EFFECT OF TIME AND RATE OF NITROGEN, SULFUR AND BORON APPLICATION ON CANOLA GROWTH IN SOUTHWESTERN QUÉBEC

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TABLE OF CONTENTS

TABLE OF CONTENTS.......................................................................................... I
LIST OF TABLES........................................................................................................ IV
LIST OF FIGURES....................................................................................................... V
LIST OF ABBREVIATIONS....................................................................................... VI
ABSTRACT................................................................................................................... VII
RESUMÉ..................................................................................................................... IX
ACKNOWLEDGEMENT............................................................................................ XI
CONTRIBUTION OF AUTHORS............................................................................. XIII

Chapter 1 GENERAL NTRODUCTION................................................................. 1
1.1 Introduction ........................................................................................................ 1
1.2 Hypothesis ......................................................................................................... 4
1.3 Objectives ......................................................................................................... 5

Chapter 2 LITERATURE REVIEW..................................................................... 6
2.1 Canola ............................................................................................................... 6
2.1.1 Variety ......................................................................................................... 6
2.1.2 History ....................................................................................................... 6
2.1.3 Usage ......................................................................................................... 7
2.1.4 Crop development ...................................................................................... 8
2.2 Nitrogen .......................................................................................................... 12
2.2.1 Nitrogen in soil .......................................................................................... 12
2.2.2 Nitrogen in canola .................................................................................... 13
2.2.3 Canola N deficiency symptoms ................................................................. 14
2.2.4 Canola responses to N fertilizer ................................................................. 15
2.2.5 Time of N fertilization .............................................................................. 15
2.3 Sulfur .............................................................................................................. 19
2.3.1 Sulfur in soil .............................................................................................. 19
2.3.2 Sulfur in canola ........................................................................................ 20
2.3.3 Sulfur deficiency symptoms .................................................................... 21
2.3.4 Canola responses to S fertilizer ................................................................. 21
2.3.5 Sulfur fertilizer placement ........................................................................ 22
2.4 Boron ............................................................................................................. 25
2.4.1 Boron in soil ............................................................................................. 25
2.4.2 Boron in canola ........................................................................................ 25
2.4.3 Boron deficiency symptoms .................................................................... 26
2.4.4 Canola responses to B fertilizer ................................................................. 27
2.4.5 Boron fertilizer placement ........................................................................ 27

Chapter 3: MATERIALS AND METHODS ....................................................... 29
3.1 Site description ............................................................................................... 29
3.2 Field experimental design ............................................................................. 29
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3 Plant material, soil preparation, and seeding</td>
<td>30</td>
</tr>
<tr>
<td>3.4 Weed and insect control</td>
<td>30</td>
</tr>
<tr>
<td>3.5 Data collection</td>
<td>31</td>
</tr>
<tr>
<td>3.6 Statistical analyses</td>
<td>33</td>
</tr>
<tr>
<td><strong>Chapter 4 RESULTS</strong></td>
<td>36</td>
</tr>
<tr>
<td>4.1 Weather conditions</td>
<td>36</td>
</tr>
<tr>
<td>4.2 Population density</td>
<td>39</td>
</tr>
<tr>
<td>4.3 Dry biomass</td>
<td>42</td>
</tr>
<tr>
<td>4.4 Leaf area</td>
<td>44</td>
</tr>
<tr>
<td>4.5 Plant height</td>
<td>46</td>
</tr>
<tr>
<td>4.6 Branches plant⁻¹</td>
<td>48</td>
</tr>
<tr>
<td>4.7 Siliques plant⁻¹</td>
<td>48</td>
</tr>
<tr>
<td>4.8 Seeds silique⁻¹</td>
<td>48</td>
</tr>
<tr>
<td>4.9 1000-seed weight</td>
<td>50</td>
</tr>
<tr>
<td>4.10 Harvest index</td>
<td>54</td>
</tr>
<tr>
<td>4.11 Yield</td>
<td>58</td>
</tr>
<tr>
<td>4.12 Oil percent</td>
<td>64</td>
</tr>
<tr>
<td>4.13 Protein percent</td>
<td>68</td>
</tr>
<tr>
<td><strong>Chapter 5 GENERAL DISCUSSION</strong></td>
<td>72</td>
</tr>
<tr>
<td>5.1 Various growth characteristics affected by N, S and B</td>
<td>72</td>
</tr>
<tr>
<td>5.1.1 Plant density</td>
<td>72</td>
</tr>
<tr>
<td>5.1.2 Dry biomass</td>
<td>73</td>
</tr>
<tr>
<td>5.1.3 Leaf area</td>
<td>74</td>
</tr>
<tr>
<td>5.1.4 Plant height</td>
<td>74</td>
</tr>
<tr>
<td>5.1.5 Branches plant⁻¹</td>
<td>75</td>
</tr>
<tr>
<td>5.1.6 Siliques plant⁻¹</td>
<td>75</td>
</tr>
<tr>
<td>5.1.7 Seeds silique⁻¹</td>
<td>75</td>
</tr>
<tr>
<td>5.1.8 1000-seed weight</td>
<td>76</td>
</tr>
<tr>
<td>5.1.9 Harvest index</td>
<td>77</td>
</tr>
<tr>
<td>5.1.10 Yield</td>
<td>78</td>
</tr>
<tr>
<td>5.1.11 Oil</td>
<td>85</td>
</tr>
<tr>
<td>5.1.12 Protein</td>
<td>86</td>
</tr>
<tr>
<td>5.1.13 Oil vs. protein</td>
<td>87</td>
</tr>
<tr>
<td>5.2 Optimum rate and timing of N fertilizer additions for canola production under southwestern Quebec soil condition</td>
<td>90</td>
</tr>
<tr>
<td>5.3 Optimum rates of application for the minor nutrient S in canola productivity under southwestern Quebec conditions</td>
<td>92</td>
</tr>
<tr>
<td>5.4 Optimum rates of application of the micronutrient boron (B) for canola productivity under southwestern Quebec conditions</td>
<td>93</td>
</tr>
<tr>
<td><strong>Chapter 6 CONCLUSIONS</strong></td>
<td>94</td>
</tr>
<tr>
<td>6.1 General conclusion</td>
<td>94</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>6.2 Acceptance or rejection of hypotheses</td>
<td>96</td>
</tr>
<tr>
<td>Chapter 7 CONTRIBUTIONS TO KNOWLEDGE</td>
<td>97</td>
</tr>
<tr>
<td>Chapter 8 SUGGESTIONS FOR FUTURE RESEARCH</td>
<td>98</td>
</tr>
<tr>
<td>REFERENCE</td>
<td>99</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>113</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Response of canola yield to S fertilizer in Manitoba and Saskatchewan</td>
<td>23</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Soil physical and chemical properties for canola fertility trials in 2011 and 2012</td>
<td>35</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Effect of rate of N, S and B application on plant characteristics (plants m$^{-2}$, dry biomass plant$^{-1}$, leaf area plant$^{-1}$, plant height, branches plant$^{-1}$, siliques plant$^{-1}$, seeds silique$^{-1}$, 1000-seed weight, harvest index, yield, oil and protein) in 2011.</td>
<td>40</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Effect of rate of N, S and B application on plant characteristics (plants m$^{-2}$, dry biomass plant$^{-1}$, leaf area plant$^{-1}$, plant height, branches plant$^{-1}$, siliques plant$^{-1}$, seeds silique$^{-1}$, 1000-seed weight, harvest index, yield, oil and protein) in 2012</td>
<td>41</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>The correlation of seed yield, oil and protein with plant characteristics (plants m$^{-2}$, dry biomass plant$^{-1}$, leaf area plant$^{-1}$, plant height, branches plant$^{-1}$, siliques plant$^{-1}$, seeds silique$^{-1}$, 1000-seed weight, harvest index, yield, oil and protein) in 2012.</td>
<td>60</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>The correlation of seed yield, oil and protein with plant characteristics (plants m-2, dry biomass plant$^{-1}$, leaf area plant$^{-1}$, plant height, branches plant$^{-1}$, siliques plant$^{-1}$, seeds silique$^{-1}$, 1000-seed weight, harvest index, yield, oil and protein) in 2011.</td>
<td>63</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>Extra expense of Split N application as compared with single application at the rate of 150 kg ha$^{-1}$ in 2011.</td>
<td>81</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 2.1  Effect of N fertilization on yield and seed quality (CCC, 2003)……………… 17

Figure 2.2  The N cycle (CCC, 2003)………………………………………………………….. 18

Figure 2.3  Simplified version of the overall S cycle (CCC, 2003)………………………… 24

Figure 4.1  Meteorological data for 2011………………………………………………………… 37

Figure 4.2  Meteorological data for 2012………………………………………………………… 38

Figure 4.3  Effect of N application on dry biomass plant$^{-1}$ in 2011……………………….. 43

Figure 4.4  Effect of N application on leaf area plant$^{-1}$ in 2011…………………………….. 45

Figure 4.5  Effect of fertilizers on plant height…………………………………………………… 47

Figure 4.6  Effect of N application on seeds silique$^{-1}$ in 2011……………………………… 49

Figure 4.7  Effect of fertilizers on 1000-seed weight…………………………………………… 51

Figure 4.8  Effect of N application on harvest index…………………………………………… 55

Figure 4.9  The relationship between N application and yield………………………………… 59

Figure 4.10 The relationship between N application and oil percent……………………… 65

Figure 4.11 The relationship between N application and protein percent………………….. 69

Figure 5.1  Correlation between protein and oil percent in 2011 and 2012……………….. 89
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Boron</td>
</tr>
<tr>
<td>CCC</td>
<td>Canola Council of Canada</td>
</tr>
<tr>
<td>GHG</td>
<td>Greenhouse gas</td>
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<tr>
<td>ha</td>
<td>Hectare</td>
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<tr>
<td>Mbp</td>
<td>Million base pairs</td>
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<tr>
<td>N</td>
<td>Nitrogen</td>
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<td>NUE</td>
<td>Nitrogen use efficiency</td>
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<tr>
<td>S</td>
<td>Sulfur</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis System</td>
</tr>
</tbody>
</table>
Abstract

Canola (Brassica napus L.) seeds have a high oil content (> 40 %), the lowest saturated fat concentration (6.8%) among the vegetable oils, plus low erucic acid and glucosinolate levels, making this crop highly valuable in the oilseed industry. Québec, a province where little canola is currently produced, has considerable potential to expand canola cultivation and, in doing so, strengthen the provincial agricultural sector. However, at this time there are no well-founded fertility recommendations for canola production in southwestern Québec; there is a lack of sufficient data on canola responses to nitrogen (N), sulfur (S) and boron (B). Therefore, field studies were conducted in 2011 and 2012 to determine the optimum rate and timing of N fertilizer additions, and also the best rates of S and B application under southwestern Québec conditions. In 2011, factorial combinations of four levels of N [0, 50, 100 (or 50 + 50) and 150 (or 50 + 100) kg ha\(^{-1}\)], two levels of S (0 and 20 kg ha\(^{-1}\)), and three levels of B (0, 0.5 foliar spray at 20% flowering stage, and 2 kg ha\(^{-1}\) soil applied before sowing) were tested. Single doses of N fertilization (50, 100, 150 kg ha\(^{-1}\)) were all applied before sowing. Split N fertilization was also evaluated at two levels: 100 kg ha\(^{-1}\) (50 + 50 kg ha\(^{-1}\)) and 150 kg ha\(^{-1}\) (50 + 100 kg ha\(^{-1}\)), where the first dose of 50 kg N ha\(^{-1}\) was applied before sowing, and the remainder was side-dressed at the 3-4 leaf stage. In 2012, two additional treatments were added, one applied as 20 kg S ha\(^{-1}\), 0.5 kg B ha\(^{-1}\) and 200 kg N ha\(^{-1}\), and the other as 20 kg S ha\(^{-1}\), 0.5 kg B ha\(^{-1}\) and 50 + 150 kg N ha\(^{-1}\). In 2011, N fertilization had positive effects on dry biomass, leaf area, plant height, seeds silique\(^{-1}\), harvest index, yield, and seed protein content, but a negative effect on seed oil content. The optimum N rate was 150 kg N ha\(^{-1}\), resulting in the highest seed yield and good seed quality. In 2012, canola did not respond as strongly to fertilizer additions as in 2011, apparently because of lower established plant densities. Application of fertilizer N affected fewer variables in 2012: 1000-seed weight, and harvest index, seed oil content and seed protein content. Of the four blocks in the 2012 experiment, only block four had a reasonably high average plant population density. For block four, the yield data was curvilinearly and positively correlated with N rate (R\(^2\) = 0.5702, P < 0.0001), with peak yield occurring at 150 kg N ha\(^{-1}\). Canola growth was often not affected by the time of N
In 2011, there was a S x B interaction for 1000-seed weight, in that the effect was positive with foliar application of 0.5 B kg ha$^{-1}$ and negative when 2 kg B ha$^{-1}$ was soil-applied. Boron alone increased 1000-seed weight in 2012. Our results indicated that S and B levels in the soils used were probably sufficient for canola production. Plant height was decreased (2.9 %) by S application at 20 kg ha$^{-1}$ in 2011. In conclusion, our data suggest that the best N application regime for canola production in southwestern Québec may be a single application at 150 kg N ha$^{-1}$; this produced the highest seed yield without sacrifice of seed quality. Sulfur and B additions may not be required for canola production in southwestern Québec.
Résumé

Les semences du canola (*Brassicanapus* L.) ont des teneurs élevées en huile (> 40%), la plus basse concentration en graisses saturées (6,8%) parmi les huiles végétales et une faible teneur en acide érucique et glucosinolates, ce qui les rend très précieux dans le secteur de la production des huiles végétales. La province de Québec, avec une faible production de l’huile de canola, a la capacité d’augmenter sa production et, effectivement, de renforcer l’agriculture provinciale. Cependant, les recommandations pour la fertilisation des plantes de canola pour la culture au Québec ne sont pas bien définies; il y a un manque d’information concernant la réponse de canola à l’azote (N), le soufre (S) et le bore (B).

Les études menées en 2011 et 2012 ont déterminé le taux et la saison idéals pour les additions de l’engrais enrichi avec de l’azote et, en plus, le taux optimal pour le soufre et le bore au Québec. En 2011, les combinaisons factorielles de quatre niveaux de N [0, 50, 100 (ou 50 + 50) et 150 (ou 50 + 100) kg ha\(^{-1}\)], deux niveaux de S (0 à 20 kg ha\(^{-1}\)), et trois niveaux de B (0, 0,5 pulvérisation foliaire à stade de la floraison de 20% et de 2 kg ha\(^{-1}\) appliqué au sol avant les semaines) ont été testées. Des doses uniques de la fertilisation d’azote (50, 100, 150 kg ha\(^{-1}\)) ont tous été appliqués avant les semaines. En même temps, la fertilisation d’azote a également été évaluée à deux niveaux: 100 kg ha\(^{-1}\) (50 + 50 kg ha\(^{-1}\)) et 150 kg ha\(^{-1}\) (50 + 100 kg ha\(^{-1}\)), où la première dose de 50 kg N ha\(^{-1}\) a été appliquée avant les semaines, et le reste été appliqué au stade de 3-4 feuilles. En 2012, deux traitements supplémentaires ont été ajoutés: le premier a été 20 kg Sha, 0,5 kg Bkg ha\(^{-1}\) et 200 Nha\(^{-1}\), et l’autre a été 20 kg Sha\(^{-1}\), 0,5 kg de B ha\(^{-1}\) et 50+ 150 kg N ha\(^{-1}\). En 2011, la fertilisation d’azote a montré des effets positifs sur la biomasse sèche, la surface foliaire, la hauteur des plantes, des graines par silique, l’indice de récolte, le rendement et la teneur en protéines des graines, mais a eu un effet négatif sur la teneur en huiles des graines. Le taux optimum de N a été 150 kg N ha\(^{-1}\), entraînant le rendement grainier élevé et une bonne qualité des semences. En 2012, le canola n’a pas bien répondu aux ajouts d’engrais comme cela a été en 2011, apparemment, en raison de la baisse des densités de plantation. L’application des engrais azotés a augmenté seulement les poids de 1000 graines, l’indice de décolte, la teneur en huiles des graines et la teneur en protéines des graines. Parmi les quatre blocs dans l’expérience de 2012, le bloc # 4 a eu
seulement une densité de population de plantes relativement élevée par rapport à la moyenne. Pour le bloc #4 les données de rendement étaient curvilignes et corrélées positivement avec le taux de N ($R^2 = 0.5702$, $p < 0.0001$), avec un rendement maximal se produisant à $150\text{kg N ha}^{-1}$. La croissance du canola n'a pas été souvent affectée par le temps d'application de N. En 2011, il y avait une interaction de S x B pour un poids de 1000 graines en ce que l'effet a été positif avec une application foliaire de $0.5\text{Bkg ha}^{-1}$ et négatif lorsque $2\text{kg Bha}^{-1}$ a été appliqué sur le sol. Seul le bore a augmenté le poids de 1000 graines en 2012. Nos résultats indiquent que les niveaux de S et B dans les sols utilisés étaient probablement suffisants pour la production de canola. La hauteur de la plante a été diminuée (2.9%) après l'application de S à $20\text{kg ha}^{-1}$ en 2011.

En conclusion, nos données suggèrent que le meilleur régime d'application de N pour la production de canola au Québec est une seule application à $150\text{kg N ha}^{-1}$, ce qui a produit un rendement grainier élevé sans sacrifice de la qualité des semences. Possiblement, les additions du soufre et du bore ne sont pas requises pour la production de canola au Québec.
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Contributions of Authors to manuscript

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Chapter 1 GENERAL INTRODUCTION

1.1 Introduction

Canola (Brassica napus) has been successfully produced in cooler agricultural regions around the world, resulting in substantial production of canola in countries such as China, India, parts of the USA, northern Europe, Australia and, of course, Canada. During 2012, total world production reached 61 million tons, Canada accounted for 21.8% of the total world production and ranked as the second highest canola producer (USDA, 2013a). Also, Canada is the leading exporter of canola oil, with exports of 7.1 million t annually (USDA, 2013b).

Canola refers to cultivars of oilseed rape, and is also a term trademarked and licensed by Canadian Canola Council to distinguish the crop, oil and meal from traditional rapeseed, which produces seed oil with less than 2% erucic acid and meals with less than 30 μmol of aliphatic glucosinolates per gram (Statistics Canada 2013). The high oil content (> 40%), lowest saturated fatty acid concentration (6.8%) among the vegetable oils, plus low erucic acid and glucosinolate levels make canola highly valuable to the oilseed industry. (Raymer, 2002). Compared to Canadian soybean production of 4.4 million t, canola yield was estimated to be 14.2 million t in 2011, making it the most produced oilseed in Canada (FAOSTAT, 2013).

In order to help meet reductions in fossil fuel consumption and greenhouse gas (GHG) emissions, the Canadian federal government has required a minimum 2%, by volume, of biodiesel blended into petro-diesel, and 5% ethanol blended into gasoline in 2011 (Environment Canada, 2012). The biodiesel requirement drives a growing market for biodiesel feedstock material in Canada. Canola ranks as the top biodiesel feedstock in Canada (Dyer et al., 2010). The Canola Council of Canada (CCC) (2003) proposed an increase of approximately 3.6 million t oilseed, or an additional 1.62 million ha of canola-cultivated land (CCC, 2003). Responding to the regulation of renewable fuel content in diesel fuel, the canola industry estimated that a 5% renewable content by 2015, without reducing canola oil export, would require Canadian farmers to increase canola production by around 2 million tons per year.
(Dyer et al., 2010). In Canada, canola is largely produced in the northern agricultural regions of the Canadian prairies (Janzen and Bettany, 1984). However, canola production is very limited in eastern regions and has very small production areas in Ontario, Québec and the Maritime Provinces. In 2011, 46% of oilseeds were exported from Canada as seed and the rest was extracted for domestic consumption (36%) or used as biodiesel feedstock (18%) (Senko and Hammond, 2012). Data from the USDA (2006) reveals that eastern Canada contributed less than 1% of Canadian canola production, while Saskatchewan, Alberta and Manitoba accounted for nearly 41, 34 and 24% of Canadian canola production, respectively (USDA, 2006). Thus, exploiting the potential for eastern Canadian canola production would be a feasible way to efficiently increase overall Canadian canola production.

Targeting the high potential market of eastern Canada by increasing the number and capacity of crushing facilities established there, and correspondingly, expanding local canola production, Twin River Technologies – Entreprise De Transformation de GrainesOléagineuses (TRT-ETGO) is now the largest canola and soybean crushing plant and oil refinery across eastern Canada; it is located in Québec, but currently operating mainly through the purchase of oilseed from western Canada and the US (TRT ETGO du Québec, 2011). The current business pattern obviously increases transportation costs and also restricts local economic development. Because of the local imbalance in demand and supply, the current plan of TRT ETGO is to target the area encompassed by the Atlantic Provinces, Québec and eastern Ontario, reaching about as far west as Belleville, for increased canola production (TRT ETGO du Québec, 2011).

These conditions bring challenges and opportunities for eastern Canada, with the potential for expanded canola cultivation and exploitation of new areas of local economic growth. Therefore, an eastern canola research network has been established to develop improved canola production practices for eastern Canada and also canola germplasm with a high seed yield, high oil content and improvements in
other agronomic traits required for successful cultivation in eastern Canada (TRT ETGO du Québec, 2011). As part of this project, our research has focused on defining appropriate fertility management under southwestern Québec soil conditions, including fertilizer types, rates, timing of application, and placement method.

There are general fertility recommendations for canola production in eastern Canada, but these lack data on N use efficiency and responses of canola to B and S in native soils (OMAF, 2011; Davison et al., 2005).

As a component of plant proteins, amino acids, nucleotides, nucleic acids and chlorophyll, N is the most limiting nutrient in canola production (Grant and Bailey 1993). Approximately 79% of the earth’s atmosphere is N in the form of N₂, but this form of N is not directly available to most plants, so that N is generally the nutrient that most restricts biological productivity (Power and Prasad, 2010). Adequate N stimulates vegetative and reproductive development, and also increases the uptake of other nutrients such as S and B (Barker and Bryson, 2006). A canola crop yielding 2000 kg ha⁻¹ requires 124 kg N ha⁻¹ in the aboveground tissue (Ukrainetz et al., 1975).

Sulfur is the fourth most important fertilizer input in agriculture systems. Sulfur deficiency often restricts canola production (Malhi and Gill, 2007). The higher the protein content, the higher the proportion of cysteine and methionine compared with cereals, contributing to a larger S requirement for canola growth (Anderson, 1975; Clandinin, 1981). Furthermore, S not only improves canola seed yield but also N use efficiency (Karamanos et al., 2007). The balance of N and S is critical to optimum seed yield when S is deficient (Malhi and Gill, 2007). Canola was reported to require 3-10 times more S than barley (Malhi and Gill, 2002).

Boron deficiency is a global problem with canola production; canola tends to be more sensitive to B levels than cereal crops (Grant and Bailey 1993; Shorrocks, 1997). A B deficiency can affect root elongation, restrict pollen tube growth and reduce pollen production, which results in severe negative effects on fertilization and seed set, and consequently decreases yields dramatically (Shorrocks, 1997).

Application of fertilizers is a globally accepted practice, which serves as a most
efficient and convenient approach to enriching soils with useful nutrients (Righi et al. 2005). Growing conditions in southwestern Québec and related production challenges differ substantially from those of the main canola producing regions of western Canada; thus, there has always been a need for southwestern Québec-specific research on canola fertilization management.

1.2 Hypotheses

1. Nitrogen application will result in a higher canola yield when S fertilization is present.

2. Nitrogen application at the rate of 150 kg ha\textsuperscript{−1} will result in the highest yield of canola, as compared with other levels of N fertilization.

3. A split application of N is more effective than a single application, resulting in higher canola seed yields.

4. Sulfur application at 20 kg ha\textsuperscript{−1} will increase canola seed yield.

5. Boron soil application at 2 kg ha\textsuperscript{−1} will increase canola seed yield.

6. Boron foliar application at 0.5 kg ha\textsuperscript{−1} will help canola overcome heat stress, resulting in higher seed yield.
1.3 Objectives:

1.3.1 General objective:

Develop the best nutrient management practices for growing canola in eastern Canada.

1.3.2 Specific objectives:

1. Determine the optimum rate and timing of N fertilizer additions for canola production in eastern Canada.

2. Determine the need and best rates of application for the minor nutrient S in canola fertility management in eastern Canada.

3. Determine the need and best rates of application for the micronutrient B for canola growth, yield, and oil content in eastern Canada.
Chapter 2: LITERATURE REVIEW

2.1 Canola

2.1.1 Variety

Canola refers to cultivars of oilseed rape, and is also a term trademarked and licensed by the Canadian Canola Council to distinguish the plant, oil and meal from traditional rapeseed; canola seed has oil that contains less than 2 % erucic acid and meals with less than 30 µmol of aliphatic glucosinolates g$^{-1}$ (Statistics Canada 2013). Canola is one of the few edible oilseeds that is well adapted to growth in cool temperature climates (Ijaz, 2012). Currently there are two species of canola, Brassica napus L, and B. rapa L. (or B. campesteris L.), utilized commercially and produced in significant amounts each year in Canada; their seeds contain at least 40% oil and produce meals with 35 to 40 % protein (Raymer 2002). Because of its high yield potential, Brassica napus is now the most widely cultivated of the two species (Gan et al., 2007). There are two types of Brassica napus: annual and biennial, which are alternatively known as spring and winter types. My study focuses on the spring form of Brassica napus and I refer to this crop as canola in the remainder of this thesis.

Brassica napus is allotetraploid, with 19 haploid chromosomes and a 1130 to 1240 megabasepair genome; it is the hybrid of the diploids Brassicarapa (n = 9, around 500 mbp) and Brassica oleracea (L.) (n = 10, around 600 mbp) (King, 2006). There is molecular evidence demonstrating that natural interspecific hybridizations have occurred several times between Brassica oleracea and Brassica. rapa (Allender and King, 2010; Palmer et al., 1983). Wild Brassica napus was found to grow in several places: Denmark, the Netherlands, New Zealand, Britain, and also Sweden (Dixon, 2007; Rakow, 2004). Visually, Brassica napus can be distinguished from other Brassicaceae by partial clasping of the stem with auricles on the lower leaves and floral buds borne above the open flowers on the terminal raceme (Bengtssonet al., 1972).

2.1.2 History

Canola cultivation and usage of canola, or at least its rapeseed precursor, can be traced back hundreds years in Asia, where it was referred to as rapeseed (Daun et al., 2011). The use of this plant in more western settings can be traced back to 500 and 700 BC, from the...
Roman age of Egypt (Colombini et al., 2005). Because it produced flowing and odourless oil (Dixon, 2007), rapeseed was a ubiquitous source of lamp oil in Europe from the 16th to 18th centuries (Kimber and McGregor, 1995). Systematic research on canola did not begin until the late 1930s (Juska and Busch, 1994), when many countries developed national policies to encourage fat and oil production (Franzaring et al., 2008). In the 1970s, responding to human health concerns, Drs. Downey and Stefansson, in Manitoba, bred high oil varieties with less than 2% erucic acid in the oil, and less than 30 µmol of aliphatic glucosinolates per gram of air-dried oil-free meal (Anstey, 1986). In 1979, production of these Canadian low erucic acid varieties began expanding rapidly and they came to be known as canola, or edible oilseed rape (Franzaring et al., 2008). Nowadays, as a major Canadian cash crop, canola yield was estimated at 14.2 million t in 2011, making it the most produced oilseed in Canada (FAOSTAT, 2013).

2.1.3 Usage

Canola oils are considered to be one of the healthiest cooking oils and the meals are widely used as an ingredient in livestock feeds (Raymer, 2002). For a fairly long time, the value of this crop was reduced by the presence of high quantities of erucic acid and glucosinolates in seeds. High levels of glucosinolates led to problems of pungent odour and sharp taste; in addition, high levels of glucosinolate reduced efficacy of canola meal when fed to livestock and poultry. For this reason, breeders searched for genetic material low in glucosinolates and, in 1969, developed the Polish spring rape variety “Bronowski”, which was low in glucosinolates (CCC, 2003). In addition to glucosinolates, erucic acid levels were a problem for rape seed, contributing to its poor reputation as a foodstuff. Research indicated that erucic acid caused cardiac necrosis and restrained oxidation of fatty acid in rats’ hearts (Christopherson and Bremer, 1972; Iqbal et al., 2011; McCutcheon et al., 1976). The second key breakthrough occurred in 1974, when the University of Manitoba first developed the low erucic acid, low glucosinolate variety “Tower”. This double low variety began the advance of canola in the following decades, and made it one of the most important oilseed crops in temperate areas (CCC, 2003).

In Canada, canola is also a possible source of plant oil for the production of biodiesel.
Because of its high oil content and low saturated fat concentration characteristics, canola is ideal as a fuel during the cold winter months in Canada; it produces high levels of oil per unit of seed, and has a relatively low temperature at which crystals form in the biodiesel (cloud point) (Daun and Hickling, 2011). The Canadian federal government requires a minimum 2% of the volume of diesel fuel to be biodiesel, blended into petro-diesel, as of 2011 (Environment Canada, 2012). This requirement has driven a rapidly growing market for biodiesel feedstock in Canada (Dyer et al., 2010).

2.1.4 Crop development

The life cycle of canola can be divided into 4 developmental stages: (1) germination and emergence, (2) leaf development and stem elongation, (3) flowering, (4) seed development and ripening (Daun, and Hickling, 2011).

(1) Germination and emergence

Canola produces orthodox, non-dormant dry seeds (Schopfer and Plachy, 1985), which are able to germinate at a soil temperature of 1 °C, but 10 °C is ideal for rapid germination and emergence (OMAF, 2011). The diameter of canola seeds ranges from 1.8 to 3 mm, with the mature seed colour ranging from dark brown to black, light brown, or reddish (Warwick 2013). The target depth for seeding is usually 2 cm below the soil surface, whereas the actual depth can be variable and depends on various environmental conditions (Harker et al. 2012). A higher emergence was observed at a depth of 4 cm than 1 cm under very dry soil conditions (Gao et al., 1999). Other extreme conditions provided the same result as normal conditions: canola had higher emergence levels and stand densities when seeded 1 cm below the soil surface than 4 cm (Harker et al., 2012; Gao et al., 1999). Harker et al. (2012) reported that canola emergence density was strongly and positively correlated with precipitation after seeding. Moreover, many canola studies have shown yield decreases correlated with decreased plant density (Ohlsson, 1972; Clarke et al., 1978; Clarke and Simpson, 1978; and McGregor, 1987). A high seeding rate and plant density contribute to a larger seed yield by reducing weed populations (Burnett, 2003). Past studies demonstrated that a non-uniform plant distribution reduces seed yield of spring canola (Brassica napus L.; Angadi et al., 2003) and winter canola (Huhn, 1999). However, a low stand density can have positive effects on
seed yield under certain specific circumstances. McGregor (1987) reported that the decreases in seed yield are proportionally less than decreases in plant density. This is probably the result of plant plasticity, which enables plants to grow larger and produce greater numbers of siliques plant$^{-1}$ (Huhn and Schuster 1975; Clarke and Simpson 1978; Clarke et al. 1978), more branches plant$^{-1}$ (Clarke and Simpson 1978; Clarke et al. 1978), more dry biomass and greater seed weight plant$^{-1}$ (McGregor, 1987).

(2) **Leaf development and stem elongation**

Once above the ground, usually 4 to 15 days after seeding, canola stems start to elongate, and quickly reach 1.25 to 2.5 cm, when the cotyledons expand and turn green, producing chlorophyll for photosynthesis. Unlike barley, the growing point of canola is exposed, which makes it more susceptible to spring frosts, soil drifting, insects and hail. The first true leaves appear 4 to 8 days after emergence (CCC, 2003). Immature leaves are glaucous, glabrous or sparsely hairy and waxy on the surface, which helps restrict water loss (Waalen et al., 2011). Under optimum conditions, canola normally produces 9-30 leaves on the main stem with 250 cm$^2$ of leaf area per plant at the maximum, although this varies somewhat with variety and environmental conditions (CCC, 2003). Stem elongation occurs at the same time as leaf development. Under optimum conditions, canola normally grows 15 to 20 internodes with 5 to 10 cm in each (Potter, 2009), resulting in a maximum final height of up to 120 cm (Kirkland, 1992). Typically, an individual plant produces 3 to 20 branches. Different branching patterns have been observed, due to genotype, light regime, and nutrients. During the rosette growth stage, the canola stem begins to thicken, but the length remains unchanged (CCC, 2003).

Photosynthetic capacity is the main determinant of plant development rate before flowering, and temperature becomes the main effect after flowering (Hodgson, 1978). Rapid leaf development was observed to increase root growth, decrease soil moisture evaporation and reduce weed populations (CCC, 2003). Larger leaf areas are generally associated with larger plants, making them able to intercept more radiation, and have higher photosynthetic levels, which are positively correlated with seed production (Dewey and Lu, 1959). Branch development is often affected by plant density and N application (Jixian and
Hua, 1997). Previous studies also found that branch number is correlated with soil moisture during vegetative growth (Halvorson et al., 2001; Saini and Sidhu, 1997).

(3) Flowering

Canola flowers normally have four free sepals and petals which are golden to pale yellow with an obovate shape (Warwick et al., 2000). Williams (1978) reported that 60 to 70% of canola flowers are self-pollinated. Among the growth stages, flowering is the most critical stage affecting silique and seed development (Zhang et al., 1991), and usually starts 50-55 days after planting and lasts 10-21 days (OMAF, 2011). Flower and silique abortion naturally occur (~45-60%) during this stage, and the level depends on the carrying capacity of leaf, stem and branches, plus effects of environmental stresses. Full plant height is usually achieved at peak flowering (CCC, 2003).

Canola studies have indicated that reduced petal size, or apetalous flowers, result in greater light interception by the leaves (Habbekotté, 1997). In addition, the longer flower duration lasts, in general, the higher the seed yield, due to greater numbers of siliques (Johnston et al., 2002; Mendham and Roberson, 2004). Conversely, longer flowering duration also increases the risk of canola encountering stressfully high temperature conditions, which results in a higher probability of flower abortion and, as a result, severely reduced seed yield (Kutcher et al., 2010; Young et al., 2004). Similarly, delayed maturity increases the risk of early frost damage, especially in Canada, which results in more green seed and low oil quality (Richards and Thurling, 1978).

Canola is most susceptible to pests, diseases, drought and heat (temperature over than 28°C) at the flowering stage (Zhang et al., 1991). Heat stress during flowering may cause flower abortion, leading to significant yield reductions (OMAF, 2011). Chen et al. (2005) observed that 20 °C is the optimum daily temperature for canola growth during the flowering stage. Canola root, stem, leaf, and total plant biomass were reduced when temperature increased from 22°C day / 18°C night to 28 °C day / 24 °C night (Qaderi et al., 2010). In some studies, reduction of canola flowers was observed even at a daily mean temperature of 25.5 °C(Oezer et al., 1999; Polowick and Sawhney, 1988). Hocking and Stapper (2001) found that high temperatures enable canola to produce higher oleic acid contents but, at same
time, lower seed oil concentration, linoleic and linolenic acid levels.

(4) Seed development and ripening

Siliques, defined as pods with one or more seeds in each (Wang et al. 2011), start elongating first at the base of the inflorescence; at the same time senesced leaves are becoming more prevalent and the stem starts to be the main source of seed filling materials from the mid-flower stage (CCC, 2003). The earliest formed siliques have competitive advantages over later formed ones, which are relatively smaller in quantity and size (Wang et al., 2011). Pod ripening occurs around 30–40 days after flower opening, when siliques become brown, and green seeds turn to brown or black (Daun and Hickling, 2011). During this stage, silique abortion and reduced seeds silique⁻¹ can be caused by internal stress, where soil water or nutrients cannot be taken up by plants, or external stress, where nutrient availability is limited or environmental stresses were extreme, ensuring suboptimal plant growth (CCC, 2003). Siliques development can be divided into heterotrophic and autotrophic phases. The main difference between these two phases is reduced carbon source: the heterotrophic phase is supported by leaf and stem photosynthesis, and in the autotrophic phase the silique mainly relies on its own photosynthesis (Jullien et al., 2011). During the maturing phase the seed coat turns to yellow or brown from green, at the same time seed moisture dramatically decreases to 2 to 3 % from 40 to 45 % (CCC, 2003). Lower siliques normally reach the fully ripened condition in 40 to 60 days after the time when the first flower opened. Some 23 to 31% of total plant dry matter is used to form seeds in canola. Canola generally produces 15 to 40 seeds silique⁻¹, seeds weighing 3.5 to 5.5 g (1000 seeds)⁻¹ (CCC, 2003). Seeds are normally 1 to 2 mm in diameter for canola (He and Wu, 2009).

Deng and Scarth (1998) reported that seed maturation can be accelerated by 10 to 15 days at 30 °C days and 25 °C nights, vs. 15 °C days and 10 °C nights. Canola plant yield is a function of 1000-seed weight, siliques plant⁻¹ and seeds silique⁻¹ (Clark and Simpson, 1978). As quantitative traits, seed yield and oil content are variables that depend on genotype, environment, and genotype-by-environment interactions (Engqvist and Becker, 1993; Gunasekera et al., 2006). Canola in western Canada contains an average of 43.8 % oil and 21.1 % protein (Canadian Grain Commission, 2012). Of the four main seed components: oil,
protein, water and residue (Hassan, et al., 2007) an increase in protein is generally at the expense of either oil alone or residue plus oil in Brassica species (Si et al., 2003); a negative relationship between oil and protein percent has been reported by many researchers (Allen and Morgan, 1972; Bhatt, 1964; Brennan et al., 2000; Hassan et al., 2007; Smith et al., 1988; Taylor et al., 1991; Zhao et al. 1993) Furthermore, the sum of seed oil and protein contents was close to fixed: 50 to 60 % for mustards, 60 to 66 % for summer rape and 64 to 70 % for winter rapeseed (Brennan et al., 2000; Holmes, 1980; Ridley, 1973). In addition, Brennan et al. (2000) also noted that each 1 % increase in level of seed protein resulted in a 1.07 % decline in protein.

2.2 Nitrogen

2.2.1 Nitrogen in soil

The earth’s atmosphere is approximately 79% N, in the form of N\textsubscript{2}; however, N is the nutrient that most often restricts biological productivity (Power and Prasad, 2010). Total N content in soil ranges from < 0.02% in subsoil to > 2.5% in organic soils. About 95% of total N in surface soils is present as organic N, with the remainder being inorganic forms such as nitrate and ammonium (Havlin et al., 2005).

(1) Organic nitrogen

Organic N in soil is in various forms: protein, amino acids, amino sugars and other complex N compounds. Approximately 20 to 40 % of total soil N is bound in amino acids; 5 to 10 % is amino sugars (such as hexosamines); < 1% is purine and pyrimidine derivatives. Protein is generally bound with clays, lignin and other materials (Troeh and Thompson, 2005).

(2) Inorganic nitrogen

Inorganic N in soil occurs as ammonium (NH\textsubscript{4}\textsuperscript{+}), nitrite (NO\textsubscript{2}\textsuperscript{−}), nitrate (NO\textsubscript{3}\textsuperscript{−}), nitrous oxide (N\textsubscript{2}O), nitric oxide (NO), and elemental N (N\textsubscript{2}). Ammonium, nitrite and nitrate are important N sources, available to plants, and account for 2 to 5 % of total soil N. They are produced through the mineralization of N-containing organic matter, or N fertilizer additions. When denitrification occurs, N loss from soil can occur as N\textsubscript{2}, N\textsubscript{2}O and NO (Havlin et al., 2005).
In soil, N is involved in various transformations: N\textsubscript{2} fixation, mineralization, nitrification, immobilization, ammonium fixation and other N oxidation or reduction processes. A major source of soil N is N\textsubscript{2} fixation by various soil microbes. Two types of N\textsubscript{2} fixation have been identified: symbiotic and non-symbiotic. In symbiotic fixation, quantities of N\textsubscript{2} are fixed by soil bacteria and actinomycetes residing in plant root nodules (Troeh and Thompson, 2005). Symbiotic N\textsubscript{2}-fixation provides enough nitrogen for adequate growth of legumes without the addition of N fertilizer. Approximately 5-8 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} are fixed through nonsymbiotic fixation, by specific prokaryotic microorganisms in soil or water; this N is incorporated into the cells of these microbes and is released into the soil environment when the cells die and are decomposed (Miller and Donahue, 1990). Mineralization is another important source of N for plants. It involves the conversion of organic N to ammonium. About 5% of organic matter is N, by weight, while only 1 to 3% of the total amount is released yearly (Troeh and Thompson, 2005). Mineralized ammonium ions have a short half-life in soil as most of it is nitrified through microbe driven oxidation to NO\textsubscript{2} and then NO\textsubscript{3}. This process is rapid and ammonium is generally nitrified within 1 or 2 days of release into the soil (Miller and Donahue, 1990). Soil N can also be involved in fixation reactions with soil, whereby ammonium is immobilized in specific clay soils; this N may eventually be used by plants or microbes (Power and Prasad, 2010). Details of the overall N cycle are given in Figure 2.2. Understanding the N cycle is essential to maximizing crop productivity while reducing the negative effects of N fertilization on the environment.

2.2.2 Nitrogen in canola

Like most crops, canola also contains large amounts of N as a component of plant proteins, amino acids, nucleotides, nucleic acids and chlorophyll (Grant and Bailey, 1993). A canola crop yielding 2000 kg ha\textsuperscript{-1} requires 124 kg N ha\textsuperscript{-1} in aboveground tissue (Ukrainetz et al. 1975). Adequate N stimulates vegetative and reproductive development, and also increases the uptake of other nutrients such as S and B (Barker and Bryson, 2006). Nitrogen is the most often limiting element in canola production (Grant and Bailey 1993) and sufficient and timely N applications are required for optimum canola production. However, excess amounts of N can cause crop lodging, reduction in canola seed soil content, increased
seed chlorophyll content and negative environmental impacts (Brennan et al., 2000; Karamanos et al., 2003 and 2007; Rathke et al., 2005).

Plants normally contain 1 to 6 % N by weight and utilize mainly \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) from the soil during growth. In general, more soil solution \( \text{NO}_3^- \) was available to plants than \( \text{NH}_4^+ \), although both are absorbed by plant roots through mass flow and diffusion (Havlin, et al., 2005). The relative N partitioning in plants varies among growth stages. During vegetative growth N is mainly present in leaves, which often contain over 75 % (and at least 25 %) of their N in chloroplasts (Barker and Bryson, 2006). The highest proportional tissue N level in leaves is observed in the early seeding stage when the majority of plant dry matter is in young leaves (CCC, 2003). The stem becomes a major N sink (~50 %) at the early flowering stage, and 40 % of the N is accumulated in silique walls and stems at the end of the flowering stage. By maturity, ~80 % of N is stored in seeds, with the rest remaining in stem and silique wall tissues (Schjoerring et al., 1995). Knowledge of N remobilization in canola plants can help growers obtain optimum N use efficiency and understanding regarding when is the best time to apply N fertilizer.

Nitrogen use efficiency (NUE) is defined as the ratio between seed N yield (kg N ha\(^{-1}\)) and N fertilizer input (kg N ha\(^{-1}\)) in canola (Gan et al., 2008). Canola NUE ranges from 12 to 40 % (depending on the cultivar and management regime). Canola is categorized as a low NUE crop (Gan et al., 2007), while NUE is between 30 and 50 % in typical grain-oilseed production systems (Raun and Johnson, 1999). At the low end, canola deposits only 12 % of available N into seeds, indicating a significant loss of N fertilizer. Approximately 50 % of fertilizer N is lost in abscised leaves, which contained 20 to 25 mg N kg\(^{-1}\), equivalent to a 17.5 kg N ha\(^{-1}\) (Schjoerring et al., 1995). Before interception by plant roots, several mechanisms contribute to fertilizer N loss from soil: denitrification, volatilization, leaching, surface runoff and incorporation into stable soil organic matter and clay colloids (Miller and Donahue, 1990).

### 2.2.3 Canola N deficiency symptoms

Nitrogen is an element that is readily retranslocated within a plant (CCC, 2003). A healthy canola plant is dark green, whereas when N is deficient, older leaves and stems will
first become greenish-yellow and may also display a purple colour. The older leaves will then slowly die. In general, canola plants grow very slowly and are small when N is limiting. In addition, N deficient plants will have only a very short flowering time and low pod numbers (Ukrainetz et al., 1975).

2.2.4 Canola responses to N fertilizer

Previous studies indicate that N application increases canola yield by increasing branches plant\(^{-1}\), buds plant\(^{-1}\), flowers plant\(^{-1}\), stem length, number of flowers, total plant weight, leaf area index (LAI), number and weight of siliques plant\(^{-1}\), seeds plant\(^{-1}\) and average seed weight (Allen and Morgan, 1972). Smith et al. (1988) showed that N is able to prolong leaf life, improve leaf area duration after the flowering stage, and enhance crop nutrient assimilation (Smith et al., 1988).

N fertilizer can boost biomass accumulation, but its application needs to be calibrated to obtain optimal plant nutrition for final seed quality. Previous research demonstrated that N application increases protein content, but at the expense of oil concentration (Brennan et al., 2000; Mason and Brennan, 1998 Malhi, 2001; Gan et al., 2007, 2008). The total seed protein content is approximately 60 to 65 % and is inversely related to oil content (Brennan et al., 2000; CCC, 2003). Seed oil content was reported to decrease 0.6 to 1.2% per additional 100 kg N ha\(^{-1}\) applied (Mendham and Roberson, 2004). Although oil content decreased at high N rates, yield increase is higher than oil content decline, resulting in a total oil yield increase per unit area (CCC, 2003). The overall response of canola to N fertilization, in terms of seed quality and yield, is indicated in Figure 2.1 (CCC, 2003). Having a temperate humid climate similar to Quebec, although somewhat warmer and dryer, Ontario recommends N application at 100 to 110 kg N ha\(^{-1}\), which, resulted in an average yield of 2863 kg ha\(^{-1}\) in 2011 (Earl, 2011; Hall, 2012).

2.3.5 Time of N fertilization

Split N application is known to make N available at more appropriate times during growth, which may be advantageous if there is potential for losing N through leaching or denitrification (Hall, 2012). Results from a field experiment on spring wheat showed that a single initial application at 40 kg N ha\(^{-1}\), and two split doses was most effective when added
N level ranges from 80 to 120 kg ha\(^{-1}\) (Power and Prasad, 2010). For a more canola related example, 50 kg N ha\(^{-1}\) applied before seeding, along with 50 kg N ha\(^{-1}\) side-dressed 5 weeks after seeding caused a 25% increase in yield in *Brassica campestris* production (Ahmad et al., 1999). Two critical environmental effects on split application effectiveness were identified as water availability and temperature. Low water availability (30 mm at the early flowering stage, which is 6 to 8 weeks after seeding) was responsible for low solubility of N fertilizer, inefficient N uptake and N translocation within canola plants. High temperatures (daytime > 25 °C and nighttime > 17°C) affect evapotranspiration, which controls uptake of soluble NH\(_4\)-N and NO\(_3\)-N (Brady and Weil, 2002). Therefore, the best time of N application needs to be identified in Quebec, to maximize canola production at the lowest cost.
Figure 2. 1 Effect of N fertilization on yield and seed quality (CCC, 2003)
Figure 2.2 The N cycle (CCC, 2003).
2.3 Sulfur

2.3.1 Sulfur in soil

Total S in soil falls within the range from a few to 1000 mg S kg\(^{-1}\) soil (0.1%) (Power and Prasad, 2010). As with the N cycle, S undergoes a cycle of oxidation in the soil and then reduction in plants (Havlin et al., 2005). The detailed S cycle is shown in Figure 2.3. Plants take S from soil as SO\(_4^{2-}\), in most cases. There is more S in fine textured soils than in coarse textured ones, since S is an integral part of organic matter. Sulfur tends to be lower in areas with greater rainfall due to increased weathering, leaching and plant cultivation. Sulfur is mainly present as organic and inorganic forms in soil (Troeh and Thompson, 2005).

(1) Organic S

As with N, S is also an integral part of soil. In non-saline soils, the N:S ratio ranges narrowly from 6:1 to 8:1 (Tisdale et al., 1985). Organic S occurs in soil mainly in two forms: C-bonded S (amino acids) and non-C-bonded S (ester sulfates) (Stevenson and Cole, 1999). Freney (1967) conducted an experiment with 24 Australian soils and found that 93 % of total S is organic S; of this, C-bonded S accounted for 41 %. Likewise, Miller and Donahue (1990) stated that 70 to 90 % of total soil S is present in organic matter.

A number of factors affect S mineralization. First, S mineralization increased as organic matter levels in soil increased. Previous research has shown that S mineralization was increased by increasing soil temperature from 20 °C and peaking at 40 °C, after which it decreased (Havlin et al., 2005). Tabatabai et al. (1988) found that S mineralization decreases as pH increases from 4 to 8, under a moderate temperature regime. More S mineralization occurs in the presence of plants than in the absence. Soil moisture content also has a strong influence on S mineralization (Havlin et al., 2005).

(2) Inorganic S

Most inorganic S in soil is in the form of SO\(_4^{2-}\) (Power and Prasad, 2010). Inorganic soil forms were subdivided into three groups: soluble, adsorbed and insoluble S.

Crops normally required 5 mg S kg\(^{-1}\) in soluble form in soil for optimal growth (Power and Prasad, 2010). The soluble S content of soil is variable and depends on factors, such as temperature, precipitation, associated cations, soil water content, and S-containing fertilizer.
Temperature plays an important role in determining the rate of organic matter mineralization. Leaching may occur if there is heavy rain, and is also affected by monovalent cations such as Na and K (Havlin et al., 2005). While rainfall may dissolve S oxides and bring sulfuric acid into soil, decreased water content increases S content in soil solution. On the other hand, S contained in solution is generally leached downward by water (Miller and Donahue, 1990). Decreased water content may be caused by high evapotranspiration rates, which leads to a movement from the soil surface to deeper soil layers, and higher sulfate concentration in the solid phase of surface layers (Power and Prasad, 2010).

Sulfur is normally adsorbed as SO\(_4^{2-}\), in one of three ways: salt adsorption by clay minerals, hydroxides and oxyhydroxides of iron and aluminum with positive charge, and soil organic matter with positive charge under certain conditions (Power and Prasad, 2010). In arid and semiarid areas, abundant Ca and Fe may limit S solubility because of precipitation as gypsum (CaSO\(_4\)) and pyrite (FeS\(_2\)) (Troeh and Thompson, 2005).

### 2.3.2 Sulfur in canola

Sulfur is the fourth most important fertilizer input in agriculture systems; its deficiency often restricts canola production (Malhi and Gill, 2007). Malhi et al (2003) reported that canola needs 3-10 times more S than barley. Typically, S concentration in plants falls within the range of 0.1 to 0.5 % (Havlin, et al., 2005). A higher protein content brings with it a higher proportion of the sulfur containing amino acids cysteine and methionine, compared with cereals, and contributes to higher S requirements for canola growth (Anderson 1975; Clandinin 1981). Glutathione is synthesized from cysteine and functions as a transient S storage repository, is an important antioxidant in plants and is also a precursor of phytochelatins, which detoxify heavy metals in plants (CCC, 2003). Sulfur is also required in chlorophyll and glucosinolate synthesis, especially in members of the Cruciferae (Marschner, 1986). Acting as a defense compound, glucosinolates are broken down, releasing various deterrents when plants are attacked by certain insect or disease organisms (CCC, 2003). Furthermore, S not only improves canola seed yield but also N use efficiency (Karamanos et al., 2007). The balance of N and S is critical to optimum seed yield and quality; seeds tend to accumulate free amino acids when S is deficient (Malhi and Gill, 2002).
Sulfur deficiency can affect plant growth, however, excess can also lead to negative consequences for canola quality by causing elevated levels of glucosinolates, an antinutritive factor in canola meal (Falk et al., 2007).

2.3.3 Sulfur deficiency symptoms

Because S is an immobile nutrient within the plant, deficiency affects all growth stages and causes significant reductions in seed yield and quality (Janzen and Bettany 1984; Malhi and Gill 2002). Mild S deficiency will reduce crop yield, but obvious symptoms may not be observed until S is severely lacking (CCC, 2003). Under S deficiency, younger plant parts initially appear yellowish, because S is required in chlorophyll synthesis (CCC, 2003). When severe S deficiency occurs, developing leaves are small and cupped, with significant purple colouring on the lower side; the stems and pods may also have a reddish-purple discolouration. Sulfur deficiency also causes delayed flowering and maturation, with only a few small, poorly filled siliques developing at the top of the plant. Good levels of S are particularly important during the bud and flowering stages, which constitute the period most sensitive to S deficiency (Grant and Bailey 1993).

2.3.4 Canola responses to S fertilizer

Sulfur application can increase the protein content of meal but can also elevate glucosinolate contents, which is undesirable. Glucosinolates increase at high S fertilizer levels, but are usually still well below the standard canola quality limit (30 µmole per gram) (CCC, 2003). A proper N:S ratio is needed for protein synthesis since amino acids are accumulated in seeds if N is applied without S on S deficient soils (Finlayson et al. 1970; Nuttall et al. 1987). Bailey (1986) found that canola tissue should have a ratio of 12:1 at flowering if it is going to achieve maximum yield. Manitoba Agriculture and Food (2011) recommended that the application of fertilizer should be in an N:S ratio between 5:1 to 8:1. Oil concentration can be increased by S application (Grant et al., 2003; Malhi and Gill, 2002; Nuttall et al., 1987; Ridley, 1973), but may also be decreased (Wetter et al., 1970) or not changed (Ridley, 1973) in some instances. In addition, S application has been found to improve seed quality by decreasing chlorophyll concentration (Grant et al., 2003). Numerous field studies conducted in the Prairie Provinces of Canada (Nuttall et al., 1987;
Ridley, 1973; Wen et al., 2003) have demonstrated that S deficient soils require 15-30 kg S ha$^{-1}$ for canola to grow and develop normally (Table 2.1).

2.3.5 Sulfur fertilizer placement

The best time to apply S largely depends on whether or not the fertilizer is applied as a form that must initially be oxidized to make it available. Sulphate-containing fertilizer is usually water soluble and so is available to plants directly after application. Applying S fertilizer at seeding can avoid S deficiency and result in maximum seed yield (Malhi and Gill, 2002). Seed yield can also be increased by top-dressing or foliar application during the early flowering stage, although this tends to be less efficient than applying the nutrient at seeding (Malhi and Gill, 2007). Spring broadcast or broadcast-incorporation application is a better choice when soil is very moist. Side banding or pre-seed banding should be utilized under dry conditions (Grant and Bailey, 1993). Sulfur fertilizer can be placed in the lower soil horizon, since plant roots tend to grow more deeply when S is limited. In order to encourage the oxidation of elemental S sources to SO$_4^{2-}$, S, fertilizer is better applied as far in advance of seeding as possible (Stevenson and Cole, 1999).
Table 2.1 Response of canola yield to S fertilizer in Manitoba and Saskatchewan

(Ridley, 1973; Nuttal et al. 1987; Wen et al. 2003).

<table>
<thead>
<tr>
<th>Province</th>
<th>Seed yield (kg ha⁻¹) at S rates (kg S ha⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Manitoba</td>
<td>280</td>
<td>2632</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>1557</td>
<td>2318</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>793</td>
<td>1165</td>
</tr>
<tr>
<td>Saskatchewan</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>606</td>
<td>925</td>
</tr>
</tbody>
</table>

Figure 2.3 Simplified version of the overall S cycle (CCC, 2003).
2.4 Boron

2.4.1 Boron in soil

Soil B levels are generally about 20 ppm, with values ranging from 2 to 100 ppm (Troeh and Thompson, 2005); less than 5 % of total soil B is usually available to plants. Boron is highly mobile in soil. Availability of B decreases with increasing soil pH, particularly at a pH greater than 6.3 to 6.5. Fine-textured soil is higher in B than coarse-textured soil and has greater B adsorption ability. Conversely, at equal concentrations of B, coarse textured soil enables plant uptake of more B (Havlin et al., 2005). There are four categories of B in soil:

(1) Present primarily in rock and minerals. The most prominent B mineral in soil is tourmaline, which is relatively insoluble and very slow to become available to plants, helping to explain increased probability of B deficiency under intensive cropping systems. Extreme-weather conditions (such as arid climates) can disrupt mineral stability and result in sufficient or even toxic levels of B in agriculture systems (Power and Prasad, 2010).

(2) Combined in soil organic matter. Organic matter is the largest potential B source available to plants, and is positively correlated with available B in soil (Miller and Donahue, 1990). Low soil moisture reduces B release from organic matter and may even result in B deficiency under arid conditions (Miller and Donahue, 1990).

(3) Adsorbed B. Boron adsorption and desorption decreases the potential for B leaching. Boron adsorption (H$_2$BO$_3^-$) capacity increases with increases in soil pH, clay content, organic matter, and Fe/Al compounds (Havlin et al., 2005).

(4) Soil solution B. Troeh and Thompson (2005) found that the form of B adsorbed is B(OH)$_3$ in acid soils and B(OH)$_4^{−1}$ in alkaline soils. Approximately 0.1 pmm B in solution is sufficient for most monocots (Havlin et al., 2005).

2.4.2 Boron in canola

Boron deficiency is a global problem, and canola tends to be more sensitive to it than do cereal crops (Grant and Bailey, 1993; Shorrocks, 1997). However, B is one of the least understood nutrients in plant nutrition (Karamanos et al, 2003). As a high B demanding crop, canola needs 2 kg B ha$^{-1}$, while wheat and corn require less than 1 kg B ha$^{-1}$ (Malhi, 2001; Gupta, 2007). Plant leaves normally contain 25 to 100 ppm B (Troeh and Thompson, 2005).
Most of our understanding about B derives from deficiency symptoms, and may involve in the following mechanisms: cell wall synthesis and structure, RNA metabolism, respiration hormone metabolism, stomatal regulation, membrane function, sugar transport and carbohydrate metabolism (CCC, 2003). As an essential nutrient for new cell growth, B is required for cell expansion, regulation of H$^+$ transport, cellular Ca$^{2+}$ retention and control of lignin production. Boron supply is needed for cell wall stability during pollen tube growth, and therefore is also essential for seed development. In addition to this, transportation of sugar photosynthesis also requires B to develop meristematic tissues (Havlin et al., 2005).

Mass flow and diffusion are two main approaches used to transfer B from soil solution into plant roots. Therefore, low soil moisture or dry weather reduces B root uptake because of low mass flow and diffusion. Interactions with other elements affect B uptake, such as Ca,K and N; for instance, high solution Ca$^{2+}$ protects crops from excess B (Havlin et al., 2005). High levels of K accelerate B deficiency. In tobacco, B deficiency symptoms are increased as the ratios of Ca:B and K:B increase (Patel and Mehta, 1966). Gupta (2007) found that liberal N application accentuates the severity of B toxicity symptoms in citrus and cereals.

2.4.3 Boron deficiency symptoms

Boron is a micronutrient and its deficiency is a leading cause of yield reduction in canola (Malhi et al., 2003; Yang et al., 2009). Boron is one of least mobile micronutrients in plants, and therefore deficiency symptoms appear first on young growing points and meristematic tissues, such as stem tips, root tips, new leaves and flower buds (Power and Prasad, 2010). Under B deficiency, the symptoms are often not visual until the blooming stage (Grant and Bailey 1993): leaves on the upper plant develop red margins and/or interveinal yellow mottling on the leaves. At the bottom of the plant, leaves senesce earlier (Grant and Bailey 1993). Boron deficiency can affect root elongation, restrict pollen tube growth and reduce pollen production, which consequently affects fertilization and seed set (Shorrocks, 1997). Boron deficiency symptoms also include thickened, cracked, and wilted leaves, petioles, and stems, plus discoulouration, cracking, or rotting of fruits, tubers or roots (Power and Prasad, 2010).
2.4.4 Canola response to B fertilization

Boron fertilization can increase plant height, branches plant⁻¹, siliques plant⁻¹, seeds silique⁻¹, oil content and final seed yield under conditions of B deficiency (Stevenson and Cole, 1999). The addition of B has a range of effects on canola yield. Boron fertilization is often reported to contribute to a small increase in seed yield, eg. 7 % (Porter, 1993) and 7 to 11 % increases (Troeh and Thompson, 2005). However, negative effects of B application have also been observed for seed yield ((Karamanos et al., 2003). In addition, uptake of Ca by canola shoots and roots can be reduced after B application (Nadian et al., 2010). Boron also has important effects on seed production and can decrease protein and increase oil percentage in seeds (Asare and Scarisbrick 1995).

2.4.5 Boron fertilizer placement

Boron can be applied either to soil or foliage. Uniform soil application is required for B fertilization because of the narrow range between deficiency and toxicity (Power and Prasad, 2010). Nuttall et al. (1987) found that application of B works best when incorporated 15 cm below the soil surface. Toxicity symptoms are observed when B fertilizer is placed too close to crop seeds; they are also caused when too much B fertilizer is applied, generally as banded applications (Follett et al. 1981). Boron toxicity may occur if soil application rates exceed 2 kg ha⁻¹ with a soil pH greater than 6.5 (CCC, 2003). Foliar application can be used when B deficiency is observed during crop growth (Grant and Bailey 1993). It appears to be efficacious at the early flowering stage when dry soil restricts root activity (Mortvedt, 1994), and should be less than 0.5 kg ha⁻¹ to avoid toxicity problems (CCC, 2003). Power and Prasad (2010) suggested one dose of 0.1 to 0.5 kg B ha⁻¹ for foliar application.

Canola has good profit potential, but is a high management crop. Heat stress is a severe problem for canola. In 2005, high temperature and insect stress led to a remarkable yield (33%) and seed quality reduction in Ontario, and the canola cultivation area subsequently decreased from 20,200 ha in 2005 to 7500 ha in 2006 (Ramsahoi, 2011). In a controlled environment experiment, canola that experienced heat stress (28 day/20 °C night) during the flowering stage had reduced heat-induced silique abortion after B foliar
application, as compared to control plants. It is also interesting to note that canola did not respond to B foliar application under normal growth conditions (Ramsahoi, 2011). According to the Crop Advances Field Crop Report (2012), Ontario trial results (2008-2011) indicated foliar B applied at flowering rarely increased yields when temperatures were cooler than normal, while increases in economic returns of up to 36% occurred in 73% of trials where canola plants were treated with B in 2010; 2012 was a year with stressfully high temperatures at flowering. OMAF (2011) also indicated that B foliar application prevents canola from blossom blast during summer heat waves (above 28 °C). Agronomic practices recommend producers apply a fungicide at the flowering stage, and therefore save the extra labour cost of B foliar application by applying B with the fungicide. The only additional cost of B foliar application at flowering is the B fertilizer itself, estimated at approximately $12 ha\(^{-1}\) at 0.3 kg B ha\(^{-1}\) (Crop Advances Field Crop Reports, 2012). Boron foliar fertilization is relatively inexpensive, but may have meaningful effects on canola production, possibly resulting in small increases in returns if there is heat stress during flowering. Therefore, it is necessary to quantify the benefits of foliar boron application for canola production in southwestern Québec.
CHAPTER 3: MATERIALS AND METHODS

3.1 Site description

A canola fertility trial was conducted at the Emile A. Lods Agronomy Research Centre of the Macdonald Campus, McGill University, from 2010 to 2012, Ste-Anne-de-Bellevue, Quebec, Canada (45°3'N, 74°11' W) from May to August of each year. In 2011, the soil at this site was a fine sandy-loam and the preceding crop was wheat as a green manure. In the following year, the trial was established adjacent to the site of the 2011 trial; the soil texture at this site was clay loam. The 2012 site was fallow in 2011. Over the long term, from 1971 to 2000, the average monthly temperature and precipitation from April to August were 15.6 °C and 81.4 mm, respectively. Details regarding temperature and precipitation from May to August in 2011 are shown in Figure 4.1a&b; the same information for 2012 is presented in Figure 4.2a&b (Environment Canada, 2012). Soil at this site is a mixed, frigid TypicEndoaquent of the Chicot series (HumicGleysol). The details of soil characteristics are given in Table 3.1 (Soil Test Laboratory McGill University, Sainte-Anne-de-Bellevue, QC, Canada).

3.2 Field experimental design

3.2.1 Experimental design

The experiments were organized following a randomized complete block design with four blocks, so that there were four replications for each treatment. Each block was comprised of 26 treatments comprised of factorial combinations of nutrient application rates: nitrogen (N ha⁻¹); sulfur (S ha⁻¹) and boron (B ha⁻¹) in 2011 (details given below), and 28 treatments in 2012 (details below), resulting in a total of 104 plots in 2011 and 112 plots in 2012 (Figure 3.2). Plot size was 2.6 x 4 m, allowing for 14 rows of canola, with 50 cm spacing between plots. There were 1.3 m wide buffer zones between blocks, and there were border plots at both ends of the experiment; border plots received the same treatments as the neighbouring plots.

3.2.2 Treatment design

In 2011, the 24 applied treatments were the result of factorial combinations of four levels of N (0, 50, 100 and 150 kg ha⁻¹) applied as urea (46-0-0 AgrocentreBelcan
Inc., Ste-Marthe, QC, Canada), two levels of S (0 and 20 kg ha\(^{-1}\)) applied as ammonium sulfate (21-0-0-24 Agrocentre Belcan Inc., Ste-Marthe, Quebec, Canada), and three levels of B (0, 0.5 kg ha\(^{-1}\) foliar spray, and 2 kg ha\(^{-1}\), soil applied). Soil application of boron consisted of sodium borate (14.3% Cameron Chemicals, Inc., Portsmouth, USA), and the B source for foliar spray was B solution (10% Alpline Plant Foods Corporation, New Hamburg, Ontario, Canada) with Agral 90 (Norac Concepts Inc., Guelph, Ontario, Canada) at the rate of 1.25 mL Agral 90 L\(^{-1}\) water. On May 12\(^{th}\), 2011, urea, ammonium sulfate and sodium borate were distributed over the plots and then manually incorporated into the soil with a rake. The last two treatments differed from the aforementioned N100-S20-B0.5 and N150-S20-B0.5 only in that the N applications were split: a first application of 50 kg N ha\(^{-1}\) on May 12, 2011, and the remainder of the N was side-dressed at 50 and 100 kg N ha\(^{-1}\) on June 20, 2011; these treatments are referred to as N50+50-S20-B0.5 and N50+100-S20-B0.5, respectively. For side-dressing, urea was placed into a narrow furrow beside each canola row and then manually mixed into the soil with a rake. The S0 plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate. In 2012, two additional treatments were added, one applied as 20 kg S ha\(^{-1}\), 0.5 kg B ha\(^{-1}\) and 200 kg N ha\(^{-1}\), and the other as 20 kg S ha\(^{-1}\), 0.5 kg B ha\(^{-1}\) and 50 + 150 kg N ha\(^{-1}\); the rest of the treatments remained the same. The times for seeding and N side dressing were on May 7, 2012 and June 13, 2012, respectively.

### 3.3 Plant material, soil preparation, and seeding

In 2011 and 2012, conventional tillage was used for soil preparation as recommended (OMAF, 2011). The canola hybrid, Invigor 5440, was used and applied as a seed treatment. Seeding was conducted with a Plotman seeder (Fabro limited, Swift Current, Saskatchewan, Canada) and seeds were planted, as recommended, at 2.0 cm below the soil surface, at 5 kg ha\(^{-1}\) (OMAF, 2011).

### 3.4 Weed and insect control

For the 2011 trial, Liberty 280SL (Bayer CropScience LP, Research Triangle Park, North Carolina) was applied as a herbicide for weed control at 2.5 L ha\(^{-1}\) and sprayed at the 2-3 leaf growth stage (June 6). On the same day as the herbicide application, Matador 120EC
Syngenta Crop Protection Canada, Inc., Guelph, Ontario, Canada) was applied at a rate of 83 mL ha\(^{-1}\) to control flea beetles. At the same plant growth stage, the same rate of Liberty and Matador as in the previous year was sprayed onto the plants on June 1, 2012.

3.5 Data collection

3.5.1 Meteorological data

Data of minimum, maximum and mean temperature, and total precipitation were obtained from the Environment Canada website, National Climate Data and information Archive (Environment Canada, 2012). The data were collected from the weather station at Ste-Anne-de-Bellevue, Québec, Canada, which is located at approximately 2.2 km from the experimental field. The data were collected from May to August during both years.

3.5.2 Growth characteristics data

Canola plants grew in the field from May to August. Plant samples were collected at three stages: (1) Germination and emergence, (2) flowering and (3) seed ripening.

(1) Germination and emergence

Stand count was conducted after canola emergence (BBCH 09 stage, Weber and Bleiholder, 1990). At this time, cotyledons emerged through the soil surface and one to three small leaves unfolded. During the first study year, the number of plants per row was counted in two randomly representative rows (4 m) for each plot on May 25, 2011. The stand count was conducted on May 24, 2012, and three rows were counted in each plot to make the data less variable.

(2) Flowering

When 20% of flowers on the main raceme opened (BBCH 62 stage, Weber and Bleiholder, 1990), sampling was done for leaf area and dry biomass measurement. On June 27\(^{th}\) 2011, four representative plants were randomly selected from each plot in the first 7 rows (right side of half plot), then leaf area per plant was measured on fresh plant material using a \(\Delta T\) Area Meter (Delta-T Devices LTD., Burwell Cambridge, UK). After that, the harvested plant biomass was dried to a constant weight at 50 °C, and the dry biomass per plant was determined. In 2012, plants were collected on June 26\(^{th}\).

(3) Seed ripening.
(3.1) 80% of siliques ripened

When approximately 80% of the siliques were ripe, dark brown and hard (BBCH 89 stage, Weber and Bleiholder, 1990), most canola characteristics were measured: plant height, branches plant\(^{-1}\), siliques plant\(^{-1}\), seeds siliques\(^{-1}\), 1000-seed weight, harvest index, yield, seed oil and protein contents.

In 2011, plant height was measured on August 24\(^{th}\), at eight locations in each plot, respectively, on inner rows of each plot. During the second year, canola matured much faster than during the first year. Plants attained full height on August 2\(^{nd}\) and were measured at five locations in each plot.

At the same stage, 5 representative plants were randomly selected from the inner rows of each right half of the plot. These samplings were done on August 17\(^{th}\), 2011 and August 6\(^{th}\), 2012. From these plants, I counted siliques plant\(^{-1}\), where a siliques was defined as containing at least one filled brown seed pod. Branches plant\(^{-1}\) (including the main stem) were also recorded, where a branch is defined as containing at least one filled siliques. The seeds were removed from the siliques, dried to constant weight at 50 \(^{\circ}\)C and weighed to give seed weight plant\(^{-1}\).

To determine other yield components, 1 m segments were randomly selected from the inner plot rows in the right half of the plot (two segments per plot) and hand harvested. Harvest of plants from 1 m segments was also conducted on August 17\(^{th}\), 2011 and August 6\(^{th}\), 2012. First, the number of plants per 1 m segment was counted, then, seeds were removed from plants and dried to a constant weight at 50 \(^{\circ}\)C, and weighed to determine seed dry weight. The remainder of the plant biomass was also dried and weighed, so that seed plus non-seed material weights allowed for an estimate of biomass m\(^{-2}\). Harvest index (HI) was then calculated as seed dry weight m\(^{-2}\) / (Seed dry weight m\(^{-2}\) + non-seed dry biomass weight m\(^{-2}\)).

(3.2) 90-100% ripened siliques

Finally, combine-harvest was conducted with a Wintersteriger Classic plot combine (Wintersteriger Inc., Saskatoon, Canada) when more than 90% of siliques were ripe (dark brown and hard). In each case, half of the plot was harvested and this was the half that had not been previously sampled (left side). The final harvests were conducted on August 25\(^{th}\),
2011 and August 8th, 2012. Fresh seed weight was recorded and fresh seeds were dried at 50 °C, to a constant weight, and then dry seed weight per half plot (5.2 m²) was determined. Yield data was calculated from the information above. In addition, 1000- seed dry weight was calculated based on the measured weight of 200 seed dry seeds from each plot. Seeds silique⁻¹ was then calculated as seed dry weight plant⁻¹ x1000/ (1000-seed dry weight x siliques plant⁻¹).

For combine-harvested seeds, grain percent oil and protein were determined at Ottawa in 2011 and Nova Scotia in 2012. In 2011, grain percent oil and protein were determined in Ottawa by using a Foss Infratec™ 1241 Grain Analyzer (Foss North America, Inc., Eden Prairie, Minnesota, USA). A small seed sample was taken from each plot and analyzed with the Foss Infratec™ 1241 using a Sample Transport Module. A top loading cuvette with a path length of 6 mm was used to hold the seeds. The cuvette was inserted into the machine, which gave an average of percent oil, protein and moisture from 10 subsamples.

During the second year, the samples were analyzed for percent oil and protein in Truro, Nova Scotia, using a SpectraStar 2500x NIR spectrometer (Unity Scientific, Inc., Purcellville, Virginia, USA). Seed samples were loaded into sample cups; small ring cups, capable of holding 3 to 5 g seeds, were used. Each sample was scanned twice. After the first scan, seed was dumped out and then put back into the cup, providing a repack of the seeds, for the second scan. The resulting percent oil, protein and moisture estimates were averaged across the two readings.

3.6 Statistical Analysis

Statistical analyses were performed with the software package SAS 9.3 (SAS Institute Inc.). A UNIVARIATE procedure was used to assess whether raw data conformed to the conventional statistical assumptions. Log and square-root transformations were used to normalize data when required. Statistical analyses were conducted following a Completely Randomized Block design and the General Linear Model (GLM) procedure. The significance of interactions and main effects were assessed based on the Type III Test of Fixed Effects. Treatments were reorganized according to rates of fertilizer application and means were compared using a protected least significant difference (LSD) test and an α value of 0.05.
Correlation analyses with Pearson’s correlation coefficient were performed to determine the relationship of various growth variables with seed yield, oil and protein percent.
Table 3.1 Soil physical and chemical properties for canola fertility trials in 2011 and 2012

<table>
<thead>
<tr>
<th>Texture</th>
<th>Loam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>493 g sand kg(^{-1}), 282 g silt kg(^{-1}), 225 g clay kg(^{-1})</td>
</tr>
<tr>
<td>SOM (g kg(^{-1}))</td>
<td>21.9</td>
</tr>
<tr>
<td>pH</td>
<td>6.13</td>
</tr>
<tr>
<td>P (mg Mehlich-3)</td>
<td>42.7</td>
</tr>
</tbody>
</table>

Note: SOM = soil organic matter; P = phosphorus, determined by Mehlich three methodology
CHAPTER 4: RESULTS

4.1 Weather conditions

Daily temperatures at the Ste-Anne-de-Bellevue site from April to August of 2011 and 2012 are given in Figures 4.1a and 4.2a, respectively. In each of these years, canola was seeded in early May when the mean daily temperature was approximately 10 °C, providing an ideal seedbed temperature for rapid crop emergence (OMAF, 2011). As the canola was growing, average daily temperature gradually increased to ~20 °C at the beginning of flowering. During the flowering stage, the most sensitive period to high temperature, the weather was not sufficiently hot to cause heat stress (> 28 °C) to canola plants in either year. As indicated in Figures 1a and 1b, the weather conditions were not stressful to canola growth and no meaningful negative temperature impact was observed.

The pattern of precipitation during the growing season varied between 2011 and 2012 (Figures 4.1b and 4.2b). In May, the seedbed was generally moist, as it received a good level of total precipitation: 115.4 mm in 2011 and 93.5 mm in 2012. Precipitation was less in June of both years (~55 mm) and temperatures were higher. In 2011, the July precipitation was not well distributed and conditions were drier (35.6 mm) than in July of 2012 (85.5 mm). High temperatures at the flowering stage can result in flower and pod abortion (OMAF, 2011). Fortunately, there was no meaningful heat stress at flowering during the dry period of July 2011, in part because the flowering stage ended in early July. Developing canola siliques reached their final size, and matured (darkened and dried) in August. It is recommended that canola be combined when seed moisture reaches 12-15% (Davison et al., 2005). In 2011, there was a large amount of precipitation (136 mm) in August and siliques reached maturity one week later than in 2012, when conditions were drier (46.9 mm).
Figure 4.1 Meteorological data for 2011.

A. Daily temperature readings (maximum, minimum, mean temperature) for the months from May to August, 2011 (growing season for the canola field trial), Ste-Anne-de-Bellevue. The temperature is presented in °C (Source: www.climate.weatheroffice.gc.ca)

B. Daily total precipitation for the months from May to August, 2011 (growing season for the canola field trial), Ste-Anne-de-Bellevue. Total precipitation is presented in mm (Source: www.climate.weatheroffice.gc.ca)
Figure 4.2 Meteorological data for 2012.

A. Daily temperature readings (maximum, minimum, mean temperature) for the months from May to August, 2012 (growing season for the canola field trial), Ste-Anne-de-Bellevue. The temperature is presented in °C (Source: www.climate.weatheroffice.gc.ca)

B. Daily total precipitation for the months from May to August, 2012 (growing season for the canola field trial), Ste-Anne-de-Bellevue. Total precipitation is presented in mm (Source: www.climate.weatheroffice.gc.ca)
4.2 Population density

Plant population density of all plots was measured at growth stage BBCH 09 (Weber and Bleiholder, 1990) in both study years. The average number of plants m\(^2\) was 110, ranging from 77 to 138, in 2011 (Table 4.1). The pattern of plant density distribution was not affected by any main effect or interaction of N, S or B (P > 0.05). A similar situation occurred in 2012. However, the average plant density (80 plants m\(^2\)) was much lower in 2012 (Table 4.2) than in 2011. In addition to this, 2012 plant population density was less uniform than in 2011, and thus there was a wider range of plot values, from 27 to 199.

In order to better understand canola growth differences, visual observations were recorded 14 days after measuring plant density, when plants were in the beginning of stem elongation (BBCH 31 stage, Weber and Bleiholder, 1990). Visual observation indicated that higher rates of N fertilization produced larger plants and darker green leaves, as shown in Appendix 1. In 2012, visual observations indicated that there were differences among blocks, where the plot coverage was only around 50% (30 days after seeding) in the first block and increased from block 1 to block 4, where the coverage was 90 – 100% at the same time. Inversely, weed populations increased from block 4 to block 1 when sampling in 2012. There were visual differences among the plants of individual plots within each block, probably due to treatment effects. Overall plant density was relatively uniform within each block. A similar trend was observed for plant height and size. Canola plants in plots with higher stand densities tended to grow taller and have a larger canopy. By comparison, in 2011, plant density was visually uniform among the four blocks and reached 90 - 100% coverage (30 days after seeding) in most plots.
Table 4.1 Effect of rate of N, S and B application on plant characteristics (plants m$^{-2}$, dry biomass plant$^{-1}$, leaf area plant$^{-1}$, plant height, branches plant$^{-1}$, siliques plant$^{-1}$, seeds silique$^{-1}$, 1000-seed weight, harvest index, yield, oil and protein) in 2011.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Range</th>
<th>Source of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitrogen</td>
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<td></td>
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<td>Sulfur</td>
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<td>Boron</td>
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<td></td>
<td></td>
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<td>N x S</td>
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<td></td>
<td></td>
<td></td>
<td>N x B</td>
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<td></td>
<td></td>
<td></td>
<td>S x B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N x S x B</td>
</tr>
<tr>
<td>Plants m$^{-2}$</td>
<td>109.63</td>
<td>77.28 ~ 138.44</td>
<td>NS</td>
</tr>
<tr>
<td>Dry biomass plant$^{-1}$, g</td>
<td>4.47</td>
<td>2.56 ~ 8.05</td>
<td>&lt;.0001 NS</td>
</tr>
<tr>
<td>Leaf area plant$^{-1}$, cm$^2$</td>
<td>379.92</td>
<td>191.20 ~ 620.60</td>
<td>&lt;.0001 NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>111.66</td>
<td>99.40 ~ 129.60</td>
<td>&lt;.0001 0.0040 NS</td>
</tr>
<tr>
<td>Branches plant$^{-1}$</td>
<td>3.65</td>
<td>2.00 ~ 14.00</td>
<td>NS</td>
</tr>
<tr>
<td>Siliques plant$^{-1}$</td>
<td>72</td>
<td>31 ~ 166</td>
<td>NS</td>
</tr>
<tr>
<td>Seeds silique$^{-1}$</td>
<td>18.87</td>
<td>14.97 ~ 28.13</td>
<td>0.0249 NS</td>
</tr>
<tr>
<td>1000-seed weight, g</td>
<td>2.79</td>
<td>2.59 ~ 3.07</td>
<td>NS</td>
</tr>
<tr>
<td>Harvest index</td>
<td>38.59%</td>
<td>32.83 ~ 4.25%</td>
<td>0.0094 NS</td>
</tr>
<tr>
<td>Yield, kg ha$^{-1}$</td>
<td>3330.00</td>
<td>2442.00 ~ 4109.00</td>
<td>&lt;.0001 NS</td>
</tr>
<tr>
<td>Oil (%)</td>
<td>47.11</td>
<td>45.00 ~ 49.40</td>
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</tr>
<tr>
<td>Protein (%)</td>
<td>23.30</td>
<td>19.99 ~ 25.58</td>
<td>&lt;.0001 NS</td>
</tr>
</tbody>
</table>

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant;
Table 4.2 Effect of rate of N, S and B application on plant characteristics (plants m\(^{-2}\), dry biomass plant\(^{-1}\), leaf area plant\(^{-1}\), plant height, branches plant\(^{-1}\), siliques plant\(^{-1}\), seeds silique\(^{-1}\), 1000-seed weight, harvest index, yield, oil and protein) in 2012.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Range</th>
<th>Source of variation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitrogen Sulfur Boron N x S Nx B S x B N x S x B</td>
</tr>
<tr>
<td>Plants m(^{-2})</td>
<td>79.95</td>
<td>26.67 ~ 199.00</td>
<td>NS NS NS NS NS NS NS NS</td>
</tr>
<tr>
<td>Dry biomass plant(^{-1}), g</td>
<td>8.26</td>
<td>2.16 ~ 21.04</td>
<td>NS NS NS NS NS NS NS NS</td>
</tr>
<tr>
<td>Leaf area plant(^{-1}), cm(^{2})</td>
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<td>80.80 ~ 1505.00</td>
<td>NS NS NS NS NS NS NS NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>117.18</td>
<td>103.20 ~ 135.40</td>
<td>NS NS NS NS NS NS NS NS</td>
</tr>
<tr>
<td>Branches plant(^{-1})</td>
<td>5.40</td>
<td>2.00 ~ 19.00</td>
<td>NS NS NS NS NS NS NS NS</td>
</tr>
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<td>Siliques plant(^{-1})</td>
<td>112</td>
<td>44 ~ 250</td>
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<tr>
<td>Seeds silique(^{-1})</td>
<td>20.72</td>
<td>12.05 ~ 101.48</td>
<td>NS NS NS NS NS NS NS NS</td>
</tr>
<tr>
<td>1000-seed weight, g</td>
<td>3.02</td>
<td>2.62 ~ 3.56</td>
<td>&lt;.0001*** NS 0.0013** NS NS NS NS</td>
</tr>
<tr>
<td>Harvest index</td>
<td>39.29%</td>
<td>35.00% ~ 47.50%</td>
<td>0.046* NS NS NS NS NS NS</td>
</tr>
<tr>
<td>Yield, kg ha(^{-1})</td>
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<td>776.62 ~ 4282.00</td>
<td>NS NS NS NS NS NS NS NS</td>
</tr>
<tr>
<td>Oil (%)</td>
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<td>39.00 ~ 46.62</td>
<td>0.0004** NS NS NS NS NS NS NS</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>23.57</td>
<td>19.07 ~ 26.20</td>
<td>&lt;.0001*** NS NS NS NS NS NS NS</td>
</tr>
</tbody>
</table>

*** P < 0.001; ** P < 0.01; * P < 0.05; NS, not significant;
4.3 Dry biomass

4.3.1 2011

There was a N rate main effect (P < 0.0001), but no effect of S (P > 0.05). In the first year, dry biomass plant\(^{-1}\) varied from 2.56 to 8.05 g and had an average dry value of 4.47 g (Table 4.1). Plant dry biomass tended to increase with increasing N application, and the highest value (5.21 g) resulted from the application of N at 150 kg ha\(^{-1}\); this treatment was not different (p < 0.05) from split N application at 50+100 N kg ha\(^{-1}\) (4.76 g). Split application of N produced a numerically higher mean dry biomass than a single application at the same amount of N application rate, but the difference was not statistically significant (P > 0.05; Figure 4.3).
Figure 4.3 Effect of N application on dry biomass plant$^{-1}$ in 2011. The Y-axis indicates dry biomass plant$^{-1}$ and the X-axis indicates the amount of N applied (The 0 kg S ha$^{-1}$ plots received 17 kg N ha$^{-1}$ to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha$^{-1}$ level includes the background N present in the soil plus the 17 kg N ha$^{-1}$ added to compensate for N added with the sulfate). Split N applications (50 kg N ha$^{-1}$ before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean dry biomass plant$^{-1}$. Columns associated with the same letters are not different at p < 0.05.
4.3.2 2012

There were neither main nor interaction effects for dry biomass plant$^{-1}$ in 2012 ($P > 0.05$). The average dry biomass plant$^{-1}$ was 8.26 g and ranged from 2.16 to 21.04 g (Table 4.2).

4.4 Leaf area

Data regarding total leaf area plant$^{-1}$ were collected at the 20% flowering stage (BBCH 62 stage, Weber and Bleiholder, 1990) in both 2011 and 2012 experiments.

4.4.1 2011

At the 20% flowering stage, N application rate had a positive effect on leaf area plant$^{-1}$ ($P < 0.0001$) (Table 4.1), whereas canola leaf area did not respond to levels of S or B fertilizer addition ($P > 0.05$). Table 3 shows that the average leaf area plant$^{-1}$ was 379.92 cm$^2$, with a range of 191.20 to 620.60 cm$^2$. In Figure 4, no data are shown for split application treatments (50+50 and 50+100 kg N ha$^{-1}$), as the N side-dress was applied immediately before sampling, so that it would not be expected to have caused any differences between the 50, 50+50 and 50+100 kg N ha$^{-1}$ treatments at the 20% flowering stage. The data demonstrated that increasing the rate of N application progressively increased plant leaf area (Figure 4.4). The lowest leaf area (320.58 cm$^2$) was for the treatment receiving 0 kg N ha$^{-1}$, while the largest value (426.52 cm$^2$) was for 150 kg N ha$^{-1}$, and resulted in a 33% increase as compared to the control. The second highest leaf area was achieved with 100 kg N ha$^{-1}$(409.51 cm$^2$), and this value was not different from the highest leaf area ($P > 0.05$).
Figure 4.4 Effect of N application on leaf area plant$^{-1}$ in 2011. The Y-axis indicates leaf area plant$^{-1}$ and the X-axis indicates the amount of N applied prior to sowing (The 0 kg S ha$^{-1}$ plots received 17 kg N ha$^{-1}$ to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha$^{-1}$ level includes the background N present in the soil plus the 17 kg N ha$^{-1}$ added to compensate for N added with the sulfate). Columns represent mean leaf area plant$^{-1}$. Columns associated with the same letters are not different at p < 0.05.
4.4.2 2012
The average leaf area plant\(^{-1}\) was 527.52 cm\(^2\) and ranged from 80.80 to 1505.00 cm\(^2\) (Table 4.2). In the present study, there was no effect of N, S or B on plant leaf area during the second study year (P > 0.05).

4.5 Plant height

4.5.1 2011
At maturity, the average final plant height reached 111.66 cm and varied from 99.40 to 129.60 cm in 2011 (Table 4.1). Differences in plant height were observed amongst the N rates (P < 0.0001). The greatest height (117.4 cm) occurred at 150 kg N ha\(^{-1}\) (Figure 4.5a), an average not different (P < 0.05) from N application at 50 +100 kg N ha\(^{-1}\) (114.4 cm). The mean value of four single N application treatments indicated that plant height increased from the lowest rate (control - 107.5 cm) to 150 kg N ha\(^{-1}\). Nitrogen application at 150 kg N ha\(^{-1}\) resulted in the highest plant height with either single or split applications. The 50+50 kg N ha\(^{-1}\) treatment decreased plant height as compared with a single application of 100 kg N ha\(^{-1}\) and was not different from the 0 kg N ha\(^{-1}\) treatment (P > 0.05). The 2011 data also demonstrated a clear influence of S on canola height (P = 0.004). The higher amount of added S reduced the mean height (Figure 4.5b). Boron did not have any effect on plant height (P > 0.05).
Figure 4.5 Effect of fertilizers on plant height.

A. Effect of N application on plant height in 2011. The Y-axis indicates plant height and the X-axis indicates the amount of N applied (The 0 kg S ha⁻¹ plots received 17 kg N ha⁻¹ to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha⁻¹ level includes the background N present in the soil plus the 17 kg N ha⁻¹ added to compensate for N added with the sulfate). Split N applications (50 kg N ha⁻¹ before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean plant height of corresponding treatment as indicated by the X-axis. Columns with the same letters are not different at p < 0.05.

B. Effect of S application on plant height in 2011. The Y-axis indicates plant height and the X-axis indicates the amount of S applied before sowing. Columns represent mean plant height. Columns with the same letters are not different at p < 0.05.
4.5.2 2012

In 2012 year, the mean canola height was 117.18 cm and ranged from 103.20 to 135.40 cm (Table 4.2). In contrast to the previous year, there was no effect of N, S or B on plant height (P > 0.05).

4.6 Branches plant\(^{-1}\)

In 2011, there was no main or interaction effect (P > 0.05) on branches plant\(^{-1}\)(Table 4.1). Branches plant\(^{-1}\) was randomly distributed from 2.00 from 14.00, and the average value was 3.65. In the second year N, S and B did not affect branches plant\(^{-1}\) (P > 0.05) (Table 4.2). The average branch number was 5.40 plant\(^{-1}\) in 2012, and ranged from 2.00 to 19.00.

4.7 Siliques plant\(^{-1}\)

In 2011, there was no effect of N, S or B onsiliques plant\(^{-1}\) (P > 0.05). In addition, the average number of siliques plant\(^{-1}\) was 72 and varied from 31 to 166 (Table 4.1). Siliques plant\(^{-1}\) did not respond to N, S or B fertilization in 2012 (P > 0.05). The mean number of siliques was 112 plant\(^{-1}\) and ranged from 44 to 250 (Table 4.2).

4.8 Seeds silique\(^{-1}\)

4.8.1 2011

In 2011, the minimum number of seeds silique\(^{-1}\) was 14.97, across the 26 treatments, and the highest value was 28.13 seeds silique\(^{-1}\). The average number of seeds silique\(^{-1}\) was 18.87. There was no effect of S and B application for seeds silique\(^{-1}\) (P > 0.05), while there was a clear effect of N application on this variable (P = 0.0249). There was an upward curvilinear effect of N rate, up to 100 N kg ha\(^{-1}\) for seeds silique\(^{-1}\) (Figure 4.6) and the maximum value was (19.53). Further increasing N application rate, to 150 kg N ha\(^{-1}\), resulted in essentially the same value, 19.51 seeds silique\(^{-1}\) (P > 0.05). Split N application did not affect seeds silique\(^{-1}\) at 150 kg N ha\(^{-1}\), while split application at 100 kg N ha\(^{-1}\) decreased seeds silique\(^{-1}\).
Figure 4.6 Effect of N application on seeds silique$^{-1}$ in 2011. The Y-axis indicates seeds silique$^{-1}$ and the X-axis indicates the amount of N applied (The 0 kg S ha$^{-1}$ plots received 17 kg N ha$^{-1}$ to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha$^{-1}$ level includes the background N present in the soil plus the 17 kg N ha$^{-1}$ added to compensate for N added with the sulfate). Split N applications (50 kg N ha$^{-1}$ before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean seeds silique$^{-1}$ of corresponding treatment as indicated by the X-axis. Columns associated with the same letters are not different at p < 0.05.
4.8.2 2012

Plants had 20.72 seeds silique⁻¹, on average, in 2012, with values ranging from 12.05 to 31.48. In addition, seeds silique⁻¹ was not affected by N, S or B application (P > 0.05; Table 4.2).

4.9 1000-seed weight

4.9.1 2011

In 2011, the average 1000-seed weight was 2.79 g and the range was from 2.59 to 3.07 g (Table 4.1). There was an interaction effect between S and B (P = 0.0431) (Figure 4.7a). When B application was increased from 0 to 0.5 kg ha⁻¹, this resulted in increased 1000-seed weight. Similarly, the single application of 2 kg B ha⁻¹ and 20 kg S ha⁻¹ also produced greater average 1000-seed weights than the control treatment, although still slightly less than that of the 0.5 kg B ha⁻¹ application. Data from the S and B interaction indicated that a small increase in 1000-seed weight occurred when S and B were applied in combination at rates of 20 kg S ha⁻¹ and 0.5 kg B ha⁻¹ (Figure 4.7a). It is also interesting to notethat 1000-seed weight decreased when S and B were applied as 20 and 2 kg ha⁻¹, respectively.
Figure 4.7. Effect of fertilizers on 1000-seed weight.

A. Interaction effect of S and B on 1000-seed weight in 2011. The Y-axis indicates 1000-seed weight and the X-axis indicates control and fertilizer treatments. Each point represents mean 1000-seed weight of the treatment indicated on the X-axis. The lines indicate departures from mean 1000-seed weight of the control treatment value due to application of B and/or S: 20 kg S ha\(^{-1}\) (S20B0), 2 kg B ha\(^{-1}\) (S0B2), 0.5 kg B ha\(^{-1}\) (S0B0.05), 20 kg S ha\(^{-1}\) plus 0.5 kg B ha\(^{-1}\) (S20B0.5) and 20 kg S ha\(^{-1}\) plus 2 kg B ha\(^{-1}\) (S20B2). Treatments followed by the same letters do not differ at p < 0.05.
4.9.2 2012

In 2012, average 1000-seed weight was 3.02 g (Table 4.2) and varied from 2.62 to 3.56 g across the 28 treatments. During this year, both N application ($P < 0.001$) and B fertilization had clear effects ($P = 0.0013$) on 1000-seed weight. In terms of N application (Figure 4.7b), the highest 1000-seed weight (3.21 g) occurred at 50+150 kg N ha$^{-1}$, which was not different from the value for N application at 50+100 kg N ha$^{-1}$ or 100 kg N ha$^{-1}$ ($P > 0.05$). These three treatments were the only ones to result in higher 1000-seed weights than the control ($P < 0.05$). Split application tended to result in higher 1000-seed weights than single applications, with the difference being clearer at larger N fertilizer application rates, especially at 50+150 kg N ha$^{-1}$, which resulted in a heavier average 1000-seed weight than treatment with 200 kg N ha$^{-1}$.

In this study, soil application of 2 kg B ha$^{-1}$ did not alter 1000-seed weight, and foliar application of 0.5 kg B ha$^{-1}$ caused a small (2.1 %) reduction in the 1000-seed weight, from 3.06 to 2.99 g ($P<0.05$) as compared with the control treatment (Figure 4.7c).
Figure 4.7. Effect of fertilizers on 1000-seed weight.

B. Effect of N application on 1000-seed weight in 2012. The Y-axis indicates 1000-seed weight and the X-axis indicates the amount of N that was applied before sowing or those that had part of the N side-dressed at the 4-6 leaf stage (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate). Split N application is represented as 50+50, 50+100, 50+150: with first application at 50 kg N ha\(^{-1}\) before sowing and the remainder was side-dressed at 50, 100, and 150 50 kg N ha\(^{-1}\) at the 4-6 leaf stage. Columns represent mean 1000-seed weight. Columns with the same letters are not different at p < 0.05.

C. Effect of B application on 1000-seed weight in 2012. The Y-axis indicates 1000-seed weight, while the X-axis indicates the amount of B applied; “0” is the control treatment, “0.5” represents 0.5 kg B ha\(^{-1}\) foliar applied at the 20 % flowering stage and “2” indicates 2 kg B ha\(^{-1}\) soil applied before sowing. Columns represent mean 1000-seed weight. Columns with the same letters are not different at p < 0.05.
4.10 Harvest index

4.10.1 2011

Increasing rates of S and B application did not increase canola harvest index (P > 0.05) (Table 4.1), whereas N application rate had a very clear influence (P = 0.0094) on this variable. Mean harvest index was affected by N rate (Figure 4.8a). Application of 50+100 kg N ha\(^{-1}\) resulted in a harvest index value of 42.25\% (Figure 4.8a), higher than the value for all other treatments (P < 0.05), and 11.8 \% greater than that of the 0 kg N ha\(^{-1}\) control treatment. The second highest harvest index resulted from application of 100 kg N ha\(^{-1}\), followed by 150 N kg ha\(^{-1}\), although the latter was not different from the control treatment. Split application resulted in a higher average harvest index at 150 kg N ha\(^{-1}\), but not 100 kg N ha\(^{-1}\).
A. Effect of N application on harvest index in 2011. The Y-axis indicates the mean harvest index of each treatment and the X-axis indicates the amount of N applied (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate). Split N applications (50 kg N ha\(^{-1}\) before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean harvest index. Columns with the same letters are not different at p < 0.05.
There was no effect of S (P = 0.8218) or B (P = 0.6577) application, but there was an indication of some N application effect (P = 0.046) (Table 4.2). Figure 4.8b presents the mean comparison of harvest index at different N levels. The 50+100 and 50+150 kg N ha\(^{-1}\) treatments resulted in the maximum mean harvest index (41.13 %) (Table 4.2), but resulted in only small increases compared with the control treatment (38.33 %). Increasing the rate of N fertilizer application tended to enhance harvest index up to 150 kg N ha\(^{-1}\); 200 kg N ha\(^{-1}\) reduced mean harvest index, to a level below that of the 0 kg N ha\(^{-1}\) control treatment. In addition, there was no difference between single and split N applications, except that treatment with 200 kg N ha\(^{-1}\) and 50+150 kg N ha\(^{-1}\) (P < 0.05), for which the split application resulted in a greater harvest index than single application.
B. Effect of N application on harvest index in 2012. The Y-axis indicates the mean harvest index and the X-axis indicates the amount of N applied (The 0 kg S ha$^{-1}$ plots received 17 kg N ha$^{-1}$ to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha$^{-1}$ level includes the background N present in the soil plus the 17 kg N ha$^{-1}$ added to compensate for N added with the sulfate). Split N applications (50 kg N ha$^{-1}$ before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean harvest. Columns with the same letters are not different at p < 0.05.
4.11 Yield

4.11.1 2011

In the first year of this study, average yield was 3330 kg ha$^{-1}$, and ranged from 2442 to 4109 kg ha$^{-1}$ (Table 4.1). Nitrogen played an important role in canola yield, while S and B did not have any effect (p > 0.05). Figure 4.9b shows that yield was positively correlated with N rate ($r^2 = 0.4662$, P < 0.0001); yield increased with increasing N input. The highest yield was achieved with the 50+100 and 150 kg N ha$^{-1}$ treatments, which were different from each other, and resulted in increases of 29.5 and 22.3 %, respectively, as compared to the control treatment. In addition, application of N at 100 and 50+50 kg ha$^{-1}$ both resulted in numerical increases in yield, although the resulting yields were not statistically different from each other (p < 0.05). The result indicated that application of N as a split application resulted in small, but probably not agronomically important yield increases, as compared to single applications applied prior to seeding.

Pearson’s correlation coefficient analysis (Table 4.3) revealed positive and significant relationships of yield with the traits dry biomass plant$^{-1}$, total leaf area plant$^{-1}$, plant height, seeds siliquae$^{-1}$, and harvest index. In addition, there was a negative correlation between yield and 1000-seed weight and oil percent.
Figure 4.9 The relationship between N application and yield.

A. Effect of N application on yield in 2011. The Y-axis indicates canola yield and the X-axis indicates the amount of N applied (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate). Split N applications (50 kg N ha\(^{-1}\) before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean yield. Columns with the same letters are not different at p < 0.05.

B. Correlation between canola yield and N rate in 2011. The Y-axis indicates canola yield and the X-axis indicates the amount of N applied before sowing: 0, 50, 100, 150 and 200 kg N ha\(^{-1}\). (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate.)
Table 4.3 The correlation of seed yield, oil and protein with plant characteristics (plants m\(^{-2}\), dry biomass plant\(^{-1}\), leaf area plant\(^{-1}\), plant height, branches plant\(^{-1}\), siliques plant\(^{-1}\), seeds silique\(^{-1}\), 1000-seed weight, harvest index, yield, oil and protein) in 2011.

<table>
<thead>
<tr>
<th></th>
<th>Yield</th>
<th>Oil (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants m(^{-2})</td>
<td>-0.069</td>
<td>-0.078</td>
<td>* 0.066</td>
</tr>
<tr>
<td>Dry biomass plant(^{-1}), g</td>
<td>0.365</td>
<td>** -0.360</td>
<td>** 0.363</td>
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<tr>
<td>Leaf area plant(^{-1}), cm(^{2})</td>
<td>0.578</td>
<td>*** -0.369</td>
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<tr>
<td>Height, cm</td>
<td>0.543</td>
<td>** -0.411</td>
<td>*** 0.553</td>
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<tr>
<td>Branches plant(^{-1})</td>
<td>0.156</td>
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<td>1000-seed weight, g</td>
<td>-0.329</td>
<td>** 0.208</td>
<td>-0.273</td>
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<tr>
<td>Harvest index</td>
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<td>** -0.310</td>
<td>** 0.314</td>
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<tr>
<td>Yield</td>
<td>1.000</td>
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<td>*** 0.638</td>
</tr>
<tr>
<td>Oil (%)</td>
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<td>*** 1.000</td>
<td>-0.602</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>0.638</td>
<td>*** -0.602</td>
<td>*** 1.000</td>
</tr>
</tbody>
</table>

*** P < 0.001; ** P < 0.01; * P < 0.05;
4.11.2 2012

The average yield in 2012 was 2814 kg ha\(^{-1}\) and ranged from 776.62 to 4282 across the 28 treatments (Table 4.2). The results also demonstrated that there was no difference in yield due to the application of N, S, and B (P > 0.05). In 2012, two more N rates were added to the set of treatments used in 2011: N at the rate of 200 and 50+150 kg ha\(^{-1}\). These new treatments did not cause an increase in yield. While it is interesting to find that yield data from block 4 was positively correlated with level of N fertilization (R\(^2\) = 0.5702, P < 0.0001) (Figure 4.9d). Seed yield increased with increasing rate of N fertilization. In addition, the average yield across blocks, which had increasing plant densities from block 1 to block 4, was linearly correlated with average plant density of block (R\(^2\) = 0.9649, P < 0.0001) (Figure 10c). Block 4 achieved highest mean yield at 3065 kg ha\(^{-1}\).

Correlation coefficients related to 2012 yields are shown in Table 4.4. There were positive relationships between yield and dry biomass plant\(^{-1}\), total leaf area plant\(^{-1}\), plant height, and seed oil percent. There was no negative correlation between yield and any measured variable.
Figure 4.9 The relationship between N application and yield.

C. Correlation between average yield of block and plant density of block in 2012. The Y-axis indicates yield and number of plants m$^{-2}$. Dots represent mean yield and plant density from the corresponding block.

D. Correlation between canola yield from block 4 and N rate in 2012. The Y-axis indicates yield data from block 4 and the X-axis indicates the amount of N applied before sowing: 0, 50, 100, 150 and 200 kg N ha$^{-1}$ (The 0 kg S ha$^{-1}$ plots received 17 kg N ha$^{-1}$ to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha$^{-1}$ level includes the background N present in the soil plus the 17 kg N ha$^{-1}$ added to compensate for N added with the sulfate.).
Table 4.4 The correlation of seed yield, oil and protein with plant characteristics (plants m$^{-2}$, dry biomass plant$^{-1}$, leaf area plant$^{-1}$, plant height, branches plant$^{-1}$, siliques plant$^{-1}$, seeds silique$^{-1}$, 1000-seed weight, harvest index, yield, oil and protein) in 2012.

<table>
<thead>
<tr>
<th></th>
<th>Yield</th>
<th>Oil (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Plants/m$^2$</td>
<td>0.113</td>
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<td>-0.117</td>
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<tr>
<td>Plants m$^{-2}$</td>
<td>0.494 *** 0.152</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Dry biomass plant$^{-1}$, g</td>
<td>0.454 *** 0.088</td>
<td>0.100</td>
<td></td>
</tr>
<tr>
<td>Leaf area plant$^{-1}$, cm$^2$</td>
<td>0.612 *** 0.276 **</td>
<td>-0.123</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>-0.030 -0.068</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td>Branches plant$^{-1}$</td>
<td>0.099 -0.038</td>
<td>0.233 *</td>
<td></td>
</tr>
<tr>
<td>Siliques plant$^{-1}$</td>
<td>-0.159 -0.059</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>Seeds silique$^{-1}$</td>
<td>-0.136 -0.404 ***</td>
<td>0.530 ***</td>
<td></td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.090 -0.131</td>
<td>0.320 **</td>
<td></td>
</tr>
<tr>
<td>Yield, kg ha$^{-1}$</td>
<td>1.000 0.257 **</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Oil (%)</td>
<td>0.257 ** 1.000</td>
<td>-0.488 ***</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>0.022 -0.488 ***</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

*** P < 0.001; ** P < 0.01; * P < 0.05;
4.12 Oil percent

4.12.1 2011

Seed oil percent was only affected by N application (P < 0.0001), but not B or S fertilization (P > 0.05) (Table 4.1). Average seed oil percent was 47.1 %, ranging from 45.0 to 49.4 %. Seed oil percent was negatively and linearly correlated with N application rate ($R^2 = 0.6561$, P < 0.0001; Figure 4.10b). The 50+100 and 50+50 kg N ha$^{-1}$ treatments resulted in the lowest oil percents, 45.63 and 46.08 %, respectively, with no difference (p < 0.05) between the two. Seed oil percent was higher for treatments receiving 150 N kg ha$^{-1}$, resulting in oil at 46.19 %. Time of N application did not affect seed oil percent at 100 kg N ha$^{-1}$ (p > 0.05), but, at 150 kg N ha$^{-1}$ split application had a negative impact, as compared with single application (P < 0.05).

Seed oil percent was negatively correlated with dry biomass plant$^{-1}$, total leaf area plant$^{-1}$, plant height, harvest index, yield and protein percent (Table 4.3). Conversely, 1000-seed weight was positively correlated with seed oil percent.
Figure 4.10 The relationship between N application and oil percent.

A. Effect of N application on the percentage of oil in 2011. The Y-axis indicates the oil percentage in the seeds and the X-axis indicates the amount of N applied (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate.) Split N applications (50 kg N ha\(^{-1}\) before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean oil percentage. Columns with the same letters are not different at p < 0.05.

B. Correlation between oil percent and N rate in 2011. The Y-axis indicates oil percent and the X-axis indicates the amount of N applied before sowing: 0, 50, 100, 150 and 200 kg N ha\(^{-1}\) (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate.)

\[ y = -0.0149x + 48.328 \]
\[ R^2 = 0.6561, P<0.0001 \]
4.12.2 2012

The average 2012 seed oil percent was 43.2 % and ranged from 39.0 to 46.6 % (Table 4.2). As in 2011, N application was the only treatment that influenced seed oil percent in 2012 (P = 0.004) (Table 4.2). We also noted that seed oil in block 4 showed a curvilinear correlation with N fertilization ($R^2 = 0.4558$, $P < 0.0001$; Figure 4.10d), but not in other blocks. As in 2011, increasing N fertilizer resulted in decreased seed oil percent. The result of oil data for all 4 blocks showed that the highest oil percent (43.9 %) was for the control treatment, and the 150, 100 and 50+100 kg ha$^{-1}$ all resulted in lower values ($P > 0.05$; Figure 4.10c). In addition, there was no difference ($P > 0.05$) due to split application of N in 2012.

In 2012 seed oil percent was positively correlated with plant height and yield (Table 4.4). However, seed oil percent was negatively correlated with 1000-seed weight and protein percent.
**Figure 4.10** The relationship between N application and oil percent.

**C. Effect of nitrogen application on the percentage of oil in 2012.**

The Y-axis indicates the percentage of oil in seeds and the X-axis indicates the amount of N applied (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate). Split N applications (50 kg N ha\(^{-1}\) before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent mean oil percentage. Columns with the same letters are not different at p < 0.05.

**D. Correlation between canola oil percent from block 4 and N rate in 2012.** The Y-axis indicates yield data from block 4 and the X-axis indicates the amount of N applied before sowing: 0, 50, 100, 150 and 200 kg N ha\(^{-1}\) (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate).
4.13 Protein percent

4.13.1 2011

In 2011 N fertilization increased the percentage of seed protein (P < 0.001), whereas S and B did not affect it (P > 0.05) (Table 4.1). Average protein percent was 23.3 %, and ranged from 20.0 to 25.6 % (Table 4.1). Protein level increased as N application rate increased ($R^2 = 0.6228$, $P < 0.0001$; Figure 4.11b). The highest protein percent (24.88 %) resulted from the 50+100 N kg ha$^{-1}$ treatment, although this was not different from the 150 and 100 N kg ha$^{-1}$ treatments, for which the protein levels were 24.44 and 24.64 %, respectively. Furthermore, the comparison of single and split applications indicated that there was no difference due to N application timing.

Protein percent was positively and correlated with dry biomass plant$^{-1}$, leaf area plant$^{-1}$, plant height, harvest index, and yield (Table 4.3). Conversely, protein percent was negatively correlated with 1000-seed weight and seed oil percent.
Figure 4.11 The relationship between N application and protein percent.

A. Effect of N application on the percentage of protein in 2011. The Y-axis indicates the percentage of protein in the seeds and the X-axis indicates the amount of N applied (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate.) Split N applications (50 kg N ha\(^{-1}\) before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns with the same letters are not different at p < 0.05.

B. Correlation between protein percent and N rate in 2011. The Y-axis indicates protein percent and the X-axis indicates the amount of N applied before sowing: 0, 50, 100, 150 and 200 kg N ha\(^{-1}\) (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate).
4.13.2 2012

In 2012, similarly to 2011, N was the only factor that affected seed protein percent (P < 0.001; Table 4.2), while S and B fertilization did not have any effect (P > 0.05). In addition, the average seed protein percentage was 23.6 % and ranged from 19.1 to 26.2 % (Table 4.2). As in 2011, seed protein was positively correlated with N fertilizer application ($R^2 = 0.4964$, P < 0.0001; Figure 4.11d); protein percent increased from 21.97 to 25.6% when N application was increased from 0 to 50+100 kg ha$^{-1}$. Protein level diminished slightly, to 25.05 %, when N was applied at 50+150 kg ha$^{-1}$, although this was not different (p < 0.05) from plants receiving N at 150, 200, 100 and 50+50 kg ha$^{-1}$. Compared to single applications, split applications did not increase seed protein percent, except for the 50+100 kg N ha$^{-1}$ treatment, which resulted in the highest seed protein percent.

Seed protein percent was positively correlated with siliques plant$^{-1}$, 1000-seed weight and harvest index (Table 4.4). In addition, there was a negative correlation between seed protein and oil percent.
Figure 4.11 The relationship between N application and protein percent.

C. Effect of N application on the percentage of protein in 2012. The Y-axis indicates the percentage of protein in the seeds and the X-axis indicates the amount of N applied (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate). Split N applications (50 kg N ha\(^{-1}\) before sowing, the remainder at the 4-6 leaf stage) are presented as 50+50, 50+100. Columns represent the mean protein percentage. Columns with the same letters are not different at p < 0.05.

D. Correlation between protein percent and N rate in 2011. The Y-axis indicates protein percent and the X-axis indicates the amount of N applied before sowing 0, 50, 100, 150 and 200 kg N ha\(^{-1}\) (The 0 kg S ha\(^{-1}\) plots received 17 kg N ha\(^{-1}\) to balance the N contained in the plots that received ammonium sulfate, thus, the effective 0 kg S ha\(^{-1}\) level includes the background N present in the soil plus the 17 kg N ha\(^{-1}\) added to compensate for N added with the sulfate).
CHAPTER 5: DISUSSION

5.1 Growth characteristics affected by N, S and B

5.1.1 Plant density

The results of this work revealed no interactions or main nutrient effects (P > 0.05) for plant density during the two study years (Tables 4.1 and 4.2). As compared to the first year, plants in the 2012 trial were less well distributed, and had a lower average plant density (110 vs. 80) and a much wider range (77-138 vs. 27-199). Brennan et al. (2000) found that plant densities of 55 to 75 plants m\(^{-2}\) allowed canola plants to reach maximum seed yield. In another study, CCC (2003) a canola population of 80 to 180 plants m\(^{-2}\) on the Canadian prairie was recommended. Thus, the established 2011 crop density was adequate to achieve optimum yield. In 2012, there was a much wider range of plant densities, 199-271, and overall plant populations were lower than the previous year, resulting in lower seed yield. In general, a higher rate of N fertilization produced larger plants with darker green leaves (Appendix 1), which is in agreement with the findings of the Canola Council of Canada (2012) and (Embleton et al. 1959).

Visual observations, at the time of initial stem elongation stage (BBCH 31 stage, Weber and Bleiholder, 1990), demonstrated that the 2011 plots had higher ground-cover levels, 90-100 %, than 2012. In 2012 plant density was reasonably uniform within blocks, but varied substantially among the four blocks. Visual observations provided a possible reason for the wide range (27-199) of plant density in 2012; this seemed to be related to non-uniform soil conditions. Moreover, our visual observations in 2012 suggested that the distribution of plant population density within blocks, and also ground coverage rate, increased from block one to block four, which seems consistent with a soil gradient across the blocks. In additional, weed populations were observed to increase from block 4 to block 1 when sampling in 2012.

Mean plant density was 44 % higher in 2011 than 2012. In parallel, canola in 2011 had a greater seed yield (3330 kg ha\(^{-1}\)) than in 2012 (2814 kg ha\(^{-1}\)). Many other canola studies have shown yield decreases with decreased plant density (Ohlsson 1972; Clarke and Simpson 1978; Clarke et al. 1978 and McGregor, 1987). Burnett (2003) found that a high seeding rate and plant density contribute to a larger seed yield by reducing weed populations. In our study, plant growth was much more uniform in 2011 than in 2012, and our data showed a linear correlation between stand density and yield across blocks in 2012. Past studies demonstrated that non-uniform plant distribution reduces seed yield in spring canola (Brassica napus L.; Angadi et al. 2003), winter canola (Huhn, 1999), sunflower (Helianthus annuus Wade, 1990), corn (Zea mays L.; Pommel and Bonhomme, 1998) and sorghum
(Sorghum bicolor (L.) Moench; Larson and Vanderlip, 1994). This could, at least in part, explain the lower seed yield in 2012 than 2011.

However, it is interesting to note that a 44% lower plant density resulted in only 18% less seed yield. This agrees with McGregor (1987), who reported that decreases in seed yield are proportionally less than decreases in plant density. This is most likely due to compensation through plant plasticity, which allows plants to compensate for suboptimal plant density by producing larger plants (Angadi et al., 2003). The plasticity of a plant depends on the availability of resources, such as light, water, and nutrients to each plant; furthermore, the greater the available resources, the greater will be the expression of plasticity (Sultan, 2000). It has been shown that seed yield of canola is a function of plant stand density, siliques plant$^{-1}$, seeds sillage$^{-1}$ and 1000-seed weight (Angadi et al., 2003). Relevant literature has demonstrated that rapeseed can compensate, to a considerable degree, minimizing potential yield losses through increases in other yield components. Increasing numbers of siliques plant$^{-1}$ was the largest response to low population density (Huhn and Schuster 1975; Clarke and Simpson 1978; Clarke et al. 1978). Our study obtained a similar result, in that mean siliques plant$^{-1}$ increased from 18.87 in 2011 to 20.72 in 2012 (Tables 4.1 and 4.2). Another important compensatory response can be in branches plant$^{-1}$ (Clarke and Simpson 1978; Clarke et al. 1978). In 2012 this variable was greater than in 2011 (5.40 vs. 3.65). It has also been reported that there is a tendency for increased and prolonged accumulation of dry biomass at reduced plant populations (McGregor, 1987). We also found more dry biomass plant$^{-1}$ at the lower plant density in 2012 than in 2011 (8.26 vs. 4.47, respectively). McGregor (1987) also pointed out that low plant population had a positive effect on seed weight of lower nodes. In our study, the 1000-seed weight was greater at the lower plant population in 2012 than in 2011 (3.02 vs. 2.79, respectively).

5.1.2 Dry biomass

Dry biomass plant$^{-1}$ was determined at the 20% flowering stage (Tables 4.1 and 4.2). Mean values for the two years revealed that dry biomass plant$^{-1}$ in 2012 (8.26g) was almost twice that of 2011 (4.47g). We also noted a much larger range of plant dry weight in 2012 (2.16 to 21.04 g) than 2011 (2.56 to 8.05g). The application of S or B did not affect plant dry biomass in either 2011 or 2012 (P>0.05). The result regarding S fertilizer was consistent with Asare and Scarisbrick (1995), whereas Nadian et al. (2010) found that plant dry biomass was clearly reduced by higher levels of S fertilization. Nadian et al. (2010) also reported that dry biomass of canola was affected by B application and that the highest value was obtained with application of 2.5 kg B ha$^{-1}$. The plant dry biomass results probably indicated
that there were sufficient levels of S and B available in our soils. Nitrogen application rate had a clear impact on plant dry biomass in the first study year (P < 0.0001), but had a less consistent influence in the second year (P > 0.05). Plants produced drier biomass as N application levels increased, and reached a maximum value at 150 kg N ha\(^{-1}\) (Figure 4.3). Similar results were reported by Asare and Scarisbrick (1995) and Allen and Morgan (1972), who found that dry matter plant\(^{-1}\) was increased by N application, but not always to a statistically significant level. In addition, in our study, plant dry biomass was not affected by single versus split application (Figure 4.3). This is in agreement with the reports of Ardakani and Normohammadi (2009) and Cheema et al. (2010).

**5.1.3 Leaf area**

In 2011, rate of N application was the only factor that affected plant leaf area (P < 0.0001), although it did not result in any effect in 2012 (P > 0.05, Table 4.1). Increasing N application rate produced larger leaf areas up to 150 kg N ha\(^{-1}\) (Figure 4.4). These results are consistent with those of Wright et al. (1988) and Allen and Morgan (1972), who found that N fertilizer enables plants to produce larger leaf areas.

**5.1.4 Plant height**

From the perspective of plant architecture, a taller plant would provide an advantage with respect to competitors such as weeds, by creating a higher crop canopy able to “shade out” developing weeds (Caldwell, 1987). Canola plants were shorter in 2012 (111.66 cm, Table 4.2) than 2011 (117.18 cm, Table 4.1). There was no difference in crop height due to application of N, S, or B in 2012 (P > 0.05). In contrast to the 2012 data, B was the only nutrient that did not have an impact on plant height (P > 0.05) in 2011; both N (P < 0.0001) and S (P = 0.004) applications increased plant height in the first year of our study. Nadian et al. (2010) found that B application did not affect canola height, as was the case with Karamanos et al. (2003) and Moradi-Telavat et al. (2008). Nadian et al. (2010) also reported that S application did not have any influence on plant height. Sulfur application at 20 kg ha\(^{-1}\) reduced crop height relative to the 0 S kg ha\(^{-1}\) controls (Figure 4.5b). Plant height generally increased as N application rate increased, but plateaued at application rates beyond 150 kg N ha\(^{-1}\), whether this was applied as a single or split application; this pattern of response is in agreement with the findings of Allen and Morgan (1972). The 100 kg N ha\(^{-1}\) treatment resulted in taller plants than the same amount of N added as a split application. Thus, N added through a single application was superior to a split application at 100 kg N ha\(^{-1}\), but this was not the case at the higher N rate, where the split application of 50+100 N kg ha\(^{-1}\) was not different from the same level of N added as a single application (P > 0.05). In the
second year, plant height was not different between single and split N applications ($P > 0.05$). Therefore, the results indicated that a split application of N generally did not increase canola height. Similar results were reported by Ardakani and Normohammadi (2009).

5.1.5 Branches plant$^{-1}$

In 2012, canola plants had more branches (5.40, Table 4.2) than in 2011 (3.65, Table 4.1); the range of values was larger in 2012 (2-19, Table 4.2) than 2011 (2-14, Table 4.1). Asare and Scarisbrick (1995) also assessed the number of reproductive branches and reported an average of 5.7 branches plant$^{-1}$. Sulfur fertilization rate did not change plant branch number in either study year ($P > 0.05$), as was found by Nadian et al. (2010). In the same experiment, Nadian et al. (2010) also reported that there was no B effect on branches plant$^{-1}$, consistent with our findings. Not only S and B, but also N fertilization, did not influence plant branch production ($P > 0.05$). However, some relevant literature has reported positive effects of N application on branch number for spring canola (Asare and Scarisbrick 1995; Allen and Morgan 1972; Yau and Thurling 1987; Jixian and Hua, 1997).

5.1.6 Siliques plant$^{-1}$

Siliques plant$^{-1}$ is one of the main yield components of canola; each silique contains a number of variable seeds and, in general, the more siliques plant$^{-1}$ the greater the grain and oil yields (Alen et al., 2000). In 2012 canola plants produced more siliques plant$^{-1}$ (average of 112, Table 4.2) than in 2011 (72, Table 4.1). (Shahrakiet al, 2012) investigated yield components of 12 spring canola hybrids originating from Canada and Australia; maximum siliques plant$^{-1}$ was for the cultivar Hyola, at 308.3, with the overall average number being 104, and a minimum 43 (hybrid 19-H). Our data also revealed no effect of B application on siliques plant$^{-1}$ in either year ($P > 0.05$), similar to the findings of Nadian et al. (2010) and Stangoulis et al. (2000). The number of siliques plant$^{-1}$ was also not influenced by the level of N or S application in either 2011 or 2012 ($P > 0.05$). The opposite result was reported by Asare and Scarisbrick (1995), who found that the number of siliques plant$^{-1}$ at final harvest was enhanced by N and S fertilization. In many other cases, N application has been reported to increase siliques plant$^{-1}$ (Nielson, 1997; Bishnoi and Singh, 1979; Bajpai et al., 1992; Chauhan et al., 1995; Arthamwar et al. 1996). Beyond nutrient additions, genetic attributes and ecosystem conditions also have large impacts on silique generation; for instance temperature, radiation level, and crop population density are important factors (Alen et al., 2000).

5.1.7 Seeds silique$^{-1}$

Another important yield component is number of seeds silique$^{-1}$; this variable is
frequently correlated with canola yield (Rawson, 1998). Number of seeds silique\(^{-1}\) is also considered a key factor in oil yield of new cultivars in Australia (Adams and Grafius, 2000).

Across the two years of our work, the mean number of seeds silique\(^{-1}\) was greater in 2012 (20.72, Table 4.2) than 2011 (18.87, Table 4.1). Cheema et al. (2001) reported seeds silique\(^{-1}\) ranging from 19.75 to 34.25 for 12 Canadian and Australian spring canola hybrids. During the two years of our study, there was no difference in number of seeds silique\(^{-1}\) due to S or B application. In 2012, increasing rates of N application affected number of seeds silique\(^{-1}\); seed number increased as applied N level increased. Similar effects of N on seeds silique\(^{-1}\) in Brassica species have been documented by others (Allen and Morgan 1972, Sen et al. 1977, Scarisbrick et al. 1980, Bajpai et al. 1992, Chauhan et al. 1995, Cheema et al., 2001). Our data also revealed a curvilinear upward response to increasing N levels (Figure 4.6), with increases extending from 0 to 100 kg N ha\(^{-1}\) and then a plateauing as N application increased from 100 to 150 kg N ha\(^{-1}\). Cheema et al. (2001) found the most seeds silique\(^{-1}\) at the second highest N application level (90 kg ha\(^{-1}\)); 120 kg N ha\(^{-1}\) did not further increase seeds silique\(^{-1}\) and could cause clear decreases relative to 90 kg N ha\(^{-1}\). Similarly, our data suggest that the most seeds silique\(^{-1}\) occurred at 100 kg N ha\(^{-1}\), after which the value plateaued or declined. Among the treatments receiving a total of 100 kg N ha\(^{-1}\), the single application produced more seeds silique\(^{-1}\) than the split application; however, split application did not increase this variable at 150 kg N ha\(^{-1}\). Thus, split N application might be beneficial when N is limiting to crop productivity, but was this was not the case at higher N levels. Cheema et al. (2001) also evaluated the effect of time of N application on seeds silique\(^{-1}\) and found that canola did not respond to different times of application.

5.1.8 1000-seed weight

Across the two years of this work, canola plants produced lighter seeds in 2012 (mean of 2.79 g per 1000 seeds, Table 4.2) than in 2011 (3.02 g, Table 4.1), and the average across years was slightly lower than the findings of Tayo and Morgan (1975) and Cheema et al. (2001), who reported an average 1000-seed weight in *Brassica napus* of 3.28 g.

The only interaction effect for the 2011 work was a S x B interaction (P = 0.0431). Individual S and B applications both increased mean 1000-seed weight as compared to the control treatment; the combination of S and B when the rates (foliar application) were 20 kg S ha\(^{-1}\) and 0.5 kg B ha\(^{-1}\), respectively (Figure 4.7a), also increased 1000-seed weight, but less than single S or B applications; conversely, a decrease of 1000-seed weight was also observed for the S and B combination when B was soil applied at 2 kg ha\(^{-1}\). The results
indicated that the highest 1000-seed weight was obtained with B foliar application at 0.5 kg ha\(^{-1}\). The S x B interaction revealed that the combination of these nutrients did not result in a larger increase than that of single applications of either S or B, and even decreased 1000-seed weight when S and B were soil applied at 20 and 2 kg ha\(^{-1}\), respectively.

The S x B interaction effect was not present in 2012 (P > 0.05), while there was a clear effect of single applications of N (P < 0.0001) and B (P = 0.0013). Figure 4.7b indicates an upward curvilinear response of 1000-seed weight in response to increasing rates of N application, as also reported by Cheema et al. (2001). However, a split application of N could result in higher 1000-seed weights than a single application; the difference was greater at higher levels of N fertilizer addition, and particularly at 50+150 kg N ha\(^{-1}\), which also resulted in a heavier mean 1000-seed weight than 200 kg N ha\(^{-1}\) (P < 0.05). Ardakani and Normohammadi (2009) found that response to time of N application by 1000-seed weight depends on canola cultivar; 1000-seed weight in cultivars Opera and Licord were increased by a 100 kg N ha\(^{-1}\) split application, while cultivar Okapi did not respond in this way. In addition, Cheema et al. (2001) reported that canola did not respond to time of N application.

In contrast to 2011, in 2012, foliar application of 0.5 kg B ha\(^{-1}\) decreased 1000 seed-weight (P < 0.05, Figure 4.7c), while soil application of 2 kg B ha\(^{-1}\) did not affect this variable (P > 0.05).

The published literature offers several suggestions regarding factors that may affect canola mean 1000-seed weight. (Scott et al. 1973) and Allen and Morgan (1972.) pointed out that the differences must develop during the short period between anthesis and maturity and would be affected by the ability of plants to translocate assimilate from leaves or siliques to seeds; nutrient availability is another critical factor affecting 1000-seed weight (Scott et al. 1973; Abrahamson (2000) and Yaniv, (2001) found that 1000-seed weight was reduced by high temperature, leading to decreased oil yield and quality.

### 5.1.9 Harvest index

The 2011 average harvest index (38.98 %, Table 4.1) was lower than in 2012 (39.29 %, Table 5), although both were higher than is often reported: 20 to 35 % (Hay, 1995). In 2011, S and B fertilization did not influence harvest index (P > 0.05), but N application did (P < 0.05). In 2012, harvest index was not affected by S or B, but there was a N application effect (P = 0.046). Comparison across the two years (Figures 4.8a and b) indicated similar patterns of response in 2011 and 2012. In the case of single application treatments, harvest index in both years increased with increased N application, up to 100 kg N ha\(^{-1}\), and was then slightly reduced with the 150 kg N ha\(^{-1}\) treatment. In 2012, the 200 kg N ha\(^{-1}\) treatment
caused a clear reduction in harvest index. This is in agreement with Cheema et al. (2001) and Zhao et al. (1993). Similarly, with split application, higher N rates elevated the canola harvest index from 0 to 50+100 kg N ha\(^{-1}\). The highest rate of single N application (200 kg N ha\(^{-1}\)) reduced harvest index as compared with the 150 kg N ha\(^{-1}\) treatment, whereas the same amount of N fertilization as a split application resulted in the highest harvest index value. The effect of application time was parallel to the single application results at total rates of 150 kg N ha\(^{-1}\) in both years. Split application of N improved harvest index more than single application and this effect was greater at higher N rates. Ardakani and Normohammadi (2009) also reported a tendency for split application to result in a higher harvest index. The harvest index concept was developed by Donald (1962, 1968) and was defined as the ratio of grain yield to total above-ground biomass production. An increase in the portion of above-ground biomass partitioned to the harvested part has been a key feature in the selection and breeding of higher yield varieties (Hay, 1995).

**5.1.10 Yield**

The average canola yield was greater in 2011 (3330 kg ha\(^{-1}\), Table 4.1) than in 2012 (2814 kg ha\(^{-1}\), Table 4.2). The same variety (Invigor 5440) yielded an average of 3149 kg ha\(^{-1}\) across a series of locations in Ontario (range of 2475 to 3880 kg ha\(^{-1}\)) (Hall, 2012). The climate of Ontario is similar to that of Québec, although generally somewhat warmer and drier.

**5.1.10.1 Nitrogen**

With sufficient nutrient supplement, particularly N, crops are able to reach higher leaf area index values and have longer leaf retention, thus receiving more solar radiation, which leads to increased silique and seed production. In the present study, N fertilization increased canola yield (P < 0.0001), while S and B had little or no effect (P > 0.05). In terms of single N applications, canola yield in 2011 was positively correlated with N rate (R\(^2\) = 0.04662, P < 0.0001; Figure 4.9b). Yield increased in response to N fertilizer application up to 150 kg N ha\(^{-1}\), which resulted in an increase of 22.3 % over the 0 N control (Figure 4.9 a and b). There was no effect of N fertilization in 2012, however, we noted that for the yield data from block 4 there was a positive relationship between yield and N rate (R\(^2\) = 0.5702, P < 0.0001; Figure 4.9d), whereas, in 2012, block 4 had the highest initial stand counts and produced the highest yields as compared with the rest of the blocks (Figure 4.9c). Yield at 200 kg N ha\(^{-1}\) from block 4 was lower than at 150 kg N ha\(^{-1}\) (Figure 4.9d). The same relationship did not exist between yield and N application rate for the other three blocks, which had lower plant populations. Burnett (2003) indicated that a low seeding rate can result in increased weed
populations, which can result in competition with canola plants for light, nutrients and water, leading to lower yields. The positive response of canola yield to N fertilizer is in agreement with many other reports (Asare and Scarisbrick., 1995; and Hocking et al. 1997; Kumar et al. 1997; Cheema et al., 2001). Maximum yield was attained at the N rate of 50+100 and 150 kg ha$^{-1}$. Gammelvind et al. (1996) found that very high rates of N fertilizer application decreased seed yield. In our study, two additional N treatments were added in 2012: 200 and 50+150 kg N ha$^{-1}$; they resulted in numerical decreases in yield over the N fertilization rate (150 kg N ha$^{-1}$) resulting in the highest yield, although there was no statistical difference between yields for these N application levels. Therefore, of the N levels evaluated, 150 kg ha$^{-1}$ was the treatment that resulted in the maximum yield, and higher N rate treatments did not result in higher yields, or more economic returns.

Split application is known to make N available at more appropriate times during crop development and growth, which could be advantageous if there is potential for N loss through leaching or denitrification (Hall, 2012). It also is important to note that soil texture and weather are critical factors for leaching or denitrification. According to previous studies denitrification occurs slowly in cold soils (2-4 kg ha$^{-1}$ day$^{-1}$ at 5°C) but rapidly in warm soils (Kunickis et al., 2010). Substantial rainfall can lead to leaching. However, clay soils behave as temporary reservoirs and minimize N loss to some extent, while sandy soils do not exhibit this behavior (MAFRI, 2013). In the present study, yield was minimally affected by split application of nitrogen, as compared to a single application prior to seeding. The observed result might also have been due to the fine loamy soil texture and favorable weather conditions. The soil texture was a fine-sandy loam in 2011 and clay loam in 2012, both of which have reasonably good water retention capacities and low redox potentials. As Figures 4.1 and 4.2 indicate, there were no heavy rainfall or heat stress events between the first fertilizer nitrogen application (prior seeding) and second (4-6 leaves stage) application. Therefore, the high nutrient storage capacity of the soils may have resulted in sufficient available nitrogen, under favorable weather conditions, which made late-season nitrogen application not beneficial to canola production at our sites in southwestern Quebec. In Western Canada, split N application did not improve canola performance over single application (Hall, 2012). Taylor et al. (1991), working in Australia, also found no difference in seed yield due to split versus single application methods.

We also made a calculation regarding the time of N application to examine its feasibility from an economic aspect, and the details are shown in Table 5.1. Take 2011 for example: treatment at the rate of N 50+100 kg ha$^{-1}$ resulted in a yield of 3812 kg ha$^{-1}$, an increase of
212 kg ha\(^{-1}\) over the same level of N application as a single treatment: 150 kg N ha\(^{-1}\), resulting in 3600 kg ha\(^{-1}\). At the same time, using a current caonla price of $0.55 kg\(^{-1}\) (OMAF, 2011), the 50+100 kg N ha\(^{-1}\) treatment resulted in an increased crop value of $116.60 ha\(^{-1}\). However, making the split application would incur several additional operating expenses, including tractor and other machine expenses, marketing fees, delivery and labour costs; with an approximate cost of $118.50 (OMAF, 2011). This means that, due to split N application, the final profit was reduced by $1.90 ha\(^{-1}\). This result indicates no increase in profit through the use of a split N application, as compared with same N rate but in a single application. In current study, there is no economic incentive for a split application, however, published literature suggests less leaching of N when split applications are used, so there may be an environmental incentive.
Table 5.1 Extra expense of Split N application as compared with single application at the rate of 150 kg ha\(^{-1}\) in 2011 (OMAF, 2011).

<table>
<thead>
<tr>
<th>Canola price (2012)</th>
<th>+ 0.55 $ /kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (N50+100)</td>
<td>+ 3812 kg/ha</td>
</tr>
<tr>
<td>Yield(N150)</td>
<td>+ 3600 kg/ha</td>
</tr>
<tr>
<td>Income[N(50+100)- N150]</td>
<td>+ 116.6 $ /ha</td>
</tr>
<tr>
<td>Tractor and Machine Expenses - Fuel (17 L) and lubricants</td>
<td>- 50.62 $/ha</td>
</tr>
<tr>
<td>Tractor and Machine Expenses - Repairs and maintenance</td>
<td>- 40.59 $/ha</td>
</tr>
<tr>
<td>Marketing fees</td>
<td>- 0.004 $/kg</td>
</tr>
<tr>
<td>Trucking</td>
<td>- 0.008 $/kg</td>
</tr>
<tr>
<td>Operator labor (self or hired)</td>
<td>- 27.28 $/ha</td>
</tr>
<tr>
<td>Total cost</td>
<td>- 118.5 $ /ha</td>
</tr>
<tr>
<td>Profit</td>
<td>= -1.9 $ /ha</td>
</tr>
</tbody>
</table>

Yield (N50+100) refers to the seed yield when N is split-applied as 50 kg N ha\(^{-1}\) before sowing, and 100 kg N ha\(^{-1}\) at the 4-6 leaf stage.  

Yield (N150) refers to the seed yield when N is singly applied as 150 kg N ha\(^{-1}\) before sowing.
5.1.10.2 Sulfur

Sulfur application, at 20 kg ha\(^{-1}\), did not affect canola yield (P > 0.05) in 2011 or 2012 (Tables 4.1 and 4.2). This is in agreement with previous reports (Nadian et al., 2010; Jan et al, 2002). There are numerous reports on the effect of S on canola grain yield. Sulfur application at 22.4 kg ha\(^{-1}\) is recommended in Ontario, depending on site specific conditions; 44.8 kg S ha\(^{-1}\) did not increase yield over 22.4 kg ha\(^{-1}\) (Hall, 2012). In the Canadian Prairie Provinces, numerous field studies suggest 15-30 kg S ha\(^{-1}\) is required for canola to grow and develop optimally in S deficient soils (Ridley, 1973; Nuttal et al., 1987; Wen et al., 2003).

Finlayson et al. (1970) and Nuttall et al. (1987) indicated that the accumulation of amino acids in seeds requires an optimal N:S ratio, as this enhances protein synthesis. Manitoba Agriculture and Food (2011) recommended N: S ratios of 5:1 to 8:1. There was no interaction between S and B or N in our work (Table 4.1 and 4.2). One possible explanation for the lack of S main and interaction effects on canola yield in the present study is sufficient amounts of S in eastern Canadian soils. This may be due to decades of S-containing “acid rain” in the Québec area.

5.1.10.3 Boron

In both 2011 and 2012, B application did not affect seed yield (P > 0.05) (Tables 4.1 and 4.2), similar to the results of Asad et al. (2003). There are a number of reports regarding the effects of B on canola yield. Boron fertilization is often reported to contribute to a small increase in seed yield, eg. a 7 % increase (Porter 1993) and 7 to 11 % increases (Troeh and Thompson, 2005). In contrast, Karamanos et al. (2003) observed a decreased grain yield with increasing B application. In addition, B application was shown to decrease the relative uptake rate of calcium by canola shoots and roots (Nadian et al., 2010). Our study suggests that the level of B present in our soils was sufficient for good levels of canola productivity.

As a crop with a high B requirement, canola needs a steady supply from vegetative growth to seed development. Foliar application has been considered an effective way to supply B when dry soil restricts root activity (Mortvedt, 1994). Seed yield was not increased by either soil or foliar application of B in our study (Tables 4.1 and 4.2). Boron limitation has been implicated in failed flower bud development and poor seed set of canola (Malhi et al., 2003). In both 2011 and 2012, canola performed well throughout its development and manifested no B deficiency symptoms. Both our sandy loam (2011) and clay loam (2012) soils provided sufficient B.

Boron foliar application has been approved as an effective method to prevent blossom
blast during summer heat waves (OMAF, 2011). Our foliar treatment did not improve yield as compared to the control treatment in either 2011 or 2012 (Tables 4.1 and 4.2). There was no heat stress during flowering in either study year (Figures 4.1a and 4.2a). One possible explanation for the lack of B effect is the lack of heat stress during flowering. This is in agreement with the Crop Advances Field Crop Reports (2012), as Ontario trial results (2008 - 2011) show that foliar B applied at flowering rarely increased profits when temperatures were cooler than normal; the only yield improvement for B treated canola in the Ontario study occurred in 2010, a year with stressfully high temperatures at flowering.

5.1.10.4 Potential yield (yield components based) vs. real yield

A large research effort, including the present experiments, has been directed at determining the best practices to allow canola to reach its maximum potential yield. It is unlikely to ever reach its full potential yield because of various physiological and environmental constraints (Addo-Quaye et al., 1986); “potential yield”, calculated based on sample yield components, and “real yield” will be different due to losses during flower and seed development, and subsequent harvest (Asare and Scarisbrick, 1995). Our 2011 data is an example; the potential yield based on 109.63 plants m$^{-2}$ x 72 siliques plant$^{-1}$ x 18.87 seeds silique$^{-1}$ x 0.00279 g seed$^{-1}$ was 4160 kg ha$^{-1}$, while average yield in 2011 was 3330 kg ha$^{-1}$. In this case, seed loss was equal to 4160 - 3330 = 830 kg ha$^{-1}$. Thus, there was a 25% seed loss in 2011. During the final stages of silique development, especially at the end of the ripening stage, siliques were very fragile and likely to break. Lutman (1993) found up to 10,000 canola seeds m$^{-2}$ on the soil surface because of shedding.

In the present study, one important source of error in the yield components was seeds silique$^{-1}$. Because seeds silique$^{-1}$ was calculated as seed weight plant$^{-1}$ divided by seed mean weight and number of siliques plant$^{-1}$, detected seed losses occurred during silique development and also during hand harvest of the fragile siliques. Canola siliques require 4 to 5 weeks to mature. Because individual plants and siliques mature at different times, a percentage of early maturing seeds, particularly from lower siliques, are lost to shattering (Asare and Scarisbrick, 1995).

In our study, additional seed losses were caused by the presence of tarnished plant bug (*Lygus lineolaris*) during silique development. Tarnished plant bug causes small lesions on siliques, with sap oozing from these feeding sites (OMAF, 2011). Young siliques may turn white and produce seeds that are shrunken or shriveled. Flea beetle was also noted on canola seedlings and minor leaf damage was observed (Appendix 2). They appeared in mid-May, feeding on leaves of young seedlings and produced a shot-hole appearance.
Canola is very sensitive to flea beetle damage in the initial 3 weeks following plant emergence, and damage is more severe with hot sunny weather (OMAF, 2011). High levels of infestation can reduce yield by up to 50% (OMAF, 2011). Tarnished plant bug and flea beetle were both present in both study years, although no significant damage was observed. As canola plants mature, thunderstorms and small birds (in most cases finches and sparrows) can cause extra silique damage and further seed loss (Asare and Scarisbrick, 1995). Fortunately, serious weather and bird damage did not occur in our two year study.

Commercial rapeseed producers know that achieving potential yield requires that all immature siliques contribute to final yield, but there is little they can do to minimize seed shedding during silique development (Asare and Scarisbrick, 1995). Canola yield may be extremely variable across years, even within the same variety and location, associated with silique seed losses during ripening.

5.1.10.5 Weather effects on seed yield

Variable weather conditions can have important effects on seed production. Asare and Scarisbrick (1995) obtained very high seed yield in one experiment compared to another; this was mainly associated with drought and warm weather in the latter case. Lower yields may be due to low soil moisture during May and June, restraining N uptake, reducing photosynthetic activity of leaves and siliques, and reducing mobilization of assimilates. Conversely, Hall (2012) linked high yields with warm April temperatures. Higher yield was obtained in trials planted during warm April conditions, which led to more mineralization of N from soil. In our study, temperatures were not warm enough to allow April seeding (Figure 1a and 1b). However, there was no extreme weather pressure during the two years of our study.

5.1.10.6 Pearson's correlation coefficient analysis

In 2011, yield was positively correlated with dry biomass plant$^{-1}$, total leaf area plant$^{-1}$, plant height, seeds silique$^{-1}$, and harvest index (Table 4.3). Similar results for seeds silique$^{-1}$ and harvest index were reported by Algan and Aygün (2001) and Golparvar (2011), who found positive relationships between seeds silique$^{-1}$, harvest index and seed yield of canola. In addition dry biomass plant$^{-1}$, total leaf area plant$^{-1}$, and plant height were consistently and positively associated with yield in our 2012 trial.

On the other hand, negative correlations existed between yield and 1000-seed weight and seed oil percent in 2011 (Table 4.3). However, in 2012 there was a positive correlation between seed oil percent and yield and no correlation between 1000-seed weight and yield (Table 4.4). Golparvar (2011) and Shahraki et al. (2012) also found a positive correlation
between seed oil percent and seed yield. Oezer et al. (1999), Sheikh et al. (1999) and Shahraki et al. (2012) all reported a positive relationship between seed yield and 1000-seed weight.

5.1.11 Oil

Seed oil percent was higher in 2011 (47.1 %, Table 4.1) than 2012 (43.2 %, Table 4.2). Using the same variety (5440), the oil value in Western Canada (data collected from Manitoba, Alberta and Saskatchewan) is 43.8 %, on average (Canadian Grain Commission, 2012). This is slightly higher than the oil percent from our 2012 samples, but was much lower than our 2011 seed oil percent.

As with yield, there was no effect of S or B treatment (P > 0.05) application on seed oil percent (Table 4.1 and 4.2). Ridley (1973) reported similar results, however, others have found that S application increased canola seed oil concentration (Ridley, 1973; Nuttal et al., 1987; Malhi and Gill, 2002; Grant et al., 2003), and one reported a decrease (Wetter et al., 1970). Our findings indicate that the soils used for our experiments contained sufficient available S.

In 2011, increasing N application was linearly and negatively correlated with seed oil percent (R² = 0.6561, P < 0.0001; Figure 4.10b). Correspondingly, increasing N application progressively lowered seed oil percent, with the highest oil percent occurring for the control treatment (Figure 4.10a and b). The N effect (P = 0.004) was also present in 2012 when the 150, 100 and 50+100 kg ha⁻¹ treatments resulted in lower oil percents than the control treatment (P > 0.05; Figure 4.10c and 4.10d). In contrast to 2011, there was no linear negative correlation between oil percent and N rate. However, it is interesting to note that there was a curvilinear negative relationship between seed oil and N fertilizer level in block 4 (R² = 0.4558, P < 0.0001; Figure 4.10d). It is possible that low plant densities in the first three of the four blocks resulted in poorer crop development and higher weed populations, with a negative impact on plant growth and yield (Burnett, 2003), this agrees with the visual observation of low plant densities and higher weed populations when sampling in blocks 1, 2 and 3 in 2012. Oil results in 2012 also indicated that seed oil percent may be more sensitive to plant density and soil gradients than other variables, so that a relationship between applied N and seed oil percent was apparent under the higher plant density conditions of block 4. The negative impact of N fertilization on oil percent has been reported by many others (Asare and Scarisbrick, 1995; Cheema et al., 2001; Dubey et al., 1994; Jackson, 2000; Kutcher et al., 2005; Rathke et al., 2005; Zhao et al., 1993). Several
reasons have been suggested for this oil vs. N fertilization pattern, one of them being that oil percent decreases with increasing N rate. Holmes (1980) felt that increasing N availability will increase the amount of N-containing protein in seeds, directing photosynthetic output to protein formation over oil synthesis. Jackson (2000) linked this situation to poor seed filling and a larger proportion of green seeds caused by higher levels of N application and resulting delayed maturity. Rathke et al. (2005) felt that high N availability has negative effects on availability of carbohydrates for oil synthesis.

There is no difference in seed oil percent between single and split applications of N, except that 150 kg N ha\(^{-1}\) resulted in a greater seed oil percent than 50+100 kg N ha\(^{-1}\) in 2011 (P < 0.05). Based on our present two-year study, there was little difference in seed oil percent due to single and split N applications, in agreement with previous research (Cheema et al. 2001). Cheema et al. (2001) reported that oil percent did not respond to different times of N application. However, Ardakani and Normohammadi (2009) found that different canola cultivars had different seed oil percent responses to time of N application; seed oil percent in cultivars Opera and Licord were increased by 100 kg N ha\(^{-1}\) split application, while cultivar Okapi was not affected in this regard.

In 2011, seed oil percent was negatively correlated with several variables: dry biomass plant\(^{-1}\), total leaf area plant\(^{-1}\), plant height, harvest index, yield and protein percents; there was also a positive relationship with 1000-seed weight (Table 4.3). Protein percent was the only variable that continued to have a negative correlation with seed oil percent in the second study year, plus there were positive relationships with total leaf area plant\(^{-1}\), plant height, and yield (Table 4.4). Bagheri et al. (2008) found positive relationships with canola oil yield and seed yield, plant height and 1000-seed weight. A positive relationship among oil yield and number of seeds plant\(^{-1}\), seed yield and 1000-seed weight was reported by Farhudi et al. (2008). More recently, Golparvar (2011) reported positive relationships between seed oil percent and plant height, seeds silique\(^{-1}\), 1000-seed weight, seed yield and harvest index.

### 5.1.12 Protein

Average protein values were very similar in 2011 (23.3 %, Table 4.1) and 2012 (23.6 %, Table 4.2). Data on the same variety, from across Western Canada (Manitoba, Alberta and Saskatchewan), produced an average protein level of 21.1 % (Canadian Grain Commission, 2012), which was lower than the current study in both 2011 and 2012.

Application of S or B had no effect (P > 0.05) on seed protein percent in either study year (Tables 4.1 and 4.2), consistent with Nadian et al. (2010). It is interesting to note that many researchers (Ahmad et al., 1999; Hassan, et al., 2007; CCC, 2003) found positive
effects of S fertilization on seed protein level. Gardner et al., (1985) speculated that a large quantity of S containing amino acids (methionine and cystine) is required in canola seed protein. Ahmad et al. (1999) found that increasing S application up to 30 kg ha\(^{-1}\) increased seed protein percent; above this level protein percent plateaued. Hassan et al. (2007) indicated that canola had a greater S response on S deficient soils. These considerations reinforce our conclusion that there was adequate S in the soils used for our experiments.

Nitrogen is a key component of amino acids, which make up the proteins of living cells, making N a prerequisite resource for protein synthesis (Grant and Bailey, 1993). In both 2011 and 2012, seed protein was curvilinearly and positively correlated with the rate of N application \(R^2 = 0.6229, P < 0.0001\) and \(R^2 = 0.4964, P < 0.0001\), respectively; Figure 4.11 b and d). The relationship between N application level and seed protein percent indicated increases in protein levels up to 150 kg N ha\(^{-1}\) (including the 50+100 kg N ha\(^{-1}\) treatment); in 2012 seed protein percent decreased slightly between 150 and 200 kg N ha\(^{-1}\) (including the 50+150 kg N ha\(^{-1}\) treatment). Similar protein responses to N were reported by other workers (e.g. Dubey et al., 1994; Kutcher et al., 2005; Ahmad et al., 1999), who reported positive relationships between level of N fertilization and seed protein percent. Our work indicated that the highest seed protein level was due to the 50+100 kg N ha\(^{-1}\) treatment in both 2011 (Figure 4.11a) and 2012 (Figure 4.11c). In 2012 there is no difference \((P > 0.05)\) between single and split N applications. In 2012, except for 150 kg N ha\(^{-1}\) (24.2%) and 50+100 kg N ha\(^{-1}\) (25.6 %), split versus single application of N (100 vs. 50+50 and 200 vs. 50+150 kg N ha\(^{-1}\)) resulted in seed protein levels that were not different \((P > 0.05)\). In general, split application of N did result in greater seed protein percent at 150 kg N ha\(^{-1}\) while, however, the differences between split and single N applications were generally small, consistent with the findings of Taylor et al. (1991).

Protein percent (2011) was positively correlated with dry biomass plant\(^{-1}\), leaf area plant\(^{-1}\), plant height, harvest index, and yield; there were also negative correlations with 1000-seed weight and oil percent (Table2). In 2012, harvest index and oil percent were the only variables to be negatively correlated with seed protein percent (Table3). In contrast to 2011, 1000-seed weight was positively correlated with seed protein percent. In addition, a positive relationship between number of siliques plant\(^{-1}\) and seed protein percent existed in 2012, but not in 2011.

### 5.1.13 Oil vs. Protein

Nitrogen application was the only evaluated factor \((P < 0.0004)\) that affected seed oil
and protein values (Tables 4.1 and 4.2). Higher rates of N fertilizer lowered seed oil percent (Figure 4.10b and 4.10d) and increased seed protein percent (Figure 4.11b and 4.11d). These results indicated a negative relationship between oil percent and protein percent ($R^2 = 0.602$, $P < 0.001$ and $R^2 = 0.488$, $P < 0.001$ in 2011 and 2012, respectively; Figure 5.1), similar to the findings of others (Bhatty, 1964; Allen and Morgan, 1972; Holmes and Ainsley, 1979; Smith et al., 1988; Wright et al., 1988; Taylor et al., 1991; Zhao et al., 1993; Brennan et al., 2000; Hassan, et al., 2007). Of the four main seed components: oil, protein, water and residue (Hassan, et al., 2007), an increase in protein was generally at the expense of either oil alone or reside plus oil in Brassicaspecies (Si et al., 2003). Results of the current study also indicated that each 1 % increase in the oil percentage resulted in 1.07 and 0.80 % decreases in protein percent in 2011 and 2012, respectively. This was very close to the value reported by Brennan et al. (2000), who noted that each 1 % increase in the level of seed protein resulted in a 1.07 % decline in protein. Although oil percent decreased at high N rates, seed yield increase was higher than oil percent decline, so that oil yield was increased. Oil percent decreased 0.6 to 1.2 % for each additional 100 kg N ha$^{-1}$ (Mendham and Roberson, 2004), which resulted in an increase in total oil yield per unit area (CCC, 2003).

In many studies, the sum of seed oil and protein percents was close to fixed (Ridley, 1973; Holmes, 1980; Brennan et al., 2000). The seed oil percent decreased with increasing seed protein percent. The sums of seed protein and oil percents on average were 70.4 and 66.8 % in 2011 and 2012, respectively. Holmes (1980) found that the sum of protein and oil percent ranged from 50 to 60 % for mustards, 60 to 66 % for summer rape and 64 to 70 % for the winter rapeseed. The oil plus protein in our study was higher than the average value in Holmes’s (1980) report; this may have been due to different cultivars and climatic conditions.
Figure 5.1 Correlation between protein and oil percent in 2011 and 2012. The Y-axis indicates protein percent and the X-axis indicates oil percent. Purple dots represent values from 2011 and green dots are from 2012.
5.2 Optimum rate and timing of N fertilizer additions for canola production under southwestern Quebec soil conditions.

In 2011, dry biomass and leaf area plant\(^{-1}\) at the 20% flowering stage were increased with increasing N application, up to a reasonably high level. Greater biomass and leaf area per plant put canola plants in a position for higher seed yield. Increases in N application caused increases in plant height, seeds silique\(^{-1}\) and harvest index at crop maturity. Increased plant height enhances canola competitiveness with developing weeds by allowing better competition for light by the crop (Caldwell, 1987). A larger number of seeds silique\(^{-1}\) could indicate a large seed reservoir and thus a potential seed yield increase (Rawson, 1988). A higher harvest index means plants allocate a greater proportion of above-ground dry biomass into economic yield (Cheema et al., 2001). Accordingly, correlation analysis demonstrated positive relationships between seed yield and the following variables: dry biomass, leaf area plant\(^{-1}\), plant height, seeds silique\(^{-1}\) and harvest index. Finally, our data indicated that there was a seed yield increase in response to N fertilizer application, with the highest yield occurring for the 150 kg N ha\(^{-1}\) treatment. In general, there was little difference between split and single N applications, particularly when economic considerations were included.

In 2012, N application increased 1000-seed weight. There was a positive relationship between 1000-seed weight and N application rate, and 1000-seed weight was often greater with a split application than a single application of N, with this difference being greater at higher N application levels. In addition, harvest index increased with increasing N application, except for 200 kg N ha\(^{-1}\). Nitrogen split application resulted in higher numerical values for harvest index than a single application, however, these numerical increases were not statistically significant. No other yield component (seeds silique\(^{-1}\), branches plant\(^{-1}\) and siliques plant\(^{-1}\)) was influenced by the level of N application. Our results indicate that, in 2012, only yield data from block 4 responded to N application level, showing a curvilinear and positive correlation with N rate (R\(^2\) = 0.5702, P < 0.0001; Figure 10c); the highest yield resulted from the addition of 150 kg N ha\(^{-1}\). We speculate that lack of a relationship when yield was considered across all four blocks may have been associated with non-uniform plant density in 2012. Relatively low plant densities were observed in the first three blocks and this may have resulted in poorer overall crop development, higher weed populations and non-uniform plant maturities as noted visually during the course of the field work. It is interesting to note that a 44% plant density reduction in 2012 only resulted in an
18% seed yield decrease. This is in general agreement with McGregor’s (1987) report that the decrease in seed yield was proportionally less than the decrease in plant density. This is likely associated with plant plasticity, which allows plants to compensate for suboptimal plant density to at least some extent (Angadi et al., 2003); an example of this would be increasing numbers of siliques plant\(^{-1}\) at lower plant population densities (Huhn and Schuster 1975; Clarke and Simpson 1978; Clarke et al. 1978), or increasing branches plant\(^{-1}\) (Clarke and Simpson 1978; Clarke et al. 1978), dry biomass (McGregor, 1987), or seed weight plant\(^{-1}\) (McGregor, 1987). All results above are reasonably in accord with our measured variables in 2011 and 2012, respectively: siliques plant\(^{-1}\) (18.87 vs. 20.72), branch number (5.40 vs. 3.65), dry biomass (8.26 vs. 4.47), 1000-seed weight (3.02 vs. 2.79). All of this supports the existence of compensatory effects at low plant population densities, which may weaken the effect of N application on 1000-seed weight and harvest index. This may explain why the effect of N fertilization on yield was only apparent in the block of the 2012 experiment with the highest plant density, block 4, but not in other blocks. If so, the 2012 results reinforce the 2011 conclusion that seed yield is generally positively correlated with N application rate; the 150 kg N ha\(^{-1}\) treatment resulted in the highest yields.

In addition to growth variables, N application also affected seed quality in terms of oil and protein percent. Higher rates of N fertilizer decreased seed oil percent and increased protein percent (Figures 12 a and b). Thus, the highest protein percent (24.88 %) was achieved at 50+100 kg N ha\(^{-1}\), which was not different from the second highest value (24.44 %) obtained at 150 kg N ha\(^{-1}\). In contrast, 150 kg N ha\(^{-1}\) resulted in a low oil percent (46.19 %) as compared with the control treatment, but was greater than the value for 50+100 kg N ha\(^{-1}\). Although oil percent decreased at high N rates, it was still well within acceptable limits, and the oil yield increase was higher than oil percent decline. Oil percent was reported to decrease 0.6 to 1.2 % per additional 100 kg N ha\(^{-1}\) applied (Mendham and Roberson, 2004), which resulted in a total oil yield increase per unit area. For example, in 2011 we found that each 100 kg N ha\(^{-1}\) addition reduced oil percent by 1.49 % and increased seed yield by 445 kg ha\(^{-1}\). We can calculate oil yield at 0 kg N ha\(^{-1}\) as yield (3330 kg ha\(^{-1}\)) x oil percent (47.11 %) = 1568.8 kg oil ha\(^{-1}\), which is lower than the oil yield obtained at 100 kg N ha\(^{-1}\): yield (3330 + 445 kg ha\(^{-1}\)) x oil percent (47.11 - 1.49 %) = 1722.2 kg oil ha\(^{-1}\). Therefore in 2011, the treatment that resulted in the highest yield was 150 kg ha\(^{-1}\), and this treatment also provided the highest oil yield (yield 1761 kg oil ha\(^{-1}\)) and the greatest profits. In addition, there was a negative correlation between oil percent and protein percent in both 2011 and 2012 (Tables 1 and 2). The total oil concentration plus protein concentration was
relatively constant at 70.4 and 66.8 % in 2011 and 2012, respectively. Thus, our optimal N rate of 150 kg ha\(^{-1}\) also achieves the highest oil and protein yields.

In our work, split application of N had a small positive effect on 1000-seed weight harvest index and yield, especially at higher rates of N application, but the difference was not sufficient to achieve statistical significance. Therefore split application of N fertilizer requires extra costs related to the second application, but did not result in meaningful yield increases or economic returns. This study demonstrated that a single N fertilizer application affected crop growth and yield components. There was also a preliminary canola fertility trial conducted in 2010 that included N application at 0, 50, 100 and 150 kg ha\(^{-1}\), and which found that 150 kg ha\(^{-1}\) resulted in the highest yields for our soil and climate conditions (data not shown). Thus, we recommend a single N application at the rate of 150 kg N ha\(^{-1}\) to produce the highest seed yield without sacrifice of seed quality. Since many effects were not constant across the two study years, more N field studies are required.

5.3 Optimum rates of application for the minor nutrient S in canola production systems under southwestern Québec conditions

Plant height was decreased (2.9 %) by S application at 20 kg ha\(^{-1}\) in 2011, but was not affected by S application in 2012. Plant height was positively correlated with seed yield. Sulfur fertilization also decreased 1000-seed weight, but only in combination with B application at 2 kg ha\(^{-1}\); it increased 1000-seed weight when in combination with B application at 0.5 kg ha\(^{-1}\) in 2011. Thousand-seed weight was decreased by the addition of combined S and B, as compared with applications of either element alone, and the combination of 20 kg S ha\(^{-1}\) and 2 B kg B ha\(^{-1}\) resulted in the lowest 1000-seed weight, a value lower than the control treatment. Clark and Simpson (1978) indicated that canola yield was a function of 1000-seed weight, siliques plant\(^{-1}\) and seeds silique\(^{-1}\). Since seeds are lost through a range of mechanisms present in “real-world” agronomy practices, real seed yield is always less than estimated potential yield. Thus, real yield per plant can be calculated as 1000-seed weight x siliques plant\(^{-1}\) x seeds silique\(^{-1}\) x (1 - proportional seed loss). Changes in the value of siliques plant\(^{-1}\) x seeds silique\(^{-1}\) x (1 - proportional seed loss) can be considered as an indication of canola compensatory effects, as at least some of these are often in the opposite direction of the 1000-seed weight changes. After including this compensatory effect, the impact of 1000-seed weight on yield may not be significant enough to reach a statistically detectable level. In our work, S application did not affect seed yield. In 2012 there was no S effect on any of the measured variables. Overall, our lack of S effects indicates sufficient S in our soils. Thus, additional S application may not be
necessary under southwestern Québec growing conditions.

5.4 Optimum rates of application of the micronutrient B for canola production systems under southwestern Québec conditions

In 2011, B fertilization affected 1000-seed weight, but only in the presence of S application. When canola received 20 kg S ha\(^{-1}\), soil application of B (2 kg ha\(^{-1}\)) reduced 1000-seed weight, and foliar application (0.5 kg B ha\(^{-1}\)) increased seed weight, as compared with the control treatment. As in 2011, B application affected 1000-seed weight in 2012, but through a main effect rather than through an interaction with S. Foliar application of 0.5 kg B ha\(^{-1}\) decreased 1000-seed weight as compared with 2 kg B ha\(^{-1}\) soil application and the control treatment. Only 1000-seed weight was affected by B fertilization. As with S fertilization, multiplying the compensatory effects of siliques plant\(^{-1}\) x seeds silique\(^{-1}\) x (1 - percentage of seed loss), indicated that the impact on 1000-seed weight was minimized and removed any potential B effects on yield. Therefore, our results indicated that B is probably present at sufficient levels in the tested soils. In addition, there was no heat stress during either 2011 or 2012 and B tends to be beneficial when heat stress is present. Although broader testing is needed, our two-year study suggests that there may be little need for canola producers to supply B fertilizer to southwestern Québec soils.
CHAPTER 6: CONCLUSIONS

6.1 General conclusion

High oil percent (> 40 %), the lowest saturated fat concentration (6.8 %) among the vegetable oils, plus low erucic acid and glucosinolate levels make canola highly valuable to the oilseed industry (Raymer, 2002). In order to help reduce fossil fuel consumption and greenhouse gas (GHG) emissions, the Canadian federal government has required a minimum 2 %, by volume, of biodiesel blended into petro-diesel (Environment Canada, 2012). This requirement drives a growing market for canola-based biodiesel feedstock material in Canada. Currently, Québec is a region of very limited canola production, however, it has the potential to become a highly productive region for this crop, especially through support from the newly established local oilseed crushing facility operated by TRT-ETGO. Proper fertilization is always a critical factor for optimum yield; however, data regarding canola responses to N, B and S fertilization have been lacking for southwestern Québec soils and growing conditions. Southwestern Québec growing conditions differ substantially from those of the main canola producing region of western Canada, thus it is necessary to develop a southwestern Québec-specific understanding of canola fertilizer requirements. Our fertility experiments provide information on canola N, S and B responses in southwestern Québec.

The first objective of this study was to determine the optimum rate and timing of N fertilizer additions for canola production under southwestern Québec conditions. In 2011, N fertilization had positive influences on dry biomass, leaf area, plant height, seeds silique\(^{-1}\), harvest index, yield and protein percent, and also had a negative effect on seed oil percent. In general, our data indicated that the N level resulting in the highest seed yield, greatest oil production and largest economic return was N at the rate of 150 kg ha\(^{-1}\). In 2012, the second year of this study, N addition only affected 1000-seed weight, harvest index, and seed oil and protein percent. However, we noted that crop population densities in the 2012 experiment were low in blocks 1-3, but not in block 4, and data from block 4 showed a generally positive and curvilinear response to N additions (R\(^2\) = 0.5702, P < 0.0001; Figure 10c). The low plant densities in the first three blocks probably resulted in some extra negative effects through high weed populations and non-uniform plant cover, leading to manifestation of plant plasticity and resulting compensation through increased numbers of branches plant\(^{-1}\), siliques plant\(^{-1}\), dry biomass plant\(^{-1}\), and 1000-seed weight. The positive correlation between N rate and block 4 yield data confirms the conclusion from 2011 that 150 kg N ha\(^{-1}\) produced the highest seed yield, at least for a well-established crop. Besides the 2011 and 2012 field experiments, a preliminary N study was conducted in 2010; the data
from this work also indicated increasing yields up to 150 kg N ha\(^{-1}\) (data not shown).

In contrast to the N rate effects, split application of N fertilizer only had a small positive effect on 1000-seed weight, harvest index and yield, and the difference was only apparent at 150 kg N ha\(^{-1}\). Economic considerations suggest that such a split application is not justified; our data indicate that split N fertilization costs extra to apply but does not result in enough yield increase to produce an economic benefit.

Therefore, we conclude that, of the treatments we evaluated, a single N application at the rate of 150 kg N ha\(^{-1}\) provided both the greatest seed and oil yields. Since most effects were not constant across the two study years, more N field studies are required.

The second objective of this study was to determine the need and best rates of application for the minor nutrient S in canola fertility management under southwestern Québec conditions. Sulfur treatment has little impact, under either N deficiency or N sufficiency conditions. Sulfur application had negative effects on plant height and 1000-seed weight in the presence of boron fertilization in 2011, but did not affect other measured canola variables, including yield. In 2012, we did not observe a S effect on any of the measured variables. The small levels of the S effect were probably due to decades of S-containing “acid rain”, resulting in sufficient S in southwestern Québec soils. Therefore, it is likely that there is no need to make S fertilizer additions to southwestern Québec soils.

The third objective of this study was to determine the need and best rates of application for the micronutrient B for canola growth, yield, and oil percent under southwestern Québec conditions. Boron application had little impact under conditions of either N deficiency or N sufficiency. In 2011, B fertilization affected 1000-seed weight, but only in the presence of S application. Thousand-seed weight was reduced with the combination of 20 kg S ha\(^{-1}\) and 2 kg B ha\(^{-1}\), both soil applied, and increased with foliar application (0.5 kg B ha\(^{-1}\)) in the presence of S fertilization. Likewise, B application affected 1000-seed weight in 2012, but through a main effect rather than an interaction with S. Compensatory effects diminished the 1000-seed weight impact on seed yield sufficiently that yield was not affected. Overall, our results indicate that B was probably present at sufficient levels in the tested soils. In addition, there was no meaningful period of heat stress during flowering in either 2011 or 2012, and B tends to be beneficial when heat stress is present. Although broader testing is needed, our two-year study suggests that there may be little need for canola producers to supply B fertilizer to southwestern Québec soils.
6.2 Acceptance or rejection of hypotheses

Hypothesis 1. Nitrogen application will result in a higher canola yield when S fertilization is also applied.

Hypothesis 1 was rejected, as there was no interaction effect of N and S (P>0.05) on canola yield in either 2011 or 2012.

Hypothesis 2. Nitrogen application at 150 kg ha\(^{-1}\) will result in the highest canola yield.

Hypothesis 2 was accepted, as in 2011 N application at 150 kg ha\(^{-1}\) resulted in the highest canola yield, and in 2012, the data from block 4 also supports this.

Hypothesis 3. Nitrogen split application is more effective than a single application, and will produce higher canola seed yields.

Hypothesis 3 was rejected, since split application had little effect on canola yield.

Hypothesis 4. Sulfur application at 20 kg ha\(^{-1}\) will increase canola seed yields.

Hypothesis 4 was rejected, as S application at 20 kg ha\(^{-1}\) did not increase seed yield as compared with the 0 kg ha\(^{-1}\) control treatment.

Hypothesis 5. Boron soil application at 2 kg ha\(^{-1}\) will increase canola seed yield.

Hypothesis 5 was rejected, as soil application of B at 2 kg ha\(^{-1}\) did not enhance seed yield.

Hypothesis 6. Boron foliar application at 0.5 kg ha\(^{-1}\) will help canola to overcome heat stress and result in higher seed yields.

Hypothesis 5 cannot be accepted or rejected, as there was no meaningful period of heat stress in either of our experimental years; more B studies are needed to determine the possible advantage of B foliar application under heat stress conditions.
CHAPTER 7: CONTRIBUTIONS TO KNOWLEDGE

The major contributions from my work are as follows:

1. My study provided the first clearly identified optimum rate and timing of N fertilizer additions for canola production under southwestern Québec soil conditions.

2. The work reported here was the first to clearly determine the lack of need for application of the minor nutrient S in canola production systems under southwestern Québec conditions.

3. The work conducted indicated that there is probably no need to apply the micronutrient B as part of canola production systems under southwestern Québec conditions.
CHAPTER 8: SUGGESTIONS FOR FUTURE RESEARCH

1. In the current study, N fertilization at 150 kg ha\(^{-1}\) resulted in the highest yields. These positive effects were not constant across the two years of the study; however, even in 2012, when there was no effect of N fertilizer on yield across four blocks of the experiment, yield values from block 4, where established populations were more optimal, did increase as N application rates rose, resulting in a significant sigmoidal relationship between N application and yield. It is suggested that additional N fertility studies be conducted to confirm our results. Nitrogen fertility by seeding rate studies might be particularly useful.

2. Because foliar B application did not result in yield increases, and because other research has shown that relatively inexpensive applications of B, often with fungicide, can enhance yield and provide positive economic returns, particularly when heat stress is present during flowering, it is suggested that the B field experiments be repeated, with a range of planting dates, to determine the potential advantage of B foliar application under heat stress conditions, such as are more likely to occur with later plantings.

3. Glucosinolate percent is an important quality aspect of canola seed and future work should include evaluation of the effects of fertilizer application, and S fertilizer in particular, on seed glucosinolate percent. (Wahid et al. 2007)

4. Analysis of N, S and B levels in canola tissues should be conducted to determine the effects of fertilization with these nutrients and their loading into specific tissues.
REFERENCES


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Ijaz M. (2012). Effect of Triazole and Strobilurin fungicides on seed yield and grain quality of winter rapeseed (*Brassica napus L.*).


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APPENDICES

Appendix 1. Differences in plant colour with low and high rates of N in 2011

Appendix 2. Minor leaf damage from flea beetle in 2012
APPENDIX 3: 2012 Field layout

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The top of the page is the north end of the field; the 2011 field layout was the same except that there were 26 treatments in each block.