogen applied.

In a study by Beverly et al. (1986), yield decreased at excessive nitrogen applications, which could indicate salt injury. They also showed that different nitrogen treatments did not affect the number of days to maturity but higher applications increased the growth rate, which resulted in higher yields. Bracy et al. (1995), Kahn et al. (1991) and Kowalenko and Hall (1987) also showed no effect on maturity rate when increasing nitrogen supply. Babik and Elkner (2002) showed that split application of high amounts of nitrogen fertilizer did not affect plant mass or the number of days to harvest. The amount of nitrogen applied however did affect the earliness of harvest. Irrigation hastened the yielding of broccoli but it only affected the yield significantly in the highest nitrogen amount (600 kg per ha) both as single or split application. Excessive water has shown to decrease yield for a given nitrogen treatment (Beverly et al., 1986). In a study made on wheat by Westerman et al. (1994), yield differences due to nitrogen application were small when water was limited but otherwise large.

An increase in nitrogen application to increase yield can be detrimental to harvest quality. Hollow stem is one of the most common physiological disorders in broccoli (Tremblay 1989). An elliptical crack is formed during the initiation of the central inflorescence in the centre of the stem. Broccoli for the fresh market is particularly sensitive to hollow stem since the base of the head is displayed to the customer. A brown discoloration of the hole prior to or after harvest may occur depending on whether the discoloration is of non-pathogenic or pathogenic origin (Tremblay 1989). Several authors have shown a relationship between hollow stem and nitrogen application in broccoli (Babik and Elkner 2002, Belec et al. 2001, Tremblay 1989, Hipp 1974, Cutcliffe 1972). Other factors affecting hollow stem are the environment (Shattuck et al. 1986) and cultivar and spacing (Cutcliffe 1975). Babik and Elkner (2002) and Scaife and Wurr (1989) (cauliflower) showed an increase in the number of hollow stem when irrigation was used together with high nitrogen application.

Another physiological disorder affected by nitrogen fertilization is head rot (Vågen 2003, Everaarts 1994, Canaday 1992). The disorder first appears as a water-soaked lesion followed by soft rotting of the tissue (Ludy and Powelson, 1997). Head rot is a serious disease of broccoli and yield losses can be as high as 100 percent (Everaarts 1994). Several bacteria, *Pseudomonas* and *Erwinias*, have been targeted as a cause of the disease (Ludy and Powelson, 1997, Everaarts 1994). Head rot is favoured by damp, wet conditions which favour bacterial growth and disease development (Ludy and Powelson, 1997).

Brown bead is a physiological disorder that causes many rejects in the region of Quebec. When the heads approach marketable maturity, sign of brown bead is yellowing followed by brown discoloration of the unopened floral buds (Jenni et al. 2001). Compared to hollow stem and soft rot, brown bead is more severe when nitrogen application is low (Jenni et al. 2001).

Nitrogen loss from the plant-soil system

Volatilization

The process where ammonium (NH_4^+) is converted to ammonia (NH_3) is known as volatilization. The ammonia gas is then lost from the plant-soil system and released into the atmosphere. Volatilization is favoured by high temperature in both soil and air, dry weather and high soil pH (>7.0) (Tremblay et al. 2001). Zhang et al. (2004), showed a volatilization loss of 15,1 percent (60 kg N per ha) in a maize-wheat rotation, during almost 1 year, on an alkaline soil (pH = 8,5). Zhou et al. (2006), could only explain a decrease in ammonium in their study by volatilization, since the pH of the soil was above 8.0.

To avoid volatilization, ammonium should be incorporated into moist, cool soil. Ammonia volatilization from crop residues has been reported for surface applied residues. When residues are incorporated immediately, losses have been minimized (De Neve & Hofman, 1998). Roelcke et al. (2002) showed that when fertilizer, in this case ammonium bicarbonate and urea, was uniformly incorporated into the topsoil (0-15 cm) for wheat and banded 10 cm deep for maize the ammonia losses decreased. For all of their treatments the ammonia fluxes ranged from 0,1 to 8,8 percent or 0,1 to 17,6 kg N per ha. Janzen and McGinn (1991) showed that volatilization losses were negligible when green manure was incorporated in the soil. When it was surface applied, a total loss of 3,6 percent of the nitrogen was lost through volatilization during the first 28 days. After re-wetting of the green manure a second flush of volatilization occurred.

Fertilizers containing a high percentage of ammonium should be avoided if conditions favouring volatilization occur. Webb et al. (2004), showed that ammonia emissions represent a very small output from cropping systems and as long as no animal manure is applied, emissions and deposition is in balance.

Denitrification

When soils are subjected to anaerobic conditions, for example bad drainage, some micro organisms are able to extract oxygen from NO_2^- or NO_3^- instead of atmospheric oxygen, O_2. Several factors affect denitrification; drainage, irrigation, precipitation, soil texture, compaction, temperature and fertilization (Tremblay et al. 2001). A review of studies have shown that denitrification is greatest in nitrogen fertilized, irrigated soils (Tremblay et al. 2001). In these studies 10 to 30 % of applied nitrogen have been lost to denitrification.

Significant denitrification generally takes place in the top 10 centimetres of soils and is only expected when more than 60 percent of soil pore space is water filled (Webb et al., 2004; Neeteson & Carton, 2001; De Neve & Hofman, 1998).

Webb et al. (2004) showed denitrification (N_2O) losses between 0,2 and 2,8 percent, overall average of 1,1 kg N per ha per year, of the total nitrogen fertilizer applied. They showed that denitrification was rapid following the re-wetting of dry soils to anaerobic conditions. Zhang et al. (2004), showed a denitrification loss of up to 2,87 percent (4,71 kg N per ha) in a maize-wheat rotation. Tremblay et al. (2001) reported that denitrification is typically between 30 and 40 kg N/ha but could be as high as 100 to 200 kg N/ha under typical vegetable crop field conditions.

Westerman et al. (1994) explained a decrease in the amounts of NO_3^- at depths below the root zone of wheat with an increase in denitrification, since saturated conditions below the root zone is common. Reducing the leaching of nitrogen will also reduce the loss through denitrification, since 2,5 percent of leached N is estimated to also be emitted as N_2O (Webb et al., 2004).

Nitrogen leaching - an environmental hazard

Leaching is one of the main factors of nitrogen loss from the soil. There are many factors affecting the leaching, among the more important are (Bartholomew & Clark, 1965):

- Form, timing and amount of nitrogen application and soil nitrogen
- Amount and timing of precipitation and irrigation
- Movement of water in the soil
- Crop characteristics

Form, timing and amount of nitrogen application and soil nitrogen

Since nitrate, the most common form of plant available nitrogen, is soluble in water its movement in the soil is closely related to that of water. Nitrate can leach below the root zone quickly when large amounts of water are applied either as irrigation or precipitation. This is especially a problem when available soil nitrogen exceeds plant needs, which can happen early in the season when plants are small or there is no crop.

Ammonium on the other hand is less mobile in soils because it attaches to soil particles. This was shown by Zhou et al. (2006), with lower nitrogen losses when ammonium was applied as compared to nitrate fertilizer. They showed that as long as the adsorption capacity of the soil was not overloaded, ammonium adsorbed to the soil colloids. They recorded that the total nitrogen leaching from the soil ranged from 5,7 % to 9,6 % in a clay loam and between 16,2 % and 30,4 % in a sandy loam (the higher number being from the nitrate fertilizer). Webster et al. (1986) recorded a total nitrogen leaching between 34 to 129 kg per ha for a sandy loam and 15 to 73 kg per ha for a clay soil, 97 percent as nitrate. During the next three winters a total of 3 and 1 percent of the fertilizer nitrogen applied the first year was detected in the drainage water in the sand and clay soil respectively. Pare et al. (2006) showed that nitrate accounted for >99 % of the nitrogen leaching in an experiment using ammonium nitrate as fertilizer. Ammonium loss was negligible, most likely because of plant assimilation, rapid nitrification and microbial immobilization in the root zone. Köhler et al. (2006) showed the same result, NH_4^+-N in the soil was always below the detection limit in their study. Webb et al. (2004), showed a 99 percent domination of nitrate in the total leaching. Kowalenko (1989) showed that within 14 days, nitrification of 120 kg NH_4^+-N was essentially completed. High amounts of ammonium in leachates, up to 23 percent of total mineralized N, have been recorded by Heumann et al. (2002) on sandy soils. Riley et al. (2001) showed that ammonium was rapidly converted to nitrate at their experimental sites.

Soils with small particles, for example a clay soil, have more surfaces for the ammonium ions to adsorb to. They can also hold more water than coarser soils, which makes the leaching of nitrate slower. This was shown by Knappe et al., (2002) who observed a decline in nitrogen leaching in soils with higher storage capacity for plant available water. Li et al. (2003) showed in a fertigation experiment with ammonium nitrate that the nitrate accumulated at the boundary of the wetted volume while the ammonium concentration was highest just below the soil surface where the application was done.

Köhler et al. (2006) showed with nitrate-leaching calculations that strongly reduced N-fertilizer applications did not result in substantially lower NO_3^- leaching to the groundwater. Yield reductions of more than 50 percent were however measured. Macdonald et al. (1989) showed that wheat grown without nitrogen fertilizer did not have lower amounts of inorganic nitrogen in the soil at harvest than plots given up to 234 kg nitrogen per ha. This indicates that most of the nitrate at risk of leaching over the winter period comes from mineralization, not from unused fertilizer applied during the season.

Several studies have shown that split application of fertilizer in wheat reduces the risk of nitrogen leaching (Raun et al., 2002; Riley et al., 2001; Alcoz et al., 1993; Ellen and Spiertz, 1980). Riley et al. (2001) showed that adding fertilizer closer to the time of maximum plant demand lowered soil nitrate concentrations substantially and lowered the risk of leaching. Raun et al., 2002, Alcoz et al., 1993 and Ellen and Spiertz (1980) showed that sidedressing wheat resulted in a higher recovery and efficiency of N compared to adding all at planting. Bowen et al. (1999) found no evidence that nitrogen applied at planting was leached below the root zone of broccoli, and concluded that the sidedressing at 2 weeks after transplanting is unnecessary.

Amount and timing of precipitation and irrigation

Excessive irrigation or precipitation is one of the major mechanisms for nitrate loss, especially on an easily drained soil. The depth of the root zone is an important factor affecting the N uptake and the leaching of nitrogen. A plant soil-system is especially prone to loss of nitrogen due to precipitation and irrigation early in the season when the root system is small and uptake is low. A split application is therefore needed to avoid leaching. Zhou et al. (2006), showed that when the amount of irrigation was low, the concentration of nitrate in the 0 to 35 cm soil depth was between 100 and 225 mg NO_3-N per kg soil. When irrigation was high, the concentration was lowered to between 50 and 100 mg per kg soil. Babik and Elkner (2002) came to the same conclusion that the optimum content of soil nitrates was different when irrigation was used. With irrigation the optimum content was between 209 and 297 mg per litre soil while on non-irrigated plots it was 112-204 mg per litre. When using a split application the optimum was even lower, between 88-179 mg per litre, and not affected by irrigation or non-irrigation. Li et al. (2003) found that NO_3^--N tended to accumulate at the periphery of the wetted volume and move downward as the wetting front moved through the profile. Both Zhou et al. (2006) and Li et al. (2003) showed high mobility of NO_3^--N. Macdonald et al. (1997) showed a positive relationship between losses of fertilizer and rainfall during the first 3 weeks following application on winter wheat, oilseed rape, potatoes and sugarbeet.

The movement of water in the soil

Consideration of the water holding capacity of the soil is important to avoid leaching losses. When the amount of nitrogen is less than the crop needs for optimal growth, the evapotranspiration by the crop is lowered allowing more water to pass through the root zone and thereby increasing the leaching of nitrate (Campbell et al., 1993). In an easily drained soil, leaching is a large problem. For example a sandy soil has less particles able to hold water than a clay soil. Simmelsgaard (1998) showed that leaching decreased with increasing amounts of clay in the soil. Leaching of nitrate from soils was 68, 44 and 26 kg per ha in soils containing 5, 12 and 20 % clay, respectively. Van Es et al. (2006) showed that drain water NO_3^--N concentrations were 2.5 times higher on a sandy loam compared to a clay loam. Webster et al. (1986) on the other hand, showed higher nitrate concentrations in early drainage with a clay soil comparing to a sand soil. This was explained by the cracks, which were common on the clay soil allowing the water to drain rapidly through the profile. Campbell et al. (1993) also found that poorly fertilized field, in this case with no phosphorous, leached more nitrate than a field receiving phosphorous. This was explained in the same way as the experiment receiving suboptimal nitrogen, weakened plants having smaller root systems were not able to take up the nitrogen. One study showed that once the evapotranspiration started rising, the probability of nitrate leaching was low (Guillard & Kopp, 2004).

Crop characteristics

There are three sources for nitrogen leaching: residual soil nitrate, nitrogen present in crop residues remaining on the field and from mineralised soil nitrogen. Leaching occurs when precipitation exceeds evapotranspiration, mainly from autumn until spring or under crop absence. Irrigated crops can also pose a problem since irrigation and precipitation often exceeds evapotranspiration even during the summer.

After the crop is harvested, large amounts of residues are left in the field, about 80 percent in broccoli (Riley and Vågen, 2003; Magnifico et al., 1979). Macdonald et al. (1989) showed that the nitrogen in wheat crop residues is the largest source of leaching, not the unused fertilizer applied the previous spring. Crop residues contain organic nitrogen that will be available for the next crop when the residues decompose. These residues will be broken down and the nitrogen will be available in the soil, either to be taken up by a new crop or subjected to leaching. Under normal summer weather conditions, 70 percent of the organic nitrogen can be available within 10 weeks after incorporation (Tremblay et al., 2001). The mineralization of nitrogen from residues can vary greatly with climate conditions. The ideal for fast mineralization is a soil being warm, moist and well aerated. Other factors that affect mineralization are the size and the ratio of carbon to nitrogen of the residues and if they are incorporated in the soil. In a study made by De Neve and Hofman (1998), leaching from broccoli started around 50 days after incorporation of crop residues to the soil. After 125 days, 45 percent of the residue nitrogen was released; this would equal about 100 kg N/ha (total uptake 260kg N/ha (Tremblay et al. 2001)). The amount of above-ground residues in broccoli increased with increasing amount of nitrogen, but not significantly. The amount of nitrogen in the residues was between 100 and 120 kg per ha (Riley and Vågen, 2003). Mean daily temperature during their study was between +16 and -3°C. After cereals and sugar beet, the amount of leaching during the winter has been in the range of 10 to 55 kg N per ha. On the other hand, these two crops usually have low amounts of residual nitrogen, not comparable to most vegetable crops (Neeteson & Carton, 2001). Everaarts et al. (1996) showed that at optimum nitrogen application around 100-120 kg per ha remained in the crop residues of cauliflower and about 50-80 kg per ha in the 0-60 cm layer of the soil. They showed that the amount of nitrogen in the crop residues increased with increasing amounts of application. When harvest is done late in the season, they proposed to leave the crop residues undisturbed, not incorporated, until after the winter to avoid leaching losses.

Diagnosing fertilizer nitrogen requirements

The possibility to optimise nitrogen application, both in quantity and timing, is still a major agricultural problem. A way to help the grower is by using instruments that diagnose nitrogen status of the crop. The ideal diagnosing instrument should be able to detect both a deficiency and an excess of nitrogen (Schröder et al. 2000). It should be easy to handle and able to inform the grower rapidly for the need of supplemental nitrogen. Results obtained by the instrument should be specific for the N status of the crop and the influence from other factors, i.e. cultivar, climatic differences between years etc., should not affect the result (Schröder et al. 2000).

Petiole sap test

Petiole sap test is a rapid test determining sap (actually crushed fresh tissues) nitrate concentration. It is most easily done using Merckoquant test strips (Merck KGaA, Darmstadt, Germany) and a Nitrachek reader (QuoMed Ltd., West Sussex, England). This method for determining sap nitrate concentration is accurate, safe, convenient, simple and cheap (Prasad and Spiers 1984).

Petiole sap test has been used in several crops to establish nitrogen needs (Westerveld et al. 2003 (cabbage, carrot and onion); Studstill et al. 2003 (pumpkin); Delgado and Follet 1998 (wheat and rye); Kubota et al. 1997 (broccoli); MacKerron et al. 1995 (potato); Beverly 1994 (tomato); Gardner and Roth 1989 (broccoli); Prasad and Spiers 1985 (tomato); Prasad and Spiers 1984 (carrot, celery, potato, sweet corn and tomato); Scaife and Stevens 1983 (cabbage)).

Gardner and Roth (1989) showed that a high degree of correlation existed between NO_3^--N concentration of broccoli midribs and yield at various sampling dates. Kubota et al. (1997) also showed that the sap test could be a valuable and a rapid technique to predict N needs of broccoli. They could not see any effect due to irrigation or crop maturity. Delgado and Follett (1998) showed that a sap test could help identify the needs for additional nitrogen fertilizer in wheat and rye. Prasad and Spiers (1984, 1985) showed a strong linear correlation between applied N and sap nitrate-N. Gardner and Roth (1989) showed a lower NO_3^--N concentration in the midribs as the season progressed.

Westerveld et al. (2003) concluded that tissue NO_3^--N concentrations were highly variable and it was difficult to match them to previously published results to indicate a critical level of NO_3^--N. MacKerron et al. (1995) showed a lack of consistent relationship between petiole sap NO_3^--N in potato and uptake of nitrogen. The variability was large between replicates, fertilizer treatments and cultivars. Scaife and Turner (1987) found no relation between sap nitrate concentration measured just before sidedressing and the optimum rates of sidedressed nitrogen in brussels sprout. They concluded that sap tests could not predict the responses to topdressing. Scaife (1988) went as far as to say that the whole approach of plotting yields against nitrogen concentration is wrong and that petiole sap analysis is an unsuitable method.

Chlorophyll concentration – SPAD

The SPAD-502 chlorophyll meter (Spectrum Technologies, Inc, IL, USA) is a hand-held spectrophotometer used to measure the relative greenness of leaves to determine N status in plants. Leaf greenness is closely related to chlorophyll, which is related to leaf N. The SPAD meter measures the difference in light transmitted by the leaf in two wavelength regions, 650 nm (peak chlorophyll absorption) and 940 nm (non chlorophyll absorption). A SPAD value is calculated by the instrument that is proportional to the relative optical density between the two wavelengths.

Villeneuve et al. (2002) showed that the chlorophyll meter was only able to detect severe deficiencies when the lowest rate of nitrogen fertilizer was applied in broccoli. They also showed that the chlorophyll index saturated at high nitrogen rates, something that shows that other factors than nitrogen limit chlorophyll production. Westcott and Wraith (1995) found that leaf SPAD readings showed linear-plateau responses when compared to stem nitrate concentration in peppermint, indicating that SPAD readings do not respond to luxury consumption of N. They concluded that the instrument is therefore promising for the detection of crop N deficiencies by using a well-fertilized plot (saturated plot) as a comparison. Wang et al. (2004) found that SPAD values correlated well with both chlorophyll content and N status of the ornamental plant peace lily. Kantety et al. (1996) showed a linear relationship between SPAD meter readings and tissue N in tall fescue, the results showed that the chlorophyll meter was an easy and efficient method of detecting N status. Wood et al. (1992) showed that the SPAD chlorophyll meter should be as reliable as leaf blade N concentration and petiole NO_3^--N for predicting supplemental N fertilization requirements of cotton. Wood et al. (1992) found that field chlorophyll measurements were highly correlated with tissue N concentration and that the measurements had excellent grain yield prediction capabilities. They concluded that the chlorophyll meter showed promise for utilization of this tool for in-season N recommendations.

Sexton and Carroll (2002) found that SPAD meter readings, in sugarbeet, lagged about two weeks behind the petiole nitrate concentration in being able to show significant differences between N treatments. SPAD meter readings were well correlated with petiole nitrate concentration at concentrations less than 10 000 mg per kg but not well correlated above 10 000 mg per kg. They concluded that SPAD readings are less sensitive measurements of plant N status than sap nitrate concentration. Bullock and Anderson (1998) concluded that the SPAD meter cannot be used to make accurate predictions of how much fertilizer N will be needed by a crop during the future growing season. They showed that the correlation between SPAD readings and N fertilizer rate were low but significant. Hoel and Solhaug (1998) showed that irradiance during measurements affects the results of the Minolta SPAD-502 chlorophyll meter and should be considered when using it for the estimation of crop N-status. Martínez and Guiamet (2004) showed that time of measurement, irradiance and plant water status must be considered when using the SPAD chlorophyll meter. Since the difference in SPAD values is small between N deficient and sufficient plants these factors are important. Himelrick et al. (1993) found inconsistent relationship between SPAD meter readings and N status of strawberry plants. They concluded that it is questionable whether the meter could be used to reliably evaluate N concentration in strawberry plants in the field. Neilsen et al. (1995) found that differences in SPAD readings and leaf N concentration in apple due to cultivar and over time were as great as those due to N treatments. They concluded that in the future, determination of critical SPAD values for apple leaves must be standardized for cultivar and sampling time. Minotti et al. (1994) showed highly significant linear and quadratic trends were obtained for the regression of N rates and SPAD readings on potato. The variety significantly affected SPAD values in several of the experiments. The results show that SPAD readings can often identify severe N deficiency in potatoes but more difficult is to establish marginal deficiencies.

Leaf polyphenolics – Dualex

A rapid way to measure polyphenolics in plant leaves is with an instrument called Dualex (Force-A, Paris, France). It is non-destructive and no preparation of the plant is necessary before measurement. The instrument is based on the transmittance of UV light through the leaf. By measuring the UV absorption at 375 nm, the amount of polyphenols in the epidermis can be established (Goulas et al, 2004). The reference light is used to determine the fluorescence detected on excitation, a light that is not absorbed by the epidermis. The instrument is portable and easy to carry in the field.

The accumulation of phenolics under nutrient stress may have an important role in the adaption of plants to difficult conditions. Hättenschwiler and Vitousek (2000), Jones and Hartley (1999) and Gershenzon (1984) propose several different roles for the build up of phenolics.

- Growth inhibitor. The phenolic compounds have the potential to slow down the plant metabolism. In reducing the growth rate, plants can be able to survive under conditions of low nutrient availability.
- Allelopathy. By producing more phenolic compounds plants can more effectively compete with neighbouring plants for limited nutrient resources.
- Defense system against predators and pathogens. By producing phenolic compounds, plants reduce the damage made by predators and pathogens at a time when the cost of recovery is high.
- Storage. Storing carbon as phenolics is a way for the plant to more effectively protect it against predators.

Chishaki and Horiguchi (1997) suggests that analysing phenolic compounds, provides a good method for diagnosing nutrient disorders prior to visible symptoms. Cartelat et al (2005) showed that both chlorophyll and phenolic content of wheat leaves were highly correlated with nitrogen concentration of leaves. Both chlorophyll and phenolic contents were found to increase along the leaf (base to tip). The phenolic content was higher in the abaxial than in the adaxial side of the leaf. The difference decreased as plants grew, but they were always highly correlated with each other. The linear correlation between chlorophyll and phenolic content shows the sensitivity to changes in leaf nitrogen concentration. They also showed that the total content of phenolics is not very sensitive to water stress, something that also Gershenzon (1984) showed; No clear relationship between water stress and phenolic content has been established. Blodgett and Stanosz (1998) showed the same, no evidence that the concentration of phenolic compounds changed on drought-stressed red pines.

Fast growing species have been shown to be the most sensitive plants to the environment regarding changes in phenolic concentration (Jones & Hartley, 1998). This would mean that these plants should be the easiest to evaluate when diagnosing phenolic compounds.

In a study on two pine and three ericaceous species total phenolic content increased between 20 and 100 percent when fertilizer decreased (Kraus et al, 2004). Király (1964) showed a decrease of phenolic compounds in wheat when nitrogen fertilization increased. In a literature review by McClure (1997) he suggests that as well as nitrogen, a deficiency of boron often leads to a high increase in phenolics. Deficiencies of nitrogen, phosphorus, potassium and sulphur usually result in higher concentrations of phenolic compounds. After adding these nutrients growth is stimulated and the formation of phenolic compounds is supressed (Gershenzon, 1984). Estiarte et al (1994) showed significantly higher content of phenolic compounds in leaf of pepper plants under low nitrogen treatments compared to high treatments. They could observe slightly higher concentrations of phenolic compounds in plants grown under low water treatments (mild stress). The difference was however, not significant.

The amount of phenolic compounds is affected by irradiance. Higher irradiance promotes higher photosynthetic capacity, which leads to higher carbon input. If this exceeds the demand for protein synthesis a stimulation of Phen synthesis is possible. (Meyer et al, 2006) The same result was obtained on plants grown on nutrient deficient soil compared to fertilized controls. The effect of nitrogen was small however, as compared to irradiance.

Saturation index to reduce the influence of factors other than nitrogen

Fertilizer requirements have been shown to vary according to season (Tremblay and Belec, 2006). The use of fixed pre-set fertilizer applications will therefore, in most cases, either over-fertilize or under-fertilize the crop. The use of nitrogen diagnosing instruments to establish N rates during the season is promising but they have shown limitations. Indeed, calibration of the chlorophyll meter to actual N status of crops has shown to be difficult. Variations in chlorophyll values arise due to soil, water supply, growing stage, sampling procedure, cultivar and seasonal effects. The use of absolute values is therefore compromised by factors other than nitrogen that can interfere in the diagnosis. Field trials to establish variety-specific correction factors have been suggested (Olfs et al. 2005; Neilsen et al. 1995). Belec et al. (2001) showed variability in sap nitrate content in broccoli by differences in plant water status. Hartz et al. (1994) suggested correcting these differences in relative moisture content of the petioles. Kubota et al. (1997) however, showed that the moisture content of broccoli petioles stayed nearly constant and that no correction was needed.

An alternative is to establish an overfertilized reference plot that will reduce or eliminate the effects of factors other than N status (Tremblay and Belec, 2006). This solution will decrease the variation of absolute diagnostic values (Tremblay and Belec, 2006). Plots within the field will receive an extra amount of nitrogen fertilizer and work as a standard for comparisons with the rest of the field. A ratio between the two measurements called a nitrogen saturation index (NSI) will be the result from such an approach (Tremblay and Belec, 2006). The higher the difference is between the non N-deficient plants and the rest of the field, the more N is required to complete the growing season.

Several authors have suggested and tested the use of a reference plot as a solution to the variation of absolute values in the diagnosing instruments. Blackmer and Schepers (1995) found that the SPAD chlorophyll meter, together with an overfertilized plot, accurately detected N deficiency that reduced corn grain yields. They concluded that using chlorophyll meter readings without a reference plot resulted in variability from such factors as soil type, growth stage and different hybrids and was of little use. Jemison and Lytle (1996) showed that normalizing chlorophyll measurements against a high-N reference plot in corn were useful at identifying sites that were deficient, but not excessive, in N. It appeared that a saturation index can correct for non-N related variation. Zebarth et al. (2002) showed only a small benefit from using a saturated plot in a study on corn. They recommended the use of leaf chlorophyll index as a preliminary screening tool, to rapidly identify fields that do not require sidedress nitrogen. They concluded however that it lacked the ability to establish rates of fertilizer N to apply to fields. Minotti et al. (1994) found that precision in interpreting the readings are improved when saturated plots are used as reference strips. Even marginal deficiencies are then possible to identify. Denuit et al. (2002) showed that using a relative value based on an over-fertilized plot as reference in winter wheat and potato is not the best one. A comparison with a non-fertilized plot however, is more promising. Hussain et al. (2000) showed a good effect from using well-fertilized reference strips in rice, between 30 and 45 kg less N/ha was needed to produce similar rice yields as pre-set splits.

Aim and Hypothesis

The purpose of this study was to evaluate sap nitrate concentrations, chlorophyll concentrations and leaf polyphenolics as indicators for plant N status. The hypothesis is that the use of N diagnosing instruments leads to N recommendations at sidedressing that limit leaching of nitrate to groundwater, preserve yield quantity, quality and uniformity.

Several aims are formulated for this project:

- Determine the effect of nitrogen rate and irrigation on marketable yield and quality.
- Determine plant uptake and the distribution of nitrogen in the soil during the season.
- Determine the effect of irrigation on nitrogen losses from the plant-soil system.
- Compare sap test, SPAD chlorophyll meter and the Dualex instrument as methods for diagnosing nitrogen requirement in broccoli.
- Determine the effect from irrigation on N diagnosing instruments.
- Determine the relationship between yield and instrument reading.
- Determine how early after fertilization the N diagnosing tests show a good estimation of crop nitrogen status.

Chapter 1: Effect of irrigation and nitrogen treatments on yield, quality, plant nitrogen uptake and soil nitrogen status

Introduction

Broccoli is an important vegetable in the province of Quebec. In 2005, the marketed production was over 26 000 t on a harvested area of 1 959 ha with a total value over 50 million $ (Statistics Canada, 2006).

Broccoli is highly dependent on nitrogen fertilization to achieve a good yield (Babik & Elkner 2002, Belec et al. 2001, Everaarts & de Willigen 1999a, Zebarth et al. 1995, Letey et al. 1983, Tremblay 1989). To insure high yield a common grower behaviour is to over fertilize (Tremblay & Beaudet, 2006). This importance of nitrogen for yield together with its relatively low cost and uncertainties about the fertility of the soil can potentially make this crop an environmental risk. Nitrogen leaching below the root zone can indeed be a problem if high amounts of precipitation and/or irrigation are brought to the crop. This happens particularly when available soil nitrogen exceeds plant uptake, which is often the case early in the season when plants are small. Several studies recommend using split applications to increase yield and avoid nitrogen loss (Ontario Ministry of Agriculture and Food 2006, Vågen 2003, Riley & Vågen 2003, Babik & Elkner 2002, Toivonen et al. 1994,). The recommendation in Quebec is a split application of 85 kg N/ha at transplanting and another 50 kg/ha, 4 to 5 weeks later (CRAAQ 2003).

Most studies have shown that the nitrogen use efficiency (NUE) of broccoli decreased with increasing amount of fertilizer (Tremblay & Beaudet 2006, Riley & Vågen 2003, Eveerarts & de Willigen 1999b, Zebarth et al. 1995, Letey et al. 1983). One study has shown the opposite (Kowalenko & Hall, 1987). Fink and Scharpf (2000) and Greenwood and Draycott (1988), both showed that the recovery rate of fertilizer nitrogen and soil mineral nitrogen was not affected by the amount of nitrogen applied. Splitting N recommendations into several applications is a way of increasing NUE and is considered a good management strategy (Tremblay 2004)

Excessive nitrogen application has been shown to decrease harvest quality. It is especially the incidence of hollow stem that increases with nitrogen rate (Babik & Elkner 2002, Tremblay 1989, Hipp 1974, Cutcliffe 1972) and especially together with irrigation (Babik & Elkner 2002). Another physiological disorder, affected by nitrogen, is bacterial soft rot (Everaarts 1994, Canaday & Wyatt 1992). Everaarts (1994) showed a sixfold increase in unmarketable heads due to head rot when nitrogen fertilizer application increased from 0 to 196 kg N/ha. Split application did not influence head rot incidence. High irrigation frequency seems to have the same negative effect on bacterial soft rot as on hollow stem (Ludy & Powelson 1997).

The status of soil nitrogen is important. Early in the season when the root system and growth is small, there is a high risk that leaching below the root zone will occur. This is especially true for irrigated fields. At harvest, there should be a minimum of nitrogen available in the soil, showing both an effective crop uptake as well as low environmental risk. This is important since broccoli is often ploughed down shortly after harvest and that the uptake of nitrogen will hence be stopped. Residual soil mineral N is often a problem when N rates exceed optimal N rate (Tremblay and Belec 2006). Tremblay and Beaudet (2006) found that NO_3-N levels were relatively low but variable prior to crop establishment in vegetable fields in the province of Quebec. After harvest, NO_3^--N levels were on average 21 kg N/ha higher than prior to planting but some extreme levels were measured which constitute a danger for ground water contamination. Broccoli was one of the crops particularly prone to increase NO_3^--N levels over a growing season. They showed in this survey that broccoli, as well as other crops, was often subjected to applications of N higher than the current recommendations for vegetables (CRAAQ 2003).

The aim of this study was to determine the effect of nitrogen rate and irrigation on marketable yield and quality, as well as plant uptake and the distribution of nitrogen in the soil during the season.

Materials and methods

The field trial was carried out at Agriculture and Agri-Food Canada's L'Acadie research substation (45º17'N, 73º20'W) during the summer and fall 2006. The soil was a clay loam with a pH of 6.3. Preceding crops were beans in 2004 and wheat in 2005.

The cultivar chosen for this project was Arcadia. It has shown good commercial performance in mid-season and late plantings, with good uniformity and is susceptible to hollow stem. The seedlings were grown in plug trays for the first 5 weeks before transplanting.

The transplants were planted in the field on the 7th of July. Phosphorus, potassium and boron fertilizer were broadcast and incorporated in the soil prior to transplanting. The application rates were based on soil analysis and recommendations by the Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ, 2003). Phosphorus was applied as P_2O_5 (0-46-0) at the amount of 165 kg/ha. Potassium was applied as K_2O (50 % 0-0-60 and 50 % 0-0-22-11Mg) at the rate of 160 kg/ha. Two kg boron per hectare was applied. The nitrogen fertilizer used was calcium ammonium nitrate at 27.5 percent N. Nitrogen fertilizer treatments were broadcast at transplanting and sidedressed at 2 and 4.5 weeks. A furrow was made for the sidedressing, 5 cm from the broccoli row and fertilizer was applied before covering. Liquid applications of boron (Solubor) were done twice during the season (6.5 and 8.5 weeks after transplanting). Application of pesticide was done during the season according to need. Azinphos-methyl (Guthion) was applied at transplanting, *Bacillus thuringiensis* (Dipel) was applied at 4.5 and 8.5 weeks with Permethrin (Pounce) applied at 6.5 weeks. To avoid plant stress, three irrigations of all plots, irrigated and non-irrigated, were carried out at transplanting and at 1 and 2 weeks.

The experiment was set up as a split plot design with four blocks. Each block was divided in two main plots, irrigated and non-irrigated. The main plots each had nine 3 by 6 meter subplots with different nitrogen treatments (table 1.1).

Table 1.1: Nitrogen fertilizer applications made during the growing season

Treatment	N applied (kg/ha)				Details
	At transplanting	At 2 weeks	At 4,5 weeks	Total N applied	
T1	0	0	0	0	Control
T2	75	30	0	105	
T3	75	30	30	135	
T4	75	30	60	165	
T5	75	30	90	195	
T6	75	30	120	225	
T7	75	0	30-120*	105-195	*According to sap test result
T8	160	65	0	225	Saturated plot
T9	225	0	0	225	Saturated plot

The treatment T7 is fertilized according to sap test results at 4.5 weeks. The rate is based on a sap petiole test previously calibrated (Bélec & Tremblay, unpublished data) to provide optimal nitrogen rate at sidedressing (table 1.2). The amount of N applied is determined by the relationship between the sap test results from treatment T7 and the saturated plot, T8 or T9. The result from treatment T7 is divided by the results from the saturated plot and the index between them is used to determine the amount of nitrogen to apply. In this experiment, the sap saturation index was 28 %, 120 kg N/ha was therefore applied at 4.5 weeks for treatment T7.

Table 1.2: Nitrogen application for treatment T7 at 4.5 weeks

Sap saturation index (Sap N-NO_3^- for T7/Sap N-NO_3^- for saturated plot) x 100	Nitrogen application kg N/ha
>91 %	30
82 - 90.9	60
73 - 81.9	90
64 - 72.9	120

Rows were spaced 0.75 meter apart and the plants 0.20 meter within the rows. The two outer rows in each subplot were guard rows.

The irrigated main plots were equipped with two pairs of tensiometers, at depths of 15 and 30 centimetres, 2 rain gauges and two lines with 3 sprinklers in each. Irrigation was started when the tensiometers at 15 centimetres, showed a tension between 30 and 40 centibars. The irrigation was split in two shots, separated by a ten minute break. This was done to gradually moisten the soil and avoid runoff of water. Irrigation was done seven times during the season. Each time 25 to 30 mm of water was applied.

Sap nitrate content was determined at 4.5 and 6.5 weeks, by taking five of the last fully developed leaves from each subplot (treatment). Leaf sampling was done before 10:00 h to minimize variations in sap nitrate concentrations due to plant transpiration later in the day (Matthäus & Gysi 2001, Coulombe et al. 1999, Kubota et al. 1997). The leaves were immediately placed in a cooler and transported to the laboratory where analysis was completed on the same day of sampling. The leaf blade was first removed and the midvein and petiole was cut into small pieces. A garlic press was used for crushing the fresh tissues and producing a juice of crushed petiole tissues hereby referred to as sap extract. The sap extract was then diluted to fit into the detection limit of the instrument. Depending on the treatment, the samples were diluted from 1:40 to non-diluted at 4.5 weeks. At 6.5 weeks the dilution was from 1:20 to non-diluted. Analysis was done using Merckoquant® test strips (Merck KGaA, Darmstadt, Germany) and a Nitrachek® 404 reflectometer (QuoMed Ltd., West Sussex, England).

One core of soil from each plot was taken in the middle of the row to a depth of 60 or 90 centimetres. This was done before transplanting and at one and seven weeks after harvest. During the growing season two soil cores from each plot were sampled to a depth of 60 centimetres, at 2, 4.5 and 6.5 weeks after transplanting. The deeper samplings were divided into increments of 0 to 30, 30 to 60 and 60 to 90 centimetres while the others were divided into 0 to 30 and 30 to 60 centimetres. Soil samples were stored in a refrigerator at 4 ºC until the next day when extraction was performed. From each sample, 13 g of the soil was extracted during agitation using a 2 molar potassium chloride for 1 hour. The $N\text{-}NO_3$ content in the samples was determined using a TRAACS Autoanalyzer (TRAACS 800 Autoanalyzer, Bran-Luebbe).

The harvest of the broccoli heads started at commercial maturity. The first harvest was done in the middle of September and continued for two weeks. An average of five to six harvests was performed, depending on fertilization treatment. The harvest parameters determined on marketable heads were: the fresh weight of heads cut to 20 centimetres long, the head diameter and the incidence of hollow stem. Non-marketable plants were examined for pathological and physiological disorders such as soft rot and brown bead. 12 days after the last harvest, the plant residue was incorporated into the soil with a rotary cultivator.

The total nitrogen uptake was determined at harvest by collecting five plants from each subplot. During the season, at 4.5 and 6.5 weeks, 6 plants were collected from three different treatments, T1, T4 and T7. The plant was cut at ground level and weighed before being cut into pieces using a grinding machine. A sample was taken for drying and later analysed for nitrate-nitrogen.
Nitrogen uptake efficiency (NUE) was determined by the ratio of nitrogen in the crop at harvest compared to nitrogen applied by subtracting the uptake made by the control plot (T1).

Meteorological data, minimum and maximum daily temperatures and precipitation (July to November), was obtained from the weather station located at the L'Acadie experimental farm (figure 1.1).

Analysis of variance for a split-plot design was performed using SAS software (SAS Institute, Cary, NC) with orthogonal polynomial contrasts to evaluate the effects of nitrogen treatments (T1-T6), irrigation and interactions on the dependent variable. Linear, quadratic and cubic orders were used. Pairwise comparisons were performed between groups using contrasts. Regression analyses were performed with linear and polynomial (second and third order) terms.

The soil sampling at seven weeks after harvest showed several samples below the detection limit (<4 kg/ha) of the method. Random numbers between 0 and 4 were therefore generated for those subplots to allow for further statistical analysis. Regression analysis was used to calculate best fit functions between marketable yield and plant uptake of nitrogen.

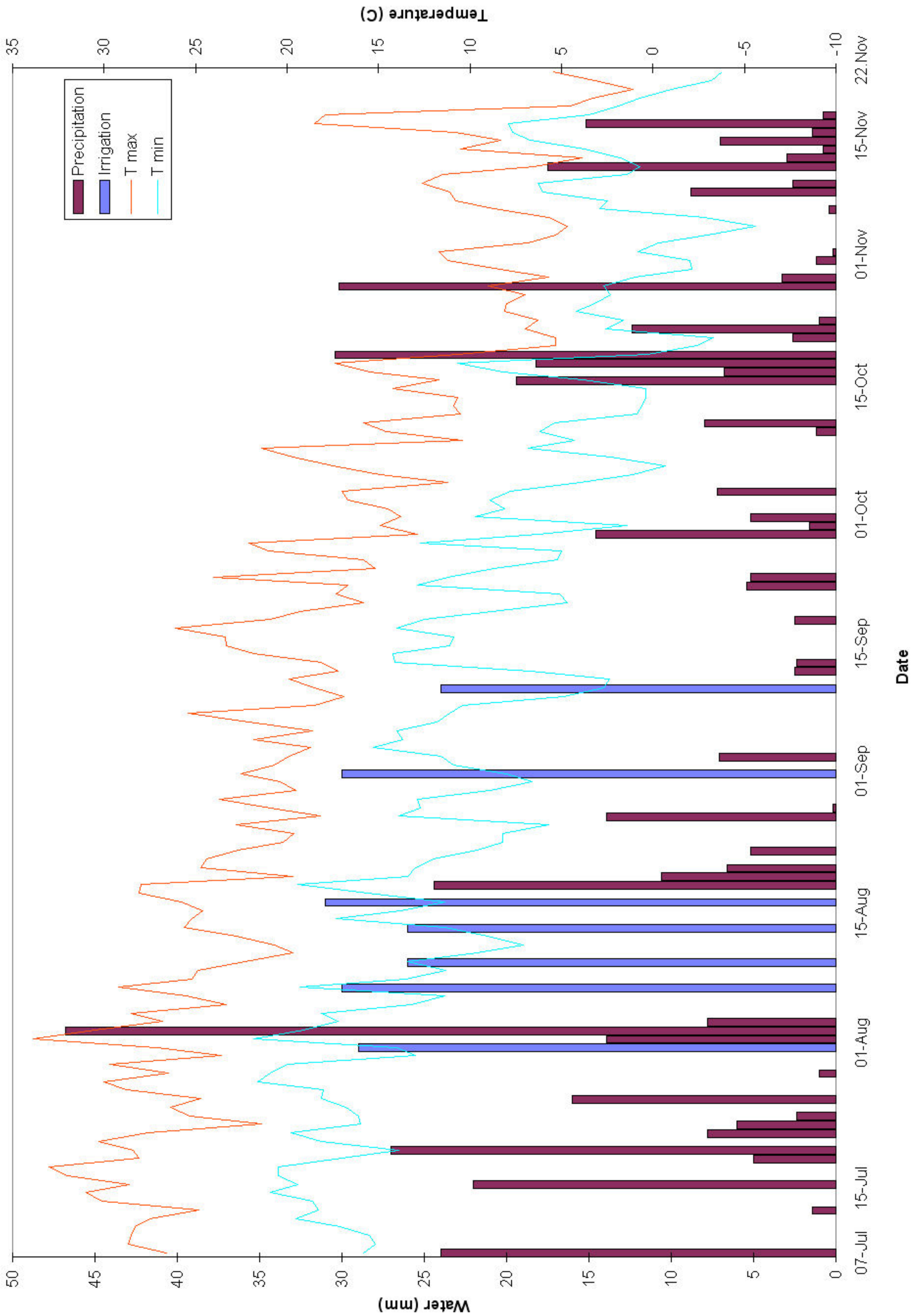

Figure 1.1: Meteorological data obtained from the weather station at L'Acadie research substation.

Results and discussion

Marketable yield and quality

Nitrogen had a curvilinear effect on marketable yield (table 1.3). Increasing the amount of nitrogen did have an effect on the yield up to 165 kg N/ha from where the response plateaued in agreement with Riley and Vågen (2003) and Bracey et al. (1995). There was no difference in yield between irrigated and non-irrigated plots. The lower mean marketable yield for irrigated plots presented in table 1.3 was mostly an effect of poor drainage in one of the irrigated plots.

Split application did not affect yield compared to a single application. Treatment T6 (75+30+120 = 225 kg N/ha) did not produce a higher yield than T9 (225+0+0 = 225 kg N/ha) ($P \leq 0.137$). Earlier studies have shown either a positive response to split application (Riley and Vågen 2003) and no, or a negative effect (Everaarts and de Willigen 1999a). The single application (T9) was the only treatment yielding lower when irrigated compared to non-irrigated ($P \leq 0.001$). This was in agreement with the results shown by Babik and Elkner (2002), that a split application was preferable when irrigation was used.

Head diameter was affected by nitrogen rate (table 1.3). Like the yield, the increase of nitrogen above 165 kg N/ha did not affect head size. The average head diameter was slightly greater for non-irrigated plants than for irrigated. There was a significant interaction (N lin x irr) on head diameter, the difference between irrigated and non-irrigated plants was larger when N applications was lower. Both saturated plots, T8 and T9, showed smaller head diameter when irrigated as compared to non-irrigated (table 1.4). Treatment T7 showed that a split application was preferable when irrigation was used, having significantly larger heads even with 30 kg N/ha less.

The incidence of hollow stem increased with increasing nitrogen application (table 1.3). Both the linear and quadratic components were significant. The number of heads affected was however, very low compared to other studies (Belec et al. 2001, Hipp 1974, Cutcliffe 1975, Cutcliffe 1972).

Broccoli heads with hollow stem were found almost only for the first three harvests. Ninety four percent of the plants with hollow stem were harvested during this period (1st harvest: 58 %, 2nd: 23 % and 3rd:13 %). This suggests that the plants with a rapid growth rate are more likely to have hollow stem, in agreement with Hipp (1974) and Scaife and Wurr (1990). Belec et al. (2001) and Tremblay (1989) showed a significant environmental effect on the incidence of hollow stem. This can explain the low amount of hollow stem in this study. Since harvest was relatively late (second half of September) the environmental factors (temperature, daylength etc.) may not have favoured rapid growth during head set as in the other studies.

Hollow stem was only noticed in treatments receiving more than 165 kg N/ha (T4 to T9, table 1.3 and 1.4). No difference in severity was however seen between the treatments, T4 to T9 This would be against the hypothesis that high growth rate during the initiation of the central inflorescence is the main reason for hollow stem (Tremblay 1989, Hipp 1974). While at mid season it was still relatively easy to see the difference in size between the larger plants in the saturated plots (T8 and T9) and the others (T1-T7), this was not the case at harvest. This means that the growth rate during the second half of the season must have been greater, especially for treatments with high nitrogen application at 4.5 weeks (T4-T7, 60-120 kg N/ha). Interesting to note is that high nitrogen application at 4.5 weeks, which favoured rapid growth, did not induce more problems with hollow stem. No difference between treatments T4 to T7 (irrigated and non-irrigated confounded) were seen in regards to hollow stem. Hollow stem was significantly lower when comparing treatment T3 with T4 to T7 but that was also the case with the yield.

Bacterial soft rot was hardly observed at harvest. No treatment had more than 2 percent of the broccoli heads affected (data not shown). Everaarts (1994) suggested that the higher dry matter percentage of the heads from low nitrogen treatments resulted in tougher tissue, making it less susceptible to bacterial attack. He also suggested that lower N rates might have resulted in lower tissue protein concentration, which would have limited the growth rate of the bacteria. Coulombe et al. (1999) observed a high incidence of head rot at higher N rates. No effect of either nitrogen or irrigation treatments on bacterial soft rot was noticed during this project.
Broccoli heads showing signs of brown bead was not observed.

Nitrogen fertilization affected the earliness of harvest and both the linear and quadratic components were significant (table 1.3). Hipp (1974), Canaday & Wyatt (1992) and Babik & Elkner (2002) also observed this. This difference (< 4 days) was small however, compared to Babik and Elkner (2002) who showed a difference of up to 10 to 12 days when comparing different amounts of nitrogen fertilizer.

Table 1.3: Effect of nitrogen treatments (T1 to T6) and irrigation on yield, head diameter, hollow stem and maturity rate.

*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.

ns = non-significant

Irrigation	Nitrogen (kg N/ha)	Yield (t/ha)	Diameter (cm)	Hollow stem (%)	Days to 50 % yield
Irrigated	T1: 0 (0+0+0)[1]	2.3	7.6	0.0	76
	T2: 105 (75+30+0)	9.2	8.8	0.0	71
	T3: 135 (75+30+30)	10.9	9.3	0.0	71
	T4: 165 (75+30+60)	13.2	10.5	4.3	70
	T5: 195 (75+30+90)	13.4	10.5	3.7	72
	T6: 225 (75+30+120)	13.5	10.5	2.8	72
Non-irrigated	T1: 0 (0+0+0)[1]	1.8	8.6	0.0	75
	T2: 105 (75+30+0)	10.2	9.4	0.0	74
	T3: 135 (75+30+30)	12.6	10.1	0.0	73
	T4: 165 (75+30+60)	13.5	10.7	0.6	73
	T5: 195 (75+30+90)	14.3	10.8	0.6	73
	T6: 225 (75+30+120)	14.7	10.9	3.8	72
Anova	Irrigation	ns	ns	ns	ns
	Nitrogen	***	***	**	*
	Irr x N	ns	**	ns	ns
Polynomial contrasts	N linear	***	***	***	**
	N quadratic	***	ns	*	*
	N cubic	ns	***	ns	ns
	N lin x irr	ns	***	ns	ns
	N qua x irr	ns	ns	ns	*
	N cub x irr	ns	ns	*	ns

[1] Between brackets are the nitrogen applications at transplanting+2 weeks+4.5 weeks

Table 1.4: Effect of nitrogen treatments (T7 to T9) and irrigation on yield, head diameter, hollow stem and maturity rate.

*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.

ns = non-significant

Irrigation	Nitrogen (kg N/ha)	Yield (t/ha)	Diameter (cm)	Hollow stem (%)	Days to 50 % yield
Irrigated	T7: 195 (75+0+120)[1]	14.0	10.6	2.1	73
	T8: 225 (160+65+0)	12.9	10.1	5.0	70
	T9: 225 (225+0+0)	12.1	10.1	6.2	69
Non-irrigated	T7: 195 (75+0+120)[1]	14.8	10.7	2.2	75
	T8: 225 (160+65+0)	14.0	10.5	1.5	73
	T9: 225 (225+0+0)	14.6	10.6	3.1	72
Anova	Irr x N	ns	**	ns	ns
Pairwise Comparisons	T7 - T8	ns	-	ns	*
	T7 - T9	ns	-	ns	**
	T8 - T9	ns	-	ns	ns
	T7irr - T7non-irr	-	ns	-	-
	T8irr - T8non-irr	-	*	-	-
	T9irr - T9non-irr	-	*	-	-
	T7irr - T8irr	-	*	-	-
	T7irr - T9irr	-	*	-	-
	T8irr - T9irr	-	ns	-	-
	T7non-irr - T8non-irr	-	ns	-	-
	T7non-irr - T9non-irr	-	ns	-	-
	T8non-irr - T9non-irr	-	ns	-	-

[1] Between brackets are the nitrogen applications at transplanting+2 weeks+4.5 weeks

Plant uptake of nitrogen

The effect of nitrogen treatments on plant uptake is shown in table 1.5. There was a linear increase in uptake with higher amounts of nitrogen at all sampling dates. The difference in uptake between irrigated and non-irrigated plots did not show until harvest. At the two first measurements, at 4.5 (irrigated twice of a total of 7) and 6.5 weeks (irrigated 5 times), the difference was small. The increase in plant uptake with increasing nitrogen rate was greater for non-irrigated plants than for irrigated ones at harvest, as shown by the linear N x irrigation component.

As shown earlier, T9 was the only treatment with significantly lower yield when irrigated. This was also the case with plant N uptake which was significantly lower for irrigated plots ($P \leq 0.0001$; table 1.6).

The ratio of nitrogen in the crop at harvest to nitrogen applied is presented in table 1.5 and 1.6. The nitrogen uptake efficiency (NUE) was consistently higher for the non-irrigated plots. Both irrigation and the interaction between irrigation and nitrogen affected NUE significantly. Irrigation seems to have aided the control plot (T1: 0 kg N/ha) to uptake nitrogen from the soil. At 4.5 weeks, while the crop had been irrigated twice, the difference was 5 kg N/ha between irrigated and non-irrigated control plots (table 1.5). At 6.5 weeks (irrigated 5 times) the difference increased to 10 kg N/ha. At harvest (irrigated 7 times) there were 12 kg N/ha more in irrigated than in non-irrigated plots. For all other treatments receiving nitrogen the pattern was the opposite, with less nitrogen taken up for irrigated plots. There was no difference in N uptake between irrigation treatments at 4.5 and 6.5 weeks but at harvest the difference was significant.

There was a similar pattern of increasing NUE with increasing nitrogen application between the two irrigation treatments, but only up to a point where increase in nitrogen application decreased NUE. The highest NUE was achieved with treatment T4: 165 kg N/ha for irrigated plots and T3: 135 kg N/ha for non-irrigated. This is different than other studies which show a consistent decline in uptake efficiency when nitrogen rates increased (Letey et al. 1983, Greenwood et al. 1989). Kowalenko and Hall (1987) however, showed an increase in uptake with increasing nitrogen application. A possible explanation of the results in this study is that the low nitrogen rates affected growth and nitrogen uptake negatively, while the high rates suffered losses through the root zone before all nitrogen was taken up.

Treatment T7 showed a higher NUE than both T8 and T9 in both irrigated and non-irrigated plots (table 1.6). This would mean that a split application of nitrogen is important for a higher NUE. When comparing treatment T6 with T8 and T9 however, no difference was observed in NUE, for neither irrigated nor non-irrigated plants (data not shown). A possible explanation to the higher NUE in treatment T7 is less root disturbance, since there was no sidedressing at 2 weeks for T7. A larger root system, and possibly a stronger plant, could have contributed to a better nitrogen uptake at the sidedressing at 4.5 weeks.

Table 1.5: Effect of nitrogen treatments (T1 to T7) and irrigation on plant uptake and nitrogen uptake efficiency measured at 4.5 and 6.5 weeks and at harvest.

*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.

ns = non-significant

Irrigation	Nitrogen (kg/ha)	Plant N at 4.5 weeks (kg/ha)	Plant N at 6.5 weeks (kg/ha)	Plant N, excluding heads, at harvest (kg/ha)	N in heads at harvest (kg N/ha)	Nitrogen upptake efficiency (NUE) (%)
Irrigated	T1: 0 (0+0+0)[1]	23	39	52	10	-
	T7: 75 (75+0+120)	55	-	see table 1.6	see table 1.6	see table 1.6
	T2: 105 (75+30+0)	57	-	98	31	66
	T3: 135 (75+30+30)	-	-	89	38	50
	T4: 165 (75+30+60)	-	127	138	48	77
	T5: 195 (75+30+90)	-	-	145	52	71
	T6: 225 (75+30+120)	-	-	149	52	63
Non-irrigated	T1: 0 (0+0+0)[1]	18	29	44	6	-
	T7: 75 (75+0+120)	50	-	see table 1.6	see table 1.6	see table 1.6
	T2: 105 (75+30+0)	59	-	120	38	104
	T3: 135 (75+30+30)	-	-	160	48	118
	T4: 165 (75+30+60)	-	123	179	54	112
	T5: 195 (75+30+90)	-	-	187	60	102
	T6: 225 (75+30+120)	-	-	203	59	95
Anova	Irrigation	ns	ns	*	ns	*
	Nitrogen	***	***	***	***	ns
	Irr x N	ns	ns	**	ns	*
Polynomial contrasts	N linear	***	***	***	***	ns
	N quadratic	ns	-	ns	**	ns
	N cubic	-	-	ns	*	ns
	N lin x irr	ns	ns	***	ns	ns
	N qua x irr	ns	-	ns	ns	ns
	N cub x irr	-	-	ns	ns	*

[1] Between brackets are the nitrogen applications at transplanting+2 weeks+4.5 weeks

Table 1.6: Effect of nitrogen treatments (T7 to T9) and irrigation on plant uptake and nitrogen uptake efficiency measured at harvest.

*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.

ns = non-significant

Irrigation	Treatment (kg/ha)	Plant N excluding heads (kg/ha)	N in heads (kg/ha)	Nitrogen upptake efficiency (NUE) (%)
Irrigated	T7: 195 (75+0+120)[1]	170	54	84
	T8: 225 (160+65+0)	128	53	54
	T9: 225 (225+0+0)	136	45	54
Non-irrigated	T7: 195 (75+0+120)[1]	223	63	121
	T8: 225 (160+65+0)	158	59	75
	T9: 225 (225+0+0)	200	63	95
Anova	Irr x N	**	ns	*
Pairwise Comparisons	T7 - T8	-	ns	-
	T7 - T9	-	ns	-
	T8 - T9	-	ns	-
	T7irr - T7non-irr	**	-	*
	T8irr - T8non-irr	ns	-	ns
	T9irr - T9non-irr	**	-	**
	T7irr - T8irr	*	-	**
	T7irr - T9irr	ns	-	**
	T8irr - T9irr	ns	-	ns
	T7non-irr - T8non-irr	***	-	***
	T7non-irr - T9non-irr	ns	-	*
	T8non-irr - T9non-irr	*	-	ns

[1] Between brackets are the nitrogen applications at transplanting+2 weeks+4.5 weeks

Marketable yield was highly correlated with % N on a dry matter basis, for both irrigated and non-irrigated plants (figure 1.2). For a given nitrogen dry matter concentration, irrigated plants generally had a higher marketable yield.

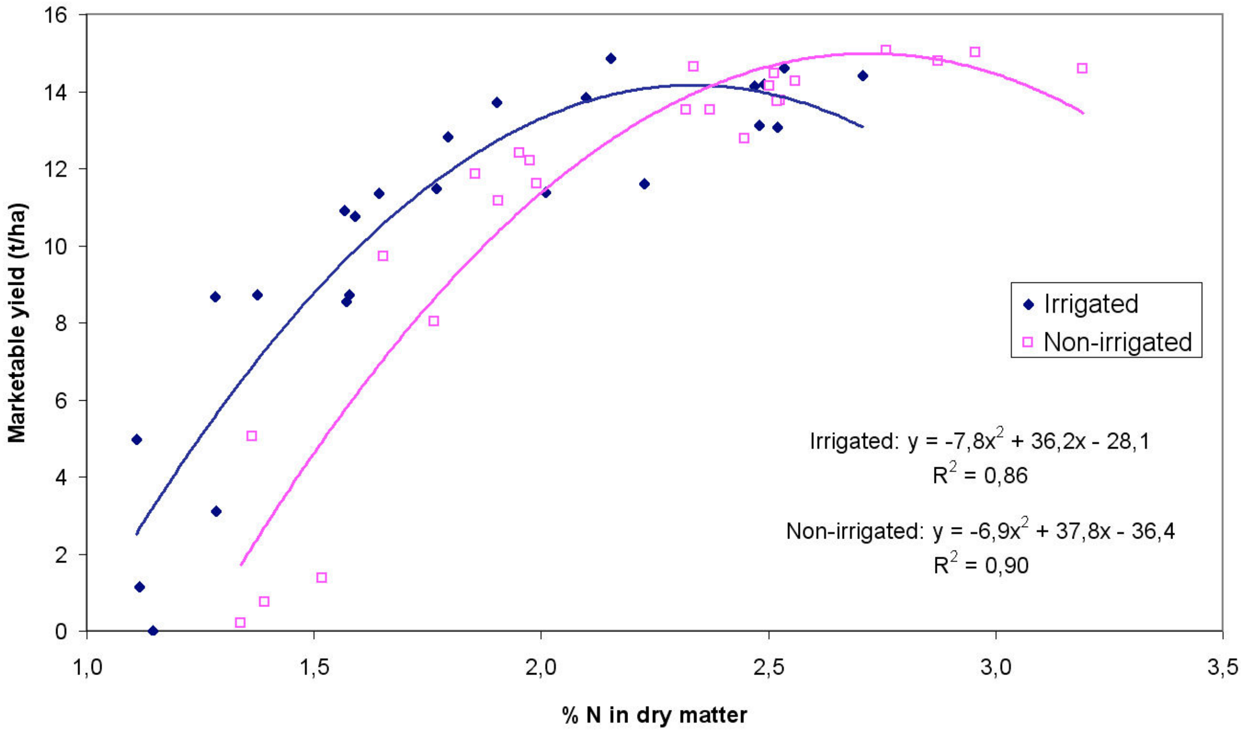

Figure 1.2: Relationship between % N in dry matter of broccoli plants and marketable yield.

Nitrate-nitrogen in the soil

Nitrate-nitrogen (NO_3-N) in the soil at 2 and 4.5 weeks was related to fertilizer rate (table 1.7). At 2 weeks, the plants showed hardly any uptake of nitrogen during the early stages of growth, as seen by the comparison of treatments T1 and T7 in table 1.7. This is in agreement with the results obtained by Bowen et al. (1999) suggesting that the increases in soil inorganic N reflects net mineralization. There is a large amount of nitrate still available in the top layer. When comparing the two nitrogen rates at 2 weeks it seems that some of the nitrogen has leached to the lower soil layer (30-60 cm). This nitrogen may be difficult for the plant to take up if it leaches further below the main broccoli root zone (60 cm) (Tremblay et al., 2001). The presence of higher NO_3-N in the 30-60 cm layer originates from the displacement of a part of the broadcast application of nitrogen at transplanting. The precipitation during July was 98 mm below the average for the month of July 2000-2005: 106 mm. The whole field was however irrigated three times, to avoid plant stress, before soil sampling at 2 weeks. With the irrigation that applied around 25 mm each time, leaching has probably occurred. The small root system of the young plants at the beginning of the season was apparently not capable of recovering all the NO_3-N present in the top soil layer. It follows that the risk of leaching early in the season is critical, especially if heavy rain occurs or irrigation is applied above field capacity. Nitrate-nitrogen was observed to deplete rapidly at the measurement at 4.5 weeks, especially in the top layer (0-30 cm), in agreement with Bowen et al. (1999). It seems that at 4.5 weeks the root system is able to take up NO_3-N more efficiently from the soil.

At 6.5 weeks and at harvest, no difference in soil nitrogen was detected (table 1.8). The analysis at harvest even showed nitrate-nitrogen content below the detection limit of the method (<4 kg NO_3-N ha^{-1}) (table 1.8 and 1.9). This was surprisingly low as compared to other studies on broccoli. Letey et al. (1983) found soil nitrate in the top 120 cm to be 36, 72 and 64 kg NO_3-N ha^{-1} for nitrogen treatments 90, 180 and 270 kg N ha^{-1} respectively. Zebarth et al. (1995) found less than 15 kg NO_3-N ha^{-1}, in the top 60 cm, for N rates of 0 and 125 kg N ha^{-1}. At an application rate of 250 kg N ha^{-1} they showed a soil nitrate content between 50 and 100 kg NO_3-N ha^{-1}. Similar results were shown by Everaarts and de Willigen (1999a) and Everaarts and de Willigen (1999b), with nitrate-nitrogen content between 12 and 34 kg NO_3-N ha^{-1} in the top 60 cm. Their results showed however, a large variation among experimental years. Tremblay and Beaudet

(2006) found an increase in the top layer of the soil (0-30 cm) between before planting and after harvest of 21 kg NO_3-N ha^{-1} for vegetable fields in the province of Quebec. This was the opposite to the results of this study which showed a decrease in the top layer of at least 22 kg NO_3-N ha^{-1}.

The difference in plant uptake of nitrogen at harvest between irrigated and non-irrigated plants were not reflected in the soil nitrate content (table 1.5 and 1.6 as compared to table 1.8 and 1.9). Even if the difference between plant uptake was over 50 kg N/ha (25 % lower) between irrigated and non-irrigated plots, no difference was observed in the soil samples. A part of the difference can possibly be explained by leaching but this would mean that almost all nitrogen, not taken up in the irrigated plots, was leached past 90 cm before harvest, which seems unlikely. Letey et al. (1983) conducted a study on broccoli with tensiometers and measured NO_3 concentrations toward the lower part of the profile. They concluded that little if any movement beyond the 120 cm depth had occurred. Other possible explanations to the loss of nitrogen are volatilisation or denitrification. Volatilisation is favoured by dry soils (Zhou et al. 2006) and this was not experienced in our study. Losses by denitrification are more likely since they are favoured by oxygen-deprived soils, which is often the case when irrigation is used (Tremblay et al. 2001). In irrigated soils, 10 to 30 % of applied mineral nitrogen is subject to denitrification and studies on vegetable fields in Europe showed a typical denitrification between 30 and 40 kg N/ha (Tremblay et al. 2001).

The relative content of soil nitrate is shown in figure 1.3 to 1.5. At transplanting and at 2 weeks, between 60 and 70 % of the measured soil nitrate was located in the top soil layer (0-30 cm, figure 1.3). At 4.5 weeks, this proportion decreased to about 50 %. This is probably due to a higher uptake of nitrate in the top soil layer compared to the 30 to 60 cm layer and leaching to lower soil layers. At 6.5 weeks the difference among treatments were variable. Between 40 and 75 % of the nitrate was in the top layer (figure 1.4). Interesting to note is the higher content of nitrate in the lower soil layer (30-60 cm) for irrigated plots. The pattern was the same for all treatments receiving nitrogen, T3-T5 (total of 135 to 195 kg N/ha). It seems like irrigation has affected the displacement of nitrogen to the lower soil layer. At harvest, no difference between layers was observed (table 1.8). Seven weeks after harvest, the mineralization of plant nitrogen content was becoming apparent in the soil samples (table 1.8). The linear component is significant for all three depths, 0-30, 30-60 and 60-90 cm but the amount can still be considered low. Low temperatures during October (mean temperature: +7.5°C) and November 2006 (+4.9°C) (figure 1.1) likely reduced mineralization (Teiz & Zeiger 2004). A higher nitrate content for non-irrigated plots was expected in the topsoil layer, due to their higher plant uptake. This was the case for T2, T3 and T6 (total of 105, 135 and 225 kg N/ha respectively) while treatment T4 and T5 showed no difference between irrigated and non-irrigated (figure 1.5).

Table 1.7: Effect of nitrogen treatments (T1 - T6) and irrigation on soil nitrate at different depths (0-30 cm and 30-60 cm) from planting to 4.5 weeks.
*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.
ns = non-significant

Irrigation	Nitrogen (kg/ha)	Soil NO_3-N at planting (kg/ha)		Soil NO_3-N at 2 weeks (kg/ha)		Soil NO_3-N at 4.5 weeks (kg/ha)	
		0-30	30-60	0-30	30-60	0-30	30-60
Irrigated	T1: 0 (0+0+0)[1]	-	-	-	-	12	12
	T7: 75 (75+0+120)	-	-	-	-	22	22
	T2: 105 (75+30+0)	-	-	-	-	20	26
	T3: 135 (75+30+30)	-	-	-	-	-	-
	T4: 165 (75+30+60)	-	-	-	-	-	-
	T5: 195 (75+30+90)	-	-	-	-	-	-
	T6: 225 (75+30+120)	-	-	-	-	-	-
Non-irrigated	T1: 0 (0+0+0)[1]	26	16	48	29	12	15
	T7: 75 (75+0+120)	-	-	139	59	21	25
	T2: 105 (75+30+0)	-	-	-	-	21	24
	T3: 135 (75+30+30)	-	-	-	-	-	-
	T4: 165 (75+30+60)	-	-	-	-	-	-
	T5: 195 (75+30+90)	-	-	-	-	-	-
	T6: 225 (75+30+120)	-	-	-	-	-	-
Anova results	Irrigation	-	-	-	-	ns	ns
	Nitrogen	-	-	***	**	*	*
	Irr x N	-	-	-	-	ns	ns
Polynomial contrasts	N linear	-	-	-	-	*	**
	N quadratic	-	-	-	-	ns	ns
	N cubic	-	-	-	-	-	-
	N lin x irr	-	-	-	-	ns	ns
	N qua x irr	-	-	-	-	ns	ns

[1] Between brackets are the nitrogen applications at transplanting+2 weeks+4.5 weeks

Table 1.8: Effect of nitrogen treatments (T1 - T6) and irrigation on soil nitrate at different depths (0-30 cm, 30-60 cm and 60-90 cm) from 6.5 weeks after transplanting to 7 weeks after harvest.
*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.
ns = non-significant

Irrigation	Nitrogen (kg/ha)	Soil NO_3-N at 6.5 weeks (kg/ha)		Soil NO_3-N at harvest (kg/ha)			Soil NO_3-N 7 weeks after harvest (kg/ha)		
		0-30	30-60	0-30	30-60	60-90	0-30	30-60	60-90
Irrigated	T1: 0 (0+0+0)[1]	4	7	<4	<4	<4	6	2	1
	T7: 75 (75+0+120)	-	-	See table 1.9			See table 1.9		
	T2: 105 (75+30+0)	-	-	<4	<4	<4	4	3	4
	T3: 135 (75+30+30)	10	16	<4	<4	<4	2	1	2
	T4: 165 (75+30+60)	4	9	<4	<4	<4	13	6	3
	T5: 195 (75+30+90)	19	15	<4	<4	<4	8	4	4
	T6: 225 (75+30+120)	-	-	<4	<4	<4	6	4	3
Non-irrigated	T1: 0 (0+0+0)[1]	4	6	<4	<4	<4	5	3	2
	T7: 75 (75+0+120)	-	-	See table 1.9			See table 1.9		
	T2: 105 (75+30+0)	-	-	<4	<4	<4	7	3	2
	T3: 135 (75+30+30)	16	14	<4	<4	<4	9	5	2
	T4: 165 (75+30+60)	11	12	<4	<4	<4	12	5	3
	T5: 195 (75+30+90)	42	14	<4	<4	<4	14	9	4
	T6: 225 (75+30+120)	-	-	<4	<4	<4	18	8	3
Anova results	Irrigation	ns	ns	ns	ns	ns	ns	ns	ns
	Nitrogen	ns	ns	ns	ns	ns	ns	ns	ns
	Irr x N	ns	ns	ns	ns	ns	ns	ns	ns
Polynomial contrasts	N linear	ns	*	ns	ns	ns	**	**	*
	N quadratic	ns	ns	ns	ns	ns	ns	ns	ns
	N cubic	ns	ns	ns	ns	ns	ns	ns	ns
	N lin x irr	ns	ns	ns	ns	ns	ns	ns	ns
	N qua x irr	ns	ns	ns	ns	ns	ns	ns	ns

[1] Between brackets are the nitrogen applications at transplanting+2 weeks+4.5 weeks

Table 1.9: Effect of nitrogen treatments (T7 – T9) and irrigation on soil nitrate at different depths (0-30 cm, 30-60 cm and 60-90 cm) at harvest and 7 weeks after harvest.

*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.

ns = non-significant

Irrigation	Treatment	Soil NO_3-N at harvest (kg/ha)			Soil NO_3-N 7 weeks after harvest (kg/ha)		
		0-30	30-60	60-90	0-30	30-60	60-90
Irrigated	T7: 195 (75+0+120)[1]	<4	<4	<4	13	4	2
	T8: 225 (160+65+0)	<4	<4	<4	10	3	3
	T9: 225 (225+0+0)	<4	<4	<4	4	3	2
Non-irrigated	T7: 195 (75+0+120)[1]	<4	<4	<4	12	4	3
	T8: 225 (160+65+0)	<4	<4	<4	5	2	2
	T9: 225 (225+0+0)	<4	<4	<4	7	3	2
Anova	Irr x N	ns	ns	ns	ns	ns	ns
Pairwise Comparisons	T7 - T8	ns	ns	ns	*	ns	ns
	T7 - T9	ns	ns	ns	**	ns	ns
	T8 - T9	ns	ns	ns	ns	ns	ns

[1] Between brackets are the nitrogen applications at transplanting+2 weeks+4.5 weeks

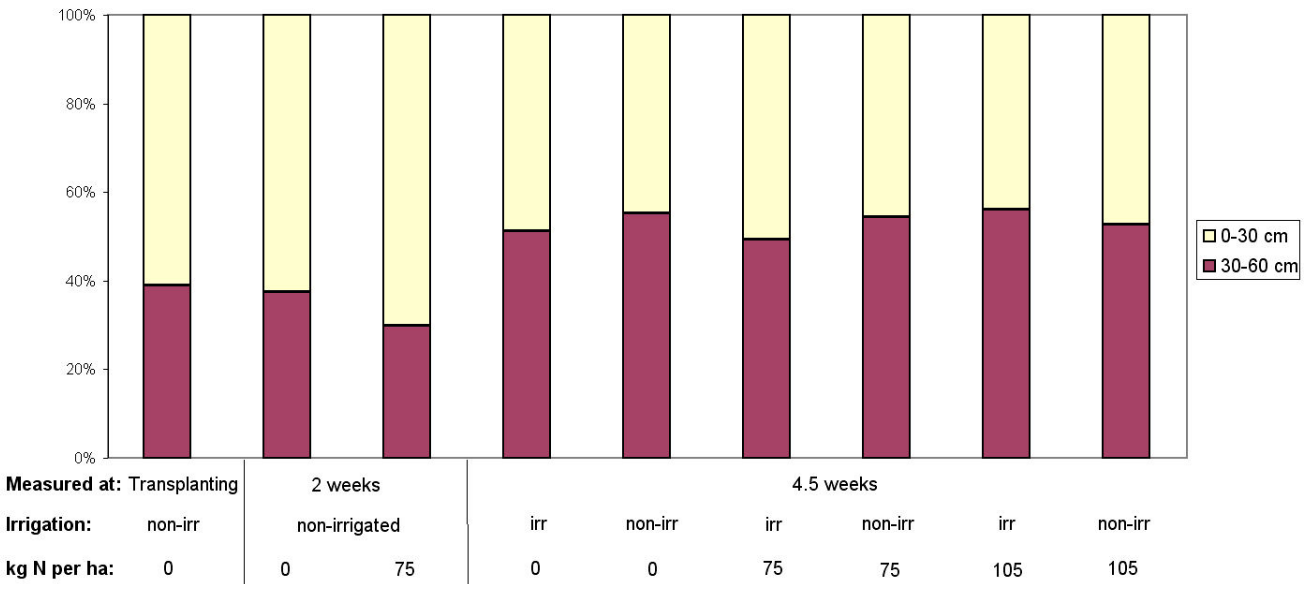

Figure 1.3: Relative contents of nitrate in the soil layers 0-30 and 30-60 cm measured at transplanting, 2 weeks and 4.5 weeks.

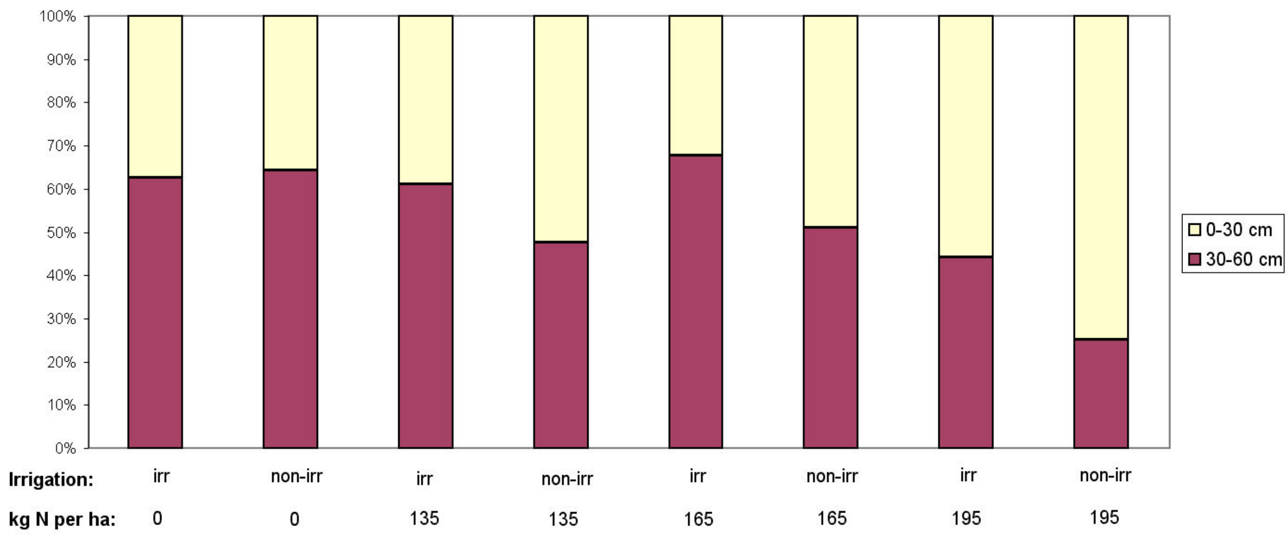

Figure 1.4: Relative contents of nitrate in the soil layers 0-30 and 30-60 cm measured at 6.5 weeks.

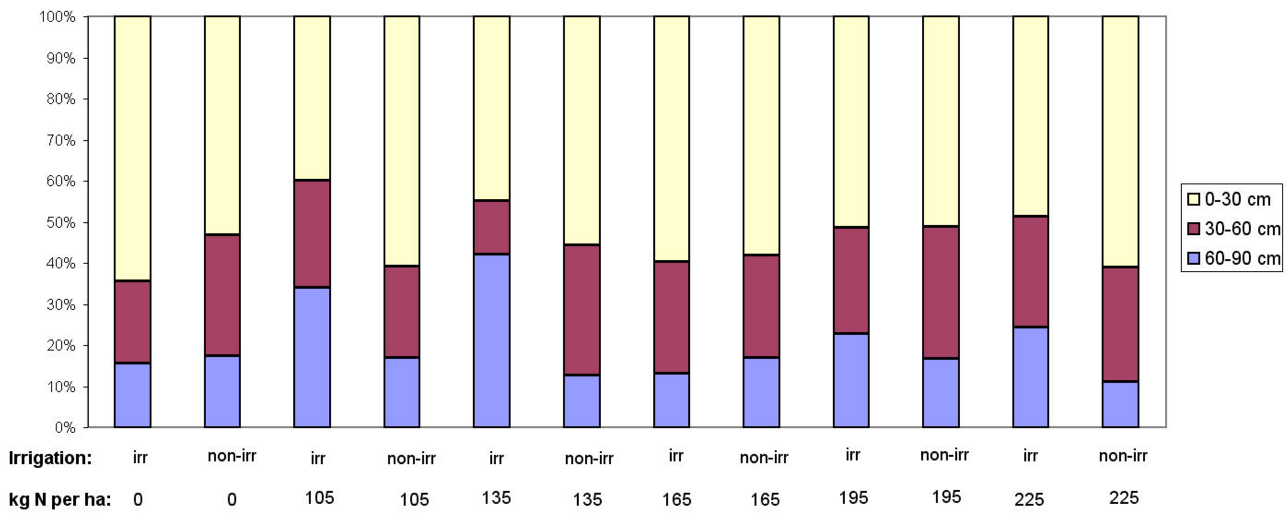

Figure 1.5: Relative contents of nitrate in the soil layers 0-30, 30-60 and 60-90 cm measured at 7 weeks after harvest.

Chapter 2: Non-destructive and rapid estimation of nitrogen status using SPAD chlorophyll meter and Dualex in comparison with sap nitrate test in Broccoli

Introduction

Fertilizer nitrogen is one of the main sources for nitrate contamination of groundwater. The need for a quick determination of plant nitrogen status has prompted the development of N-testing devices. The sap test has been used for a long time but due to the tedious procedure involved its actual use by growers is limited. The sap test was also criticised for its spatial and temporal variability in a potato crop (MacKerron et al., 1995) and the fact that it did not relate well to either short-term or seasonal uptake of nitrogen. Other authors have shown good results when using the sap test for fine-tuning the sidedressing at 5 weeks for broccoli and cabbage (Villeneuve et al. 2002, Kubota et al. 1997, Gardner & Roth 1989, Scaife & Stevens 1983,).

A chlorophyll meter can be used as an alternative to the sap test as a quick non-destructive and inexpensive method to determine leaf N concentration. Many studies have been done with the SPAD chlorophyll meter on several different crops (Swiader & Moore 2002, Sunderman et al. 1997, Piekielek et al. 1995, Westcott & Wraith 1995). The problem with the SPAD meter is the saturation of the chlorophyll index (Westerveld et al. 2004, Villeneuve et al. 2002). Indeed, chlorophyll readings reach a plateau in adequately fertilized crops and the difference between SPAD meter readings in two different applications can be small.

Due to changes in instrument reading over time and the effect of cultivar, leaf selection, soil type, climate, irradiance, leaf water status and plant stress, the use of a reference plot will be necessary to obtain reliable results. The reference plot is a way to keep the confounding factors constant and focus on the distinctive effect of nitrogen status at measurement. The idea is that supplementary fertilization needed is based on the difference between the reference plot and the rest of the field. Associating different fertilizer rates for different threshold values between the field and the reference plot will lead to more adequately fertilized crops and less risk of environmental problems. Both well-fertilized (Scharf et al. 2006 (corn), Westerveld et al. 2004 (cabbage, onion and carrot), Wiesler et al. 2002 (sugar beet), Swiader & Moore 2002 (pumpkin), Zebarth et al. 2002 (corn), Hussain et al. 2000 (rice), Varvel et al. 1997 (corn), Sunderman et al. 1997 (corn), Jemison & Lytle 1996 (corn), Blackmer & Schepers 1995 (corn), Westcott & Wraith 1995 (peppermint), Piekielek et al. 1995 (corn), Minotti et al. 1994 (potato), Schepers et al. 1992 (corn) and unfertilized plots ((Olivier et al. 2006 (potato), Denuit et al. 2002 (wheat, potato)) are recommended as reference plots.

The Dualex is an instrument estimating epidermial phenolic concentration in leaves. When available nitrogen decreases, the phenolic compound in leaf epiderms increases. Measuring the phenolic compounds to determine crop nitrogen status is suggested in several studies (Cartelat et al. 2005, Kraus et al. 2004, Chishaki and Horiguchi 1997). There are reasons to believe that the production of phenolic compounds is affected by several factors in the same way as the chlorophyll content. Fast growing species have been shown to be more sensitive to the environment (e.g. temperature, radiation, fertilization, precipitation) regarding phenolic concentration (Jones & Hartley, 1998). In a study made by Meyer et al. (2006), higher irradiance was shown to increase the production of phenols. The use of a reference plot to determine a saturation index is therefore recommended.

The aim of this study was to compare sap test, SPAD chlorophyll meter and the Dualex instrument as methods for diagnosing nitrogen requirement in broccoli. The relationship between yield and instrument reading and how early after fertilization the N-tests show a good estimation of crop nitrogen status will also be evaluated.

Materials and methods

See chapter 1 for experimental layout, application rates of fertilizer and irrigation.

N-testing devices

Sap test

Sap nitrate content was determined at 4.5 and at 6.5 weeks, by taking five of the last fully developed leaves from each subplot (treatment). The sampling of the leaves was done before 10:00 to minimize variations in sap nitrate concentrations due to plant transpiration. The leaves were immediately placed in a cooler after they were collected. They were then transported to the laboratory where the analysis was completed the same day. The leaf blade was first removed and the midvein and petiole were cut into small pieces. A garlic press was used to extract the sap. The sap extraction was then diluted to comply with the detection limit of the instrument. Depending on the treatment, the samples at 4.5 weeks were diluted at 1:40, 1:20 or non-diluted. At 6.5 weeks the dilution was 1:20 or non-diluted. Analyzing was done using Merckoquant® test strips (Merck, Darmstadt, Germany) and a Nitrachek® 404 reflectometer (QuoMed Ltd., West Sussex, England).

Dualex

The plant polyphenolic content was determined at 3.5, 4, 4.5 and 6.5 weeks after transplanting by using the Dualex (Force-A, Paris, France). Measurements were done on both the abaxial and adaxial side on the 20 last fully developed leaves from each experimental unit. Measurements were done at the middle of the length of the leaf blade, avoiding the midribs. Dualex value is defined as the sum of the readings obtained from the abaxial and adaxial side.

SPAD

Minolta SPAD-502 was used to determine the amount of chlorophyll in the leaves. Measurements were done at the same time as the Dualex 3.5, 4, 4.5 and 6.5 weeks after transplanting. The same leaf and the same position on the leaf were used as the Dualex. Measurements however, were only done on the abaxial side of the leaf.

Saturation index

Since it is not only nitrogen application that affects sap, SPAD and Dualex readings, relative values for these measurements were calculated. The average results obtained by the N-tests were divided by the average of the fully fertilized plot (T8 = 160+65+0 kg N/ha at transplanting+2+4.5 weeks) and expressed as a percentage. T8 was measured at all times (3.5, 4, 4.5 and 6.5 weeks) and was therefore used in the calculation of the saturation index.

Statistical analysis

Analysis of variance for a split-plot design was performed using SAS software (SAS Institute, Cary, NC). Trend analyses were performed using orthogonal polynomial contrasts to evaluate the effects of nitrogen treatments (T1-T6), irrigation and interactions on the dependent variable. Trends of the linear, quadratic and cubic orders were used. Pairwise comparisons were performed between groups using contrasts. Regression analyses were performed with linear and polynomial (second and third order) terms. Regression analysis was used to calculate best fit functions between marketable yield, uptake of nitrogen and results obtained with N-testing devices.

Results and discussion

Sap test

Nitrogen fertilization, at transplanting and at 2 weeks, were linearly related to the sap test at 4.5 weeks (figure 2.1 and table 2.1). Irrigation had no effect at 4.5 weeks however, but it was provided only twice at this stage. The high nitrogen application on the saturated plot (T8 = 160+65 kg N/ha) showed an equally effective uptake as treatments T1, T7 and T2 (T1 = 0+0, T7 = 75+0 and T2 = 75+30 kg N/ha applied at transplanting + 2 weeks). The sap test at 4.5 weeks was used to determine the sidedressing for T7 (75+0+X kg N/ha applied at transplanting + 2 weeks + 4.5 weeks). As can be seen in figure 2.1 the mean saturation index between T8 and T7 was around 25 percent. Therefore 120 kg N/ha was applied at 4.5 weeks according to previously calibrated amounts of nitrogen fertilizer (see materials and methods in chapter 1). The large difference between the saturated plots and the other treatments was the demonstration of a significant effect of nitrogen during this growing season. The sap test however, does not show if the nitrate taken up was actually used by the plant. It could very well reflect luxury consumption of N, which means that a percentage of the nitrogen taken up was not utilized for plant growth.

At 6.5 weeks after transplanting the sap nitrate concentration was much lower and the difference between high and low fertilized plots was smaller as compared to what it was at 4.5 weeks. This has also been shown by Schröder et al. (2000) in corn production. Regardless of nitrogen supply, nitrate concentration drops when plants develop. At this stage, irrigation had a significant effect on sap nitrate concentration. The increase in sap nitrate concentration with increasing nitrogen application was greater without irrigation than with irrigation (Irr x N **).

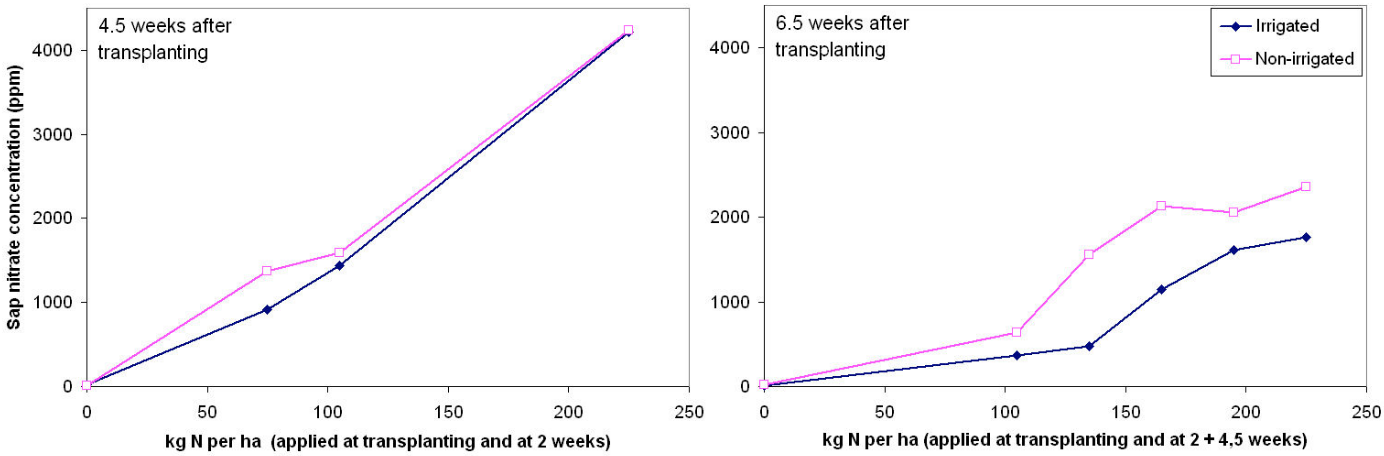

Figure 2.1: Effects of nitrogen fertilization and irrigation on sap nitrate concentration at 4.5 weeks (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks). At 6.5 weeks after transplanting (T1 = 0+0+0, T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).

Table 2.1: Analysis of variance and contrasts for sap nitrate concentration at 4.5 weeks (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks). At 6.5 weeks after transplanting (T1 = 0+0+0, T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).

*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.

ns = non-significant

		Sap N at 4.5 weeks	Sap N at 6.5 weeks
Anova results	Irrigation	ns	*
	Nitrogen	***	***
	Irr x N	ns	**
Polynomial contrasts	N linear	***	***
	N quadratic	ns	**
	N cubic	ns	**
	N lin x irr	ns	*
	N qua x irr	ns	*
	N cub x irr	ns	ns

Dualex

Nitrogen fertilization had a significant effect on Dualex readings (figure 2.2 and table 2.2). At the first two readings, at 3.5 and at 4 weeks, both the linear and quadratic components were significant. At the second sidedressing, 4.5 weeks after transplanting, the Dualex value decreased linearly with increasing N. This shows that the high nitrogen application of T8 (160+65 kg N/ha at transplanting + 2 weeks) had a significant effect on plant stress. This means that we cannot attribute the high sap nitrate concentration in treatment T8 (figure 2.1) just to luxury consumption. Nitrogen was as effective at high rates as at low rates in decreasing polyphenolic production. Build up of polyphenolics was sensitive to both low and high nitrogen rates and this suggests that the Dualex is appropriate to compare a field to a reference plot.

The results from 6.5 weeks show a curvilinear relationship between applied nitrogen and Dualex value. At this stage, the linear N irrigation component was significant. Increasing the amount of nitrogen had a larger effect on decreasing the Dualex value when irrigation was used (N lin x irr*).

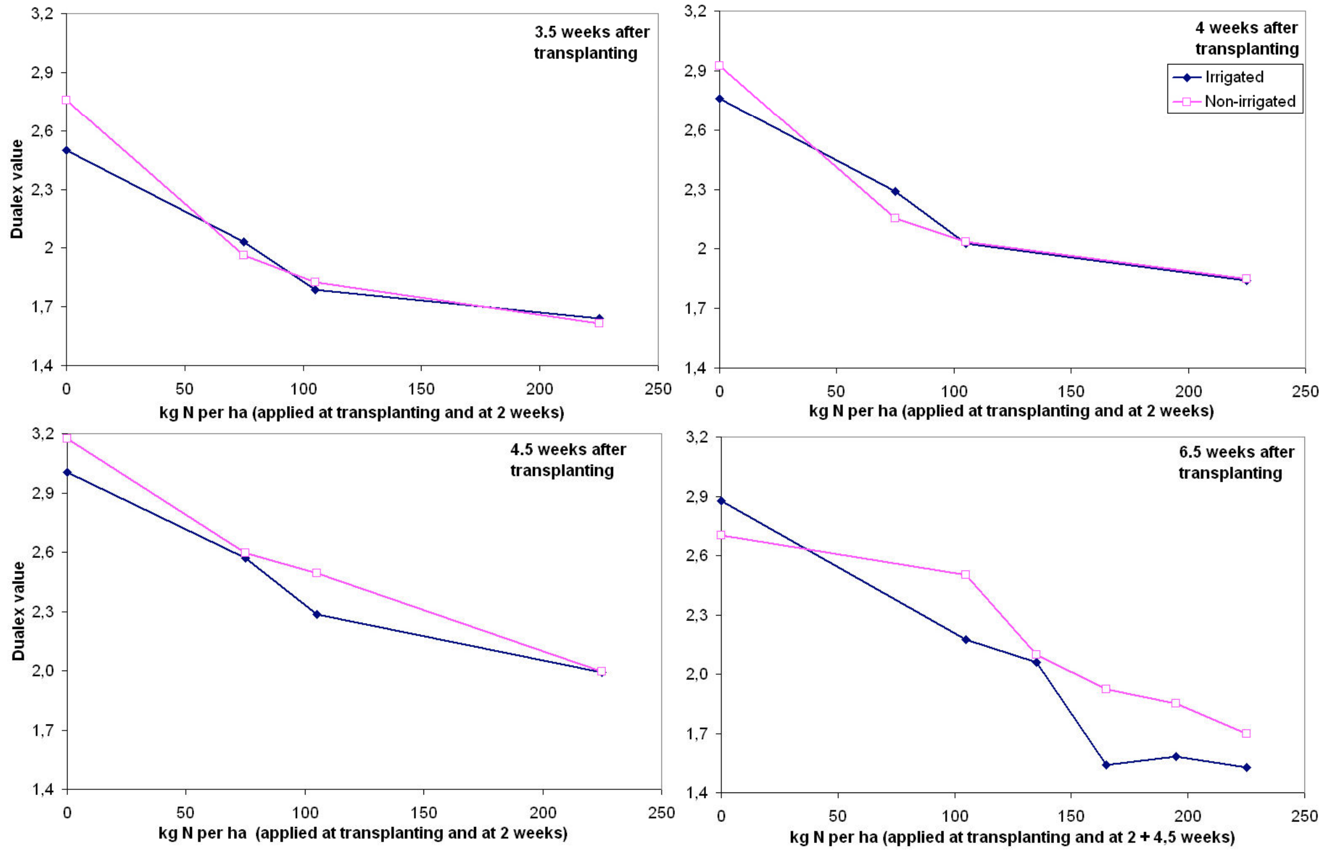

Figure 2.2: Effects of nitrogen fertilization and irrigation on Dualex reading at 3.5, 4 and 4.5 weeks (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks). At 6.5 weeks after transplanting (T1 = 0+0+0, T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).

Table 2.2: Analysis of variance and contrasts for Dualex reading at 3.5, 4, 4.5 weeks (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks). At 6.5 weeks after transplanting (T1 = 0+0+0, T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).
*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.
ns = non-significant

		Dualex reading at 3.5 weeks	Dualex reading at 4 weeks	Dualex reading at 4.5 weeks	Dualex reading at 6.5 weeks
Anova results	Irrigation	ns	ns	ns	ns
	Nitrogen	***	***	***	***
	Irr x N	ns	ns	ns	ns
Polynomial contrasts	N linear	***	***	***	***
	N quadratic	***	***	ns	ns
	N cubic	ns	ns	ns	*
	N lin x irr	ns	ns	ns	*
	N qua x irr	ns	ns	ns	ns
	N cub x irr	ns	ns	ns	ns

SPAD

SPAD meter value increased curvilinearly at 3.5, 4 and 4.5 weeks, with increasing amounts of nitrogen (figure 2.3 and table 2.3). At 6.5 weeks, the relationship between SPAD value and applied nitrogen was linear. At this stage, irrigation had a significant effect. The SPAD meter did not present a linear relationship with nitrogen application at 4.5 weeks as the sap test and the Dualex did. This means that the chlorophyll concentration in the leaves was not increasing at the higher nitrogen amounts. This could reflect luxury consumption by the plant or a slow response on the measurable chlorophyll concentration to nitrogen application. According to Schröder et al. (2000), SPAD meter readings are poor indicators of excess N since not all N was converted to chlorophyll. They suggested however that nitrogen deficiency was immediately affecting the chlorophyll concentration negatively, to which the SPAD meter was sensitive. They also suggested that SPAD meter values would decrease when crops were subjected to water stress. This was not the case in our study. Actually, the SPAD meter showed the opposite, with higher values for non-irrigated plants at 6.5 weeks.

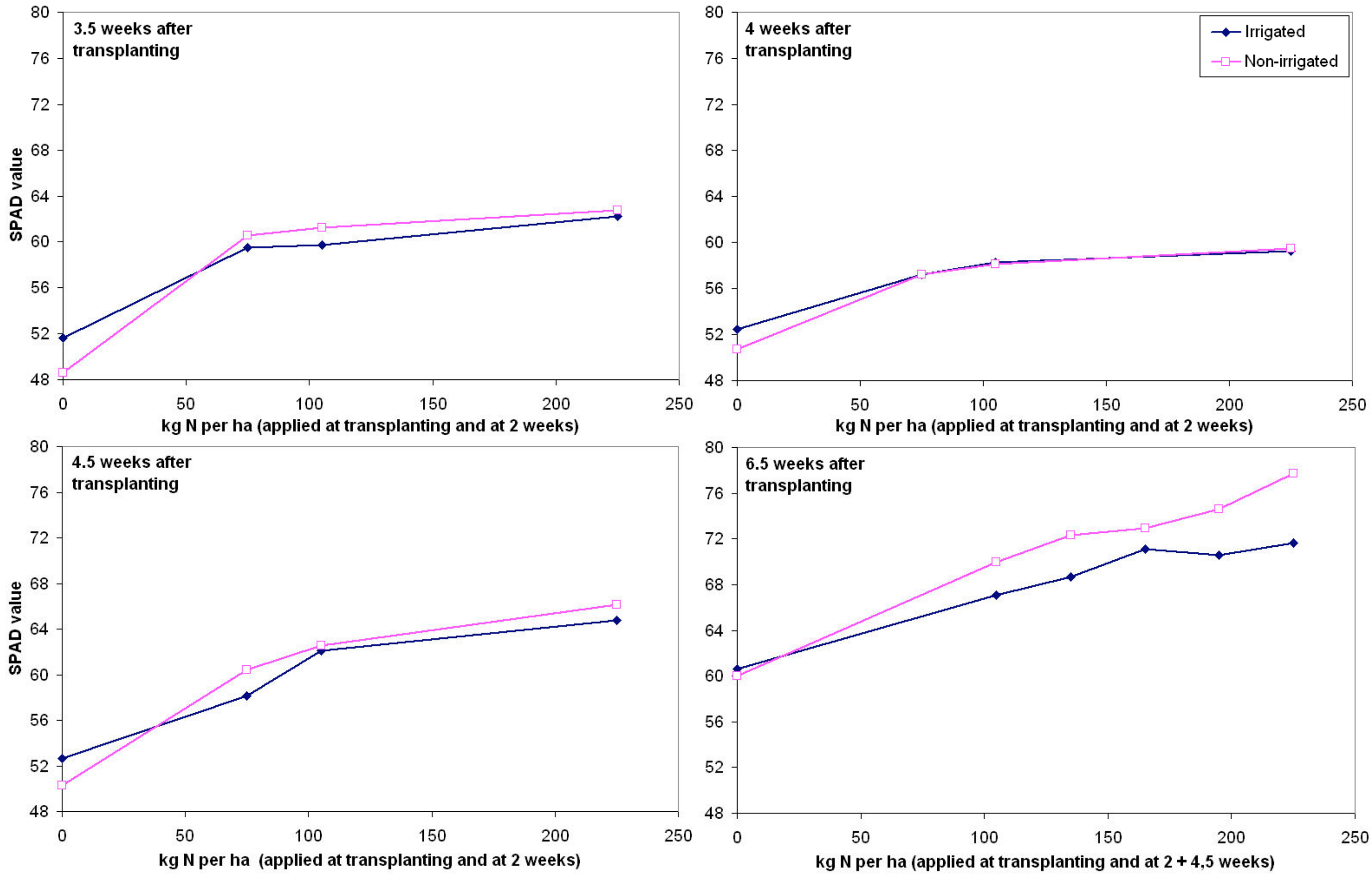

Figure 2.3: Effects of nitrogen fertilization and irrigation on SPAD reading at 3.5, 4 and 4.5 weeks (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks). At 6.5 weeks after transplanting (T1 = 0+0+0, T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).

Table 2.3: Analysis of variance and contrasts for SPAD reading at 3.5, 4, 4.5 weeks (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks). At 6.5 weeks after transplanting (T1 = 0+0+0, T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).

*, **, *** Statistically significant at the 0.05, 0.01 and 0.001 levels, respectively.

ns = non-significant

		SPAD reading at 3.5 weeks	SPAD reading at 4 weeks	SPAD reading at 4.5 weeks	SPAD reading at 6.5 weeks
Anova results	Irrigation	ns	ns	ns	*
	Nitrogen	***	***	***	***
	Irr x N	ns	ns	ns	ns
Polynomial contrasts	N linear	***	***	***	***
	N quadratic	***	***	***	ns
	N cubic	ns	ns	ns	ns
	N lin x irr	ns	ns	ns	ns
	N qua x irr	*	ns	ns	ns
	N cub x irr	ns	ns	ns	ns

Relationship between the results from the N-testing devices at 6.5 weeks and yield

When comparing results from N-testing devices at 6.5 weeks, both the Dualex and SPAD meter showed a better relationship between instrument reading and marketable yield as compared to sap nitrate concentration (figure 2.4). A change in Dualex and SPAD values made a difference on the regression curve compared to the sap nitrate concentration. The sap test showed a relationship with yield up to a nitrate concentration of about 1000 ppm. SPAD and Dualex showed a steady difference in yield with each change in instrument reading. The quadratic shape of the regression curve for the SPAD value was affected mostly by one measurement. If this observation had been located more closely to the others, linear relationships between SPAD value and marketable yield would have likely been obtained. The SPAD chlorophyll meter had most of its observations between values of 70 and 75 as compared to the Dualex, which showed a larger variation. It appears that the sidedressing at 4.5 weeks had more effect on the Dualex as compared to the SPAD meter during the 2 weeks following N application.

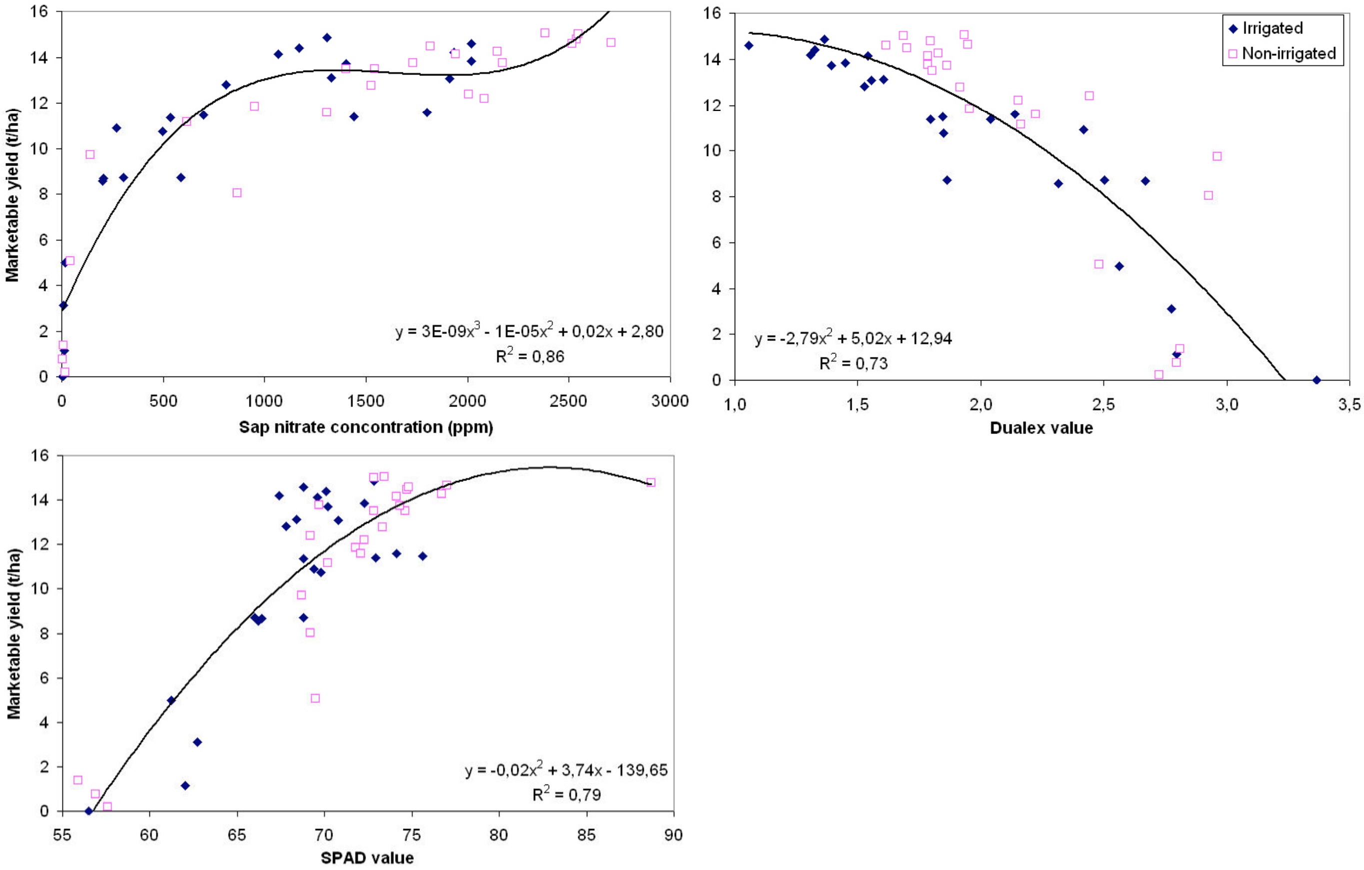

Figure 2.4: Relationship between marketable yield and sap nitrate concentration, Dualex and SPAD readings at 6.5 weeks after transplanting for treatments T1-T6 (T1 = 0+0+0, T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).

Relationship between N-testing devices and plant uptake of nitrogen

The relationship between nitrogen uptake and marketable yield was shown in chapter 1. Since the N-testing devices where shown related to N uptake, their readings may be related to yield as well. The N-testing devices all showed a linear relationship with uptake of nitrogen at 4.5 weeks (figure 2.5). The variation in the response was best explained by the Dualex (R^2 = 0.74). At 6.5 weeks only the Dualex presented a linear relationship with uptake of nitrogen (R^2 = 0.80) (figure 2.6). How sensitive these devices are and how fast they react to nitrogen application could possibly explain the difference seen at 6.5 weeks between instruments. Considering the results at 6.5 weeks, it seems that the Dualex is faster to react to changes in nitrogen uptake. At 4.5 weeks there was enough time, from the broadcast application of nitrogen at transplanting and the sidedressing at 2 weeks, to show a good relationship between all instrument readings and actual uptake. At 6.5 weeks however, only 2 weeks after sidedressing, neither sap nitrate concentration nor SPAD presented a linear relationship to nitrogen uptake.

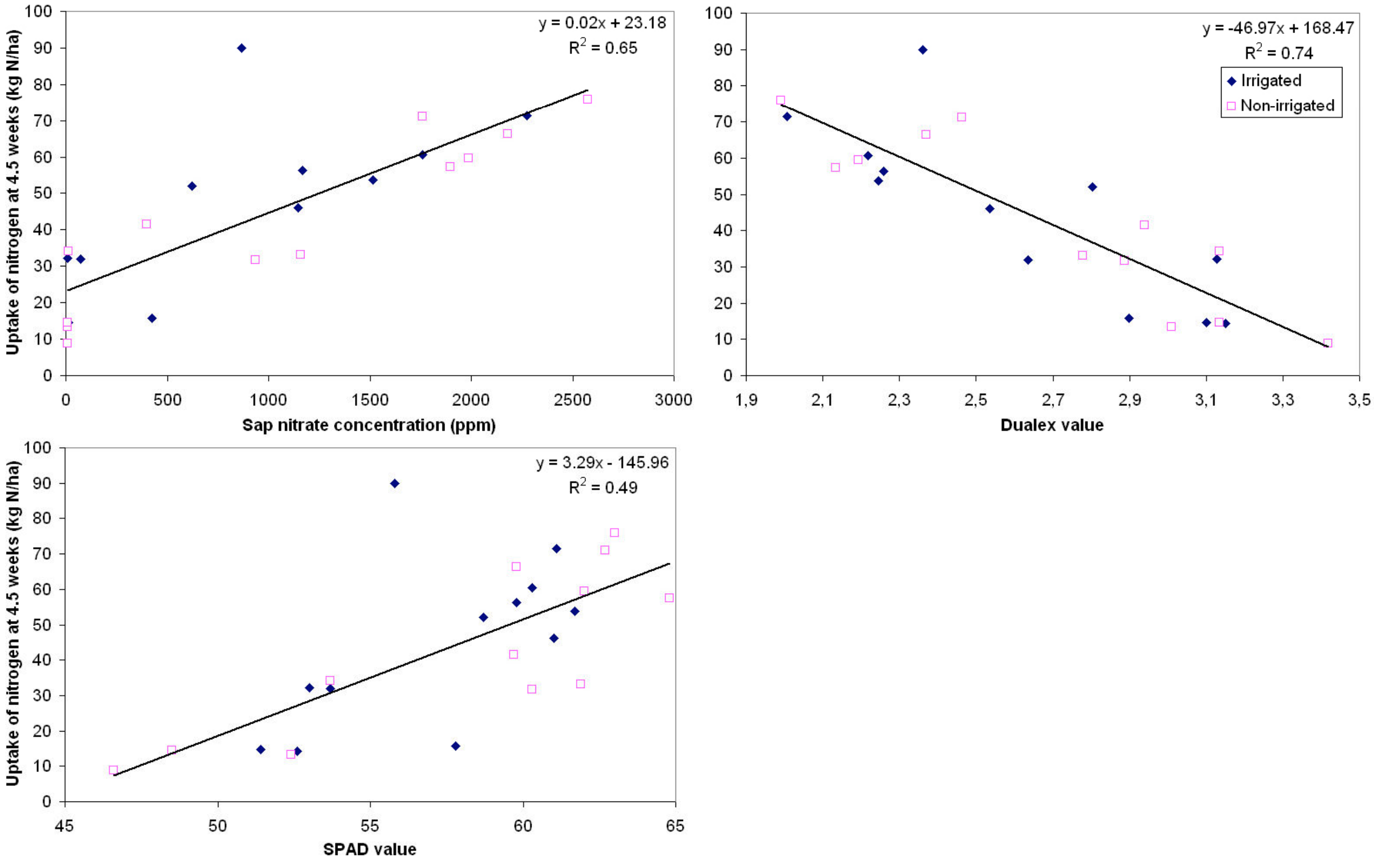

Figure 2.5: Relationship between uptake of nitrogen at 4.5 weeks and readings of nitrogen diagnosing instruments (sap-test, Dualex and SPAD) for treatments T1, T4 and T7 (T1 = 0+0, T4 = 75+30, T7 = 75+0 kg N/ha applied at transplanting + 2 weeks).

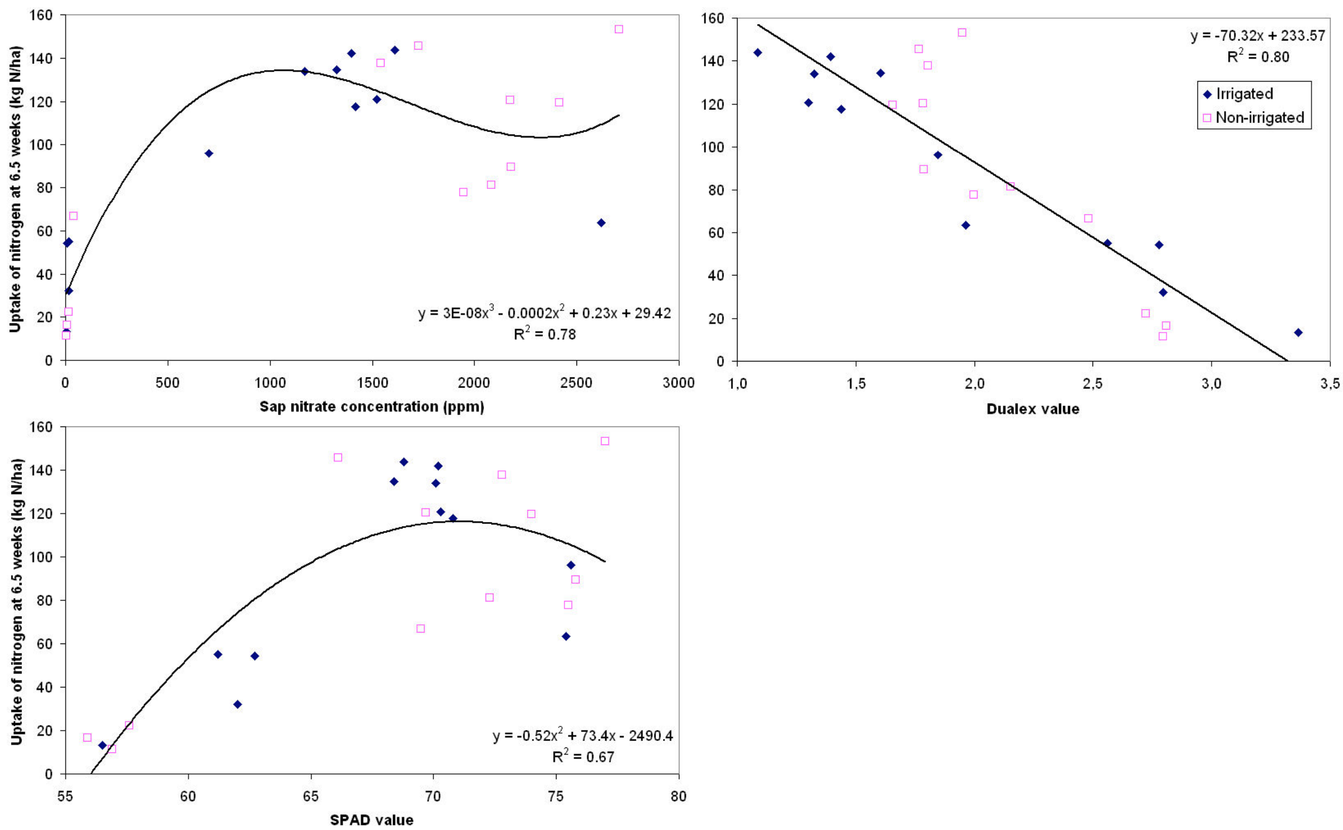

Figure 2.6: Relationship between uptake of nitrogen at 6.5 weeks and readings of nitrogen diagnosing instruments (sap-test, Dualex and SPAD) for treatments T1, T4 and T7 (T1 = 0+0+0, T4 = 75+30+60, T7 = 75+0+120 kg N/ha applied at transplanting + 2 + 4.5 weeks).

Relationship between N-testing devices and yield

The good relationship between Dualex and nitrogen uptake, as shown in figure 2.6, can also be observed with Dualex and yield. It should be possible to establish this relationship using treatment T2 (75+30+0 kg N/ha at transplanting + 2 weeks + 4.5 weeks) as a reference and comparing the increase in yield with the response from the N-testing devices in treatment T3-T6 (all treated the same until second sidedressing at 4.5 weeks). At 6.5 weeks, only 2 weeks after sidedressing, the Dualex showed a linear relationship between reading and yield increase (R^2 = 0.80) (figure 2.7). Dualex values for irrigated and non-irrigated plots were located almost exactly on top of another (irrigated: y=1.41+5.65 R^2=0.77 and non-irrigated: y=1.41+5.36 R^2=0.83). Sap test and SPAD were characterized by a curvilinear relationship with yield and a poor adjustment (R^2 = 0.49 and R^2 = 0.41, respectively). The quadratic shape of the trend line for the SPAD meter was affected by one observation far to the right in the graph. If this observation had been located together with the others, a linear relationship between increase in SPAD value and increase in marketable yield should have been obtained. The SPAD meter however, would still not show a significant relationship with yield.

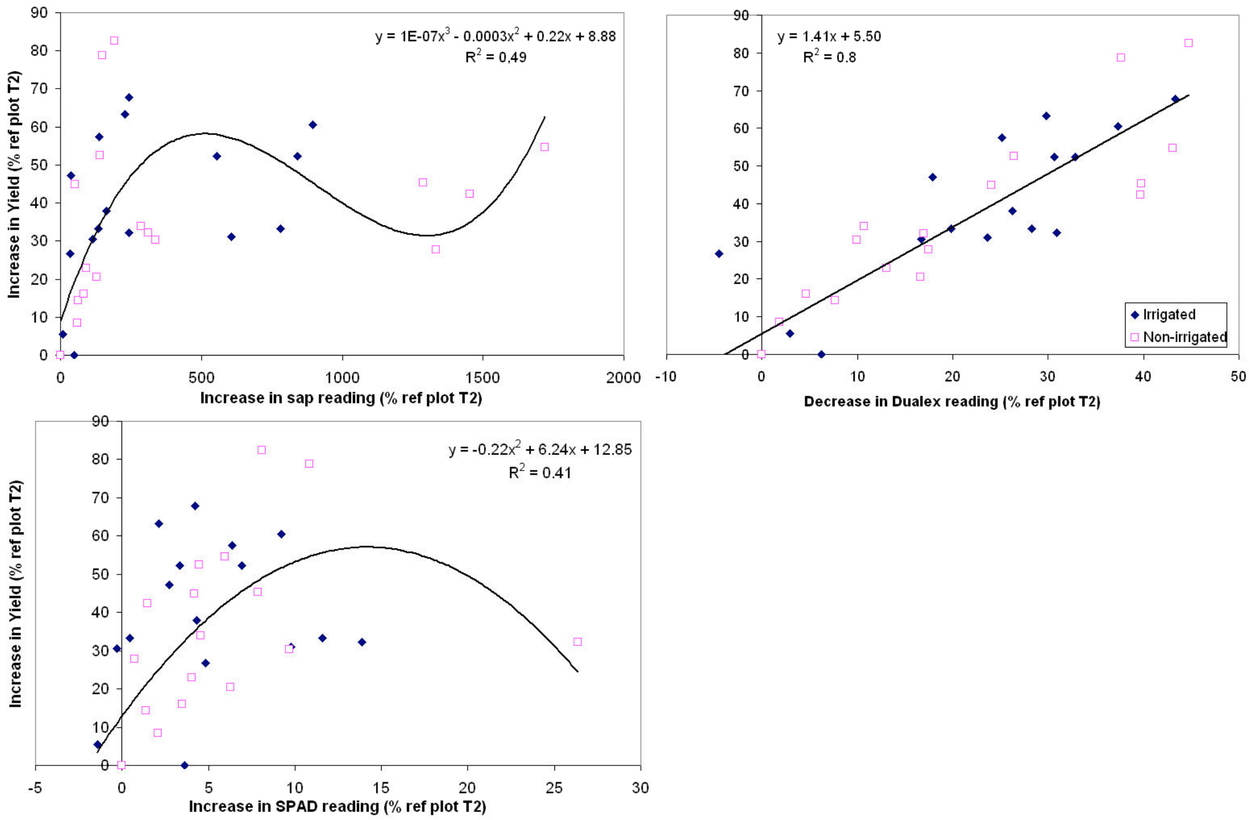

Figure 2.7: Relationship between change in yield and results from nitrogen diagnosing instruments comparing treatment T2 with T3-T6 at 6.5 weeks. T2 = 75+30+0, T3 = 75+30+30, T4 = 75+30+60, T5 = 75+30+90 and T6 = 75+30+120 kg N/ha applied at transplanting + 2 + 4.5 weeks.

Using N-testing devices and a reference plot to determine nitrogen sidedressing rate

The use of a reference plot for determining sidedressing rate is a promising method since broccoli is strongly affected by nitrogen fertilization. When the results between reference plot and the field are small it is difficult to establish the amount of nitrogen to apply. The results obtained in our study with the N-testing devices on a reference plot were significantly different compared to the rest of the field. The studies recommending an unfertilized plot did not show a significant difference with the use of an over-fertilized reference plots. From this study and for broccoli in general, it was not a problem to find a significant difference with either unfertilized or overfertilized reference plots as compared to the rest of the field (table 2.5 and table 2.6). The easier management of an overfertilized reference plot should make it the better choice. Broadcast application of nitrogen before transplanting can be done over the whole field with an extra application on the reference plot.

The overfertilized plot will also show the effective use of nitrogen application at that particular year, while that is more difficult to determine when using an unfertilized plot. An unfertilized plot however, provides information about soil mineral nitrogen compared to the crop need. Since it is almost always necessary to apply nitrogen fertilizer to broccoli, this information is not so critical.

Sap nitrate concentration was the test showing the largest difference between unfertilized and overfertilized plots, with a saturation index 200 times higher than the one of overfertilized plants (table 2.6). This was a huge difference as compared to SPAD values which only increased by 26 % with 225 kg N/ha compared to the control plot (T1 = 0 kg N/ha). Martínez & Guiamet (2004) showed, in a study on wheat and corn, that the SPAD chlorophyll meter was very sensitive to differences in irradiance, leaf water status and time of measurement. They showed that the result could differ by 2-3 units and since it has quite a narrow span from high to low fertilized plants, the use of a reference plot was vital.

The SPAD test was however, very sensitive to differences in nitrogen application, which was shown by the significant change in SPAD values between 75 and 105 kg N/ha (P = 0.015 for absolute SPAD value and P = 0.0085 for relative SPAD value). The SPAD meter showed a significant difference between the two treatments, something that neither the sap test nor the Dualex was able to achieve. This was in contradiction with Villeneuve et al. (2002) who showed that the sap test was more sensitive to changes in nitrogen application as compared to the SPAD meter.

The measurements made with the Dualex showed a good correlation between abaxial, adaxial and total values. There should therefore be no need for measuring both sides of the leaf. The best choice of leaf side is abaxial, since the difference between unfertilized and overfertilized plots was largest, at 96 %.

The Dualex was the instrument showing the most potential as a nitrogen diagnosing tool for broccoli (table 2.4). It showed a linear relationship with nitrogen at the time of sidedressing, at 4.5 weeks. The ease of use and the accurate measurement, both regarding uptake and yield in relation with instrument reading, makes it the best choice for this purpose.

Table 2.4: Pros and cons with sap, Dualex and SPAD instruments

	Sap	Dualex	SPAD
Pros	Well tested over a long time. Linear relationship between nitrogen application and instrument reading at sidedressing, at 4.5 weeks.	Quick, non-destructable. Inexpensive measuring. Irrigation had no effect on results. Fast reaction to N application. Significant relationship between yield and instrument reading. Significant relationship between uptake of nitrogen and instrument reading at both 4.5 and 6.5 weeks. Linear relationship between nitrogen application and instrument reading at sidedressing, at 4.5 weeks.	Quick, non-destructable. Inexpensive measuring. Significant relationship between yield and instrument reading. Sensitive to differences in nitrogen application.
Cons	Time consuming. Expensive analysis. Effected by irrigation. Non-significant relationship between yield and instrument reading.	New instrument with few tests.	Effected by irrigation. Poor predictor of excess N applications.

The use of the SPAD/Dualex ratio was proposed by Cartelat et al. (2005). Since both SPAD and Dualex value change from the base to the tip of the leaf, a ratio will decrease the standard deviation for the whole leaf. The opposite effect of nitrogen on chlorophyll and polyphenolics will mean a larger difference between N-deficient and N-sufficient plants. Table 2.5 shows that the difference between T1 and T8 was 95 %, compared to SPAD alone, which was only 26 %. As well as a larger difference, there was still a significant difference between the two applications 75 and 105 kg N/ha. An instrument that measures both chlorophyll and polyphenolics at the same time would be ideal. Using the ratio between SPAD and Dualex AB the difference between unfertilized and overfertilized plots was 140 % (data not shown).

Table 2.5: Absolute values at 4.5 weeks for sap, SPAD, Dualex and Ratio (SPAD/Dualex AB+AD) for treatments T1, T7, T2 and T8 (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks).

Variable	T1 = 0 kg N/ha	T7 = 75 kg N/ha	T2 = 105 kg N/ha	T8 = 225 kg N/ha
Sap ppm	17.6a	1147.2b	1516.0b	4227.1c
SPAD value	51.5a	59.3b	62.3c	65.4d
Dualex AB+AD value	3.1a	2.6b	2.4b	2c
Dualex AB value	1.1a	0.9b	0.8b	0.6c
Dualex AD value	1.9a	1.7b	1.6b	1.4c
Ratio	16.8a	23.3b	26.4c	33.3d

* Data on the same line with the same letters do not differ significantly at p = 0.05

Table 2.6: Relative values, using saturation index with treatment T8 (SI T8), at 4.5 weeks for sap, SPAD, Dualex and Ratio (SPAD/Dualex AB+AD) for treatments T1, T7, T2 and T8 (T1 = 0+0, T7 = 75+0, T2 = 75+30 and T8 = 160+65 kg N/ha applied at transplanting + 2 weeks).

Variable	T1 = 0 kg N/ha	T7 = 75 kg N/ha	T2 = 105 kg N/ha	T8 = 225 kg N/ha
Sap ppm SI T8	0.5a	27.8b	38.7b	100.0c
SPAD value SI T8	78.9a	90.7b	95.4c	100.0d
Dualex AB+AD value SI T8	156.9a	130.1b	121.1b	100.0c
Dualex AB value SI T8	196.4a	149.7b	133.0b	100.0c
Dualex AD value SI T8	140.9a	122.3b	116.6b	100.0c
Ratio SI T8	51.2a	70.1b	80.6c	100.0d

* Data on the same line with the same letters do not differ significantly at p = 0.05

Conclusion

The purpose of this project was to use N diagnosing instruments to evaluate their capacity to adapt nitrogen fertilization to better suit the crop and limit the effect on the environment.

The importance of nitrogen on marketable yield and quality was evident. For every increase in nitrogen application, up to 165 kg N/ha, the yield increased. Above 165 kg N/ha the response plateaued. Irrigation was expected to have a larger impact on yield and quality then what was the case. It can possibly be explained by low amounts of water stress on non-irrigated plants and the fact that no long drought periods were recorded in our trial.

Plant uptake of nitrogen increased linearly with an increase of fertilizer application. No effect from irrigation was seen on plant N uptake until the measurements done at harvest. Then however, the difference was large. Higher soil residual nitrogen content was expected in the irrigated plots since N uptake decreased, but that was not observed. Leaching losses early in the season and possibly denitrification during the season may explain the loss of nitrogen.

All three instruments were showing potential for N management of broccoli, especially the SPAD and Dualex for their ease of use. The sap test was showing the largest difference between different nitrogen fertilizer applications, but also the largest variation within the same treatment. It seems that the sap test was less stable than the other instruments. This is especially true when comparing marketable yield with instrument reading. The Dualex was the instrument with the best correlation between diagnosing status and yield and it also showed a very fast response to nitrogen fertilization. It could predict yield much better than the other two instruments could, in as short time as two weeks after fertilization. Dualex was the only instrument not affected by irrigation itself at any instance, interaction effects was however seen.

The next step is to provide fertilizer recommendations from SPAD and Dualex measurements and based on an overfertilized reference plot. This method looks very promising when trying to reduce the impact from nitrogen on the environment.

References

Alcoz M. M., Hons F. M. & Haby V. A. (1993) Nitrogen fertilization timing effect on wheat production, nitrogen uptake efficiency, and residual soil nitrogen. Agronomy Journal 85: 1198-1203

Babik I. & Elkner K. (2002) The effect of nitrogen fertilization and irrigation on yield and quality of broccoli. Acta Horticulturae 571: 33-43

Bartholomew W. V. & Clark F. E. (1965) Soil Nitrogen. Academic Press Inc, USA

Belec C., Villeneuve S., Coulombe J. & Tremblay N. (2001) Influence of nitrogen fertilization on yield, hollow stem incidence and sap nitrate concentration in broccoli. Canadian Journal of Plant Science 81: 765 - 772

Beverly R. B., Jarrell W. M. & Letey J. Jr. (1986) A nitrogen and water response surface for sprinkler-irrigated broccoli. Agronomy Journal 78: 91-94

Beverly R. B. (1994) Stem sap testing as a real-time guide to tomato seedling nitrogen and ptassium fertilization. Communications in Soil Science and Plant Analysis 25(7&8): 1045-1056

Blackmer T. M. & Schepers J. S. (1995) Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. Journal of Production Agrigulture 8(1): 56-60

Blodgett J. T. & Stanosz G. R. (1998) Monoterpene and phenolic compound concentrations in water-stressed red pine inoculated with Sphaeropsis sapinea. Phytopathology 88: 245-251

Bowen P. A., Zebarth B. J. & Toivonen P. M. A. (1999) Dynamics of nitrogen and dry matter partitioning and accumulation in broccoli (Brassica oleracea var. italica) in relation to extractable soil inorganic nitrogen. Canadian Journal of Plant Science 79: 277-286

Bracey R. P., Parish R. L. & Bergeron P. E. (1995) Sidedress N application methods for broccoli production. Journal of vegetable crop production, Vol 1(1): 63-71

Bullock D. G. & Anderson D. S. (1998) Evaluation of the Minolta SPAD-502 chlorophyll meter for nitrogen management in corn. Journal of Plant Nutrition 21(4): 741-755

Campbell C. A., Zentner R. P., Selles F. & Akinremi O. O. (1993) Nitrate leaching as influenced by fertilization in the brown soil zone. Canadian Journal of Soil Science 73: 387-397

Canaday C. H. & Wyatt J. E. (1992) Effects of nitrogen fertilization on bacterial soft rot in two broccoli cultivars, one resistance and one susceptible to the disease. Plant Disease 76: 989-991

Cartelat A., Cerovic Z. G., Goulas Y., Meyer S., Lelarge C., Prioul J.-L., Barbottin A., Jeuffroy M.-H., Gate P., Agati G. & Moya I. (2005) Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum L.*). Field Crops Research 91: 35-49

Chishaki N. & Horiguchi T. (1997) Responses of secondary metabolism in plants to nutrient deficiency. Soil Science and Plant Nutrition 43: 987-991

Coulombe J., Villeneuve S., Belec C. & Tremblay N. (1999) Evaluation of soil and petiole sap nitrate quick tests for broccoli in Quebec. Acta Horticulturae 506: 147-152

CRAAQ (2003) Guide de référence en fertilisation. 1st edition. Quebec, Canada.

Cutcliffe J. A. (1972) Effects of plant spacing and nitrogen on incidence of hollow stem in broccoli. Canadian Journal of Plant Science 52: 833 - 834

Cutcliffe J.A. (1975) Cultivar and spacing effects on incidence of hollow stem in broccoli. Canadian Journal of Plant Science 55: 867 - 869

De Neve S. & Hofman G. (1998) N mineralization and nitrate leaching from vegetable crop residues under field conditions: A model evaluation. Soil Biology & Biochemistry 30(14): 2067-2075

Debaeke P., Rouet P. & Justes E. (2006) Relationship between the normalized SPAD index and the nitrogen nutrition index: Application to durum wheat. Journal of Plant Nutrition 29: 75-92

Delgado J. A. & Follett R. F. (1998) Sap test to determine nitrate-nitrogen concentrations in aboveground biomass of winter cover crops. Communications in Soil Science and Plant Analysis 29(5&6): 545-559

Denuit J.-P., Olivier M., Goffaux M.-J., Herman J.-L., Goffart J.-P., Destain J.-P. & Frankinet M. (2002) Management of nitrogen fertilization of winter wheat and potato crops using the chlorophyll meter for crop nitrogen status assessment. Agronomie 22: 847-853

Ellen J. & Spiertz J. H. J. (1980) Effects of rate and timing of nitrogen dressings on grain yield formation of winter wheat. Fertilization Resources 1(3): 177-190

Estiarte M., Filella I., Serra J. & Peñuelas J. (1994) Effects of nutrient and water stress on leaf phenolic content of peppers and susceptibility to generalist herbivore Heliocoverpa armigera (Hubner). Oecologia 99: 387-391

Everaarts A. P. (1994) Nitrogen fertilization and head rot in broccoli. Netherlands Journal of Agricultural Science 42-3: 195-201

Everaarts A. P., de Moel C. P. & Van Noordwijk M. (1996) The effect of nitrogen and the method of application on nitrogen uptake of cauliflower and on nitrogen in crop residues and soil at harvest. Netherlands Journal of Agricultural Science 44: 43-55

Everaarts A. P. & de Willigen P. (1999a) The effect of nitrogen and the method of application on yield and quality of broccoli. Netherlands Journal of Agricultural Science 47: 123-133

Everaarts A. P. & de Willigen P. (1999b) The effect of the rate and method of nitrogen application on nitrogen uptake and utilization by broccoli (*Brassica oleracea* var. *italica*). Netherlands Journal of Agricultural Science 47: 201-214

Fink M. & Scharpf H. C. (2000) Apparent nitrogen mineralization and recovery of nitrogen supply in field trials with vegetable crops. Journal of Horticultural Science & Biotechnology 75(6): 723-726

Gardner B. R. & Roth R. L. (1989) Midrib nitrate concentration as a means for determining nitrogen needs of broccoli. Journal of Plant Nutrition 12(1): 111-125

Gershenzon J. (1984) Changes in the levels of plant secondary metabolites under water and nutrient stress. Recent Advances in Phytochemistry 18: 273-320

Goulas Y., Cerovic Z. G., Cartelat A. & Moya I. (2004) Dualex: a new instrument for field measurements of epidermal ultraviolet absorbance by chlorophyll fluorescence. Applied Optics Vol. 43, no. 23: 4488-4496

Greenwood D. J. & Draycott A. (1988) Recovery of fertilizer-N by diverse vegetable crops: Process and models. In: Nitrogen efficiency in agricultural soils (Jenkinson D. S. and Smith K. A., Eds) Elsevier, London and New York, 46-61

Greenwood D. J., Kubo K.-I., Burns I. G. & Draycott A. (1989) Apparent recovery of fertilizer N by vegetable crops. Soil Science and Plant Nutrition 35 (3): 367-381

Guillard K. & Kopp K. L. (2004) Nitrogen fertilizer form and associated nitrate leaching from cool-season lawn turf. Journal of Environmental Quality 33: 1822-1827

Hartz T. K., Smith R. F., Schulbach K. F. & Le Strange M. (1994) On-farm nitrogen tests improve fertilizer efficiency, protect groundwater. California Agriculture 48: 29-32

Heumann S., Böttcher J. & Springob G. (2002) N mineralization parameters of sandy arable soils. Journal of Plant Nutrition and Soil Science 165: 441-450

Himelrick D. G., Dozier JR. W. A., Wood C. W. & Sharpe R. R. (1993) Determination of strawberry nitrogen status with SPAD chlorophyll meter. Advances in Strawberry Research 12: 49-53

Hipp B. W. (1974) Influence of nitrogen and maturity rate on hollow stem in broccoli. Hortscience 9: 68 - 69

Hoel B. O. & Solhaug K. A. (1998) Effect of irradiance on chlorophyll estimation with the Minolta SPAD-502 leaf chlorophyll meter. Annals of Botany 82: 389-392

Hussain F., Bronson K. F., Yadvinder-Singh, Bijay-Singh & Peng S. (2000) Use of chlorophyll meter sufficiency indices for nitrogen management of irrigated rice in Asia. Agronomy Journal 92: 875-879

Hättenschwiler S. and Vitousek P. M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. Tree vol. 15, no. 6: 238-243

Janzen H. H. & McGinn S. M. (1991) Volatile loss of nitrogen during decomposition of legume green manure. Soil Biology & Biochemistry 23(3): 291-297

Jemison J. M. & Lytle D. E. (1996) Field evaluation of two nitrogen testing methods in Maine. Journal of Production Agriculture 9(1): 108-113

Jenni S., Dutilleul P., Yamasaki S. & Tremblay N. (2001) Brown bead of broccoli. I. Response of the physiological disorder to management practices. Hortscience 36(7): 1224-1227

Jones C. G. and Hartley S. E. (1999) A protein competition model of phenolic allocation. Oikos 86: 27-44

Jones C.G. & Hartley S.E. (1998) Global change and plant phenolic concentrations: Species level predictions using the protein competition model. In: Responses of Plant Metabolism to Air Pollution and Global Change (eds LJ de Kok and I Stulen). Backhuys Publishers, Leiden, pp 23-50

Kahn B. A., Shilling P. G., Brusewitz G. H. & McNew R. W. (1991) Force to shear the stalk, stalk diameter, and yield of broccoli in response to nitrogen fertilization and within-row spacing. Journal of the American Society for Horticultural Science 116(2): 222-227

Kantety R. V., van Santen E., Woods F. M. & Wood C. W. (1996) Chlorophyll meter predicts nitrogen status of tall fescue. Journal of Plant Nutrition 19(6): 881-899

Kiraly Z. (1964) Effect of nitrogen fertilization on phenol metabolism and stem rust susceptibility of wheat. Journal of Phytopathology 51: 252–261.

Knappe S., Haferkorn, U. & Meissner R. (2002) Influence of different agricultural management systems on nitrogen leaching: results of lysimeter studies. Journal of Plant Nutrition and Soil Science 165: 73-77

Kraus T. E. C., Zasoski R. J. & Dahlgren R. A. (2004) Fertility and pH effects on polyphenol and condensed tannin concentration in foliage and roots. Plant and Soil 262: 95-109

Kowalenko C. G. & Hall J. W. (1987) Nitrogen recovery in single- and multiple- harvested direct-seeded broccoli trials. Journal of the American Society for Horticultural Science 112(1): 4-8

Kowalenko C. G. (1989) The fate of applied nitrogen in a fraser valley soil using 15N in field microplots. Canadian Journal of Soil Science 69: 825-833

Kubota A., Thompson T. L., Doerge T. A. & Godin R. E. (1997) A petiole sap nitrate test for broccoli. Journal of Plant Nutrition 20(6): 669-682

Köhler K., Wilhelmus H., Duynisveld M. & Böttcher J. (2006) Nitrogen fertilization and nitrate leaching into groundwater on arable sandy soils. Journal of Plant Nutrition and Soil Science 169: 185-195

Letey J., Jarrell W. M., Valoras N. & Beverly R. (1983) Fertilizer application and irrigation management of broccoli production and fertilizer use efficiency. Agronomy Journal 75: 502-507

Li J., Zhang J. & Ren L. (2003) Water and nitrogen distribution as affected by fertigation of ammonium nitrate from a point source. Irrigation Science 22:19-30

Ludy R. L. & Powelson M. L. (1997) Effect of sprinkler irrigation on bacterial soft rot and yield of broccoli. Plant Disease 81(6): 614-618

Macdonald A. J., Poulton P. R., Powlson D. S. & Jenkinson D. S. (1997) Effects of season, soil type and cropping recoveries, residues and losses of ^{15}N-labelled fertilizer applied to arable crops in spring. Journal of Agricultural Science, Cambridge 129: 125-154

Macdonald A. J., Powlson D. S., Poulton P. R. & Jenkinson D. S. (1989) Unused fertiliser nitrogen in arable soils-Its contribution to nitrate leaching. Journal of the Science of Food and Agriculture 46: 407-419

MacKerron D. K. L., Young M. W. & Davies H. V. (1995) A critical assessment of the value of petiole sap analysis in optimizing the nitrogen nutrition of the potato crop. Plant and Soil 172: 247-260

Magnifico V., Lattanzio V. & Sarli G. (1979) Growth and nutrient removal by broccoli. Journal of the American Society for Horticultural Science 104(2): 201-203

Martínez D. E. & Guiamet J. J. (2004) Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 24: 41-46

Matthäus D. & Gysi C. (2001) Plant-sap analysis in vegetables – a tool to decide on nitrogen top dressing. Acta Horticulturae 563: 93-102

McClure J. W. (1977) The physiology of phenolic compounds in plants. In T. Swain, J.B. Harbourne and C.F. Van Sumere, eds. Biochemistry of plant phenolics, Vol. 12. Plenum Press, New York, pp. 525-556.

Meyer S., Cerovic Z. G., Goulas Y., Montpied P., Demotes-Mainard S., Bidel L. P. R., Moya I. & Dreyer E. (2006) Relationship between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: a signature of the carbon-nitrogen balance within leaves? Plant, Cell and Environment 29: 1338-1348

Minotti P. L., Halseth D. E. & Sieczka J. B. (1994) Filed chlorophyll measurements to assess the nitrogen status of potato varieties. Hortscience 29(12): 1497-1500

Neeteson J. J. & Carton O. T. (2001) The environmental impact of nitrogen in field vegetable production. Acta Horticulturae 563: 21-28

Neilsen D., Hogue E. J., Neilsen G. H. & Parchomchuk P. (1995) Using SPAD-502 values to assess the nitrogen status of apple trees. Hortscience 30(3): 508-512

Nkoa R., Desjardins Y., Tremblay N., Querrec L., Baana M. & Nkoa B. (2003) A mathematical model for nitrogen demand quantification and a link to broccoli (*Brassica oleracea var. italica*) glutamine synthetase activity. Plant Science 165: 483-496

Olivier M., Goffart J.-P. & Ledent J.-F. (2006) Treshold value for chlorophyll meter as decision tool for nitrogen management of potato. Agronomy Journal 98: 496-506

Ontario Ministry of Agriculture and Food (2006) Vegetable production recommendations 2006-2007. Publication 363, Ministry of Agriculture, Food and Rural Affairs

Pare K., Chantigny M. H., Carey K., Johnston W. J. & Dionne J. (2006) Nitrogen uptake and leaching under annual bluegrass ecotypes and bentgrass species: A lysimeter experiment. Crop Science 46: 847-853

Piekielek W. P., Fox R. H., Toth J. D. & Macneal K. E. (1995) Use of a meter at the early dent stage of corn to evaluate nitrogen sufficiency. Agronomy Journal 87: 403-408

Prasad M. & Spiers T. M. (1984) Evaluation of a rapid method for plant sap nitrate analysis. Communications in Soil Science and Plant Analysis 15(6): 673-679

Prasad M. & Spiers T. M. (1985) A rapid nitrate sap test for outdoor tomatoes. Scientia Horticulturae 25: 211-215

Raun, W. R., Solie J.B., Johnson G.V., Stone M.L., Mullen R.W., Freeman K.W., Thomason W.E. and Lukina E.V. (2002) Improving nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. Agronomy Journal 94:815-820

Riley W. J., Ortiz-Monasterio I. & Matson P. A. (2001) Nitrogen leaching and soil nitrate, nitrite, and ammonium levels under irrigated wheat in Northern Mexico. Nutrient Cycling in Agroecosystems 61: 223-236

Riley H. & Vågen I. (2003) Critical N-concentration in broccoli and cauliflower, evaluated in field trials with varying levels and timing of fertilizer. Acta Horticulturae 627: 241-249

Roelcke M., Li S. X., Tian X. H., Gao Y. J. & Richter J. (2002) *In situ* comparisons of ammonia volatilization from N fertilizers in Chinese loess soils. Nutrient Cycling in Agroecosystems 62: 73-88

Scaife M. A. (1988) Derivation of critical nutrient concentrations for growth rate from data from field experiments. Plant and Soil 109: 159-169

Scaife M. A. & Stevens K. L. (1983) Monitoring sap nitrate in vegetable crops: Comparison of test strips with electrode methods, and effects of time of day and leaf position. Communications in Soil Science and Plant Analysis 14(9): 761-771

Scaife M. A. & Turner M. K. (1987) Field measurement of sap and soil nitrate to predict nitrogen top-dressing requirements of Brussels sprouts. Journal of Plant Nutrition 10: 1705-1712

Scaife A. & Wurr D. C. E. (1990) Effects of nitrogen and irrigation on hollow stem of cauliflower (*Brassica oleracea* var *botrytis*). Journal of Horticultural Science 65: 25-29

Scharf P. C., Brouder S. M. & Hoeft R. G. (2006) Chlorophyll meter readings can predict nitrogen need and yield response of corn in North-Central USA. Agronomy Journal 98: 655-665

Schepers J. S., Francis D. D., Vigil M. & Below F. E. (1992) Comparison of corn leaf nitrogen concentration and chlorophyll meter readings. Communications in Soil Science and Plant Analysis 23(17-20): 2173-2187

Sexton P. & Carroll J. (2002) Comparison of SPAD chlorophyll meter readings vs. petiole nitrate concentration in sugarbeet. Journal of Plant Nutrition 25(9): 1975-1986

Shattuck V. I., Shelp B. J., Loughton A. & Baker R. (1986) Environmental stability of yield and hollow stem in broccoli (*Brassica oleracea* var. *Italica*). Canadian Journal of Plant Science 66: 683 - 688

Shelp B. J. and Liu L. (1992) Nutrient uptake by field-grown broccoli and net nutrient mobilization during inflorescence development. Plant and Soil 140: 151-155

Shröder J. J., Neeteson J. J., Oenema O. & Struik P. C. (2000) Does the crop or the soil indicate how to save nitrogen in maize production? Reviewing the state of the art. Field Crops Research 66: 151-164

Simmelsgaard S. E.(1998) The effect of crop, N-level, soil type and drainage on nitrate leaching from Danish soil. Soil use and management 14: 30-36

Statistics Canada (2006) Fruit and vegetable production. Vol 75, no 1.
Available at: http://dsp-psd.communication.gc.ca/Pilot/Statcan/22-003-XIB/22-003-XIB-e.html

Studstill D. W., Simonne E. H., Hutchinson C. M., Hochmuth R. C., Dukes M. D. & Davis W. E. (2003) Petiole sap testing sampling procedures for monitoring pumpkin nutritional status. Communications in Soil Science and Plant Analysis 34(15&16): 2355-2362

Sunderman H. D., Pontius J. S. & Lawless J. R. (1997) Variability in leaf chlorophyll concentration among fully-fertilized corn hybrids. Communications in Soil Science and Plant Analysis 28(19&20): 1793-1803

Swiader J. M. & Moore A. (2002) SPAD-chlorophyll response to nitrogen fertilization and evaluation of nitrogen status in dryland and irrigated pumpkins. Journal of Plant Nutrition 25(5): 1089-1100

Teiz L. & Zeiger E. (2004) Plant Physiology. 3rd ed. Sinhauer Associates Inc. USA

Toivonen P. M. A., Zebarth B. J. & Bowen P. A. (1994) Effect of nitrogen fertilization on head size, vitamin C content and storage life of broccoli (*Brassica oleracea* var. *Italica*). Canadian Journal of Plant Science 74: 607-610

Tremblay N. (1989) Effect of nitrogen sources and rates on yield and hollow stem development in broccoli. Canadian Journal of Plant Science 69: 1049 - 1053

Tremblay N. (2004) Determining nitrogen requirements from crops characteristics. Benefits and challenges. Recent Research Development in Agronomy & Horticulture 1(2004): 157-182

Tremblay N. & Beaudet P. (2006) Soil N_{min} content and P_2O_5 export from vegetable crops in Quebec (Canada). Acta Horticulturae 700: 185-189

Tremblay N. & Belec C. (2006) Adapting nitrogen fertilization to unpredictable seasonal conditions with the least impact on the environment. Horttechnology 16(3): 408-412

Tremblay N., Scharpf H.-C., Weier U., Laurence H. & Owen J. (2001) Nitrogen management in field vegetables: A guide to efficient fertilization. Agriculture and Agri-Food Canada

Van Es H. M., Sogbedji J. M. & Schindelbeck R. R. (2006) Effect of manure application timing, crop, and soil type on nitrate leaching. Journal of environmental quality 35: 670-679

Varvel G. E., Schepers J. S. & Francis D. D. (1997) Ability for in-season correction of nitrogen deficiency in corn using chlorophyll meters. Soil Science Society of American Journal 61: 1233-1239

Villeneuve S., Coulombe J., Belec C. & Tremblay N. (2002) A comparison of sap nitrate test and chlorophyll meter for nitrogen status diagnosis in broccoli (*Brassica oleracea* L. ssp. *italica*).

Vågen I. M. (2003) Nitrogen uptake in a broccoli crop. 1: Nitrogen dynamics on a relative time scale. Acta Horticulturae 627: 195-202

Wang Q., Chen J. & Li Y. (2004) Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. Journal of Plant Nutrition 27(3): 557-569

Webb J., Ellis S., Harrison R. & Thorman R. (2004) Measurement of N fluxes and soil N in two arable soils in the UK. Plant and Soil 260: 253-270

Webster C. P., Belford R. K. & Cannell R. Q. (1986) Crop uptake and leaching losses of ^{15}N labelled fertilizer nitrogen in relation to waterlogging of clay and sandy loam soils. Plant and Soil 92: 89-101

Westcott M. P. & Wraith J. M. (1995) Correlation of leaf chlorophyll readings and stem nitrate concentrations in peppermint. Communications in Soil Science and Plant Analysis 26(9 & 10): 1481-1490

Westerman R. L., Boman R. K., Raun W. R. & Johnson G. V. (1994) Ammonium and nitrate nitrogen in soil profiles of long-term winter wheat fertilization experiments. Agronomy Journal 86: 94-99

Westerveld S. M., McKeown A. W., Scott-Dupree C. D. & McDonald M. R. (2003) How well do critical nitrogen concentrations work for cabbage, carrot, and onion crops? Hortscience 38(6): 1122-1128

Westerveld S. M., McKeown A. W., Scott-Dupree C. D. & McDonald M. R. (2004) Assessment of chlorophyll and nitrate meters as field tissue nitrogen tests for cabbage, onions and carrots. Horttechnology 14(2): 179-188

Wiesler F., Bauer M., Kamh M., Engels T. & Reusch S. (2002) The crop as indicator for sidedress nitrogen demand in sugar beet production – limitations and perspectives. Journal of Plant Nutrition and Soil Science 165: 93-99

Wood C. W., Reeves D. W., Duffield R. R. & Edmisten K. L. (1992) Field chlorophyll measurements for evaluation of corn nitrogen status. Journal of Plant Nutrition 15(4): 487-500

Wood C. W., Tracy P. W., Reeves D. W. & Edmisten K. L. (1992) Determination of cotton nitrogen status with a hand-held chlorophyll meter. Journal of Plant Nutrition 15(9): 1435-1448

Zebarth B. J., Bowen P. A. & Toivonen P. M. A. (1995) Influence of nitrogen fertilization on broccoli yield, nitrogen accumulation and apparent fertilizer-nitrogen recovery. Canadian Journal of Plant Science 75: 717-725

Zebarth B. J., Younie M., Paul J. W. & Bittman S. (2002) Evaluation of leaf chlorophyll index for making fertilizer nitrogen recommendations for silage corn in a high fertility environment. . Communications in Soil Science and Plant Analysis 33(5&6) 665-684

Zhang Y.-M., Chen D.-L., Zhang J.-B., Edis R., Hu C.-S. & Zhu A.-N. (2004) Ammonia volatilization and denitrification losses from an irrigated maize-wheat rotation field in the north China plain. Pedosphere 14(4): 533-540

Zhou J.-B., Xi J.-G., Chen Z.-J. & Li S.-X. (2006) Leaching and transformation of nitrogen fertilizers in soil after application of N with irrigation: A soil column method. Pedosphere 16(2): 245-252