

**NITROGEN FERTILIZATION AND SOIL  
MINERAL NITROGEN DYNAMICS TO OPTIMIZE  
CANOLA NUTRITION AND YIELD  
IN QUEBEC**

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## ABSTRACT

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Canola is an ideal feedstock for biodiesel production because of its high oil and low saturated fat concentrations. There is interest in producing more canola in Québec, but producers lack fertilization guidelines to optimize high oilseed yield and quality in canola. Nitrogen (N) is the most important determinate of oilseed yield and quality and N fertilization is important for biomass accumulation during the early vegetative stage and for oil synthesis during the reproductive stage. The first objective of this study was to monitor soil mineral N ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) dynamics and canola straw nutrition in response to N fertilization. Two fertilization methods - a pre-plant and split application of fertilizer N were studied at the Emile A. Lods Agronomy Research Centre on the Macdonald Campus of McGill University at Ste-Anne-de-Bellevue, Québec, using a fractional factorial experimental design. The second objective was to evaluate N use efficiency (NUE) and harvest index (HI) of canola grown in pots containing soils from Ste-Anne-de-Bellevue, St-Augustin-de-Desmaures and Ottawa using a completely randomized design. Split application of a sidedressed N fertilizer did not increase the post-harvest soil mineral N concentration or increase straw nutrition compared with the pre-plant N application. There was considerable spatio-temporal heterogeneity in soil mineral N dynamics, so additional field trials are warranted. The pot study showed inconsistent correlations between straw N concentration and yield in canola grown in the soils collected from

Ste-Anne-de-Bellevue (not related), St-Augustin-de-Desmaures (negative), and Ottawa (positive). Straw N concentrations were related to low straw and oilseed yield, indicating there is an optimal straw N concentration to achieve target yields. Seeding in late May and disease occurrence close to the end of flowering stage reduced the oilseed yield more than straw yield. Future research on the pattern of N translocation (e.g.: from leaf to pod, then to oilseed) under Québec climatic conditions will contribute to the development of an N fertilization guideline. Since some soils in Québec have an appreciable soil N supply, knowledge of how much soil N is used to meet canola N requirement will keep N fertilizer costs low while optimizing oilseed yield and quality.

## RÉSUMÉ

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Le canola est une matière première idéale pour la production de biocarburant car il a une teneur élevée en huile et basse en gras saturés. Au Québec, les producteurs sont intéressés à cultiver davantage de canola, mais font face à un manque de directives en matière d'engrais nécessaire afin d'obtenir un haut rendement d'huile de qualité à partir du canola. L'azote (N) est chez les oléagineux le facteur le plus important déterminant le rendement et la qualité de l'huile; l'engrais azoté est important pour l'accumulation de biomasse pendant le premier stade végétatif et pour la production d'huile pendant le stade reproductif. Le premier objectif de la présente étude a été de suivre l'évolution de la dynamique de l'azote minéral du sol ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) et la nutrition de la paille de canola en réponse à l'engrais azoté. Deux méthodes de fertilisation – fertilisation en présemis et fertilisation partagée fractionnée ont été étudiées au Centre de recherche agronomique Emile A. Lods, au campus Macdonald de l'université McGill à Ste-Anne-de-Bellevue, Québec, utilisant un plan d'expérience factoriel fractionnel. Le deuxième objectif a été d'évaluer l'efficacité d'utilisation de l'azote (NUE) et l'indice de récolte (HI) du canola cultivé en pots avec du sol provenant de Ste-Anne-de-Bellevue, de St-Augustin-de-Desmaures et d'Ottawa, utilisant un plan d'expérience entièrement aléatoire (conception de bloc complètement randomisé). La fertilisation fractionnée et l'application d'azote en bande avec de l'engrais azoté de couverture n'a pas augmenté la concentration d'azote

minéral du sol après-récolte, ni la nutrition de la paille de canola comparativement à la fertilisation azotée précoce (see correction above for précoce). Dû à l'importante hétérogénéité spatio-temporelle de la dynamique de l'azote minéral du sol, des études sur le terrain additionnelles sont à recommander. L'étude des plants en pots a démontré une corrélation linéaire négative entre la concentration en azote de la paille et le rendement de la paille. Les concentrations en azote de la paille ont été corrélées avec des bas rendements de paille et d'huile, indiquant qu'il existerait une concentration idéale de l'azote de paille pour obtenir les rendements visés. Certains facteurs réduisent la qualité de l'huile ainsi que le rendement de la paille, soit de semer vers la fin mai ainsi que les maladies qui apparaissent lors de la floraison. D'autres études sur la translocation de l'azote (p. ex. de la feuille à la gousse, puis à la graine) sous les conditions climatiques du Québec contribueront au développement des directives en matière de fertilisation azotée. Puisque certains sols du Québec ont des réserves appréciables d'azote du sol, connaître la quantité d'azote du sol nécessaire afin de satisfaire aux besoins en azote du canola permettra de maintenir de bas coûts pour l'engrais azoté, ainsi que d'optimiser le rendement et la qualité de l'huile des oléagineux.

## **CONTRIBUTION OF AUTHORS**

This thesis consists of a literature review and two manuscripts. Literature review, preceded by a general introduction, establishes the research context, and presents the hypothesis and objectives of the study. Chapters 2 and 3 presented two separate experiments that were designed to answer the research questions from different aspects. Both chapters 2 and 3 were co-authored by the candidate, her supervisor Dr. Joann Whalen and co-supervisor Dr. Bao-Luo Ma.

The research work of the candidate was part of a larger project initiated by Eastern Canada Oilseed Development Alliance (ECODA). Field design and sampling work plan were provided by Dr. Bao-Luo Ma. Overall direction for the thesis research, guidance in soil and plant sampling and analysis, data interpretation and editorial assistance came from Dr. Joann Whalen. The candidate was responsible for data collection, laboratory analysis, statistics, data interpretation, and manuscripts writing.

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## GENERAL INTRODUCTION

Canola (*Brassica napus*) yield was estimated to be 13.4 million tonnes in 2012, ranking as the most produced oilseed in Canada, compared to soybean's production of 4.4 million tonnes (Canadian Oilseed Processors Association, 2012). The high oil content (>40%) and low saturated fat concentration (6.8%) of canola, compared with soybean (23% oil and 12% saturated fat), makes canola highly valuable in the oilseed industry. In 2010, the Canadian federal government mandated a minimum 2% of the volume of biodiesel blend with diesel and 5% of ethanol blend with gasoline to meet the demands of lowering fossil fuel consumption and GHG emissions (Environment Canada, 2012c). In order to fulfill the mandate within the next 3 years, the Canola Council of Canada (CCC) (2012) estimated approximately 3.6 million tonnes more oilseed or an additional 1.62 million hectares of land cultivated with canola is required (CCC, 2012).

Until 2011, 46% of the oilseeds were exported from Canada as seed, 36% was extracted for domestic consumption, including cooking oil and animal meal, and 18 % was used as biodiesel feedstock (Senko and Hammond, 2012). Québec contributed to less than 1% of the total revenue (cultivation, oilseed crushing and manufacture, and exporting), which came from oil crushing and livestock meal manufacture. The increasing number and capacity of crushing facilities in Québec is driving the expansion of canola production in Québec, especially the establishment of Twin River Technologies – Entreprise De Transformation De Graines Oléagineuses (TRT-ETGO, the biggest canola (60%) and soybean (40%) crushing facility across Eastern Canada.

Currently, 90% of the oilseed processed by TRT-ETGO is purchased from the Prairie Region and US (TRT ETGO du Québec, 2011). This increases transportation costs, restricts local economic growth, creates an unbalanced demand and supply chain, and does not support the sustainable economic development. The challenge for Québec is to expand canola cultivation to meet local demand and to contribute to nationwide demand for oilseed production.

Currently, Québec canola growers are creating a fertilization program, to verify the seeding rate and time, fertilizer application rate and method, pesticide, to maximize canola yield (TRT ETGO du Québec, 2011). For instance, farmers may need to sow in early May so canola can bloom before the humid and hot season (over 28 °C) in the June and July months, to avoid flower and seed abortion attributed from heat stress (Gan et al., 2007). Therefore, local refinement of the recommended fertilization, including fertilizer types, rates, and timing of application and placement method, is crucial to achieve profitable canola cultivation in Québec at greater scale.

Like other non-legume crops, N is the most limiting nutrient in canola production (Grant and Bailey, 1993). Nitrogen fertilizer is recommended at 110 to 120 kg N ha<sup>-1</sup> for the maximum yield in the Prairie Provinces and 100 to 110 kg N ha<sup>-1</sup> in Ontario (Thomas, 2003; Government of Saskatchewan, 2008). At some locations, canola can respond to N over 200 kg N ha<sup>-1</sup> (Ahmad et al., 1999; Cutforth, et al., 2009). Although higher shoot biomass can be achieved at higher rates of N fertilizer, it lowers the oil concentration of the oilseed, reduces seed quality and results in a lower nitrogen use efficiency (NUE) (Grant and Bailey, 1993). Also, high N fertilizer supply mineral N

that is prone to loss to the environment (primary from nitrate) and transformed to other plant-unavailable forms (ammonia, nitrous oxide, and organics) by microorganism (Thomas, 2003). Canola requires and assimilates N at different rates throughout its growth and development. According to Saskatchewan Soil Conservation (2000), 75 to 80 % of the N required by canola is taken up in the period within 5 weeks after emergence, with greatest uptake rate between 3 and 5 weeks after seeding. Therefore, synchronizing canola N demands with soil mineral N supply can minimize N exposure to the environment, increase canola N uptake from applied N fertilizer and decrease the amount of fertilizer required for maximum economics.

The objective of the study were to

- i. To monitor soil mineral N ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) dynamics and canola straw nutrition using two fertilization methods - a pre-plant and split-application of fertilizer N at Agronomy Research Centre on the Macdonald Campus of McGill University at St-Anne-de-Bellevue, Québec, using a fractional factorial experimental design;
- ii. To compare the harvesting index (HI) of canola grown in experimental units in sandy clay loam soils located in Ste-Anne-de-Bellevue, and in coarse-textured sandy loam soils located in Ottawa and in St-Augustin-de-Desmaures, using a completely randomized block design.

## CHAPTER ONE: LITERATURE REVIEW

### 1.1 Canola history and characteristics:

Cultivation and usage of canola dates back to hundreds years ago in Asia when it was called rapeseed and extracted to obtain lubricants and lamp oil (Daun, 2011). During World War II, rapeseed was used as cooking oil. However, rapeseed was high in erucic acids, which are detrimental to human health. In the 1970s, canola was bred from rapeseed by Dr. Downey and Dr. Stefansson in Manitoba, to produce an oilseed with lower erucic acid and glucosinolate concentrations (Anstey, 1986). The name “canola” was derived from “Canadian oil, low acid” (Anstey, 1986). According to the international standards, only rapeseed containing less than 30  $\mu\text{moles g}^{-1}$  of glucosinolates and less than 2% erucic acid is certified as canola (Thomas, 2003). Nowadays, canola is considered one of the healthiest cooking oils and an ingredient for livestock meal (Daun, 2011). There has been increasing interest in using canola for biodiesel production due to its high oil content and low saturated fat concentration. A high oil content produces more oil per unit of seed, and low saturated fat concentrations lowers the temperature where crystals form in the biodiesel, which is ideal for fuel burned during cold winter months in Canada (Daun, 2011).

Canola belongs to one of the most widely cultivated families, the Brassicaceae or Cruciferae. *Brassica napus*, *B. rapa*, and *B. juncea* are the commonly cultivated canola species in Canada (Daun, 2011). *Brassica napus* is most widely cultivated and has the highest oilseed yield potential (Gan, et al., 2007), so the rest of the review will focus on this species and refer it to canola. Canola requires 90 to 120 growing days to

reach maturity (Thomas, 2003). As a typical Brassicaceae, it has greyish-green leaves and 9 to 30 leaves on the main stem (Hammond, 2011). According to the 2011 canola performance trials conducted in Western Canada (Manitoba, Saskatchewan, Alberta, and British Columbia), canola height averages from 0.9 to 1.5 m with oilseed yields between 3051 and 5403 kg ha<sup>-1</sup> (Senko and Hammond, 2012).

Canola's growth can be divided into 5 stages according to morphology: (1) germination and emergence, (2) leaf development and stem elongation, (3) flowering, (4) seed development, and (5) ripening (Daun, 2011). Among the growth stages, the early flowering stage (i.e.: 20% of all buds raceme flowering, about 6 to 8 weeks after seeding) is the most critical for pod and seed development. This is when canola is most susceptible to pests, disease (e.g. Sclerotinia stem rot), heat stress (when temperature is over 28°C), and drought. The early- to mid- flowering stage is also characterized by substantial leaf abscission due to nitrogen (N) remobilization and translocation from leaves to pods and seeds (Zhang, 1991; Hammond, 2011). Seed development and ripening stage lasts 40 to 60 days in total, during which buds convert to green pods, and the yellow and green seeds ripen to brown or black (Daun, 2011).

## 1.2 Role of nitrogen in canola

Nitrogen is the most important determinant of canola's optimum nutrition and high yield due to its high N demand and an inadequate N supply in unfertilized soils (Grant and Bailey, 1993; Jackson, 2000; Malhi, 2001; Masclaux-Daubresse et al.,

2010). Nitrogen (1) is an integral part of amino acid, genetic materials, and proteins, enzymes, vitamins and hormones, (2) regulates and stimulates vegetative and reproductive developments and (3) enhances the uptake of other nutrients (e.g. phosphorous and boron) (Barker and Bryson, 2006). The majority of N is stored in leaves. At least 25% and more often over 75% of the leaf N is contained in the chloroplasts - the hub of photosynthesis (Barker and Bryson, 2006). Canola N uptake is highest before flower initiation when canola shoots contain approximately 24 g N kg<sup>-1</sup> (Thomas, 2003). Shoot N concentration is correlated with oilseed yields due to the fact that most of the shoot N is translocated to the reproductive organs. Apart from the visible roles of N to increase biomass and oilseed yield, the composition of nitrogenous compounds (protein or amines) determines oilseed quality (Grant and Bailey, 1993; Malhi, 2001; Chamorro et al., 2002). Conversely, excessive N applications are attributed to lodging and inferior quality oilseeds with oil concentration less than 35% (Thomas, 2003). Consequently, the challenge for agronomists and soil scientists is ensuring an optimum supply of N to the canola crop, particularly at the early vegetative growth stage, to achieve target yields of oilseed with 17-26 % protein content (Malhi, 2001).

#### 1.2.1 Nitrogen assimilation, accumulation and distribution in canola

Once N is intercepted by plant roots, redox transformations and remobilization takes place throughout the canola plant's life cycle (Barker and Bryson, 2006). Plants directly synthesize ammonium (NH<sub>4</sub>-N) to amino acids, while nitrate (NO<sub>3</sub>-N) needs



to be reduced to  $\text{NH}_4\text{-N}$  before being utilized. This two-step reduction involves the 1) reduction from nitrate to nitrite in the cytoplasm of the roots and shoots and 2) nitrite reduction to ammonium in the chloroplasts (Barker and Bryson, 2006; Masclaux-Daubresse et al., 2010). About 70% of the N required by canola is taken up before flowering, then translocated from the leaves and stems to pods and seeds (Hocking et al., 1997; Saskatchewan Soil Conservation Association. 2000; Mendham and Roberson, 2004; Malagoli et al., 2005). Before the rosette stage (about a month after planting, during the leaf development and stem elongation stage), the leaf is the main N reservoir (75%) regardless of N fertilizer application (Schjoerring et al., 1995). The stem becomes a major N sink (50%) at the early flowering stage (around 6 to 8 weeks after seeding). At the end of the flowering stage, pod wall and stem are the main N pool, at 40% each. At the maturity, seed is the main N storage pool (80%), with 10% of the N remaining in stem and 10% N in pod wall (without leaves) (Schjoerring et al., 1995). Knowing canola's ability to utilize N remobilized from leaves, stems and pod walls, and allocating to oilseed production informs producers when to apply N fertilizer to achieve high N use efficiency.

In summary, canola N concentration is highest before peak vegetative growth and declines as biomass accumulates during the later stages of crop development. Consequently, we expect that the petiole  $\text{NO}_3\text{-N}$  concentration at various growth stages is related to oilseed yield as demonstrated in Figure 1.

### 1.3 Soil nitrogen supply

### 1.3.1 Mineral nitrogen

Nitrate ( $\text{NO}_3\text{-N}$ ) and ammonium ( $\text{NH}_4\text{-N}$ ) are the soluble N forms that are immediately available to canola with  $\text{NO}_3\text{-N}$  being the dominant form in aerobic soils. These two ions percolate through the rooting zone, and are conveyed by transport systems within the plant. They are then assimilated and transported to targeted organs, where they are synthesized into various biological compounds (Masclaux-Daubresse et al., 2010). Although the metabolic cost of absorption and assimilation of  $\text{NO}_3\text{-N}$  is far greater than that for  $\text{NH}_4\text{-N}$ , canola achieves optimal growth with a mixed N fertilizer, such as ammonium nitrate and calcium ammonium nitrate (Thomas, 2003).  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations fluctuate greatly during the growing season because they are affected by various abiotic (temperature, precipitation) and biotic (microbial) factors and soil physical properties (Barker and Bryson, 2006). Sharifi et al. (2007) reported two to three orders of magnitudes difference in soil  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations during the growing region in Atlantic Canada, which gives an idea of the variability in soil mineral N to canola in a humid temperate climate like Eastern Canada. Leaching, immobilization, denitrification, volatilization, erosion and adsorption on clay colloids are transformations that reduce  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  pools that are accessible to canola (Hammond, 2011)

The challenge for canola producers is to ensure that there is adequate amount of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  in the soil solution for crop uptake during the growing season, and to minimize N losses so as to maximize profits. The N inputs that increase soluble N forms and pathways that lead to  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  losses from the soil-plant system

are summarized in Figure 2.

### 1.3.2 Organic nitrogen

Between 95% and 99% of the soil nitrogen is found in organic forms such as proteins, amino acids, and amino sugars (Brady and Weil, 2002). Although organic N cannot be used by plant directly, it serves as a residual source of soil fertility. Mineralization is the process of organic N conversion to  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ , through microbial-mediated reactions of ammonia oxidation and nitrification. These microbial-mediated reactions are influenced by soil temperature, moisture content, and substrate availability (e.g.: C/ N ratio of organic fertilizer) (Brady and Weil, 2002). The mineralization rate is generally low, 0.4% to 5% of the soil organic N pool per year (Thomas, 2003). However, in the humid temperate regions of Eastern Canada, in-season N mineralization can contribute to greater crop production than in the prairie region (St. Luce et al., 2011). Ma et al. (1999) reported that 20 kg N ha<sup>-1</sup> were mineralized from stockpiled and rotted dairy manure applied at 800 kg N ha<sup>-1</sup> for maize production under a Gleysol soil in southern Ontario. In 40 potato fields in New Brunswick and Prince Edward Island, Canada and Maine, USA, the N mineralized from organic matter during growing seasons contributed to 4% to 75% (average of 60%) of total plant N uptake (Sharifi et al., 2007). To benefit from mineralized N and achieve maximum oilseed yields in canola cultivation, while minimizing inorganic N inputs, farmers need to ensure that N mineralization was in synchrony with crop N requirements (Ma et al., 2005; Barker and Bryson, 2006).

#### 1. 4 Canola's response to nitrogen fertilizers

Multiple studies demonstrate improved canola plant growth and development, and oilseed production in response to N fertilizer application (Grant and Bailey, 1993; Malhi, 2001; Thomas, 2003). A canola plant's leaf area, the number of leaves per plant, plant height, number of flowering branches, number of pods, and oilseed yield all increase with increasing rates of N fertilizer (Table 1, Table 2) (Ogunlela et al., 1989; Ozer, 2003).

In Ontario, which has a similar temperate humid climate to Québec, the recommended N fertilization for canola is 100-110 kg N ha<sup>-1</sup>, which gave an average yield of 2863 kg ha<sup>-1</sup> in 2011 (Earl, 2011; Hall, 2012). Table 3 summarizes maximum oilseed yields in response to different N fertilizer rates in studies under different soil types and climate conditions (details on precipitation and temperature are not provided). The rates are not the provincial recommended rates as fertilizer and oilseed prices are not taken into considerations.

Soil texture can affect canola's responses to N fertilizers as soil texture impacts water holding capacity, organic matter content, and susceptibility to erosion, eventually will influence the fate of applied N. Nitrogen loss through leaching of medium and fine soil was 10% to 15% less than coarse soil in maize field in south-eastern U.S. (Brady and Weil, 2002). However, there is no adjusted N fertilizer recommendation based on soil texture in Québec.

##### 1.4.1 Nitrogen fertilizer and oilseed quality

Although N fertilizer application boosts biomass accumulation of canola, the oilseed's oil concentration declines with the increasing levels of N application because there is an increasing protein concentration at the expense of the oil concentration (Mason and Brennan, 1998; Brennan et al., 2000; Malhi, 2001; Gan, 2007, 2008b). The sum of the oil and protein concentration is 60% to 65% (Brennan et al., 2000; Thomas, 2003). Mendham and Roberson (2004) reported that the oil concentration decreases by 0.6% to 1.2% per additional 100 kg ha<sup>-1</sup> N applied. As canola is grown primarily for oil extraction, N fertilizer recommendation needs to be calibrated to achieve optimal plant nutrition and maximize the oil concentration in harvested seed.

#### 1.4.2 Canola nitrogen use efficiency

Canola is characterised by low nitrogen use efficiency (NUE) (between 12% and 40%, depending on the cultivar) (Gan et al., 2007). In canola, the NUE is generally defined as the ratio between seed yield (kg N ha<sup>-1</sup>) and N fertilizer input (kg N ha<sup>-1</sup>) (Gan et al., 2008a) or N accumulated in the whole plant (Svečnjak and Rengel, 2006), although there are other methods of expressing NUE (Hocking et al., 1997). Low NUE can be the consequence of extensive leaf abscission during pod development stages. Hocking et al. (1997) reported that dead leaves contained 20 to 25 mg N kg<sup>-1</sup>, which was equivalent to 17.5 kg N ha<sup>-1</sup> yield lost. About 50% of the N lost in abscised leaves originated from the applied N fertilizer (Schjoerring et al., 1995). Masclaux-Daubresse et al. (2010) explained that the massive leaf senescence was the

consequence of negatively enzyme-regulated N uptake during the transition from the vegetative to the reproductive growth. Canola is rather inefficient in utilizing fertilizer N to produce oilseed, therefore better N fertilizer management, which based on the soil N transformations, N uptake rates, and remobilization within canola plant could be helpful.

## 1.5 Indicators of soil nitrogen availability to canola

### 1.5.1 Soil mineral nitrogen test

A soil N test can estimate the soil fertility status of N, and provide farmers a reasonable guide to adjust N fertilizer inputs to achieve optimum yield. The most common test uses 2M KCl, a neutral salt solution, to extract  $\text{NO}_3\text{-N}$  and exchangeable  $\text{NH}_4\text{-N}$  from the soil (Maynard et al., 1993). The  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations can be quantified by methods such as colorimetry, ion-selective potentiometry, steam distillation, microdiffusion, ion chromatography, and ultraviolet (UV) spectrophotometry (Mulvaney, 1996). Among them, colorimetric methods and steam distillation are the most widely used. Colorimetric methods are advantageous over steam distillation because 1) there are less environmental impacts by replacing copperised Cd with Devarda's alloy (mainly consists of Cu, Zn, and Al) than, the chemical is to reduce  $\text{NO}_3\text{-N}$  to  $\text{NH}_4\text{-N}$ ; and 2) there are fewer carryover contaminations between analyses compared to batch analysis (Sims et al., 1995).

#### 1.5.1.1 Pre-plant mineral nitrogen test

Pre-plant mineral N test is used to adjust N application rates in Manitoba and other semi-arid regions in Canada according to the expected oilseed yields (Table 5). Pre-planting soil N test can estimate N credits from the previous growing seasons and N mineralization in the early season (Ma and Wu, 2008). However, it cannot consider spatial and temporal heterogeneity in soil  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  or predict N available to canola from in-season mineralization, especially in humid climate regions such as Eastern Canada. As unpredictable rainfall, in late April or early May, can aggravate N loss by leaching and runoff (Ma et al., 2005; Zebarth et al., 2005), in-season soil N test and post-emergence application is an alternative (Thomas, 2003). However, there is no pre-plant soil N test calculated from crops grown in Québec, and this is a topic where research is going (St. Luce et al., 2011)

#### 1.5.1.2 Pre-sidedress soil mineral test and consequent nitrogen split application

The pre-sidedress soil  $\text{NO}_3\text{-N}$  test has been proven to be successful in maize cultivation in New Jersey (USA) (Salardini et al., 1992) and southern Ontario (Canada) (Vyn et al., 1999). For a more canola related example, an application of  $50 \text{ kg N ha}^{-1}$  applied before seeding and  $50 \text{ kg N ha}^{-1}$  sidedressed 5 weeks after seeding led to a 25% increase in yield in *Brassica campestris* production in India. However, soil  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations were not reported before sidedressing in that study, so soil mineral N status cannot be used to predict oilseed yields (Ahmad et al., 1999).

Split application of N fertilizer did not increase total oilseed yields or other

components of yield (e.g.: oil concentration, number of pods per plant, number of seeds per pod) in Australia or Pakistan (Taylor et al., 1991; Cheema et al., 2001). Low water availability (30 mm at the early flowering stage which is 6 to 8 weeks after seeding) and high temperatures (daytime  $> 25^{\circ}\text{C}$  and nighttime  $> 17^{\circ}\text{C}$ ) were the primary reasons for inefficient N uptake and N translocation from leaves to pods for N split application (Taylor et al., 1991; Thomas, 2003). Water availability affects the solubility of N fertilizer and temperature affects evapotranspiration which controls uptake of soluble  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  (Brady and Weil, 2002). Therefore, site-specific N fertility management is essential for better NUE in canola production systems in Québec because of large variation in soluble  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  pools in this humid temperate climate.

#### 1.5.2 Plant tissue analysis as an indicator of plant nitrogen status and soil nitrogen supply

Plant tissue analyses demonstrate the crop nutritional profile in a more direct way than soil N tests. Total N is an essential indicator of N status in the plant. The Dumas technique, converting all forms of N to molecular  $\text{N}_2$ , is the most extensively used method (Jones and Case, 1990). Flash dynamic combustion, a modified Dumas approach, is more popular because it is fast, precise and accurate. LECO<sup>TM</sup> CHN and Flash EA<sup>TM</sup> NC soil analyzer are the equipment that is based on this theory. Although these two apparatus are primarily used for total N of soil, they can be used to measure plant materials after being calibrated.



Hocking et al. (2002) studied the effects of N fertilizer (0 to 150 kg N ha<sup>-1</sup>) on N uptake by canola during 1991 to 1992. They reported at the early flowering stage N concentrations averaged at 26.0 mg N g<sup>-1</sup> and varied between 20.6 and 34.8 mg N g<sup>-1</sup> in a late sowing year (1991) compared to 23.8 mg N g<sup>-1</sup> and varied between 16.8 and 29.9 mg N g<sup>-1</sup> when sowings were conducted at the recommended date (1992). At harvest, shoot N concentrations varied between 3.7 mg N g<sup>-1</sup> and 7.2 mg N g<sup>-1</sup> in two growing seasons, and mean N concentration in 1991 was 0.6 mg N g<sup>-1</sup> higher than that of 1992. Interestingly, biomass (kg ha<sup>-1</sup>) at the early flowering stage and harvest were negatively correlated to straw N concentrations. The highest biomass (14040 kg ha<sup>-1</sup>) (shoots and oilseeds) was achieved in 1992 when the straw N concentration was 22.8 mg N g<sup>-1</sup> at the early flowering stage and 5.6 mg N g<sup>-1</sup> after harvest. Hocking et al. explained that the dry condition in 1991 stimulated the premature ripening of canola and 2 °C lower temperatures at pod and seed filling stage in 1992 increased the oilseed potential. Similar canola N fertility studies were conducted by Qian and Schoenau (1999); Jackson (2000); Svecnjak and Rengel (2006). Jackson reported that canola shoots contained 12.4 mg N g<sup>-1</sup> with a yield of 2452 kg N ha<sup>-1</sup>. Table 5 presents shoots and oilseeds N concentrations at early flowering and harvesting, and in response to dry matter accumulation and oilseed yields.

The major shortcoming of plant tissue test is its “hysteresis”, because it may be too late to correct deficiencies for in-season crops due to the time needed for plant sampling and analysis (Scherer, 2001; Malhi and Gill, 2007). Therefore, agronomists suggested testing young tissue at vegetative growth stages, between stem elongation

and floral initiation, when N assimilation rate is high (Thomas, 2003).

#### 1.6: Balanced nutrition management with sulfur and boron

Scientific literature indicates that the ratios of N and S fertilizers or plant available N and S concentrations in the soil are important in canola production (Grant and Bailey, 1993; Jackson, 2000; Zhao et al., 1992). For optimum plant nutrition, canola has high sulfur (S) demand, accounting for 30% of the plant total N, compared to 9% to 15% in other crops (Thomas, 2003). Sulfur is contained in the biologically active compounds, such as biotin, glutathione, thiamine and coenzyme A, and is important in energy transfer and protein structure (Scherer, 2001). Sulfur is particularly necessary in the synthesis of secondary S-metabolites in Brassica species, such as glucosinolates (McGrath and Zhao, 1996; Jackson, 2000). Sufficient S is necessary through all growth stages as N metabolism can be disrupted by S deficiency (Duke et al., 1986; Malhi, 2001; Malhi and Gill, 2007; Jamal et al., 2010). Likewise, N application rates affect canola's S uptake, and vary according to S fertilizer rates. At the low S rates (e.g.: 5 kg S ha<sup>-1</sup>), N application reduced S uptake reflecting the suppressed seed yields. At the higher S rates (40 kg S ha<sup>-1</sup>), however, N application increased S uptake because of the enhanced dry matter accumulations (Janzen and Bettany, 1984). Manitoba Agriculture and Food (2001) suggests that application of fertilizer in an N:S ratio between 5:1 to 8:1 will supply adequate S. Table 6 illustrates reduced seed yields when N applied at the absence of S, and maximum yields with appropriate ratios of N and S.

Canola is considered to be a high B demanding crop, with a greater B fertilizer recommendation ( $2 \text{ kg B ha}^{-1}$ ) than wheat and corn ( $<1 \text{ kg B ha}^{-1}$ ) (Malhi, 2001; Gupta, 2007). Boron is critical for flower formation, pollination, and at the seed formation stage (Grant and Bailey, 1993). Canola's accessibility to  $\text{H}_3\text{BO}_3$  and  $\text{H}_2\text{BO}_3^-$ , the major soluble B forms that are immediately available to crop, depends on high water availability. Droughty soil also reduces B release from organic matter, which is the major B reservoir. Plant-available B ranged from  $0.38$  to  $4.67 \text{ mg B kg}^{-1}$  in Eastern Canada (Gupta, 2007), which is sufficient to meet the needs of most crops except alfalfa in Ontario (Minister of Agriculture, Food, and Rural Affairs, 2011). According to Karamanos et al. (2003), boron didn't increase oilseed yields in any of the 22 locations they studied in the prairie regions, even when the soil plant-available B ( $0.15 \text{ mg B kg}^{-1}$ ) was considered insufficient for canola production. In Ontario, Earl (2010) reported foliar applied B was effective on alleviating heat stress at the early flowering stage and resulted in 5.7% greater yield in a drought year. Given that canola's responses to B fertilizer may depend on climate and region, site-specific validations should be done to clarify its feasibility in Québec.

### 1.7 Conclusion and future research direction

Nitrogen is the most essential nutrient in canola production that directly contributes to yield and seed quality and end-of-season profits. Soil N is difficult to manage as it is influenced by soil physical and chemical properties, various abiotic (rainfall, temperature), and biotic (microorganisms) factors. In Québec, there is no

adjusted fertilizer recommendation that integrates weather and soil nutrition status in the early growing season (St. Luce et al., 2011). In other words, N fertility management (fertilizer types, rates, placement methods) may increase NUE at one site but be less effective in another system. Given the importance of S and B on canola, balanced nutrition management involving S and B fertilizers requires more site-specific validations because their application depends on background soil nutrient status and changing weather affects crop's response to fertilizers.

Further research that is relevant for expanding and improving canola production in Qu bec should be to: 1) define the critical soil and plant shoot concentrations of  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and  $\text{SO}_4\text{-S}$  during important growth stages (i.e. five weeks after sowing when N uptake rates accelerate, and the early flowering stage); 2) investigate the contribution of soil organic matter for higher soil mineral N status to canola field; 3) correlate canola's uptake capacity of N, S, and B with soil water availability and temperature within humid temperate climate of southern Qu bec; 4) identify the optimal N, S, and B fertilizers rates for maximum yield and oilseed quality. For this project, I will focus on: 1) soil  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  changes at the critical growth stages at the field scale; 2) optimize canola nutrition for maximum yield.

Based on this literature review, five hypotheses can be proposed:

In the field setting I would like to test:

- 1) soil mineral N ( $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$ ) concentration decrease during the growing season;
- 2) split application of N fertilizer results in higher soil mineral N concentrations

compared to the pre-plant N fertilization.

In the pot study I would like to see:

- 3) straw and oilseed yields of canola are highly responsive to increasing N fertilizer inputs;
- 4) Residual soil mineral N concentration is positively correlation to straw N concentration and yield;
- 5) Canola grown in clayey soil is more responsive to N fertilizer than those grown in sandy soil.

Table 1: Increased canola leaf area per plant ( $\text{dm}^2$ ) and number of leaves during the generative growth using N fertilizer in a hydroponic study (Ogunlela et al., 1989)

N supply (ppm)	Leaf area plant <sup>-1</sup> ( $\text{dm}^2$ )			Number of leaves per plant		
	Early flowering	10 DAF	27 DAF	Early flowering	10 DAF	27 DAF
30	12	13	10	20	19	19
100	36	34	27	25	28	26

\*30 ppm is equivalent to  $30 \text{ kg N ha}^{-1}$ , and 100 ppm is equivalent to  $100 \text{ kg N ha}^{-1}$

\*DAF denotes days after flowering

Table 2: Effect of N fertilizer rate on some agronomic characters of canola grown in Turkey (Ozer, 2003)

Treatment (kg N ha <sup>-1</sup> )	Plant height (cm)	Branch number	Pod number	Oilseed yield (kg N ha <sup>-1</sup> )
0	106 <sup>d</sup>	4.97 <sup>d</sup>	18.8 <sup>c</sup>	788 <sup>d</sup>
80	112 <sup>c</sup>	5.06 <sup>c</sup>	222 <sup>b</sup>	1103 <sup>c</sup>
160	114 <sup>b</sup>	5.37 <sup>b</sup>	247 <sup>a</sup>	1295 <sup>b</sup>
240	116 <sup>a</sup>	5.63 <sup>a</sup>	254 <sup>a</sup>	1325 <sup>a</sup>

Within a column, letters (a, b, c, and d) indicate whether treatments are different from each other ( $P < 0.05$ ), starting with the treatments with highest doses.

Table 3: Canola maximum oilseed yields in relation to nitrogen fertilizer rates at temperate regions

Location	N applied (kg ha <sup>-1</sup> )	Yield (kg ha <sup>-1</sup> )	Soil texture classification	Reference
Conrad (Montana, USA)	200	2650	Clay-Loam Argiustolls	(Jackson, 2000)
Saskatoon (Saskatchewan, Can.)	135	1684	Clay loam Dark Brown	(Gan et al., 2007)
Wongan Hills South Wales (Aus.)	138	1801	Sandy loam Red-brown	(Mason and Brennan, 1998)
Swift Current (Saskatchewan, Can.)	217	2308	Orthic Brown Chernozem	(Cutforth et al, 2009)



Table 4: Nitrogen sufficiency levels of shoot and oilseed at the early flowering and harvest stage

Growth Stage		Sufficient	Reference
		(g N kg <sup>-1</sup> )	
Early flowering	shoot	~24	Hocking et al. (2002)
			Thomas (2003)
Harvest	shoot	~16	Janzen and Bettany (1984)
		>12.4	Jackson, (2000)
		5 ~15	Thomas (2003)
		>14.6	Svečnjak and Rengel (2006)
	oilseed	2.72~6.16	Aider and Barbana (2010)
		3.4~4	Thomas (2003)

\*total N concentration of oilseed is calculated by dividing protein concentration by

6.25

Table 5: Nitrogen fertilizer recommendations based on soil NO<sub>3</sub>-N concentration after canola harvest in relation to expected seed yields in Manitoba

Fall soil NO <sub>3</sub> -N (0-60cm)	Expected seed yield (kg ha <sup>-1</sup> )			
	2290	2036	1781	1527
	Recommended N fertilizer rate (kg N ha <sup>-1</sup> )			
22.4	179	151	118	84
33.6	162	129	95	62
44.8	140	106	78	45
56.0	123	90	62	28
67.2	112	78	45	17

Source: Manitoba Agriculture, Food and Initiatives (2004)

Table 6: Effect of N and S fertilizer supply on oil seed yield

Treatment	Seed yield (g pot <sup>-1</sup> )			
	500 mg N		1000 mg N	
	NIKLAS	TOPAS	NIKLAS	TOPAS
Control	0	0	0	0
25 mg S	2.10	0.9	0	0
50 mg S	3.15	2.85	1.25	0.35
75 mg S	2.55	2.65	5.30	5.85
100 mg S	3.05	2.50	6.70	7.50

\*NIKLAS and TOPAS are two of commercial varieties.

Source: Thomas (2003)

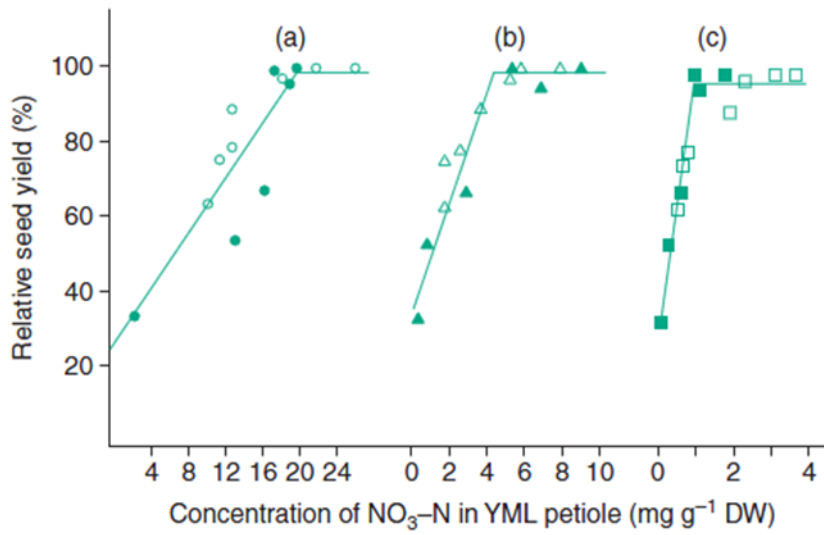


Figure 1: Correlation between  $\text{NO}_3\text{-N}$  ( $\text{mg g}^{-1}$  DW) in dried petioles of the youngest mature leaf (YML) of canola at three stages of development: (a) 5 to 6 leaf rosette, (b) flower buds are visible, and (c) initiation of flowering, and relative seed yield expressed as a percentage of the best treatment at each of the two sites. (Hocking et al., 1997).

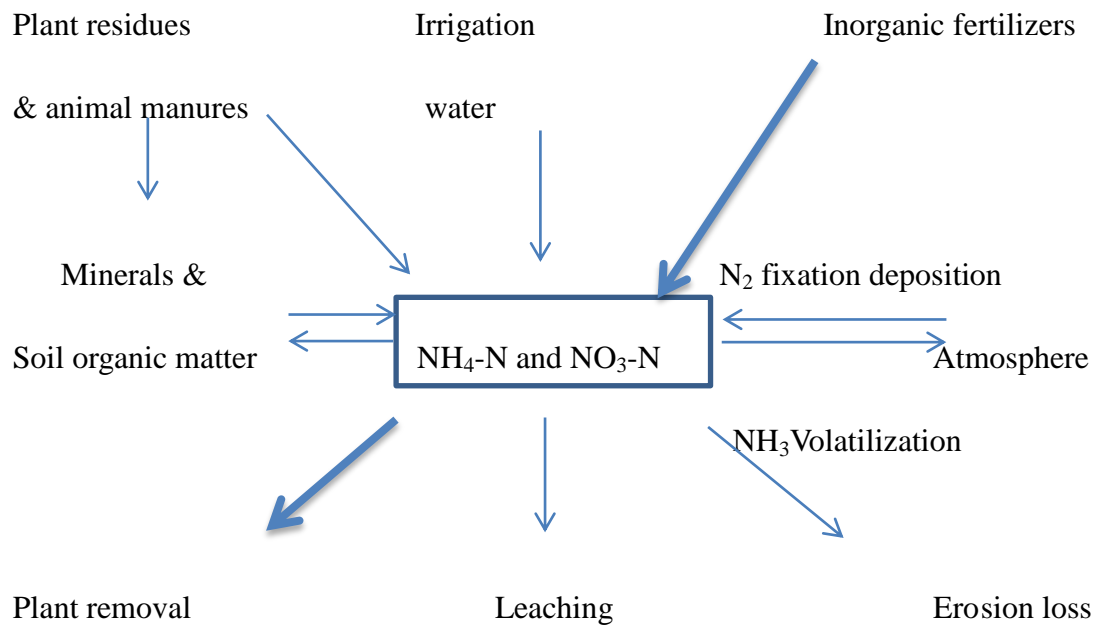


Figure 2: Soil mineral nitrogen inputs and outputs regulated by various approaches. Although crops are much less competitive than microbes in accessing the soil mineral N, N removed by crops at the end of season accounts for more soil nitrogen depletion than other outputs. Canola can remove  $34 \text{ kg N ha}^{-1}$ , basically from inorganic N fertilizer and in-season mineralized N, by the end of the season (Brady and Weil, 2002)

## **CHAPTER TWO: SOIL AND PLANT ANALYSIS TO INDICATE CANOLA NITROGEN RESPONSE TO VARIABLE RATES AND METHODS OF NITROGEN FERTILIZATION**

### **2.1 Abstract**

Canola (*B. napus*) requires higher nitrogen (N) inputs than other macronutrients (P, K, S) for optimum yield. However, little information is available on the response of this crop to N fertilizer in relation to soil mineral N and plant tissue analysis within the temperate humid region of southern Québec. Accordingly, a two-year study was initiated to monitor the soil mineral N dynamics during the growing season to test whether the soil mineral N concentration can be correlated to straw nutrient composition. The field experiment was conducted in Ste-Anne-de-Bellevue, Québec in 2011 and 2012. Urea was applied at rates of 0, 50, 100 and 150 kg N ha<sup>-1</sup> at sowing, and 50 + 50 and 50 + 100 kg N ha<sup>-1</sup> as split application with the second dose being sidedressed six weeks after sowing. Plots receiving high N fertilizer input (>100 kg N ha<sup>-1</sup>) had higher residual soil mineral N concentration but not higher straw N concentrations (LSD, NS) in the 2011 trial. There was no difference between single and split N applications. In the 2012 trial, the NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations in plots fertilized with 150 kg N ha<sup>-1</sup> were good indicators of changes in the soil mineral N status during the growing season ( $r=0.96$  and  $r=0.79$ ). However, additional field validation is recommended due to high spatio-temporal variability within the field site.

## 2.2 Introduction

Canola production in Eastern Canada is low (approximately 11 000 t per year) compared to Saskatchewan (43 000 t per year) (Ruel and Tardif, 2012; Senko and Hammond, 2012). The two primary causes for lower canola production in Eastern Canada are: (1) canola breeding programs are concentrated in the prairie region of Canada (Daun, 2011); therefore, there are not many cultivars that are well adapted to the humid growing conditions in Eastern Canada (TRT ETGO du Québec, 2011), and (2) historically, there was little canola cultivation in Eastern Canada. Lack of information on canola production, including fertilizer rates and application methods, pest and disease prevention, and management throughout the life cycle, limits producers' ability to achieve profitable canola yields (Ruel and Tardif, 2012). For instance, fertilizer guidelines provided for Québec are based on recommendations from the Prairie Region. Without site-specific validations, producers may insufficiently or excessively supply nutrients to canola grown in Québec. Therefore, an appropriate fertility program that considers the best fertilizer types, application rates and methods is imperative before expanding cultivation of the canola crop in Eastern Canada.

Among the fertilizers that are essential to balanced canola nutrition, nitrogen (N) is crucial because N fertilizers increase yield more than any other element, and N accounts for the highest canola farming input (Grant and Bailey, 1993). According to Centre de Référence en Agriculture et Agroalimentaire du Québec (CRAAQ) (2010), N is recommended to be applied at the rate of 100 to 150 kg ha<sup>-1</sup>, compared to P and S

at 20 kg ha<sup>-1</sup> each. However, achieving synchrony between plant N demand and soil plant-available N for optimum oilseed yield is complicated. Particularly in the humid region of southern Québec, leaching can contribute to greater N loss than experienced in the Prairie Region (St. Luce et al., 2011). Information on soil mineral N dynamics in canola fields can help develop N fertilizer application strategies to maximize the yield, improve N use efficiency (NUE) and the producers' profits while protecting the environment. However, we need soil mineral N data from canola cropping systems since the soil mineral N dynamics under the common field crops grown in Québec (e.g.: corn, cereals) are not useful due to their different growth habits, root architecture, and N uptake patterns compared to canola (CRAAQ, 2010).

Compared to traditional N broadcasting prior to seeding, N split application offers the opportunity to match a plant's nutrient demand with the timing and the quantity required for local conditions (Ma et al., 2005). According to the Saskatchewan Soil Conservation Association (2000), canola biomass accumulation accelerates from the third week after germination and slows down at the week six. Nitrogen uptake during this period accounts for 75% to 80% of the N required for oilseed production biomass accumulation. Therefore, a split application of N fertilizer between the third and sixth week after seeding can be highly beneficial for canola. In corn production in southern Ontario, Vyn et al. (1999) reported positive correlations of  $r=0.61$  to  $0.93$  between soil nitrate (NO<sub>3</sub>-N) concentrations prior to the N split application and corn grain. Canola could respond in a similar manner in Québec since climate conditions are similar to those in southern Ontario. However split application



of N fertilizer did not increase total oilseed yields or other canola yield parameters (e.g.: oil concentration, number of pods per plant, number of seeds per pod) in semi-arid regions of Australia or Pakistan (Taylor et al., 1991; Cheema et al., 2001). Therefore canola's response to split application of N fertilizer may depend on weather conditions, particularly soil moisture. If N-split application is successful in Québec, the trade-off between labour, time and magnitude of increased yield should still be considered before recommending this practice for canola production systems.

The objectives of this study were to (1) record changes in soil mineral N ( $\text{NH}_4\text{-N}$  +  $\text{NO}_3\text{-N}$ ) during canola growth in field plots located in Québec, (2) evaluate the efficacy of N-split application in maintaining high soil  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations during the growing season and higher straw N concentration at harvest.

## **2.3 Materials and methods**

### **2.3.1 Site description**

The experimental site is located at the Emile A. Lods Agronomy Research Centre on the Macdonald Campus of McGill University in Ste-Anne-de-Bellevue, Québec (45°3' N, 74°11' W). Long term (from 1971 to 2000) average monthly temperature and precipitation from April to August are 15.6 °C and 81.4 mm respectively (Environment Canada, 2012). Soil at this site is a mixed, frigid Typic Endoaquent of the Chicot series (Humic Gleysol). Soil characteristics are provided in Table 1.

### 2.3.2 Experiment design

The experiments were conducted from May to August 2011 and 2012. The 2011 trial followed a wheat (*Triticum aestivum* L.) crop, and the 2012 trial was in a nearby plot that was fallow in the previous year. The experiment was a randomized complete block design with 4 replicates (Appendix 1). Each block had 26 treatments in 2011 and 28 treatments in 2012, which gave a total of 104 plots in 2011 and 112 plots in 2012. Plot dimensions were 8 m by 3 m, allowing 14 rows of canola, with 20 cm spacing and 10 cm for buffer zones at the edge of each plot. There were 3 m wide buffer zones between blocks, and border plots at two sides of the field. Border plots received the same treatments as the neighboring plots.

In 2011, the 24 treatments received four levels of N (0, 50, 100 and 150 kg ha<sup>-1</sup>) applied as urea (46-0-0), crossed with two levels of S (0 and 20 kg ha<sup>-1</sup>) applied as ammonium sulfate (21-0-0-24), crossed with three levels of B (0, 0.5 foliar spray, and 2 kg ha<sup>-1</sup> soil applied) applied as alpine boron. The 24 treatments were tilled into the soil on May 7, 2011 and the seeding was done the same day. Last two treatments differed from the aforementioned N100-S20-B0.5 and N150-S20-B0.5 only in the way that the N applications were split into two doses: first doses at 50 and 100 kg N ha<sup>-1</sup> each on May 7, 2011, and the remainder of the N was side-dressed on June 13, 2011. The S0 plots received 17 kg N ha<sup>-1</sup> to balance the N contained in the plots that received ammonium sulfate. In 2012, two more treatments, N200-S20-B0.5 and N150+50-S20-B0.5, were added; the rest of the treatments remained the same.

### **2.3.3 Soil sampling and analysis in the 2011 trial**

In 2011, soils were sampled on May 30, three weeks after sowing (May 7), and September 1, after canola harvest. As the monthly rainfall (149 mm) in May was approximately twice as high as the long term average (74.1 mm) (Figure (3(a))), samples were taken from the plots only fertilized with N fertilizers (0 S kg ha<sup>-1</sup> and 0 B kg ha<sup>-1</sup> treatments) to investigate whether heavy rain leached the same soil mineral N status across the field. Soils (0-20 cm in depth) were taken from random positions in each plot with a soil probe (2.5 cm diameter), passed through a size 4 mess Tyler<sup>®</sup> sieve, and kept in Ziplock<sup>®</sup> bags. Samples were stored in 4 °C refrigerator before extraction. Within a week after sampling, soils were extracted with 2M KCl for the mineral N (NO<sub>3</sub>-N and NH<sub>4</sub>-N) concentration (Maynard and Kalra 1993). Briefly, for each sample, 50 mL 2M KCl was added to approximately 5g field moist soil that had been weighed into a 250 mL Erlenmeyer flask, shaken for 1 hour and filtered into acid washed plastic bottles using Fisher<sup>®</sup> Q5 filter paper (Fisher Scientific). Moisture content was determined from oven-dried samples (105 °C for 48 h). NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations were quantified via a Lachat Quick-Chem flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI, USA).

### **2.3.4 Soil sampling and analysis for the 2012 trial**

In 2012, soils were sampled (1) prior to the second dose of N split application on June 13 (5th week after seeding), (2) 20% early flowering stage June 26 (6th week after seeding), and (3) after harvesting on August 10 (26th week after seeding). Soils

were only taken from selected plots that received the following treatments: N0-S0-B0, N0-S20-B0, N0-S0-B2, N0-S0-B0.5, N150-S0-B0, N150-S20-B0, N150-S0-B0.5 and N150-S0-B2, from Blocks 1 and 4 (Appendix 1). The same soil sampling strategy (depth, sampling and sieving) and extraction methods were applied as in section 2.3.3., mineral N concentration was determined by the modified indophenol blue technique (Sims *et al.*, 1995), and spectrophotometric measurements of samples were done in triplicate using an EL312 Model microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

### **2.3.5 Plant sampling and analysis:**

Canola plants were harvested for nutrient analysis on August 17, 2011. Five plants that represented the overall growth status were cut as close as possible to the soil surface in the fifth row from the left facing the plot. Oilseeds were separated from the shoots. Shoots were dried in the oven at 60 °C for 48 hours until constant weights were reached. They were ground by a Milley® grinder to less than 1mm. Between 8 and 12 mg of ground shoots was weighed into tin capsules and the N concentration was determined with the ThermoFinnigan Flash EA 1112 CN analyzer (Carlo Erba, Milan, Italy). Other macronutrients, including P, K, Ca, and Mg, were determined by total wet digestion, following the procedures of Jones and Case (1990), based on the method of Parkinson and Allen (1975). Briefly, 0.16 g of ground plant tissue (<1 mm) was weighed directly to glass tubes where 4.4 ml of digestion reagent (H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>, Se and Li<sub>2</sub>SO<sub>4</sub>H<sub>2</sub>O as catalyst) was added to each sample. The tubes were

placed into digestion blocks pre-heated to 200 °C. An hour later, the temperature was increased to 340 °C and the samples were left for approximately 2 hours to complete digestion. After reaching room temperature, deionized water was added until a total volume of 100 mL was reached, and the mixture was homogenized by a Vortex<sup>®</sup>. The P concentrations were quantified by the Lachat Quick-Chem flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI, U.S.), and K, Ca, Mg concentrations were determined by a 2380 model atomic absorption spectrophotometer (PerkinElmer Inc. Waltham, MA.USA)

#### **2.3.6 Statistical Analysis**

. UNIVARIATE procedures in the SAS package (SAS System 9.2, SAS Institute, Inc, Cary, NC) were used to assess the normality and homogeneity of variance for each treatment. One-way ANOVA was performed to compare the averages of soil mineral N and straw N concentrations using the General Linear Model (GLM) procedure, and an  $\alpha$  value of 0.05 was considered significant. Least Significant Difference was used to separate the means. Contrast was used to compare the efficacy of N single and split application. Correlation analyses with Pearson's correlation coefficient were performed to see whether there were relations between soil mineral N concentration at the early growing and post-harvesting stages in straw N concentration

## **2.4 Results**

### **2.4.1 Weather conditions**

In both 2011 and 2012, the average maximum and minimum temperatures were 0.5 to 1.5 °C higher than the long term average, with a mean maximum temperature of more than 25 °C from mid-June to mid-August (Figure 1 (a)). Rainfall in the growing seasons had different patterns in the two study years. In 2011, plots received total rainfall of 656 mm from April to August, which was 1.6 times more than the long term average (407 mm) (Figure 1 (b)). However, the rainfall was not well distributed and July was dry (59 mm) compared to long term average of 91 mm. Canola was in the mid- to late- flowering stage in July and was most sensitive to heat stress at this time. Drought occurred in 2012 with a total rainfall of 375mm. June received 74mm which was less than the average rainfall of 83 mm during the early flowering stage of the 2012 trial. The 2012 trial reached maturity 1 week earlier than the 2011 trial.

### **2.4.2 Soil mineral N concentration from fertilizer application after heavy rain in the 2011 trial**

There were increasing mineral N concentrations in respond to the increased N fertilizer applications (Appendix 2). Total mineral N concentration from N150 plots (35.5 mg N kg<sup>-1</sup>) were almost three times higher than that from N0 plots (12.4 mg N kg<sup>-1</sup>), with 20.18 mg N kg<sup>-1</sup> from NO<sub>3</sub>-N. The NH<sub>4</sub>-N concentrations were lower than NO<sub>3</sub>-N in all treatments and the differences between NH<sub>4</sub>-N and NO<sub>3</sub>-N was less when N fertilizer rates increased. According to one-way ANOVA analysis, N fertilizer

applications had no effects on the soil total mineral N concentration ( $P=0.077$ ),  $\text{NO}_3\text{-N}$  ( $P=0.056$ ), and  $\text{NH}_4\text{-N}$  ( $P=0.13$ ).

#### **2.4.3 Residual soil mineral N concentration in the 2011 trial**

Overall, soil mineral N concentrations after harvest were half of those at the early growing stage, ranging between 6.1 to 12.2 mg N kg<sup>-1</sup> (Appendix 3). The highest total mineral N concentration occurred in N50+50 plot with the largest standard deviation of 7.98. The magnitudes of decreases were higher in plots with higher N fertilizer inputs. There is no correlation between mineral N concentrations during the early growing season and post-harvest (data not shown). Soil total mineral N concentration,  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations were poorly correlated with N fertilizer inputs (Data not shown).

#### **2.4.4 Nutrient composition of canola straw**

N concentration increased from 5.66 to 6.39 mg N g<sup>-1</sup> in response to N fertilizer input except that N0 resulted in 0.01 mg N g<sup>-1</sup> higher N concentration than N50 (Appendix 4). Despite the wide range of N inputs, magnitude of increments on straw N concentration was small with a slope of 0.005. Plots fertilized with more than 100 kg N ha<sup>-1</sup> fertilizers (both single and split applications) had higher straw N concentration ( $P<0.05$ ) than the control plots. The correlation coefficient of the relationship between straw N content and N fertilizer rates was positive ( $r=0.16$ ), but relatively higher than the correlation coefficient of the relationship between residual

soil mineral N and straw N concentration (Appendix 5).

There were no differences in straw concentrations on P, Mg, and K among all treatments (Table 2), except there was a declining trend of Ca concentrations in response to elevated N fertilizer inputs. All the tested nutrients were close to the averages of Alberta Agricultural and Rural Development (1996) except the significantly low P concentrations.

#### **2.4.5 Soil mineral N dynamics in the 2012 trial**

Overall,  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  both decreased during the growing season. In the N0 plots,  $\text{NO}_3\text{-N}$  fluctuated between 0 and 117 mg N kg<sup>-1</sup> during the growing season, with the average peak ( $52.0 \pm 39.8$  mg N/kg) at the early flowering stage (Table 3). Compared to the highly fluctuating  $\text{NO}_3\text{-N}$  concentrations,  $\text{NH}_4\text{-N}$  decreased from 38.4 to 3.6 mg N kg<sup>-1</sup>. The average  $\text{NO}_3\text{-N}$  concentration was 71.3% of the total mineral N concentrations cross the field during the growing season. In the N150 plots,  $\text{NO}_3\text{-N}$  varied between 0 and 119.4 mg N kg<sup>-1</sup> and  $\text{NH}_4\text{-N}$  reduced from 58.7 to 5.0 mg N kg<sup>-1</sup>.  $\text{NO}_3\text{-N}$  in the N150 plots varied less than those in the N0 plots, but still the majority of soil mineral N was in the  $\text{NO}_3\text{-N}$  form. Despite of the high standard deviation, N150 had higher  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations than the N0 plots ( $P < 0.05$ ) according to LSD (t-test) (data not shown).

Despite the poor correlation between the  $\text{NO}_3\text{-N}$  concentration from the N0 plots ( $r = 0.0938$ ) with the sampling time (i.e.: pre-sidedress, early flowering, and post-harvest), the  $\text{NO}_3\text{-N}$  concentration from the N150 plots, and the  $\text{NH}_4\text{-N}$



concentrations from both N0 and N150 plots were negatively related to the sampling time, with Pearson correlation coefficients of 0.96, 0.89 and 0.79 respectively.

## **2.5 Discussion**

### **2.5.1 Effect of N fertilizer treatment on soil mineral N concentration**

#### **2.5.1.1 Soil mineral N dynamics in the 2011 trial**

Although no pre-plant soil N information is available for the 2011 trial, N0 at the early growing stage could be a reference. NO<sub>3</sub>-N was 9.8 ( $\pm 1.3$ ) mg N kg<sup>-1</sup>, which was within the range (7.5 to 10.5 mg N kg<sup>-1</sup>) of soil from fields that cultivated wheat in the previous year in New Brunswick (Sharifi et al. 2007) and slightly lower than corn fields (12.5 mg N kg<sup>-1</sup>) in Ottawa (Ma and Wu, 2008). Therefore, the twice more than long term average rainfall did not lower NO<sub>3</sub>-N concentration significantly. One possible reason was the addition of NH<sub>4</sub>-N from S fertilizer, i.e.: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, which can be quickly transformed from NH<sub>4</sub>-N to NO<sub>2</sub>-N and NO<sub>3</sub>-N (Brady and Weil, 2002). The relative abundance of NH<sub>4</sub>-N (21%) over previous findings in Chicot soil (0 to 10%) (Liang and MacKenzie, 1994) may be evidence of this.

Autumn NO<sub>3</sub>-N concentrations returned to the background level for all of the treatments, though N150 (both single and split application) had 2 mg N kg<sup>-1</sup> higher than plots receiving less than 100 kg N ha<sup>-1</sup>. The concentrations were lower than the values of 14.5 to 40.8 mg N kg<sup>-1</sup> reported by Liang and Mackenzie (1994) under corn production at the Lods Agronomy Research Centre. One possible reason is that canola removes approximately twice as much N than corn and wheat per kg ha<sup>-1</sup> of grain

(Canadian Fertilizer Institute, 2001), which resulted in low residual soil N concentration (Hocking et al, 2002). On the other hand, rainfall was more than twice the long term average rainfall in August, which could have led to excessive NO<sub>3</sub>-N leaching, since NO<sub>3</sub>-N losses via leaching increase with higher N fertilizer input (Malhi et al, 1990; Elmi, et al, 2004).

#### **2.5.1.2 Soil mineral N dynamics in the 2012 trial**

The background NO<sub>3</sub>-N concentration (6.8 mg N kg<sup>-1</sup>) was lower than the early season concentration in the 2011 trial and the critical values (12.5 mg N kg<sup>-1</sup>) for maximum corn yields in Ottawa (Ma and Wu, 2008). Man and Wu explained the critical NO<sub>3</sub>-N concentration for maximum yield can be lower for fields that have not been fertilized for a long time or lower spring temperatures, which is the case in our study as the 2012 trial was conducted in the plot that was fallow in the previous year.

The N0 plot experienced a drastic NO<sub>3</sub>-N increase in N0 plots from sowing time to the early flowering stage, with a slope of 22.6 (r=0.96). This may be attributed to a large amount of NO<sub>3</sub>-N released from soil organic matter (SOM) with rising temperature (Figure 1(a)) (Breschini and Hartz, 2002; Ma and Wu, 2008). Meanwhile, NO<sub>3</sub>-N concentration prior to sidedress was four times more than that of the 2011 trial (two weeks before sidedressing). One possible reason for the low soil mineral N concentrations in the 2011 trial was NO<sub>3</sub>-N loss attributed to the twice more than averages rainfall in May in 2011.

The declining trends of NH<sub>4</sub>-N in both the N0 and N150 plots, and NO<sub>3</sub>-N in

N150 were consistent throughout the two study years. Overall, the soil mineral N concentrations in the 2012 trials were 1 to 2 times higher than those of the 2011 trial. This increase is likely attributed to much less rainfall, and then followed by increased temperature.

In the case of corn, a pre-sidedress  $\text{NO}_3\text{-N}$  test giving a value of 20-30 mg  $\text{NO}_3\text{-N}$   $\text{kg}^{-1}$  is considered to be the critical threshold, which means no yield response to N fertilizer is expected if soil  $\text{NO}_3\text{-N}$  concentration is at this level or higher (Magdoff, 1991). This range is confirmed with 33 mg  $\text{NO}_3\text{-N}$   $\text{kg}^{-1}$  in south coastal British Columbia for corn Zebarth et al. (1997), 21 mg  $\text{NO}_3\text{-N}$   $\text{kg}^{-1}$  in New York by Klausner et al. (1993) for corn, and 25 mg  $\text{NO}_3\text{-N}$   $\text{kg}^{-1}$  in New Brunswick for potato production by Bélanger et al. (2001). In our case, both N0 and N150 plots met this critical value despite the high variations across the field. This may indirectly explain the similar straw N concentration in all the treatments.

### **2.5.2 Effect of N fertilizer on straw nutrient concentration in the 2011 trial**

Despite the wide range of the N fertilizer inputs, straw N concentration increased at much less magnitudes with a slope of 0.005 ( $r=0.160$ ) (Appendix 5). Nitrogen split application did not enhance straw N concentration compared to N broadcasting. The range of N concentration is in accordance with the results of Soon et al. (2002), (5.86 and 7.04 mg N  $\text{g}^{-1}$  in two consecutive years) and Hocking et al. (2002) (3.2 to 9.5 mg N  $\text{g}^{-1}$  in two years at four locations). According to Hocking et al. (2002), straw N concentration between 5.0 to 7.2 mg N  $\text{g}^{-1}$  was correlated to the lower total dry matter

(oilseed and straw) of two-year field trial. However, Janzen and Bettany (1984) and Jackson (2000) had higher straw N concentration for up 16 mg N g<sup>-1</sup> for maximum yield. Similarly, the explanations of low critical soil NO<sub>3</sub>-N concentration for maximum yield based on soils having low fertility (Ma and Wu, 2008), it is possible that the critical straw N concentration for maximum yield was lower in Québec.

Other macronutrients (Ca, Mg, K) were consistent with the averages from Alberta Agricultural and Rural Development (1996), except for P concentrations being significantly low and under the critical levels of P (0.7 to 3.4 mg P g<sup>-1</sup>) in whole shoot (Rashid and Bughio, 1993). However, the residual soil P concentration was high, ranging between 40.7 and 92.5 mg Mehlich-3 P kg<sup>-1</sup> at the site (data not shown). This may indicate canola was incapable of extracting P from the soil solution. Generally, straw P uptake increases with N application rates (Soon et al., 2002; Thomas, 2003), which was not the case in the 2011 trial. Rashid and Bughio (1993) proposed rapeseed, the precursor of canola, lacked the mycorrhizal assistants needed to capture plant available P. If there was a P deficiency in canola grown in this trial, it may explain why straw N concentration did not respond to N fertilizer inputs over 100 kg N ha<sup>-1</sup>.

### **2.5.3 Predicting canola N requirement with soil N test**

In the 2012 trial, NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations were negatively correlated with the timing of samplings ( $r=0.79$  to  $0.96$ ,  $P<0.05$ ) at N150 plots but not in the unfertilized N0 plots for NO<sub>3</sub>-N ( $r=0.09$ , NS) (Figure 3). Soil mineral N test before seeding failed to be useful in determining the N fertilizer requirements because the N0

plots got mineral N from SOM and reached the critical soil  $\text{NO}_3\text{-N}$  concentration prior to sidedress for maximum yield. This is not unusual in humid climate in Eastern Canada, depending on the weather conditions and the specific factor like the SOM level. Zebarth et al. (2003) suggested that pre-plant soil mineral N tests can be best used in combination with other soil and/or plant tests.

## **2.6 Conclusion**

Both the soil and plant results demonstrated that an application of N over  $100 \text{ kg N ha}^{-1}$  did not improve canola nutrition compared to lower N inputs. To reduce the costs, labor, and time for fertilization, N can be applied before sowing in one dose. Repeated measurement of straw canola N and monitoring soil mineral N status is warranted because 1) N split application may be beneficial to fields having low organic reservoir; 2) the variations in weather, especially rainfall, which can result in  $20\text{-}30 \text{ mg N kg}^{-1}$  differences in the soil mineral N concentration. I recommend doing plant tissue analysis at the critical early growing stage, such as prior to sidedress, which can be correlated to soil mineral N test to solidate soil and crop-based N indicator; 3) plant N uptake is constrained by the availability of other nutrients, such as P and S, therefore further trials are warranted to confirm the efficiency levels of other essential macronutrients on canola growth.

Table 1: Soil physical and chemical properties at the study site for canola fertility trial

Texture	Loam
	493 g sand kg <sup>-1</sup> , 282 g silt kg <sup>-1</sup> , 225 g clay kg <sup>-1</sup>
SOM (g kg <sup>-1</sup> )	21.9
pH	6.13
P (mg Mehlich-3 kg <sup>-1</sup> )	42.7

Table 2: Macronutrients concentrations in canola straw in the 2011 trial.

Treatment	P (mg g <sup>-1</sup> )	Ca (mg g <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	K (mg g <sup>-1</sup> )
N0 <sup>a</sup>	0.38 (0.05) <sup>b</sup>	12.16 (1.77)	16.4 (0.21)	18.70 (3.35)
N50	0.36 (0.04)	12.24 (1.84)	17.2 (0.28)	18.42 (4.21)
N100	0.36 (0.06)	12.02 (1.3)	16.2 (0.21)	20.17 (2.24)
N150	0.36 (0.07)	11.69 (1.10)	16.0 (0.35)	22.10 (3.06)
N50+50	0.36 (0.05)	11.47 (1.17)	16.1 (0.24)	17.73 (2.15)
N100+50	0.35 (0.01)	10.63 (0.65)	14.5 (0.07)	19.26 (0.15)
LSD (P<0.05) <sup>c</sup>	NS <sup>d</sup>	NS	NS	NS
Average				
Analysis	1.2 <sup>e</sup>	14.3	19	8

a: For N single application, treatment N0, N50, N100 and N150 values are the mean of 24 replicates; for N split application, treatment N50 + 50 and N50 + 100, values are the mean of 4 replicates;

b: Values in parenthesis are standard deviation of the mean;

c: LSD with 95% confidence interval;

d: There were no significant differences among treatments;

e: Average macronutrient of canola straw analysis published by Alberta Agricultural and Rural Development (1996).

Table 3: Soil mineral N dynamics during the growing season in the 2012 trial.

N level (kg N ha <sup>-1</sup> )	Pre-sidedress		Early flowering		Post-harvesting	
	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N
0	37.4 <sup>a</sup> (25.4) <sup>b</sup>	20.6 (8.7)	52.0 (39.8)	8.9 (2.8)	10.7 (1.73)	6.0 (3.25)
150	63.4 (30.8)	25.4 (16.7)	50.4 (38.1)	24.3 (13.4)	27.9 (16.7)	7.4 (2.4)
LSD <sup>c</sup>	** <sup>d</sup>	NS <sup>e</sup>	NS	**	**	NS

a: Values are the mean of 8 replicates;

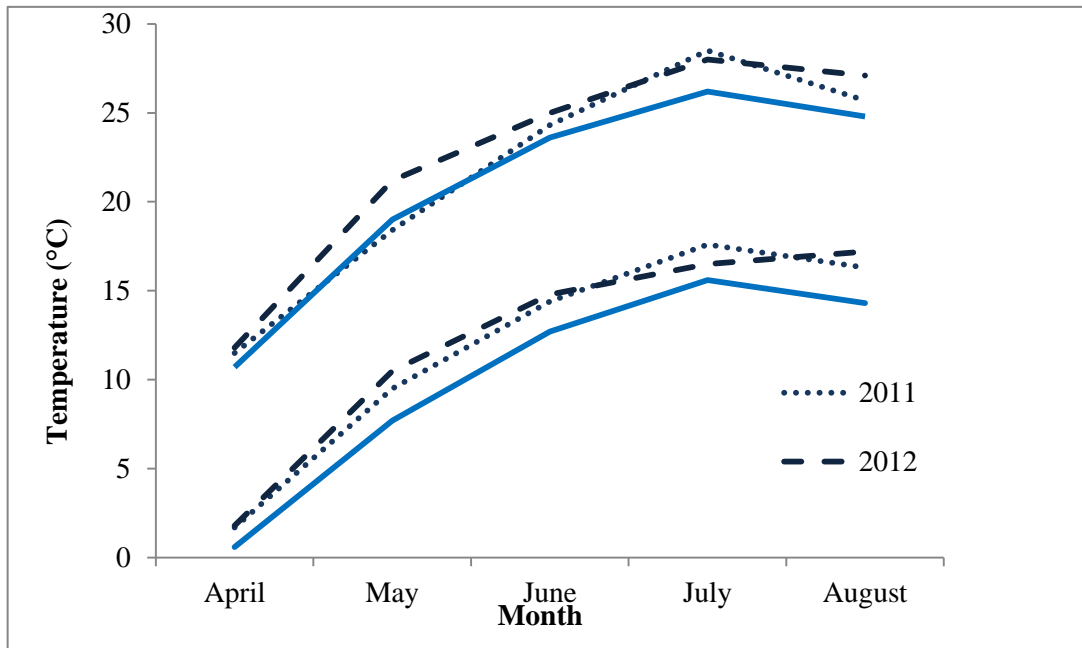
b: Values in the parentheses present standard deviation;

c: LSD with 95% confidence interval;

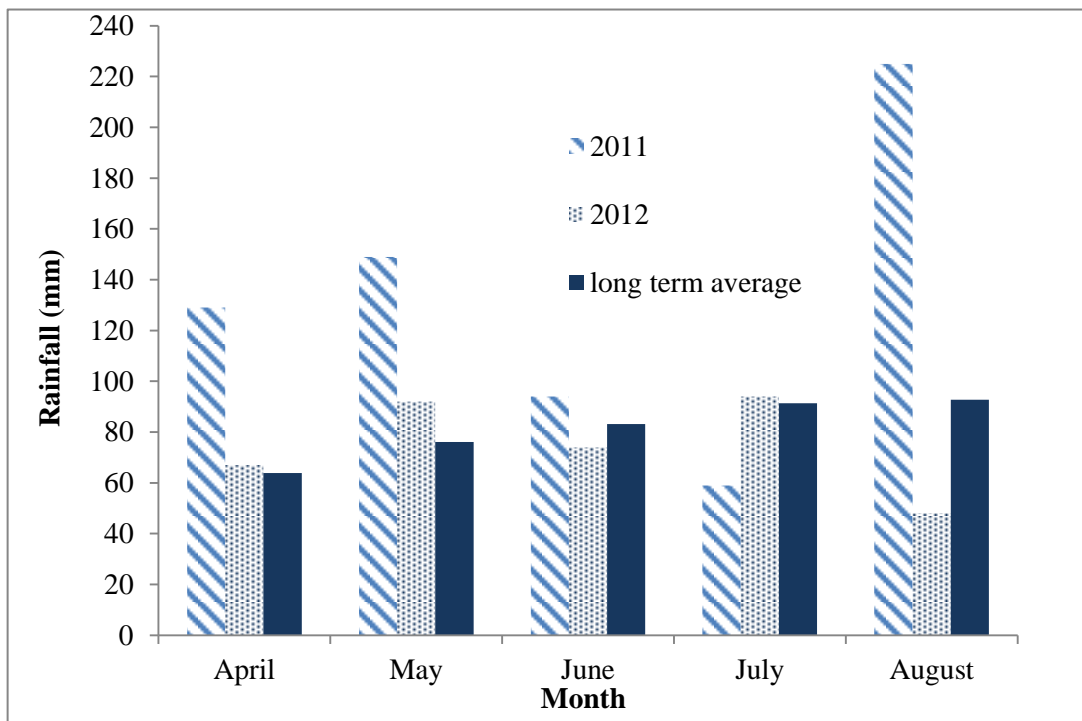
d: \*\* indicates the values are significantly different;

e: NS indicates no significant differences between the treatments;





(a)



(b)

Figure 1: Mean maximum and minimum temperature (a) and average rainfall (b) during the growing season near Ste-Anne-de-Bellevue, Qu ébec (Environment Canada, 2012a)

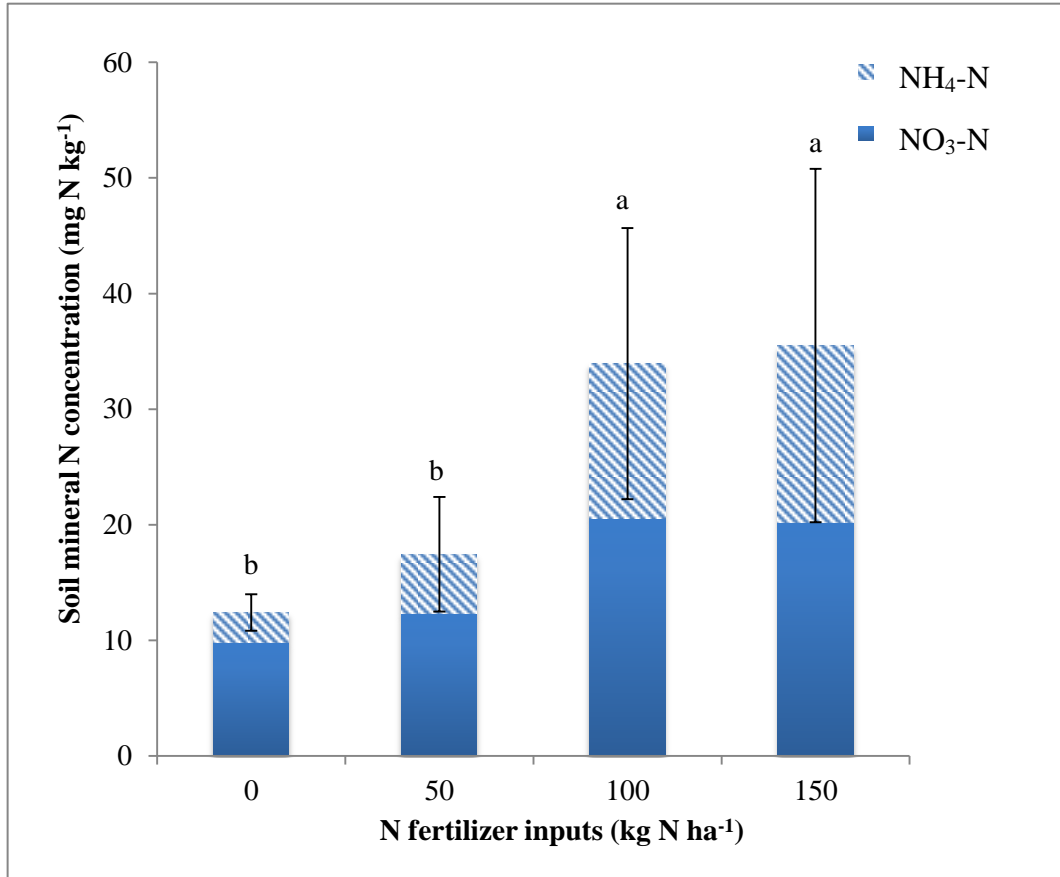


Figure 2: Mean  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations in soil from canola plots receiving N fertilizers, measured on May 30, 2011, about three weeks after seeding. Bars associated with each column are standard deviations of the mean total mineral N ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) concentration. Columns labelled with the same letter were not significantly different (LSD test,  $P < 0.05$ )

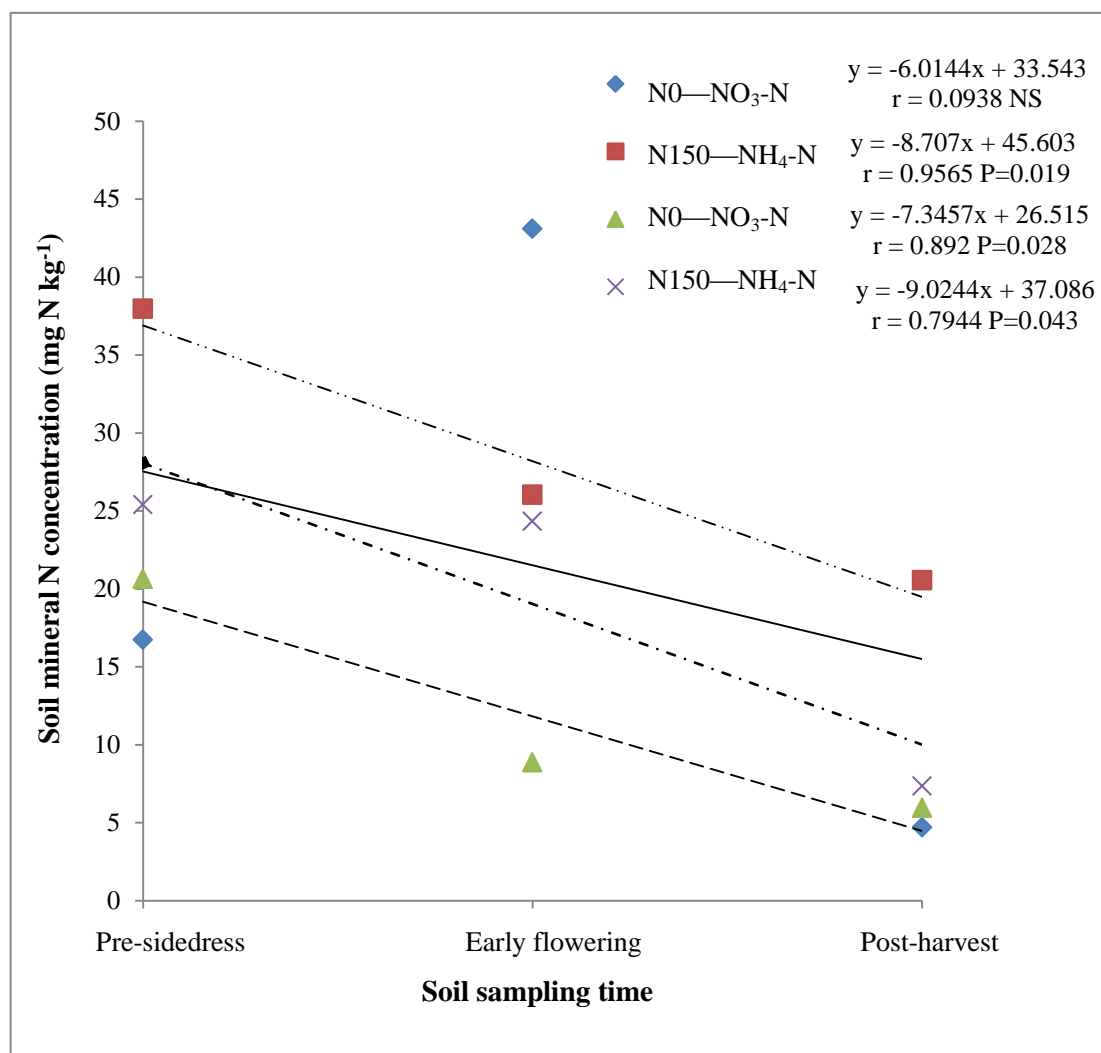


Figure 3: Relationship between the mean soil mineral N concentration and sampling time in canola plots, 2012. Each data point is the mean of 8 samples. Standard deviations are provided in Table 3.

## CONNECTING PARAGRAPH

Preliminary soil and straw N concentration results indicate that N fertilizer should be applied at the rate of 100 kg N ha<sup>-1</sup> which is in accordance with the recommendations of the Ontario Canola Growers Association (Hall, 2012). However, maximum yield may not be achieved because weather conditions can affect the efficacy of fertilizer (Nuttall, et al., 2002; Gan, et al., 2008a) and optimum growth may not be achieved due to P deficiency based on the straw analyses. Furthermore, soil mineral N concentrations were highly varied due to spacio-temporal heterogeneity. In order to precisely optimize N fertilizer for canola production in Eastern Canada, a supplemental pot experiment was conducted to validate yield and oilseed quality response to N fertilizer, with the advantage of overcoming the limitations of time and spaces.

## **CHAPTER THREE: YIELD AND OILSEED QUALITY OF CANOLA IN RESPONSE TO N FERTILIZER: A POT STUDY WITH THREE AGRICULTURAL SOILS FROM EASTERN CANADA**

### **3.1 Abstract**

A pot experiment was conducted to investigate the effects of N fertilizer on straw and oilseed yield, oilseed protein concentration and nitrogen use efficiency (NUE), and harvest index (HI) of canola. The experiment was designed as a factorial study with four rates of N (0, 75, 150 and 300 kg N ha<sup>-1</sup>) and soils from three research sites (Ste-Anne-de-Bellevue and St-Augustin-de-Desmaures, Qu ébec, and Ottawa, Ontario) was used. Canola grown in the Ste-Anne-de-Bellevue soil was most responsive to the N fertilizer ( $r=0.83$   $P<0.0001$ ), resulting in the highest straw (6.7 g plant<sup>-1</sup>) and oilseed yields (1.8 g plant<sup>-1</sup>) in this study. Oilseed protein concentration (20.9 to 22.6 %) was close to the average of the canola field trials in Qu ébec. Correlations between straw N concentration and yield differed among soils: there was no relationship between these values for soil from Ste-Anne-de-Bellevue, a negative relationship for St-Augustin-de-Desmaures soil and a positive for Ottawa soil. Overall, all the indicators, including straw and oilseed yield, straw N concentration (1.66 to 3.69 mg N g<sup>-1</sup>), HI (8.8 to 20.3 %) and NUE (6.4 to 10.3% for St-Augustin-de-Desmaures only) were lower in the pot study than field averages, suggesting canola did not reach its potential growth. Further straw nutrient analysis is warranted to identify nutrient limitations that may have impacted canola production grown in the pots.

### 3.2 Introduction

Understanding canola's yield and oilseed quality in response to N fertilizer is required to optimize N fertilizer application in Eastern Canada so producers can maximize net returns and minimize N losses to the environment. Insufficient N nutrition slows canola growth and development resulting shorter stems, fewer branches, and a smaller canopy (Ogunlela et al., 1989). However, excessive N supply leads to lodging and reduces oil contents to less than 35% (compared to the average at 40%) (Grant and Bailey, 1993). As the sum of the oil and protein concentration of an oilseed is roughly constant, ranging between 60 to 65%. Therefore, a protein concentration greater than 26% is an indicator of inferior oilseed quality because the oil concentration decreases (Brennan et al., 2000; Brandt et al., 2002). Mendham and Roberson (2004) reported that the oil concentration decreases by 0.6 to 1.2% per additional 100 kg N ha<sup>-1</sup> applied. In Manitoba, canola farmers are more likely to apply more than the recommended dose of N fertilizer than the recommended dose to obtain higher grain yield because producers are paid on a weight basis without penalties for low oil content (Holzapfel, 2007). However, when canola is grown for oil extraction, it is preferable to optimize canola plant nutrition to get a high oil concentration.

Regardless of the rate of N fertilizer applied, approximately 12% to 40% of applied N was recovered in the oilseed (Gan et al., 2007), which tend to be lower than cereal crops, which have about 33% of N fertilizer recovered

in grains (Raun and Johnson, 1999). Nitrogen use efficiency is the ratio between oilseed yield ( $\text{kg ha}^{-1}$ ) and total N accumulated in the plant (Svečnjak and Rengel, 2006). This ratio decreases significantly when N fertilizer rates increase or soil mineral N supply exceeds crop's requirement (Chamorro et al., 2002). Therefore, it is important to find the threshold where NUE decreases, so producers can apply judicious amounts of N to maintain economic viability and minimizing losses. Provided canola receives sufficient N for optimum nutrition, growers also need to consider the trade-offs between the potential yield increase when higher than recommended N fertilizer rates are applied and the net returns when considering the cost of fertilizer inputs. The inherent soil N supply can differ among soil types, due to texture, historical manure application and preceding crops in the field, which influences a crop's response to N fertilizers. Therefore, it is necessary to know whether soils from certain regions in Eastern Canada have a greater capacity to supply N than others, which can help agronomists provide better information to canola producers.

The objectives of this study was to 1) investigate NUE of canola grown in soil from three locations in Eastern Canada; 2) compare canola straw N concentration and yield in response to N fertilizer; and 3) compare the residual soil mineral N ( $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$ ) concentrations at harvest as related to N fertilizer input in soil from three locations in Eastern Canada.

### 3.3 Materials and methods

#### 3.3.1 Site descriptions and soil collection

Soils used for the pot experiment was collected from the Emile A. Lods Agronomy Research Centre on the Macdonald Campus of McGill University in Ste-Anne-de-Bellevue, Québec, the experimental farm of Laval University at Saint-Augustin-de-Desmaures, Québec, and the Central Experimental Farm in Ottawa, Ontario. Background information for the Emile A. Lods Agronomy Research Centre was provided in Table 1 of Chapter 2.

The St-Augustin-de-Desmaures site (46°44' N 71°31' W) is a Humic Gleysol (609 g sand kg<sup>-1</sup>, 290 g silt kg<sup>-1</sup> and 101 g clay kg<sup>-1</sup>) sandy loam, with a pH of 5.95, an organic matter content of 27 g kg<sup>-1</sup> and a Mehlich-3 extractable-P of 66.2 mg kg<sup>-1</sup>. The site had a rolling topography and the plot where the soil was collected for the experiment was at the bottom of a hill. The mean annual temperature at the site is 4.4 °C, with an annual precipitation of 1231 mm (Environment Canada, 2012b). The preceding crop was wheat (*Triticum aestivum* L).

The Ottawa site (45°23'N 75°43'W) is a sandy loam Orthic Humic Gleysol (621 g sand kg<sup>-1</sup>, 288 g silt kg<sup>-1</sup> and 90 g clay kg<sup>-1</sup>), with a pH of 6.44, an organic matter content of 29 g kg<sup>-1</sup> and a Mehlich-3 extractable-P of 200 mg kg<sup>-1</sup>. The mean annual temperature is 6.3 °C, with an annual precipitation of 914 mm (Environment Canada, 2012b). The preceding crop



was soybean (*Glycine max* (L.) Merr.).

Soils (0 to 20cm depth) were collected using a shovel in the months of April and May, 2012. Soils were passed through a size 4 mesh Tyler® sieve to remove plant residues and stored in plastic containers in an unheated barn where the temperature was around 8 °C before the experiment began.

### **3.3.2 Experiment design and treatment**

The experiment was a factorial design consisting of four rates of N (0, 7.5, 15 and 30 g N m<sup>-2</sup>), three rates of S (0, 2 and 4 kg S m<sup>-2</sup>) and three rates of B (0 and 0.05 g B m<sup>-2</sup> in foliar spray and 0.2 g B m<sup>-2</sup> soil applied). Each combination of rates was replicated four times, for a total of 144 experimental units. The dose of 7.5 g N m<sup>-2</sup> is equivalent to 75 kg N ha<sup>-1</sup> at the field scale and this conversion applied for all other treatments. For the rest of this chapter, the field rates were used. Nitrogen was applied with laboratory grade ammonium nitrate (39.5-0-0), S was applied as potassium sulfate (0-0-50-19), and B was applied as boric acid (17.5%). All chemicals were made into stock solutions and different treatments were achieved by doubling or tripling the amount of the stock solutions. Phosphorus was added as a basal nutrient at the rate of 50 kg P ha<sup>-1</sup> with monopotassium phosphate.

The experimental unit was a plastic pot (15 cm top diameter, 12 cm bottom diameter, 10.5 cm tall) with an approximate volume of 1.7 L. A Fisher Q8 (coarse porosity) filter paper was placed at the bottom of the pot to avoid

soil loss from watering. Each pot was filled with 9 cm of soil (about 1 kg of soil). Seeding started with soils from Ste-Anne-de-Bellevue and St-Augustin-de-Desmaures on April 27, 2012 in a greenhouse. The greenhouse had an average day temperature of 25 °C and night temperature of 15 °C. The photoperiod was maintained at 14-hour daytime by artificial light. Between 6 and 8 seeds were sown after adding fertilizer solutions. Pots were covered with transparent plastic to maintain soil moisture and help germination. Saucers were placed under the pots to ensure sufficient water supply. Most seeds germinated within 3 to 5 days after seeding in all the treatments except for the treatments where N was applied at 300 kg N ha<sup>-1</sup> and S at 40 kg S ha<sup>-2</sup> for soil from Ste-Anne-de-Bellevue, even after re-seeding. Seedlings were thinned to two plants per pot at the three-leaf stage and moved outdoors at the five-leaf stage on May 17, 2012 when the weather was frost-free. The Ottawa trial was seeded on May 28, 2012 due to limited greenhouse space. The same seeding and thinning practices as the Ste-Anne-de-Bellevue and St-Augustin-de-Desmaures trials were applied.

Boron foliar fertilization was done at the early flowering stage in the last week of June with Oligo-B<sup>TM</sup> 10% boron solution containing surfactant (Axter Agrosience Inc.).

### **3.3.3 Plant growth monitor, harvesting and analysis**

Many canola plants grown on soil from the Ste-Anne-de-Bellevue and

St-Augustin-de-Desmaures sites were suspected of experiencing phosphorous deficiency starting in mid-June due to the appearance of purplish stems and leaves. Crone's solution, a modified Hoagland solution without the addition of N, S and B, was added to all the pots to correct the deficiency in early July (Appendix 2) (Jones, 1983).

As rainy weather was forecast close to the harvest time, each pot was covered with a transparent plastic bag covering the top of the plant and tied to stem to avoid seed loss. Holes were punched on the underside of the plastic bags to ensure air exchange and minimize rain capture.

The Ste-Anne-de-Bellevue and St-Augustin-de-Desmaures trials were harvested the first week of August, and the Ottawa trial was harvested the last week of August. Oilseed was separated from pods, and the oilseed and straw yields were recorded. The same straw drying, grinding and analytical strategies were applied as in section 2.3.4. Total N of the straw and oilseed were measured by a ThermoFinnigan Flash EA 1112 CN analyzer (Carlo Erba, Milan, Italy). The protein concentration of the oilseed was calculated by multiplying the total N of the oilseed by 6.25.

### **3.3.4 Soil sampling and analysis**

Soil was sampled after harvest for mineral N ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) analysis. The same soil sampling strategy, extraction method, and quantifying techniques were applied in section 2.3.2.

### 3.3.5 Harvest index (HI) calculation

Harvest Index was calculated as:

$$HI = \text{oilseed yield} / (\text{oilseed yield} + \text{straw yield})$$

where HI is the harvest index (%) and oilseed yield ( $\text{kg ha}^{-1}$ ) produced per unit of biological yield ( $\text{kg oilseed yield} + \text{straw yield ha}^{-1}$ ) (Cheema et al., 2001).

### 3.3.6 Statistical Analysis

Data was tested for normality and found to be normally distributed, so data analysis was done using parametric statistics. Two way ANOVA model  $x = u + (\text{N fertilizer input})_i + (\text{soil type})_j + (\text{N fertilizer} \times \text{soil type})_{ij} + \text{replicates} + \epsilon_{ij}$  was used to assess the effects of N fertilizer and soil type on soil mineral N concentration after harvesting and various crop indicators. The Xpred procedure was used to predict the missing values conducting a mean separation test (LSD test,  $P < 0.05$ ) for the main effects. Tukey procedure was used to compare the means when the N fertilizer inputs  $\times$  soil type effect was significant. Values presented in the tables and figures are the means  $\pm$  standard deviation of untransformed data. Correlations between various dependent variables were made on the untransformed data using Pearson correlation coefficients. All the procedures were from the SAS package (SAS System 9.2. SAS Institute, Inc, Cary, NC).

### **3.4 Results**

#### **3.4.1 Observations**

Overall, canola grown in the pots had smaller canopies than those in a field trial in the same season (observations). Canola grown in the soil from Ste-Anne-de-Bellevue had more branches and bigger stems than canola grown in soil from the other two sites. Close to the late flowering stage, canola grown in soil from Saint-Augustin-de-Desmaures had smaller roots (suspected root rot) and therefore the plants were more susceptible to wind and rain as they could be tipped more easily. The onset of flowering for canola grown in the Ottawa soil was the least consistent among three sites. Some canola flowered 1 to 2 weeks earlier than the average. However, the pod filling and seed maturing was prolonged for plant growth in soil from Ottawa and floral and pod abortions were more abundant than other two sites (Appendix 3). Meanwhile, White Rust (Staghead) (*Albugo candida*) and Downy Mildew (*Peronospora parasitica*) diseases were suspected for pots with soil from the Ottawa site.

#### **3.4.2 Soil mineral N concentration at harvest**

According to the two-way ANOVA test, the soil types significantly influenced the soil mineral N ( $P=0.0002$ ),  $\text{NO}_3\text{-N}$  ( $P<0.0001$ ) and  $\text{NH}_4\text{-N}$  ( $P=0.027$ ) (Table 1). Soil from Saint-Augustin-de-Desmaures had a greater total mineral N concentration than other two sites, about 3.5 to 10.5 mg N kg<sup>-1</sup>

<sup>1</sup> more than the other soils that received the same N fertilizer input. The N fertilizer did not increase soil  $\text{NH}_4\text{-N}$  concentration at harvest, although  $\text{NH}_4\text{-N}$  was the dominant mineral N form for soil from Ottawa (60% to 90% of soil mineral N) (Figure1). The interaction between N fertilizer rate and soil type affected soil  $\text{NO}_3\text{-N}$  concentration ( $P=0.029$ ). Furthermore, the  $\text{NO}_3\text{-N}$  concentration of soil from Ste-Anne-de-Bellevue was correlated to straw yields ( $r=0.38$ ,  $P=0.01$ ) (Figure 4), but not for the other two sites (data not shown).

### **3.4.3 Indicators of canola plant growth**

#### **3.4.3.1 Straw and oilseed yield, and harvest index**

Fertilizer rate, soil type and the interactions between these two factors all significantly influenced canola straw and oilseed yields ( $P \leq 0.0001$ ) (Table 1). Canola grown in the soil from Ste-Anne-de-Bellevue was most responsive to N fertilizer ( $P=0.043$ ), resulting in the highest straw ( $1.35 \text{ g pot}^{-1}$ ) and oilseed ( $2.60 \text{ g pot}^{-1}$ ) yield (Figure 2 and 3). Canola grown in the soils collected from Ottawa was least responsive to N fertilizer and had the lowest straw and oilseed yield (Figure 2 and 3).

In terms of the ability to produce oilseed per unit of biological yield (straw yield), Saint-Augustin-de-Desmaures had the highest HI (20.3%) when N was applied at  $300 \text{ kg N ha}^{-1}$  (Table 2). Ste-Anne-de-Bellevue had a relatively constant HI (14.8 to 17.4%), regardless of N fertilizer input.

Ottawa soil had reliably low HIs (8.8 to 12.9%), which was in accordance with the observations of floral and pod allocation.

#### **3.4.3.2 Straw N and oilseed protein concentration**

Straw N concentration ranged between 1.6 to 3.5 mg N g<sup>-1</sup> for most treatments (Figure 5, 6 and 7), except that high values were measured in straw from canola grown in Ottawa soil (up to 12.4 mg N g<sup>-1</sup>). Within soils sampled from the same site, straw N concentration did not respond to N fertilizer (data not shown). Oilseed protein concentration from canola grown in the soil from St-Augustin-de-Desmaures increased from 20.9 % to 22.6% with the increasing N fertilizer inputs (P=0.015). But the protein concentration was not correlated with straw N concentration (r=0.08) at this site (data not shown). The protein concentrations were within the range of the averages from field-grown canola in Québec (21.7% to 24.1%) (Ruel and Tardif, 2011), but higher than those from Western Canada (19.4% to 19.6%) (Canadian Grain Commission, 2011).

### **3.5 Discussion**

#### **3.5.1 Canola yield in response to N fertilizer input**

The overall trend of straw yield in response to N fertilizer was similar to the results of Nuttall et al. (1992) and Brandt et al. (2003), which had high rates of biomass accumulation when the N fertilizer rates were lower (under

100 kg N ha<sup>-1</sup>), and the rate slowed down with N fertilizer applied higher than 150 kg N ha<sup>-1</sup>. The increasing trend was most significant in canola grown in the soil from Ste-Anne-de-Bellevue, whose straw yields increased rapidly up to 150 kg N ha<sup>-1</sup>. The threshold was about at 75 kg N ha<sup>-1</sup> for soil from St-Augustin-de-Desmaures and Ottawa. In the pot study where plants were watered regularly, there is a tendency for soluble N fertilizer (e.g.: NH<sub>4</sub>NO<sub>3</sub>) to be leached or lost through gaseous emission (e.g.: denitrification). The loamy soil from Ste-Anne-de-Bellevue may have greater N retention capacity through cation and anion exchange, allowing a prolonged and steady supply of NO<sub>3</sub>-N during the growing season. This is supported by the correlation between straw N concentration and soil NO<sub>3</sub>-N concentration at the end of the growing season (Figure 4). In contrast, sandy loam soil from St-Augustin-de-Desmaures and Ottawa probably had lower NO<sub>3</sub>-N retention capacity and thus the soil N supply may have been depleted during the growing season. Regular repeated measurement of the soil mineral N concentration is not feasible in a pot study, but could be done in the field to determine if the proposed mechanism explains the experimental findings in this study.

### **3.5.2 Correlation between straw N concentration and yield**

The inconsistent correlations between straw N concentration and yield in canola grown in soil from three sites was considered another



indicator of limitations to crop growth and development. Hocking et al. (1997, 2002) showed negative relationships between straw yield and N concentration in two seasons under different weather conditions. The drought year had relatively high straw N concentration and low yield, but the difference on yields were less in the regions with higher soil fertility. One possible explanation is that the remobilization of N from the vegetative to the reproductive organs depends on water availability (Taylor et al., 1991; Thomas, 2003). Under-developed crops are less capable of translocating N to the pods, which results in a higher N concentration in straw than expected. This explanation is consistent with the positive relationship between straw yield and N concentration in canola grown in the soil from Ottawa. When the negative relationship occurred in canola grown in the soil from St-Augustin-de-Desmaures, it suggested that the plants were under stress and “stole” N away from the straw to complete its lifecycle (Barker and Bryson, 2006). Therefore, we want to have a constantly low N concentration in straw which indicates the entire N used for oil synthesis.

### **3.5.3 Effects of cropping history and seeding time on canola production**

Both plant indicators (straw and oilseed yield, and HI) suggested that canola did not reach its potential growth in this pot study. Two possible reasons for the low yield under Ottawa soil are that soybean was the preceding crop and Ottawa pots were seeded late (late May). The Association

des Centres Locaux de Développement de Québec warned canola growers that a soybean - canola rotation increases the possibility of disease transmission (Thomas, 2003; Ruel and Tardif, 2012). This may explain the higher frequencies of disease encountered in the soil from Ottawa. Secondly, late seeding, often accompanied by higher temperatures during the pod filling stage, can lower oilseed yields by 15% to 58 % (Gan et al., 2007). Lastly, prolonged daytime length, brought by the late seeding, stimulates the advanced floral initiation (Thomas, 2003). As 75% to 80 % of canola N was assimilated before the flowering stage (Schjoerring et al., 1995) and is translocated to floral structures including pod and seed for reproductive development. Overall, the stress are expected to cause an imbalance in canola nutrient status, which can be verified by calculating DRIS (diagnosis and recommendation integrated system nutrient ratios) or CND (compositional nutrient diagnosis) norms based on nutrients concentrations (N, P, K, Mg etc.)

#### **3.5.4 Low canola production in a pot study with three soils, compared to other studies**

Although the straw and oilseed yields of canola were highly responsive to N fertilizers, all the crop parameters, except protein concentration, suggested the low production compared to the previous studies (Schjoerring et al., 1995; Chamorro et al., 2002; Brandt et al., 2003; Gan et

al., 2007). One canola plant produced 7.33 g oilseed under favorable growing condition, 3.57 g when it was under heat stress (maximum day time temperature was 35 °C) and 5.47g under 50% water stress (Gan et al; 2007). The oilseed yields in this pot study were 50% to 90% less than the yields when canola was under 50% water stress, which indicates inferior physiological performance of canola. The straw N concentrations were 16 mg N g<sup>-1</sup> by Janzen and Bettany (1984), 12 mg N g<sup>-1</sup> by Jackson (2000) and 15 mg N g<sup>-1</sup> by Svečnjak and Rengel (2006), which was about three to five times higher than the straw N concentration found in this pot study. Low production was in accordance with the observations of smaller canopy, and purplish stems and leaves, during the growing season. Although hydroponic solution was used to correct the deficiency, the negative effects it had exerted may have occurred for too long to be offset.

### **3.6 Conclusion**

Canola grown in soil from Ste-Anne-de-Bellevue were most responsive to N fertilizer inputs at the rate up to 300 kg N ha<sup>-1</sup>. However, optimum growth and maximum oilseed yield were not reached in canola grown in the Ste-Anne-de-Bellevue soil or any other soil compared to field grown canola. Heat stress and insufficient disease prevention appeared to limit the canola yield, particularly when it was grown in soil from St-Augustin-de-Desmaures and Ottawa. Although no measurements were

available for the yield loss due to late sowing, it is strongly suggested that canola sowing should be done as early in May as possible. Attention should be paid to the sequence of crops grown in rotation with canola, such as soybean. Soybean residue in particular may be the host for fungal pathogen that affect canola growth adversely, while wheat residue could also be a host for pathogen on immobilizing N and other nutrients, thereby limiting canola nutrition. Further research on the effect of preceding crops on canola production in Québec and Ontario is warranted.

Table 1: Harvest index (%) in response to N fertilizer input. Harvest index is expressed as the ratio between oilseed yield (g pot<sup>-1</sup>) and biological yield (g straw + oilseed pot<sup>-1</sup>) yield.

N input kg N ha <sup>-1</sup>	fertilizer		Soil sampling site	
	Ste-Anne-de- Bellevue	St-Augustin-de- Desmaures	Ottawa	
0	15.9 ( $\pm 4.5^a$ )	20.3 ( $\pm 6.7$ )	12.9 ( $\pm 9.8$ )	
75	17.4 ( $\pm 5.4$ )	19.3 ( $\pm 4.1$ )	8.8 ( $\pm 4.4$ )	
150	14.8 ( $\pm 6.0$ )	17.4 ( $\pm 3.9$ )	10.6 ( $\pm 8.0$ )	
300	16.4 ( $\pm 6.1$ )	20.3 ( $\pm 9.3$ )	10.5 ( $\pm 6.0$ )	

a: numbers in the parenthesis are standard deviations and apply for all the following tables.

Table 2: Protein concentration of oilseed in response to N fertilizer input.

Values are the mean  $\pm$  standard deviation.

N fertilizer input (kg N ha <sup>-1</sup> )	Protein %	Sample number
0	20.9 ( $\pm 1.50$ )	20
75	21.40 ( $\pm 1.75$ )	22
150	21.83 ( $\pm 1.67$ )	22
300	22.61 ( $\pm 2.52$ )	21
Average: Québec <sup>a</sup>	21.7 ~24.1	
Canadian Grain Commission <sup>b</sup>	19.4~19.6	

a: averages from Québec canola trials (Ruel and Tardif, 2011)

b: averages from Canadian Grain Commission based on canola trials in Western Canada (2011).

Table 3: Analysis of variance of the effects of N fertilizer input and soil type on residual soil mineral N concentration at post-harvest and crop yield parameters

Parameter	Source of variation		
	N input	fertilizer Soil type	N fertilizer input x soil type
NO <sub>3</sub> -N	0.0093	<0.0001	0.0287
NH <sub>4</sub> -N	NS	0.027	NS
Total mineral N	0.030	0.0002	NS
Straw yield	<0.0001	<0.0001	0.0001
Oilseed yield	<0.0001	<0.0001	0.0001
Straw N concentration	0.030	NS	NS
Protein <sup>a</sup>	0.02	_____	_____

a: Protein results are only available for St-Augustin-de-Desmaures site

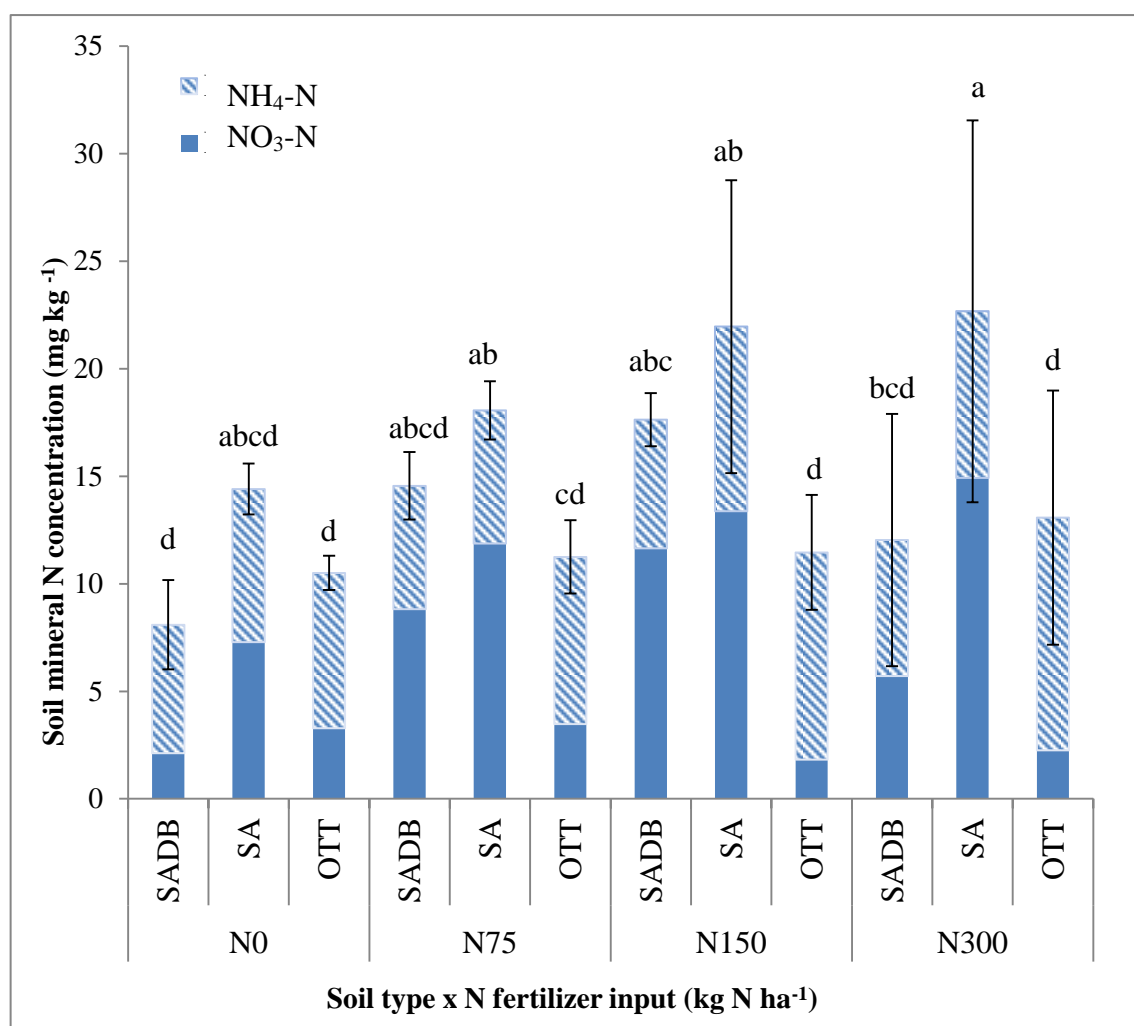


Figure 1: Soil mineral N concentrations at pot harvest as affected by the soil type x N fertilizer input interaction. SADB stands for Ste-Anne-de-Bellevue, SA for St-Augustin-de-Desmaures and OTT for Ottawa. Values in the bar graph are the mean (n=4) with standard deviations. Bars with different letter are significantly different (Tukey test,  $P < 0.05$ ). The same applied for the following figures.



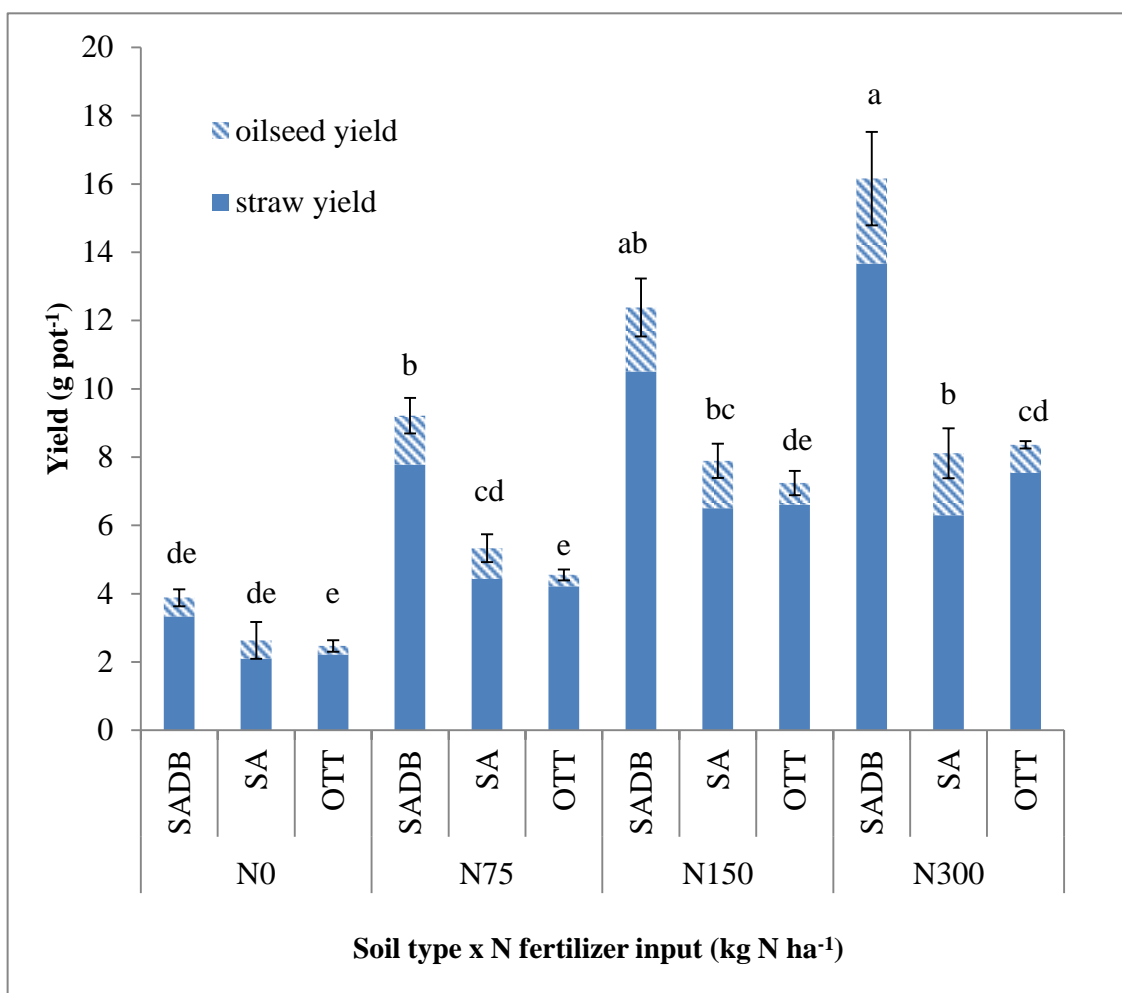


Figure 2: Straw and oilseed yield in response to the interaction of soil type x N fertilizer. Values are the mean based on 16 to 32 samples. The error bars are standard deviations of the oilseed yields. Bars with different letter are significantly different (Tukey test,  $P < 0.05$ ). See Figure 1 for abbreviation.

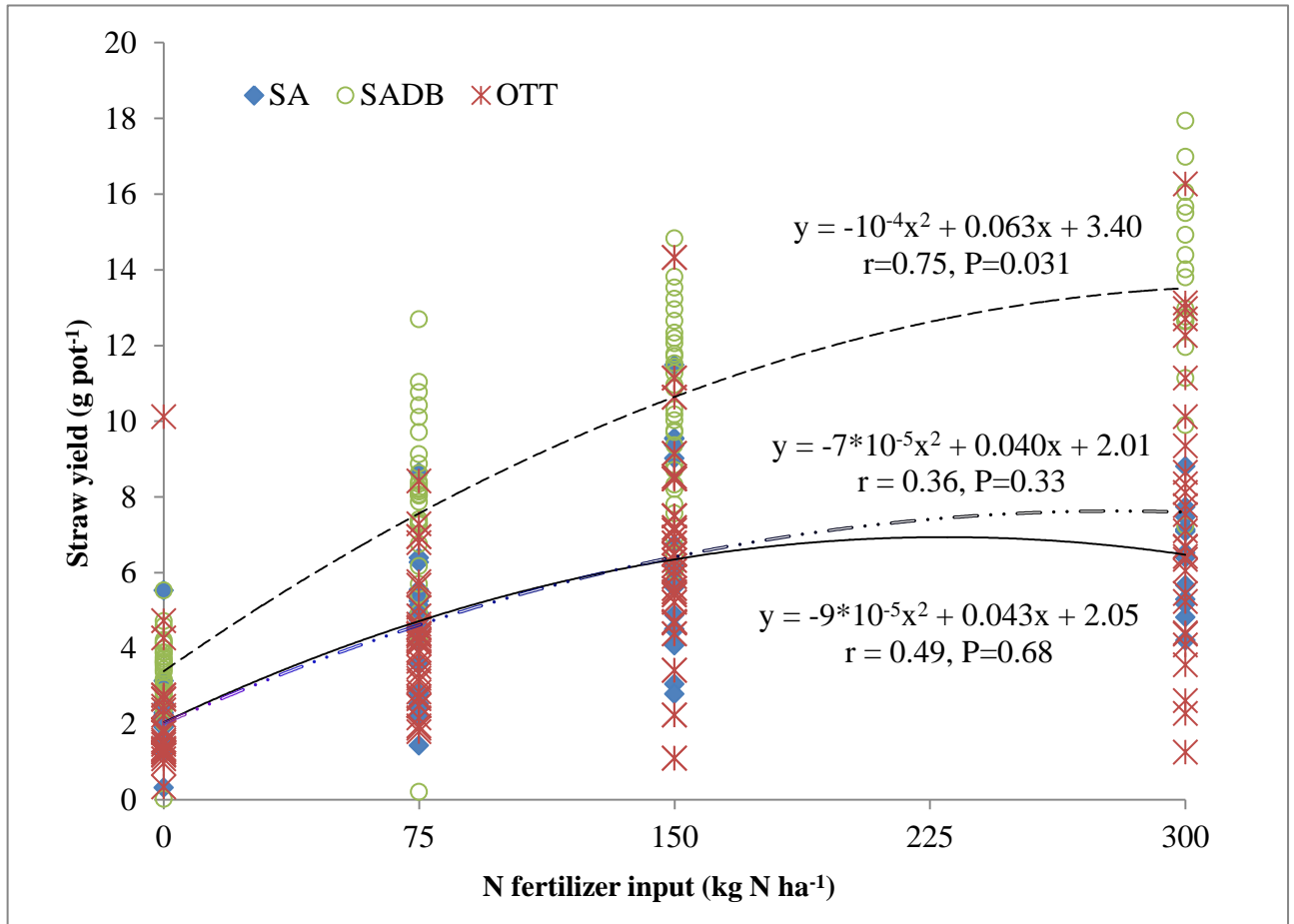


Figure 3: Straw yield in response to N fertilizer input of canola grown in soil from three locations in Eastern Canada. The strength of the association between these variables are calculated as a Pearson correlation coefficient (r value). See Figure 1 for abbreviation.

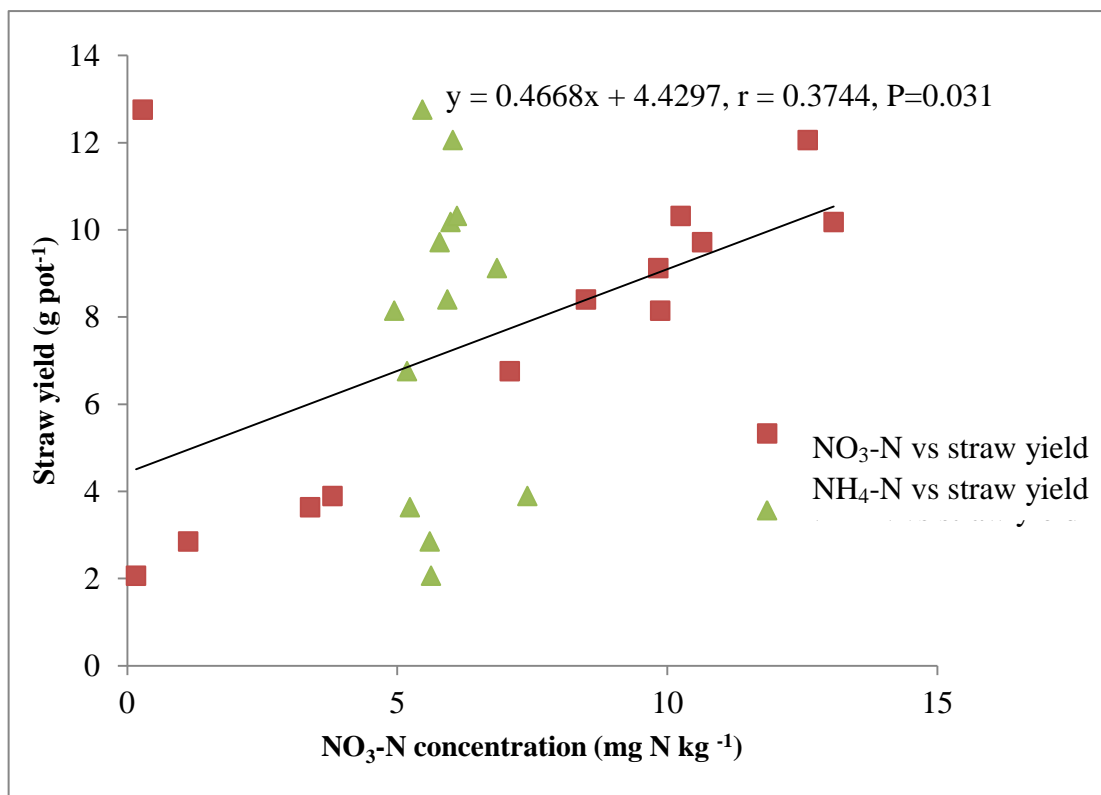


Figure 4: Canola straw yield in response to soil NO<sub>3</sub>-N concentration at harvest (Ste-Anne-de-Bellevue). The strength of the association between these variables is calculated as a Pearson correlation coefficient (r value). See Figure 1 for abbreviation.

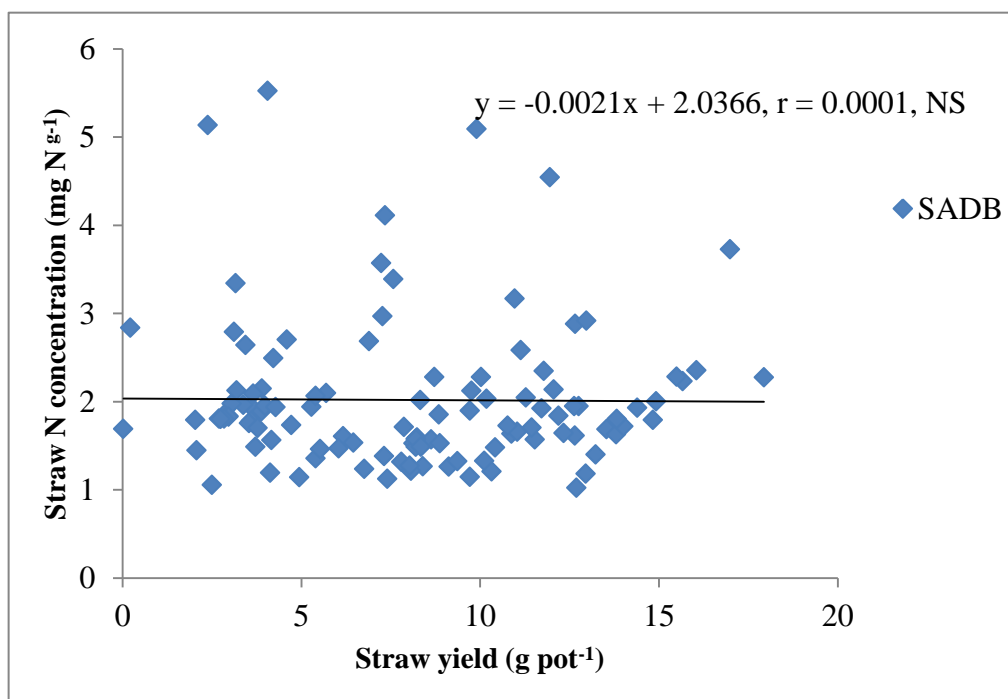


Figure 5: Correlation between straw N concentration and yield of canola grown in soil from Ste-Anne-de-Bellevue. The strength of the association between these variables is calculated as Pearson correlation coefficient (r value)

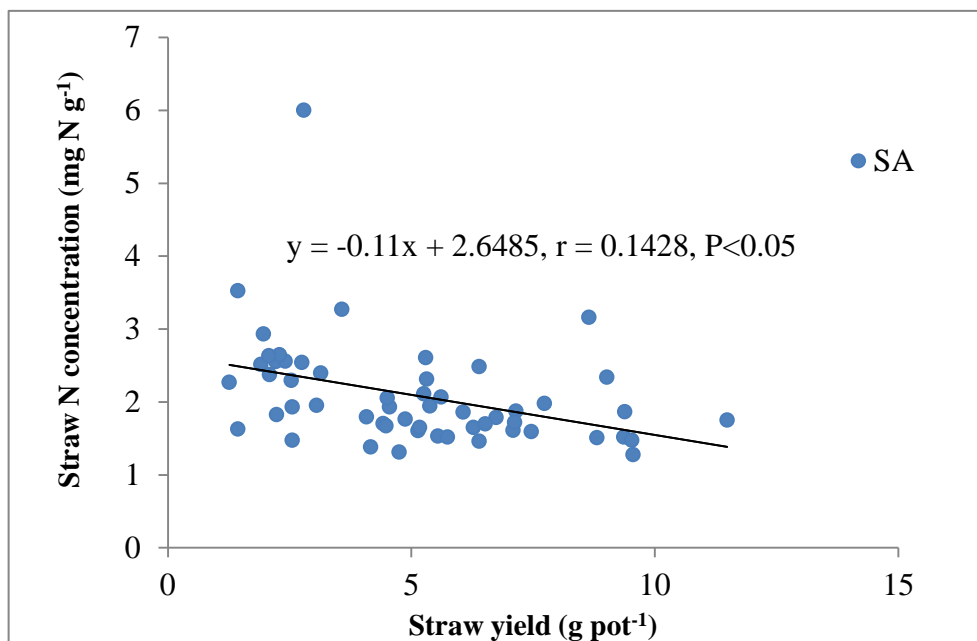


Figure 6: Correlation between straw N concentration and yield of canola grown in soil from St-Augustin-de-Desmaures. The strength of the association between these variables is calculated as Pearson correlation coefficient (r value).

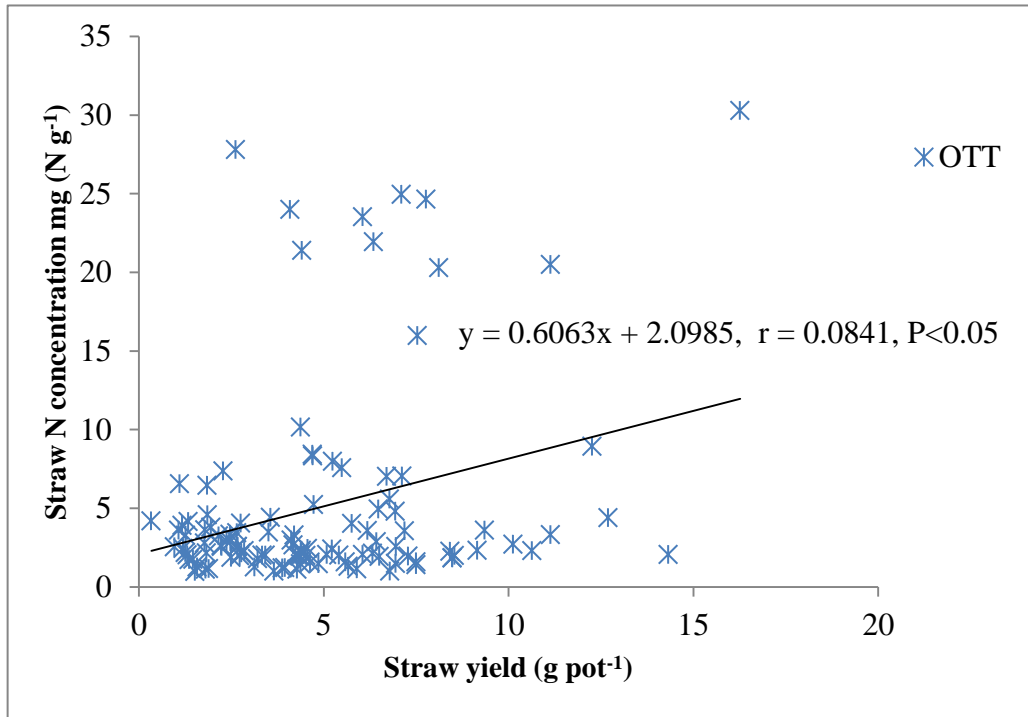


Figure 7: Correlation between straw N concentration and yield of canola grown in soil from Ottawa. The strength of the association between these variables is calculated as Pearson correlation coefficient (r value).

## GENERAL CONCLUSION

The Canadian government has made a commitment to reduce GHG emission by increasing biofuel (biodiesel and ethanol) blend among fossil fuels. Canola-based biodiesel provides an alternative to lower GHG emission from burning fossil fuel. Expanding canola cultivation in Québec arises to be a good strategy to increase the oilseed production and supporting the newly established local oilseed crushing industry (e.g.: TRT- ETGO). Nitrogen fertilization is the most decisive factor for optimum yield for non-legume crops like canola. Meeting the N requirement of canola for optimum yield and increasing the use efficiency of applied N fertilizer are the keys to the viability and sustainability of the canola industry.

According to my study, for canola cultivation in southern Québec, N fertilizer can be applied less than 100 kg N ha<sup>-1</sup>, compared with 100 to 110 kg N ha<sup>-1</sup> in the prairie region, without affecting the straw nutrition. Plant available N mineralized from soil organic matter (SOM) under the humid Québec climate contributes to canola N requirement. Split application of N fertilizer should not be used when soil mineral N concentrations increase rapidly between the sowing time and the 5<sup>th</sup> or 6<sup>th</sup> week after sowing. Fertilizer rates may need to be adjusted based on soil texture, as the loam soil from Ste-Anne-de-Bellevue gave higher straw yields than the sandy loam soil from St- Augustin-de-Desmaures, but this may have been related to cropping history (the fields were previously under fallow or wheat respectively). Previous crop of soybean could make canola susceptible to diseases and pests. In this regard, further research should be conducted to identify the abiotic (temperature,

moist content and substrates) factors that favor microbial activities that produce mineral N from SOM. So farmers could take the credits from SOM and apply less N fertilizers. It is also worthy to evaluate the influence of N fertilizer on residue decomposition and nutrients release from the common crops that are grown in rotation with canola in Québec soil, which will help farmers make full use of the crop-derived nutrients and prevent disease.

In a long run, it is necessary to formulate interpretative criteria, such critical value or sufficiency range for soil N test at various growing stages and plant tissue analysis of elements that contribute to optimum canola nutrition should also be encouraged. These tests will explicitly identify any nutrients that may be limiting the yields, so farmers can adjust fertilization to correct any deficiency accordingly. Multiple approaches such as the critical value approach (CVA), compositional nutrient diagnosis (CND) and diagnosis and recommendation integrated system (DRIS) have been formulated for many crops, but I am not aware of such a method that has been calibrated for canola grown in Québec. The development of these diagnostic tools relies on data collected from many factorial-designed field or greenhouse experiments, which captures the spatio-temporal heterogeneity present in the study region and to ensure validity and accuracy of the implications.



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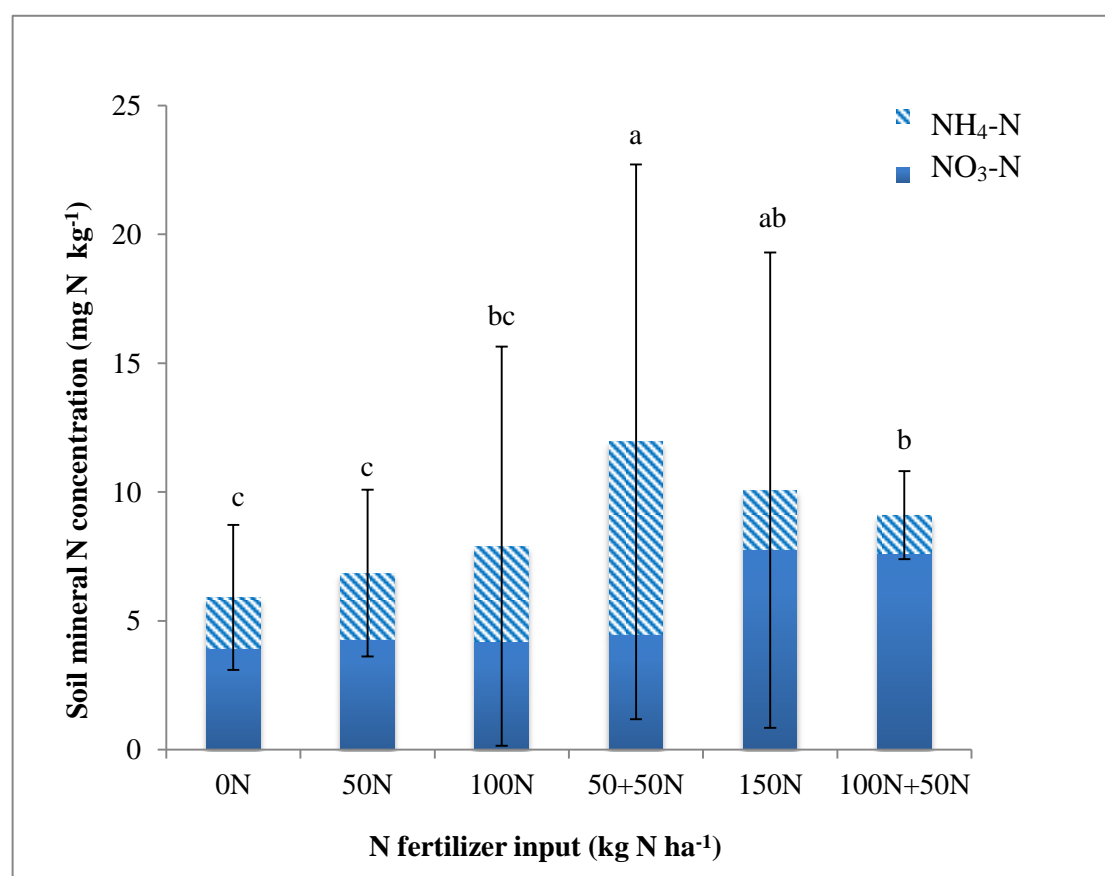
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# APPENDIX1: 2012 Field layout

Block1		Block2		Block3		Block4	
Border		Border		Border		Border	
101		201		301		401	
102		202		302		402	
103		203		303		403	
104		204		304		404	
105		205		305		405	
106		206		306		406	
107		207		307		407	
108		208		308		408	
109		209		309		409	
110		210		310		410	
111		211		311		411	
112		212		312		412	
113		213		313		413	
114		214		314		414	
115		215		315		415	
116		216		316		416	
117		217		317		417	
118		218		318		418	
119		219		319		419	
120		220		320		420	
121		221		321		421	
122		222		322		422	
123		223		323		423	
124		224		324		424	
125		225		325		425	
126		226		326		426	
127		227		327		427	
128		228		328		428	
Border		Border		Border		Border	

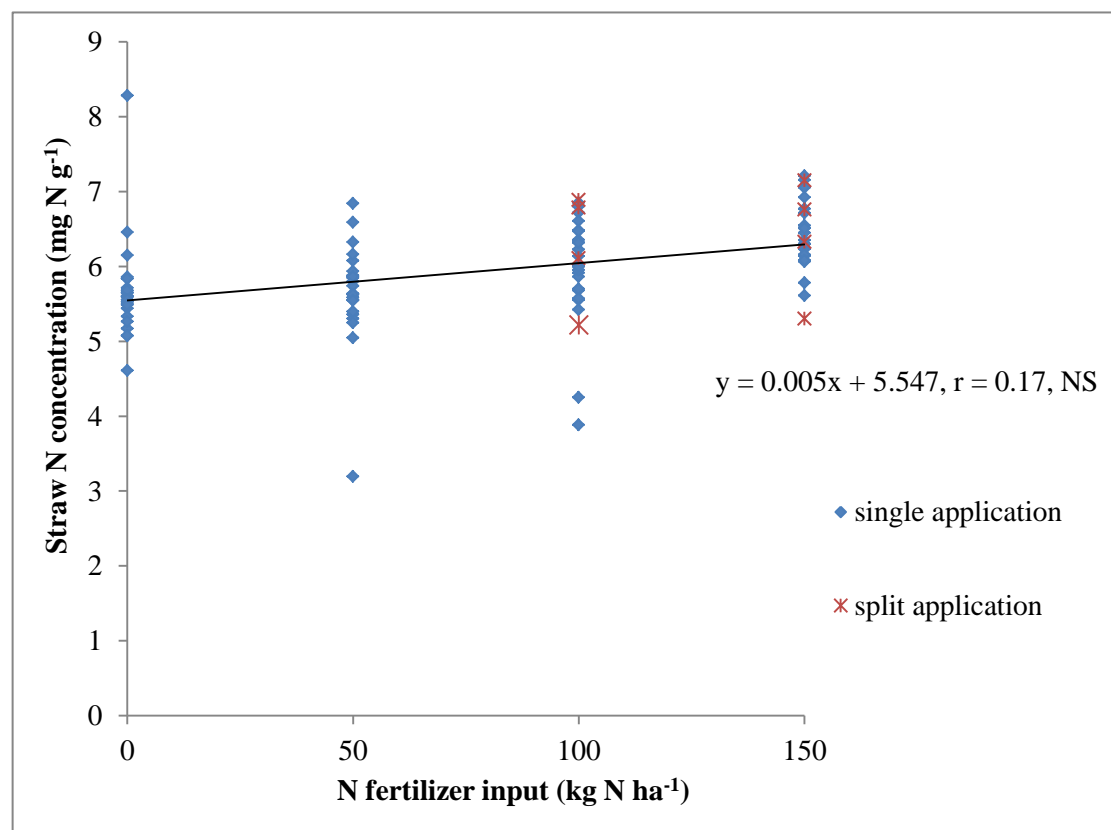
Upward was equivalent to the north in the field; 2011 field layout is the same except that there were 26 treatments in each block. Soils were sampled after harvesting at all plots; plots in shadow are where soils were sampled before side-dressing, at early flowering and harvesting in 2012 trial; combinations of other treatments were not listed.

**Appendix 2: Mean  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations in soil from canola plots, measured after harvest on September 2, 2011. Bars associated with each column are the standard deviation of the mean mineral N ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) concentration.**



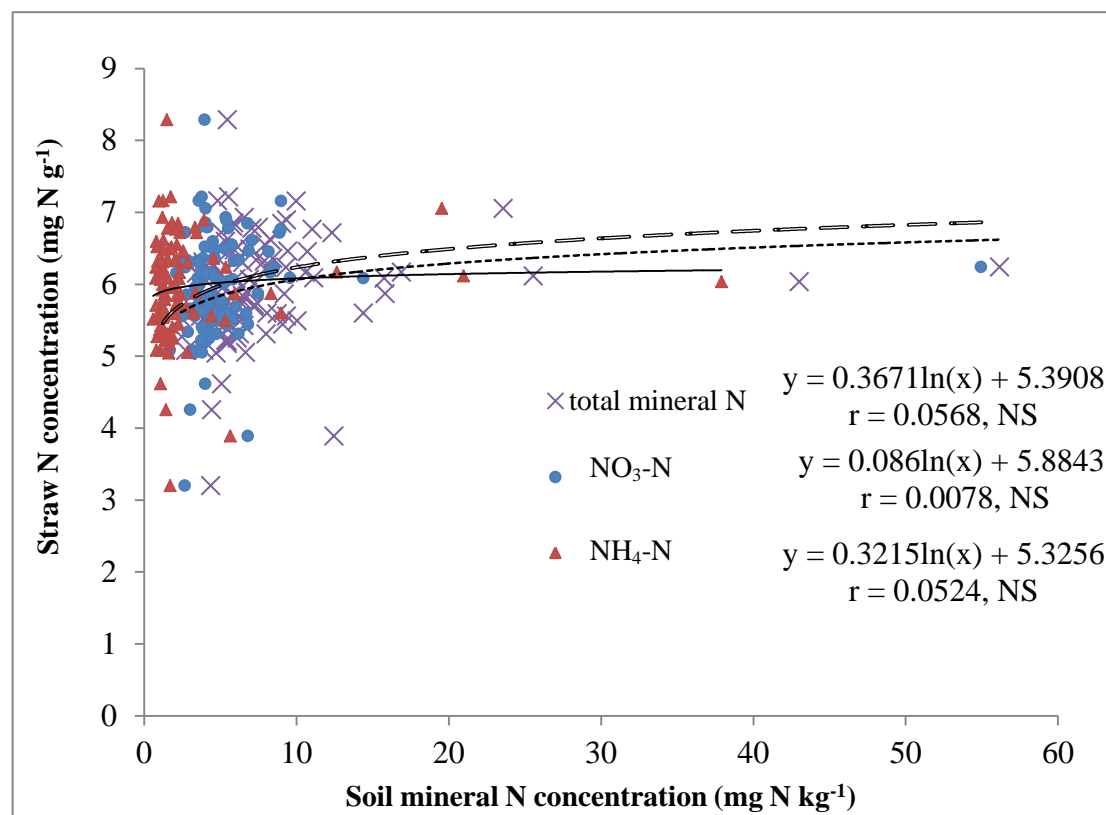
### Appendix 3: Canola straw N concentration related to N fertilizer input in 2011.

Fertilizer was applied in a single dose at seeding (50, 100 and 150 kg N ha<sup>-1</sup>) or a split application (50 + 50 and 100 + 50 kg N ha<sup>-1</sup>) with the second dose applied at the fifth week after seeding.



#### Appendix 4: Relationship between soil mineral N concentration and canola straw

N concentration at harvest in 2011.





**APPENDIX 5: Ingredients of the Crone's solution (modified Hoagland solution  
without the addition of N, S and B to correct nutrient deficiency)**

Ingredient	Rate g L <sup>-1</sup>
KCl	0.737
CaCO <sub>3</sub>	0.44
CaHPO <sub>4</sub>	0.39
MgCl <sub>2</sub> 6H <sub>2</sub> O	0.205

**APPENDIX 6: Seed abortions and pod shattering of premature canola in soil  
from Ottawa**

