Evaluation of industrial hemp yield and quality in the province of Québec

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July 2014

A thesis submitted to McGill University in partial fulfillment of the requirements of the Degree of Master of Science

DEDICATION

This thesis is dedicated to my strong little man, for his patience, understanding, curiosity, and for always believing in me. Je dédie aussi cette thèse à mes parents pour leur appui dans tout ce que j'entreprends.

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ABSTRACT

Industrial hemp (*Cannabis sativa L.*) is a multipurpose crop for which there is growing interest. However, there is currently limited information on the adaptability of commercial cultivars in eastern Canada. The present project assessed the adaptability of eleven cultivars (Anka, Alyssa, CanMa, CFX-1, CFX-2, CRS-1, Delores, Férimon, Finola, Jutta, and Yvonne) in four contrasting regions of Québec, in terms of hemp seed and fiber yield and quality. Average seed and fiber yields were respectively 1315 and 3226 kg ha^{-1}. Férimon, Jutta, Anka and CanMa showed superior and stable seed yields across the environments. Férimon stood out from the others in terms of fiber yield having the highest yields followed by Anka and Jutta. Seed crude protein (CP) concentrations varied between cultivars and averaged 237 g kg^{-1}. Cultivars with lower agronomical yield also had higher CP concentration. Cellulose, hemicellulose and lignin concentrations of stems respectively averaged 564, 123 and 93 g kg^{-1}. Limited variations were observed among cultivars. In addition, fertilization trials were performed with CRS-1 and Anka (N & K: 0, 50, 100, 150 and 200 kg ha^{-1} and P: 0, 25, 50, 75 and 100 kg ha^{-1}). A positive linear response of seed and fiber yields and crude protein concentration was observed following nitrogen fertilization, whereas no response was observed for phosphorus and potassium.

RÉSUMÉ

Le chanvre industriel (*Cannabis sativa L.*) est une culture à multiples usages pour laquelle il y a un intérêt croissant. Cependant, l'information sur l'adaptabilité des cultivars commerciaux dans l'est du Canada est actuellement limitée. Le présent projet a donc évalué l'adaptabilité d'onze cultivars (Anka, Alyssa, CanMa, CFX-1, CFX-2, CRS-1, Delores, Férimon, Finola, Jutta, et Yvonne) dans quatre régions administratives du Québec, en termes de rendement et de qualité de la graine (chènevis) et de la fibre (chènevotte et filasse) de chanvre. Les rendements moyens en grains et en fibres étaient respectivement de 1315 et 3226 kg ha^{-1}. Férimon, Jutta, Anka et CanMa ont démontré des rendements en grain supérieurs et stables à travers les environnements. Férimon s'est démarqué en ayant les plus hauts rendements en fibre, suivi d'Anka et Jutta. La concentration en protéine brute (CP) des grains a varié entre les cultivars et était en moyenne de 237 g kg^{-1}. Les cultivars avec un rendement agronomique plus faible avaient aussi une CP plus élevée. Les concentrations moyennes de cellulose, d'hémicellulose et de lignine étaient 564, 123 et 93 g kg^{-1}, respectivement, et très peu de variations entre les cultivars ont été observées. De plus, des essais de fertilisation ont été réalisés avec CRS-1 et Anka (N & K: 0, 50, 100, 150 et 200 kg ha^{-1} et P: 0, 25, 50, 75 et 100 kg ha^{-1}). Une réponse linéaire positive du rendement en grain et en fibre, ainsi que de la concentration en protéine brute a été observée suivant une fertilisation azotée. Par contre, aucune réponse n'a été observée pour le phosphore et le potassium

ACKNOWLEDGMENTS

I am sincerely grateful to my co-supervisor Dr. Philippe Seguin who offered me the opportunity to work on this appealing project. I want to thank him for his support and guidance throughout the venture and for the confidence he has shown in me. I also want to thank my co-supervisor Dr. Jean-Benoit Charron for his constant help and support in this project with a crop that was unfamiliar to most of us. I thank Dr. Alan K. Watson for his contribution in the advisory committee. I thank Dr. Anne Vanasse from Laval University for her collaboration which enabled us to expand the scope of our research sites. I am also grateful to Mr. Boisjoly for having hosted us on his lands for the third research site. I have learned a lot discussing with him. I want to write a special thanks to Michael J. Lewis, Amelie Roy Desilets and Jim Straughton from the Emile A. Lods Agronomy Research Centre for their help and dedication into work. Thanks to Mr. Olivier Lalonde from the CÉROM for sharing results with us. I also want to acknowledge the cooperation of Dr. Mustafa's laboratory team for sharing their expertise and material with me. Thanks to Dr. Gaëtan Tremblay and Mr. Mario Laterriere from Agriculture and Agri-Food Canada for the development of a calibration equation to determine hemp material composition using NIRS technology. I want to express my gratitude to the Ministère de l'Agriculture, des Pêcheries et de l’Alimentation du Québec (MAPAQ) for their financial support. Finally, I want to say a huge thanks to all my lab mates and students who took a bit of their time and energy to help me with the field work. It would not have been possible to achieve this project without their help.

LIST OF ABBREVIATIONS

AAFC – Agriculture and Agri-Food Canada

ADF – Acid Detergent Fiber

ADL – Acid Detergent Lignin

ANOVA – Analysis of Variance

AOAC – Association of Official Analytical Chemists

BCMAF – British Columbia Ministry of Agriculture and Food

C – Cultivar

CHTA – Canadian Hemp Trade Alliance

CHUs – Corn Heat Units

CP – Crude protein

DM – Dry Matter

E – Environment

F – Fertilization

FAOSTAT – Statistics Division of the Food and Agriculture Organization

G – Genotype

GLA – gamma-linolenic acid

GLM – General Linear Model

Ha – Hectare

K – Potassium

LAN – Lanoraie

LAP – La Pocatière

LSD – Least Significant Difference

MAPAQ – Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec

MDDEFP – Ministère du Développement Durable, de l'Environnement, de la Faune et des Parcs

N – Nitrogen

NCSL – National Conference of State Legislatures

NDF – Neutral Detergent Fiber

NIRS – Near Infra-Red Spectroscopy

P – Phosphorus

QC – Québec (Refers to Saint-Augustin-de-Desmaures)

RPD – Ratio of Prediction to Deviation

SAB – Sainte-Anne-de-Bellevue

SMB – Saint-Mathieu-de-Beloeil

THC – Delta-9-tetrahydrocannabinol

CONTRIBUTION OF AUTHORS

This thesis has been written in the form of manuscripts. This format has been approved by the Faculty of Graduate Studies at McGill University as described in the "Guidelines for Thesis Preparation and Submission". This research was designed by Dr. Philippe Seguin, thesis co-supervisor and co-author of all manuscripts, and Dr. Jean-Benoît Charron, thesis co-supervisor and co-author of all manuscripts. The candidate conducted all field studies and data collection, compiled and analyzed the results and wrote the manuscripts and thesis under the supervision of Dr. Philippe Seguin. Laboratory analyses were conducted by the candidate under the supervision of Dr. Philippe Seguin, Dr. Arif Mustafa from the Department of Animal Science, McGill University, and Dr. Gaëtan Tremblay from Agriculture and Agri-Food Canada. The present thesis is composed of 6 chapters. The first and second chapters are the General Introduction and Review of Literature, respectively. Chapters 3, 4 represent the field and laboratory experiments and were written in the form of manuscripts to be submitted for publication to peer-reviewed journals in the future. Chapters 5 and 6 highlight the conclusions, summaries and recommendations for future research in that area of study.

Both manuscripts are co-authored by the candidate, Dr. Philippe Seguin, Department of Plant Science, McGill University and Dr. Jean-Benoît Charron, Department of Plant Science, McGill University. Marie-Pier Aubin, the primary author, performed all experiments, data analysis and manuscript writing. Dr. Philippe Seguin and Dr. Jean-

Benoit Charron provided the funds and support for the research, including supervisory guidance and reviewing of the manuscript. All authors were involved in the editing of the manuscripts.

CHAPTER 1

GENERAL INTRODUCTION

In Canada, specialty crop is the term used to define crops that are not included into the major crops categories such as grain, oilseed or horticultural crops (AAFC, 2013). In 2009, specialty crops represented 3% of the total Canadian crops production (AAFC, 2010). The establishment of new crops enhances biodiversity and promotes market diversification, allowing producers to stay competitive on the local and international markets. Among specialty crops, industrial hemp has made a comeback in 1998. After a 60 years ban, this crop has generated a lot of interest among agricultural producers looking for alternative crops. This promising crop has so far demonstrated a good potential under Canadian temperate climate and can be used as a dual purpose crop as both its seeds and fibers can be harvested and used in a range of products (AACF, 2013). The Canadian production area has increased continuously since 2008 and it is still gaining popularity (Anum Laate, 2012); in 2013, total licensed hectares totalized 26 980 ha (CHTA, 2014). Canada's total hemp exports, almost entirely consisting of hemp seeds, represented a value of approximately $8.8 million in 2009 (AAFC, 2010). Western Europe and China were the biggest producers in 2013. Numerous other countries now produce industrial hemp including Canada, Chile, Poland, Hungary and Russia, to name only a few (FAOSTAT, 2013). Hemp seeds are recognized to be a particularly nutritive food with a high concentration of proteins and a good ratio of essential fatty acids (House et al. 2010; Deferne and Pate, 1996; Chen et al., 2010). Industrial hemp also represents an interesting alternative as a biomass crop, its fiber being highly resistant and valuable. This dual use of hemp could reduce the controversy around land use for industrial purpose instead of food production and might be an advantage compared to other biomass/biofuel crops. In Canada, the average seed yield is 800 kg DM per hectare, while the oil yield is 200 liters per hectare (AAFC, 2007). As for the biomass yield, the average yield represents 6000 kg of straw per hectare, giving about 1500 kg of transformed fiber (AAFC, 2007).

Industrial hemp is a particularly good crop to include in crop rotations. Not only its deep root system enhances soil structure, but its fast growth and broad leaves also efficiently control weeds without any herbicide application. Indeed, under good growing condition, hemp has been reported to impact positively the following crop (Bócsa and Karus, 1998). The use of hemp as a breaking crop on continuous soybean cultivation is one example where it has been shown to increase soybean yield in the subsequent year by 10.8% (Liu et al., 2012).

Hemp has the capacity to grow under various environmental conditions (AACF, 2013). Its high plasticity is in part attributable to a high genotypic variability. So far, only a few breeding programs worked on the development and improvement of Canadian cultivars since the re-legalization of the species. Despite the interest for industrial hemp in eastern Canada, there is very limited information on most adapted cultivars, and their performance and quality has not been assessed under these conditions. In addition, there is limited information on most appropriate agronomic practices in that region. There is then a need to characterize cultivars that are currently available on the market.

With the progressive increase of hemp acreage, agronomical recommendations have been formulated for Western Canada, as well as Ontario (BCMAF, 1999; Mooleki et al., 2006; Manitoba Agriculture, Food and Rural Initiatives, 2013; Baxter and Scheifele, 2000). Such guidelines are nonetheless lacking for the Province of Québec and Atlantic Canada. Experimental fertilization trials are therefore essential for this region in order to maximize the yields and quality of seeds and fibers.

1.1 Objectives

- To characterize legally approved cultivars produced under Québec conditions in terms of yield and quality of both seeds and fibers.
- To determine optimal N, P and K fertilization rates for industrial hemp as a dual purpose crop.

1.2 Hypotheses

- Specific cultivars will have a better adaptation than others under Québec growing conditions in terms of yield and quality of both seeds and fibers.

- Certain cultivars will be more suited than others for use as a dual purpose crop (i.e., both seeds and fibers).
- N, P and K fertilization will increase hemp yield and quality up to a certain point, but the benefits encountered will reach a plateau at certain fertilization rates.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Origin, taxonomy and botany

Evidence of hemp utilization goes back to the 5th century BC. It appears to start in China and spreads towards other parts of Asia, Africa, and Europe to finally reach America (Ranalli, 1999). *Cannabis sativa* is from the Cannabaceae family and is essentially divided into two subspecies: *sativa* and *indica*. Whereas taxonomy is often questioned, *Cannabis sativa* ssp. *sativa* generally refers to low Δ-9-tetrahydrocannabinol (THC) cultivars containing only trace of THC, while *Cannabis sativa* ssp. *indica* refers to high THC cultivars (Small and Cronquist, 1976). Selection of low THC varieties overtime has led to the development of today's industrial hemp cultivars. Hemp is an annual C_3 broad leaf species. It is generally found in temperate regions, and it has shown a great capacity to adapt to various growing conditions. Plants height usually ranges between 1.8 to 4.6 m, which gives this crop a good potential for biomass production (AAFC, 2007). Hemp is a photosensitive plant, as its flowering is triggered by the diminution of the photoperiod (Lisson et al., 2000). The growing season can then last 70 to 140 days, depending on the cultivar, the region as well as the final use planned for the crop (AAFC, 2007). Hemp is an obligate outbreeder. Most naturally occurring hemp individuals are dioecious, although monoecious plants do occur occasionally. Breeding programs usually aim at developing stable monoecious varieties where both male and female parts are found on the same plant (Ranalli, 1999). Since male plants senescence occurs rapidly after flowering and seeds only develop from female flowers, males are of little interest for agricultural production. Certified monoecious cultivars allow producer to harvest seed and fiber from a uniform population without encountering non-productive male plants.

2.2 Legal issue

Throughout the years, industrial hemp production (ssp. sativa) has been hindered due to its similarity with the illegal marijuana (ssp. indica). Because of this high similarity with the THC containing cultivars, the reputation of this crop was marred despite all the possibilities that it offers as a multi-purpose crop. Hemp production was greatly restricted in Canada through the *Opium and Narcotic Drug Act* in 1929. As a result of this law reinforcement, Canadian industrial hemp production totally disappeared in 1938 (AAFC, 2007). Hemp was further banned on a larger scale as it fell under the legislation of the international *Single Convention on Narcotic Drugs* in 1961 (Baxter and Scheifele, 2000). In the late nineties, due to lobbying from the agricultural industry actors, *Industrial Hemp Regulations* were generated following the creation of the *Controlled Drugs and Substances Act*. Consequently, hemp was added to the list of controlled drugs and substances in Canada to become commercially legal once again in 1998 (Baxter and Scheifele, 2000). It was henceforth possible for producers to grow hemp (containing ≤ 0.3% THC) with the authorization of Health Canada (AAFC, 2007). In 2014, industrial hemp production is still banned in the USA, which makes this country the first importer of Canadian hemp derived products (AAFC, 2010). An amendment to the US Controlled Substances Act has been proposed vainly to the House of Congress several times in the past to “exclude industrial hemp from the definition of marijuana” (H.R. 525, 2013), the last time being in June 2013 (United States Legislative Information, 2013). *The Industrial Hemp Farming Act* would result in the legalization of hemp production in the United States with regulations similar to those in Canada. Although the federal government did not authorize any amendment yet, 8 states have independently created laws and policies to take position toward industrial hemp production (NCSL, 2014; Serecon Management Consulting Inc., 2012).

2.3 Seeds

Hemp seeds, shelled seeds, flour and oil are a valuable source of food and feed (AAFC, 2007). Whole seeds protein content from hemp cultivars grown in Western Canada was reported to range between 24 and 30% (House et al., 2010). Comparing the amino acids profile of the hemp seed protein to the common references in human nutrition, the profile of hemp is similar to the one of soybean and white egg, which makes hemp a source of complete protein (Callaway, 2004). Essential fatty acids play many important roles in the human body, such as preventing cardiovascular disease, supporting brain development and modulating inflammatory response (Simopoulos, 1988; Brown, 2008). Polyunsaturated essential fatty acids are not synthesized by the human body, thus the necessity to get linoleic acid (omega 6) and *α*-linolenic acid (omega 3) through an appropriate diet (Stuchlík, and Zak, 2002; Surette, 2008). Furthermore, a suitable ratio of omega 6 to omega 3 for human requirements has been set around 3:1 (Erasmus, 1999). The oil content of hemp seeds has been identified to be exceptionally high and properly balanced. A study evaluating Chinese hemp cultivars revealed an oil content over 50% from which the unsaturated fatty acids content could reach over 90% (Chen, He et al., 2010). Although this study displayed a particularly abundant percentage of oil content, hemp seeds were reported several times to contain an average oil concentration of 30-35% (House, Neufeld et al., 2010; Callaway, 2004; Deferne and Pate, 1996). The great potential of hemp as a vegetable oil in human diet was often underlined, with its average 3:1 ratio of linoleic acid to *α*-linolenic acid (Chen, He et al., 2010; Leizer, et al., 2000; Deferne and Pate, 1996). In addition, the presence of γ-linolenic acid (GLA; 18:3, *cis*-6,9,12), a derivative of the omega 6 fatty acid has been reported, which could be a factor of interest in the context of manufacturing dietary supplement (Mölleken and Theimer, 1997). The composition of the fatty acid profile has moreover been demonstrated to vary according to the plant cultivars and climate, the cultivars from the cooler regions displaying a higher concentration of GLA (Mölleken and Theimer, 1997). The latter information is once again an incentive to conduct further studies on a wide range of cultivars and locations.

2.4 Fibers

Hemp stems contain primary and secondary fibers, the primary bast fibers being found in the phloem, and the secondary fibers, or hurds, being found in the core of the stems (Bócsa and Karus, 1998). Bast fibers are sought for their flexibility and high breaking strength. They form the outer portion of the stems and have a lower lignin concentration than the woody core of the plants. Primary bast fibers are also longer than secondary fibers, which makes them more suitable for the textile industry (Bócsa and Karus, 1998). Other bast fiber market outputs include various byproducts such as automotive light panels, paper and pulp, bioplastics and insulation mats (Serecon Management Consulting Inc., 2012). In addition to the environment and cultivar effects, agronomic factors can also influence fiber yield and quality. For instance, an increased plant density can impact positively the length of internodes, and thus the length of bast fibers (Struik et al., 2000; Amaducci et al., 2008). The location of the fibers on a stem has also been reported to influence the quality. Indeed, less secondary fibers were found on the top part of stems, indicating a more flexible and better quality material on the upper portion of the stem (Amaducci et al., 2008). The bast fibers are clustered in bundles and enclose cellulose, the compound of interest, which is gradually incrusted by lignin as the plant matures (Bócsa and Karus, 1998; Struik et al., 2000; Amaducci et al., 2008). The harvest date can then affect the quality of the resulting fiber and the level of processing required to separate the different fiber types. The optimal time for harvest is cultivar dependent, as it is generally related to the flowering period (Bocsa and Karus, 1998; Struik et al., 2000). The lignin content from the beginning to the end of the flowering period has been reported to increase, although not significantly. In addition, total fiber yield has been reported to increase by approximately 25% during the flowering period, which could be a worthwhile reason to delay the harvest (Amaducci et al, 2008). In another line of thinking, Struik et al. (2008) reported reduced radiation use efficiency after the initiation of flowering, and suggested the used of late-maturing cultivars in order to maximize the growing season length before severe lignification. Bearing in mind that the quality of industrial hemp fiber is closely linked to plant growth stage at harvest, a modelling system for predicting phenology would be an excellent asset (Amaducci et al., 2012). A phenological model taking into account the environment as well as agronomic practices has indeed been evaluated in Europe. Although it has been reported to have a reasonable accuracy for determining optimal seeding and harvesting periods of specific cultivars, it still needs to be refined regarding decisions based on hemp flowering (Amaducci et al., 2012).

Secondary fibers with a high lignin concentration can represent up to 2 times the volume of the primary bast fiber (Li et al., 2013; Karus and Vogt, 2004). Formerly considered as a waste by-product, hurd is now seen as a value added to hemp production. Indeed, this material can be used as animal bedding or construction material such as wall insulator panels (Karus and Vogt, 2004; BRE, 2002). In 2002, the majority of the European hemp production was oriented toward fiber production, a small portion of hemp seeds being mainly sold as animal feed (Karus and Vogt, 2004). On the opposite, until very recently, Canadian hemp production mostly focused on seed production (AAFC, 2010). New incentives to market the hemp fiber are nevertheless gradually developing. The main limiting factor responsible for the slow development of this part of the market is the limited number of fiber processing plants. Indeed, only a few installations based in Alberta and Manitoba have the facilities to undertake straw decortication (Serecon Management Consulting Inc., 2012). A new processing facility is also expected to open in the province of Québec, in the Lanaudière region (Lamontagne and Sicard, 2012). In an attempt to improve crop rotation plan, hemp trials have also proliferated in the Montérégie region, in association with textile manufactures and ethanol production industries that have manifested their interest (Fontaine, 2011).

2.5 Hemp germplasm and its particularities

Only certified seeds from cultivars approved by Health Canada are marketable in Canada. Some of the cultivars originate from Europe, and some have been developed in Canada (Baxter and Scheifele, 2000). In 2013, there were 39 industrial hemp cultivars approved by Health Canada (Table A.1). The most widely grown cultivars in Canada are currently Alyssa, Anka, CRS–1, CFX–1, CFX–2, Delores and Finola (CHTA, 2014). An important factor to consider when looking at prospective cultivars to breed is the THC content, as Health Canada only allows the use of varieties containing less than 0.3% THC. Small and Marcus (2003) evaluated the Canadian hemp monitoring system and concluded it was a reliable procedure to carry on industrial hemp production at THC levels consistently below 0.3%. They stipulated that European germplasm THC content when grown under Canada conditions was frequently higher than the Canadian limit of 0.3% and would therefore not be ideal for a breeding program in North America. This may be due to the origin of the gene pool. Indeed, THC content was reported to vary according to latitude and climatic condition, the cultivars from southern regions being more likely to synthesize higher levels of THC (Bócsa and Karus, 1998). Besides the influence of cultivars and growing conditions, the plant parts and age have also been reported to impact THC concentrations (Bócsa et al., 1997; UNODC, 2009).

Depending on the breeding method, three outputs are possible for the progeny sexual type: dioecious, monoecious, and predominant female also called unisexual individual. The latter corresponds to a dioecious generation with approximately 70-85% of female plants, which generally gives higher seed yields (Bócsa and Karus, 1998; Baxter and Scheifele, 2000). Consequently, some varieties are more suitable for seed production, others for fiber production, while some may serve as a dual purpose crop (Table A.2). Since hemp is a photosensitive plant flowering as day length decreases, the selection of early versus late-maturing cultivars is a factor to consider depending on the region and expected utilization of the crop (Lisson et al. 2000; Amaducci et al, 2008).

Hemp is a wind-pollinated crop with large variations observed for a given cultivar (Ranalli, 1999). Likewise, genetic diversity is found across cultivars from different environments. Consequently, it is essential that the adaptability or the performance of a specific cultivar be assessed based on its location as well as on the agronomical practices the crop is subjected to (Watson et al., 2012). Such information represents a primary tool for producers to select the proper cultivar regarding their market of interest. Better knowledge of the profile of cultivars also guides breeders towards potential improvement of existing germplasm.

In Québec, some specific cultivars including Alyssa, Anka, Jutta and Yvonne have been reported to be better suited for warmer regions including Montérégie, Estrie and Lanaudière (Beaulieu and Doucet, 2013). On the opposite, Finola, Férimon, CFX-1, CFX-2, CRS-1 and CanMa have been identified to better perform in colder regions such as Abitibi-Témiscamingue, Bas-Saint-Laurent, Chaudières-Appalaches, Gaspésie and Saguenay–Lac-Saint-Jean.

2.6 Fertilization guidelines

There is currently limited information available in Canada on optimal fertilization regimes for local production. Fertilization recommendations have been suggested for British Columbia, the prairies, and Ontario; none existing at the moment for Québec. Recommended maximum nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) soil levels in British Columbia are currently 120, 229 and 193 kg ha^{-1}, respectively (BCMAF, 1999). Soils must then be fertilized to approximate these values according to a recent soil test. Suggested maximum fertilization rates for Alberta are similar with 135, 259 and 211 kg ha^{-1} for N, P_2O_5, and K_2O, respectively (Stanford, 1998). In Saskatchewan, 100 kg N ha^{-1}, 50 kg P_2O_5 ha^{-1} and 67 kg K_2O ha^{-1} are recommended, and banded applications of N and P_2O_5 are favored. Studies conducted at Agriculture and Agri-Food Canada however demonstrated that fertilizer placed directly with the seed could impact negatively seeds germination when in dry conditions (Mooleki et al., 2006). Seeding and fertilizer application should therefore be done separately. In Manitoba, hemp total nutrients requirements, accounting for soil nutrients and fertilizer application, are similar to those of wheat (90-134 kg N ha^{-1} , 103 kg P_2O_5 ha^{-1} and 81 kg K_2O ha^{-1}) (Manitoba Agriculture, Food and Rural Initiatives, 2013). It has also been demonstrated locally that hemp nutrient uptake is 224, 122 and 284 kg ha^{-1} for N, P_2O_5 and K_2O respectively (Heard, 2001). In Ontario, recommendations for hemp fertilization are also based on wheat guidelines with a maximum of 110 kg N ha^{-1}, 48-108 kg K_2O ha^{-1}, and P_2O_5 level depending on initial soil levels (Baxter and Scheifele, 2000). The determination of appropriate nutrient requirements for hemp grown in Québec would be useful, among other things, to calculate the annual phosphorus balance; this information being required by the provincial government. The annual Phosphorus Report, mandatory for Québec agricultural producers, takes in consideration the phosphorus applied, the land areas as well as the estimated phosphorus uptake by each crops. It ensures a sustainable management of the P_2O_5 input versus the soil capacity that is set in the Agricultural Operations Regulation (MDDEFP, 2014).

2.7 Yield response to fertilization

2.7.1 Nitrogen

Under low initial nitrogen soil levels and adequate soil moisture, nitrogen fertilization was reported to have a positive impact on height, biomass and seed yields (Vera et al., 2004; 2010). Vera et al. (2010) also reported an interaction between nitrogen fertilization levels and cultivars, response to nitrogen differing between the cultivars Crag and Finola. Crag is a dual purpose cultivar used for both fiber and seed production, whereas Finola is a high seed yielding cultivar. In that study, Finola was more responsive to nitrogen application than Crag in terms of seed production. Overall, they estimated that the optimal nitrogen fertilization rate is 150 kg ha^{-1} to maximize height, fiber and seed production. The latter optimal rate was higher than the one previously reported by Vera et al. (2004) who tested the response of the cultivars Fasamo and Finola. With these cultivars, optimal seed yield was reached with 100 kg N ha^{-1} for Finola, and 120 kg N ha^{-1} for Fasamo, a dual purpose cultivar.

2.7.2 Phosphorus

Hemp total biomass yield can be responsive to phosphorus fertilization under certain growing conditions. Coffman and Gentner (1977) determined that *Cannabis sativa* (marijuana) from Afghanistan grown under greenhouse conditions gave higher total biomass production with higher phosphorus application rates. Increasing phosphorus fertilization also increased industrial hemp height according to Vera et al. (2004). Nevertheless, this latter industrial hemp trial conducted in Canada showed that seed-placed phosphorus fertilizer could result in poor seed germination, and thus, reduced plant density, biomass and seed yield. This seed toxicity was observed in soils with a low moisture content (Vera et al., 2004). The inconsistency of yield response to phosphorus application was observed once again by Vera et al. (2010). Although no significant effect of phosphorus fertilizer was reported in general, an interaction between cultivars and phosphorus fertilization was reported to have an impact on plant density. Crag density was indeed negatively affected by decreasing phosphorus fertilizer rates, which was not the case for Finola. The effect of phosphorus fertilization rates on industrial hemp then appears to be dependent on growing conditions and cultivars selection.

2.7.3 Potassium

According to Finnan and Burke (2012), potassium fertilization had no effect on hemp density, total biomass and seed yield for all of three cultivars tested (‘Férimon’, ‘Futura 75’ and ‘Felina 32’). In terms of the potassium intake of mature plants, they determined that Futura 75, a late-maturing cultivar, had a greater potassium uptake than the two other earlier maturing cultivars. In addition, they found most of the used potassium was allocated to the stems.

2.8 Qualitative response to fertilization

2.8.1 Crude protein concentration

A positive correlation between nitrogen fertilization and crude protein (CP) concentration of hemp seeds has been reported (Vera et al., 2004; Mihoc et al., 2013). Moreover, cultivars such as Finola and Fasamo responded to a different magnitude following nitrogen application rates (0 to 120 kg N ha^{-1}), Finola having a higher CP concentration than Fasamo (Vera et al. 2004). Cultivar effect on CP concentration was also observed with Romanian cultivars (Mihoc et al., 2013). On the opposite, phosphorus fertilization did not have any effect on the seed CP content (Vera et al., 2004; Izsáki and Ivány, 2008). It was stated that although phosphorus and potassium did not directly influence CP content, these nutrients were necessary for proper nitrogen absorption (Mihoc et al., 2013). Very limited information is however available in the literature on the effect of potassium on hemp seed composition.

2.8.2 Oil concentration

The effect of nitrogen fertilization on oil concentration is variable among hemp cultivars. The oil concentration of seeds of the cultivar Fasamo was negatively correlated with nitrogen fertilization, whereas Finola oil concentration increased with nitrogen fertilization (Vera et al, 2004). This negative correlation between nitrogen and oil concentration has been reported in several other crops including canola and soybean (Bhatty, 1964; Johnston et al., 2002). In these studies, nitrogen fertilization enhanced crude protein concentration while systematically lowering oil concentration. Furthermore, nitrogen fertilization coupled with phosphorus fertilization has been reported to increase oil content of some hemp cultivars in Romania (Mihoc et al., 2013). However, nitrogen and phosphorus fertilization did not have any effect on oil content of Chinese hemp cultivars in a trial conducted in Hungary (Izsáki and Ivány, 2008). Seed composition is thus affected by nutrient supply to a certain extent, and also by other external factors such as environmental conditions.

2.8.3 Fiber quality

Nitrogen supply is known to increase hemp biomass production and thus, cellulose and lignin yield. Hessler (1947) denoted that the higher the lignin content in stems, the weaker the fibers in terms of flexibility. In this study, fiber fineness was the parameter of interest. Coarse fibers were considered as poor quality fibers, whereas flexible fibers implying low lignin content were described as good quality and strong fibers. The study showed that stems with high level of lignin required a lower breaking strength and thus, were considered as poor quality material. This correlation was in agreement with Jordan et al. (1946) who previously obtained similar results. As opposed to lignin, no correlation was find between nitrogen supply and cellulose content in fields with high initial nitrogen content (Struik et al., 2000). The increase in yield following nitrogen application was negligible. Besides, it is noteworthy to mention that when looking at fiber composition after retting, very limited differences were found among the cellulose and lignin levels (Hessler, 1947). The fertilization plan should then be established according to the post-harvest treatment projected and the desired utilization of the plant material.

CONNECTING TEXT FOR CHAPTER 3

The following study characterized eleven industrial hemp cultivars in different regions of the province of Québec. It examined agronomic parameters such as seed and fiber yields, as well as seed crude protein concentrations and stem fiber cellulose, hemicellulose and lignin concentrations.

Chapter 3 was written as a manuscript which will serve as a basis for eventual submission to a publication. The manuscript was co-authored by the candidate, Dr. Philippe Seguin, thesis co-supervisor, and Dr. Jean-Benoît Charron, thesis co-supervisor, both from the Department of Plant Science, McGill University. All authors contributed to editing the manuscript. Laboratory analyses were conducted by the candidate under the supervision of Dr. Philippe Seguin, Dr. Arif Mustafa from the Department of Animal Science, McGill University, and Dr. Gaëtan Tremblay from Agriculture and Agri-Food Canada.

CHAPTER 3

CHARACTERIZATION OF INDUSTRIAL HEMP CULTIVARS GROWN IN QUÉBEC

3.1 Abstract

Industrial hemp (*Cannabis sativa L.*) is a multipurpose crop for which there is growing interest. However, there is currently limited information on the adaptability of commercial cultivars in eastern Canada. The present project assessed the adaptability of 11 hemp cultivars (Anka, Alyssa, CanMa, CFX-1, CFX-2, CRS-1, Delores, Férimon, Finola, Jutta, and Yvonne) in terms of seed and fiber yield and quality, in seven environments in the province of Québec. Despite the presence of genotype × environment interactions, Férimon, Jutta, Anka, and CanMa showed superior and consistent seed yields across environments, having an average of 1878, 1408, 1397 and 1386 kg ha^{-1} respectively. Férimon, Anka and Jutta also showed superior and stable fiber yields, with an average of 5827, 4591 and 4096 kg ha^{-1} across environments. Finola, CFX-2, CFX-1, Yvonne and CRS-1 had higher and more stable crude protein concentration across environments with averages of 257, 252, 244, 240 and 238 g kg^{-1}. Limited variations were observed among cultivars in terms of fiber composition. Results from this study could serve as guidance for further research on industrial hemp production in the province of Québec.

3.2 Introduction

Interest for industrial hemp is growing as this promising crop offers many potential applications. Accordingly, total Canadian acreage dedicated to hemp production has nearly sevenfold between 2008 and 2013 (Anum Laate, 2012; CHTA, 2014). Since the legalization of industrial hemp in Canada in 1998, new hemp cultivars have been developed to satisfy market needs. In 2013, Health Canada's approved list of legal cultivars included 39 cultivars for commercial production (Beaulieu, 2013). These Canadian and European cultivars are either suitable for seed production, or both seed and fiber production. Selection of cultivars for commercial production is based on three major criteria. First, the botany of the cultivar is considered. Monoecious cultivars and dioecious cultivars with a predominance of females are often favored as they generally offer higher yield due to the absence or limited presence of male plants loss after flowering (Bócsa and Karus, 1998). Monoecious cultivars also tend to have a more homogeneous maturity than dioecious cultivars, which brings up the second criteria of late maturity. Late-maturing plants represent an advantage in terms of yield. The longer the growing season, the greater the total biomass yield (Faux et al. 2013). This characteristic is particularly important in the northern hemisphere where flowering is triggered as day length shortens after the summer solstice (Lisson et al., 2000). The last aspect considered in the selection of hemp cultivars is the potential fiber and/or seed production. The production of hemp or either seed or fiber is primarily driven by market demand and the accessibility to processing facilities.

The adaptation of the various cultivars approved for growth in Canada, varies according to the region. For example, in Québec, the cultivars Alyssa, Anka, Jutta and Yvonne have been reported to have a better performance in warmer southwest regions including Montérégie, Estrie and Lanaudière (Beaulieu and Doucet, 2013). On the opposite, Finola, Férimon, CFX-1, CFX-2, CRS-1 and CanMa have been reported to better perform in colder northern and eastern regions such as Abitibi-Témiscamingue, Bas-Saint-Laurent, Chaudières-Appalaches, Gaspésie and Saguenay–Lac-Saint-Jean.

There is currently very limited information on the performance and adaptation of industrial hemp cultivars in eastern Canada and especially in the province of Québec. The aim of this study was to characterize cultivars approved by Health Canada when grown in Québec. The main parameters studied were seed yield, DM fiber yield, seed oil and crude protein concentrations, as well as stem cellulose, hemicellulose and lignin concentrations.

3.3 Material & methods

3.3.1 Experimental sites and treatments

Experiments were conducted in three environments in Québec, Canada in 2012 and four in 2013. The sites were located in Sainte-Anne-de-Bellevue (SAB :45° 26' N, 73°

55' W; 2950 CHUs), Saint-Mathieu-de-Beloeil (SMB : 45° 34'N , 73° 15' W; 2900 CHUs), Saint-Augustin-de-Desmaures (QC: 46°43' N, 71°30' W; 2400 CHUs), and La Pocatière (LAP :47° 22' N, 70° 2' W; 2150 CHUs). Corn heat units (CHUs) are a measure of heat accumulation over a season considering minimum and maximum daily temperatures (Brown and Bootsma, 1993). The following ten cultivars were tested in 2012: Anka, Alyssa, CanMa, CFX-1, CFX-2, CRS-1, Delores, Finola, Jutta, and Yvonne. Yvonne was replaced by Férimon in 2013 due to a lack of seed availability. The soil type was a Chicot light sandy loam (Gleysol Melanic Brunisol) at SAB in 2012 and 2013, a loamy clay (Melanic Brunisol) in 2012 and a sandy loam (Gleysol) in 2013 in QC, a Saint-Urbain clay loam (Orthic Humid Gleysol) in SMB in 2013 and a sand (Orthic Dystric Brunisol) in LAP in 2013. Two experiments were conducted in SAB in 2012 and are later referred to as SAB-a and SAB-b. Fertilization was based on results of soil tests conducted at each site following general recommendations for hemp production in Ontario (Baxter and Scheifele, 2000). Experiments in SAB were fertilized with 110 kg N ha^{-1} incorporated at seeding. In QC, 110 kg N ha^{-1}, 20 kg P ha^{-1} and 20 kg K ha^{-1} were applied and incorporated at seeding. Finally, in SMB and LAP, 120 kg N ha^{-1} and 18 kg P ha^{-1} were split-applied, 50% of the fertilizer being incorporated at seeding and the rest being side-banded at the 4-6 leaves growth stage. Seeding was done in early June at all sites using a cereal seeder with an 18 cm row spacing, except for experiment SAB-b, which was seeded in mid-June. The seeding rate was 43 kg ha^{-1} of pure live seeds in SAB-a, SAB-b and QC, and a targeted 150 plants m^{-2} adjusted according to the 1000 seeds weight and the germination rate at SMB and LAP. Dimension of plots ranged between 5.4 and 9.2 m^2 depending on the site. Monthly average temperature and precipitations from May to September at meteorological stations nearby the experimental sites (<10 km) are provided in Table 3.1.

3.3.2 Data Collection

Plant density was determined at the 4 leaves stage by counting plants along 1 meter, in two non-border rows per plot. The average height of 10 plants per plot was measured at harvest. Plants were harvested at maturity (late August to early September) and the area harvested was approximately 3 m^2, the exact area depending on the site. Only the middle rows of plots were harvested to avoid border effects. At harvest, plants were manually cut approximately 2 cm from the ground. Following harvest, whole plants were dried at 45ºC for 48-72 hours. Whole plants were then threshed using a stationary combine to separate seeds from the rest of the total biomass. Total dry matter (DM) fiber yield was calculated by subtracting seed yield from total biomass. Harvest index (%) was calculated by dividing seed yield by total biomass yield.

3.3.3 Chemical analyses

Seeds from SAB and QC were ground to a fine powder (approximately to 1 mm) using a standard coffee mill (SmartGrind™ CBG100S, Black & Decker, Middleton, WI), followed by the use of a pestle and mortar. Seeds from LAP and SMB were ground using a homogenizer with steel balls calibrated for cereals (custom built homogenizer, Equi-Lab Inc., Neuville, QC, Canada). Seed crude protein concentration was determined for all samples.

3.3.3.1 Seed crude protein concentration

Crude protein concentration of a random set of samples was first quantified following elemental nitrogen analysis by combustion (Truspec Nitrogen Determinator System, Leco Corp., St. Joseph, MI) and using a nitrogen to protein conversion factor of 6.25. All the samples were further scanned by near infrared reflectance spectroscopy (NIRS) using a NIRSystem 6500 monochromator (Foss, Silver Spring, MD) in the range from 400 to 2500 nm. Calibration equations were developed using a modified least squares regression method of the WinISI III software (Infrasoft International LLC, State College, PA) with the use of the previously determined chemical values and their respective spectrum. The prediction equation for the crude protein concentration was successfully validated with a ratio of prediction to deviation (RPD) of 3.70 for all samples, except samples from SMB and LAP environments. The grinding technique used for the later sites differed from the one used in SAB and QC which affected NIRS results for SMB and LAP. Therefore, crude protein concentration of all samples from SMB and LAP was determined using elemental nitrogen analysis as described earlier.

3.3.3.2 Fiber composition

For each plot, a composite sample of hemp stems was ground through a 1 mm screen using a Wiley mill (standard model 3; Arthur H. Thomas Co., Philadelphia, PA). All samples were first scanned by near infrared reflectance spectroscopy (NIRS). From the resulting spectra, 74 samples were selected for chemical analysis to provide reference values to the software for the development of a calibration equation. Neutral detergent fiber (NDF) (Van Soest et al. 1991), acid detergent fiber (ADF) and acid detergent lignin (ADL) (AOAC, 1990) concentrations, were quantified in the 74 samples using an Ankom Fiber Analyzer (Ankom Technology Corp., Fairport, NY). Moisture content was determined using the oven method at 105°C (AOAC, 1990). The NDF, ADF and ADL concentrations of the entire population were then predicted using the developed calibration equation. NIRS predictions for NDF and ADF concentrations were considered moderately successful and moderately useful with RPD of 2.33 and 2.17 respectively. However, no prediction for ADL concentration was possible due to a RPD of 0.99 (data not shown). In addition, cellulose concentrations were estimated using NIRS with a RPD of 2.43. Hemicellulose and lignin concentration were determined by calculation.

3.3.4 Statistical analyses

The analysis of variance (ANOVA) was performed for each year separately due to the evaluation of different cultivars in both years. Indeed, 10 cultivars were tested in 2012 and 2013, but Yvonne was replaced by Férimon in 2013 because of the unavailability of Yvonne seeds in 2013. Cultivars were assigned in each environment to a randomized complete block design with three replicates. The general linear model (GLM) procedure in SAS (SAS Institute, 2008) was used for data analysis. Genotype × environment interactions were significant for most parameters of interest; data were thus reanalyzed separately for each environment. Replicates were considered random effects, while genotypes and environments were considered fixed effects. Comparisons between cultivars means was made using the least significant difference (LSD). Statistical significance was assumed at $P \leq 0.05$. Only significant effects are later presented and discussed.

3.4 Results and discussion

3.4.1 Seed yield

Genotype × environment interaction was observed for seed yield in 2012 (Table 3.2). Seed yield variation among genotypes at a given site ranged between 35 and 81% across all environments (Table 3.4). Despite the overall inconsistent performance of genotypes across environments, Férimon and Jutta both ranked among the three highest yielding genotypes in four environments, ranking first and second across all of these environments (Table 3.4). However, it is important to note that Férimon was evaluated only in 2013 in a total of four environments (Table 3.4). Seed yield varied between 47 and

3181 kg DM ha^{-1} across all environments and cultivars, with means of 1173 and 1398 kg ha^{-1} observed in 2012 and 2013, respectively (Tables 3.2-3.3). The average seed yields observed herein were above those previously reported, which generally range between 800 and 1000 kg ha^{-1} in Canada (AAFC, 2007; Beaulieu and Doucet, 2013).

The performance of genotypes across environments is also illustrated by the use of Francis and Kannenberg's descriptive method (Francis and Kannenberg, 1978), which plot the coefficient of variation of the genotypes relative to their corresponding average yield across environments (Figure 3.1). In this representation, genotypes clustering in quadrant I are the most desirable as they have the most stable and highest performance across environments. Five genotypes among the 11 evaluated clustered in quadrant I (i.e., Férimon, Jutta, Anka, CanMa and CFX-1). Indeed, despite a generalized high variability in 2012 and 2013, and a G × E interaction for seed yield in 2012, genotypes in quadrant I were consistently superior to the other genotypes. The top three performing cultivars are all monoecious, which generally have higher seed yield when compared to dioecious ones (Bócsa and Karus, 1998; Baxter and Scheifele, 2000).

Regarding comparisons between environments, the highest seed yields were observed in Saint-Mathieu-de-Beloeil (SMB) (Table 3.4). A clay loam with high organic matter content at that site may have benefited hemp plants by providing a better water holding capacity and by optimizing nutrient availability during critical stages of plant development. Disparities in field management practices may also be responsible for the greater yield. As opposed to a single fertilizer application at seeding, fertilizer application was split in Saint-Mathieu-de-Beloeil (SMB) and Lapocatière (LAP), half the fertilizer being applied at seeding, and the second half sided-banded at the 4-6 leaves growth stage. A better nutrient use efficiency may have therefore impacted positively seed yield at these sites.

3.4.2 Fiber yield

Genotype × environment interaction were observed for fiber yield in 2012 and 2013 (Tables 3.2-3.3). Fiber yield variation between genotypes in a given environment ranged from 66 to 88%. Fiber yield ranged between 172 and 8837 kg ha^{-1} across all environments, with overall means of 2705 and 3451 kg ha^{-1} in 2012 and 2013, respectively (Table 3.5). The average fiber yields observed in our study were below the average fiber yield reported in western Canada. Whereas fiber yields of about 20 000 kg ha^{-1} were reported in Europe, yields in Canada generally approximate 6000 kg ha^{-1} (Werf et al., 1996; Struick et al. 2000; AAFC, 2007). Apart from environmental factors, one possible cause for this lower biomass production could be an inadequate seeding time. Indeed, recommended seeding dates in Saskatchewan are from May 1st to May 31, and from late-April to late-May in Ontario, or as soon as soil temperature is adequate (Mooleki et al., 2006; Baxter and Scheifele, 2000). Hemp is known to germinate at 4-6°C, and ideal temperatures are set at 8-10°C (Baxter and Scheifele, 2000). Furthermore, it has been reported that earlier seeding (in late-April) as well as early-maturing cultivars were associated to higher seed and fiber yields (Faux et al, 2013). In this experiment, Alyssa, Anka, Jutta and Yvonne were late-maturing cultivars, while the rest of the selection were early-maturing cultivars (Girouard et al., 1999).

Férimon, Anka and Jutta ranked, across the seven environments, among the three highest yielding genotypes consistently having higher fiber yields (Table 3.5; Figure 3.2). These three genotypes are later maturing monoecious cultivars. They are also considered to be dual purpose cultivars, which means they have the potential to produce high yields of both seeds and fibers (Beaulieu and Doucet, 2013). Their dual purpose use potential was reflected in our study, as Férimon, Anka and Jutta stood out from the eleven genotypes tested in terms of having both high seed and fiber yields.

Highest fiber yields were observed in SMB. Although fiber yields at high latitude locations were sometimes superior to fiber yields at low latitude locations, it was inconsistent. Therefore, similarly to the seed yield, it was not possible to correlate fiber yield to corn heat unit accumulation of a specific environment.

3.4.3 Other agronomical parameters

Genotype and environment main effect as well as genotype × environment interactions were observed for harvest index, height, density and maturity in at least one of the two growing seasons studied (Tables 3.2-3.3). Harvest index as well as height values showed great variability because dual purpose cultivars were pooled with shorter cultivars developed solely for seed production (Table 3.2-3.3). Large variations were also observed regarding density between cultivars, which reflects the effect of environment as well as external factors such as weather conditions and seed quality (Table 3.2-3.3). For instance, seedling emergence was particularly low (data not shown) in SAB-a due to soil crusting following seeding, whereas seedling emergence was better in the SAB-b trial which was seeded later on in the season. Greater seed yield was also observed in SAB-b with 700 kg ha^{-1} more than for SAB-a. The growing season (i.e., days from seeding to harvest) lasted approximately 69 days in 2012, and 110 days in 2013, which suits well Québec climate conditions (Tables 3.2-3.3).

3.4.4 Seeds crude protein concentration

A genotype × environment interaction for seed crude protein concentration was observed in 2013 only. Crude protein concentration differences among genotypes varied between 12 and 18% at a given site (Table 3.6). Actual concentrations ranged between 201 and 283 g kg^{-1} with means of 241 and 233 in 2012 and 2013, respectively (Tables 3.2-3.3). Similar values have been reported for crude protein concentration of hemp seeds of a range of cultivars (House et al., 2010; Mihoc et al. 2013). The cultivars Finola, CFX-2 and CanMa ranked first, second, and third, respectively, across all environments (Table 3.6). Although Finola and CFX-2 had the highest concentrations of crude protein, they nevertheless showed low seed and fiber yields coupled with high coefficients of variation (Figures 3.1, 3.2 and 3.3).

3.4.5 Stem cellulose, hemicellulose and lignin concentration

The ADF, NDF and cellulose concentrations were predicted using NIRS. However, ADL (lignin concentration) and hemicellulose could not be predicted through NIRS and were thus calculated (lignin = ADF – cellulose; hemicellulose = NDF - ADF). Inaccurate predictions of ADL concentrations were also previously reported, the ADL concentration of samples being inadequate for near infra-red spectroscopy (Toonen et al, 2004). A genotype × environment interaction was only observed for cellulose concentration in 2012 (Table 3.2). However, this interaction was mainly due to generally lower cellulose values at QC (Table 3.7). Cellulose concentrations did not fluctuate considerably among cultivars, and the cultivar effect was only found to be significant in 2012. Similarly, significant differences in hemicellulose concentrations among cultivars were only observed in 2012 (Table 3.2). This is in agreement with Toonen et al. (2004) who only found significant differences between cultivars for NDF and ADF concentrations, and did not observed large variations among these values. Cellulose, hemicellulose and lignin concentrations respectively averaged 569, 123 and 93 g kg^{-1} in 2012, and 558, 123 and 93 g kg^{-1} in 2013 (Tables 3.2 and 3.3). The associated NDF, ADF and ADL average values to calculate these fiber concentrations (778, 655 and 93 g kg^{-1}) are comparable to the ones determined in the literature (743, 650, and 117 g kg^{-1}) for mature hemp plants (Toonen et al. 2004).

3.5 Conclusion

The performance of the eleven genotypes varied upon the environment in which they grew, except for the seed yield and height values in 2013 that showed no significant G × E interaction. The effect of genotype on seed and fiber yield was only significant in 2013, when the cultivar Férimon replaced Yvonne in four environments. In terms of seed yield, Férimon, Jutta, Anka and CanMa demonstrated the more consistent performance and highest yields among the genotypes evaluated. Férimon, Jutta and Anka also showed the highest and more stable fiber yields of all genotypes evaluated. Finola, CFX-1, CFX-2, CRS-1 and Yvonne showed the highest and more stable crude protein concentration across the seven environments. However, their associated seed and fiber yields were inconsistent across environments and often lower than other genotypes evaluated. The significance of variations in fiber composition among cultivars were not consistent across the two growing seasons. In addition, very limited differences were observed among cultivars regarding fiber composition. The present experiment demonstrated the potential of industrial hemp under Québec growing conditions. Certain cultivars showed a better adaptability than others, and data suggest that Férimon, Jutta and Anka would be well suited to serve as dual-purpose cultivars.

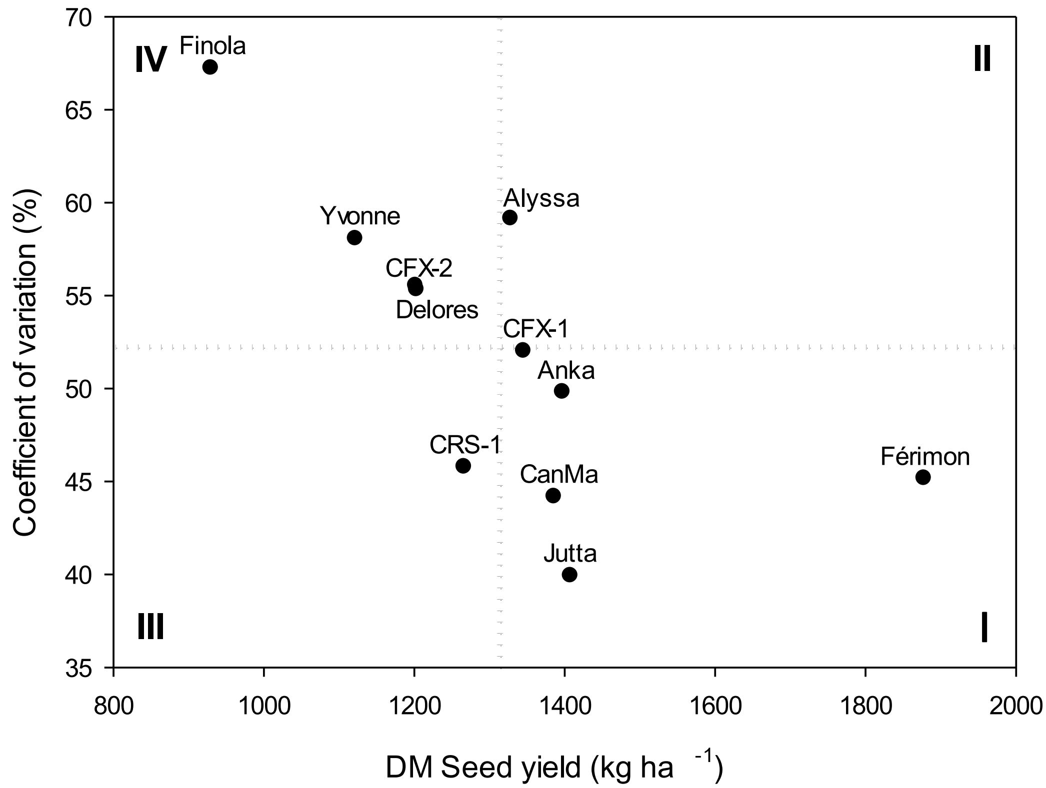

Figure 3.1 Average industrial hemp seed yields and coefficients of variation of 11 genotypes grown in 7 environments.

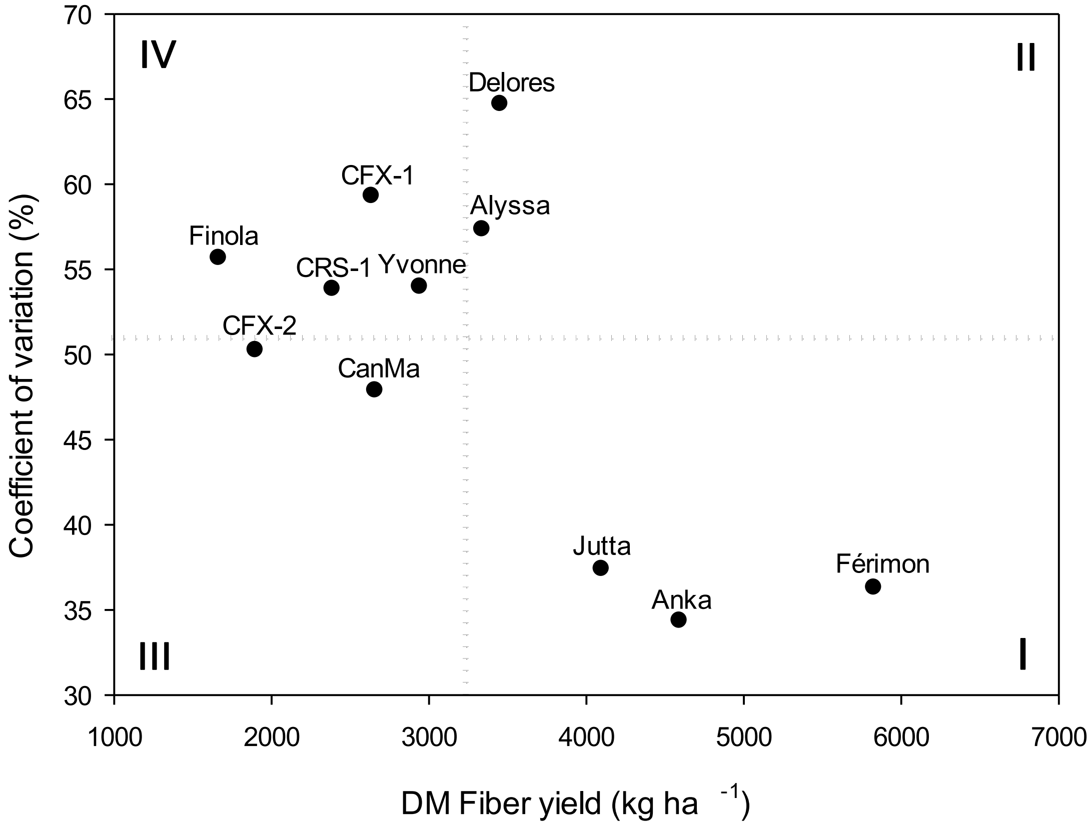

Figure 3.2 Average industrial hemp fiber yields and coefficients of variation of 11 genotypes grown in 7 environments.

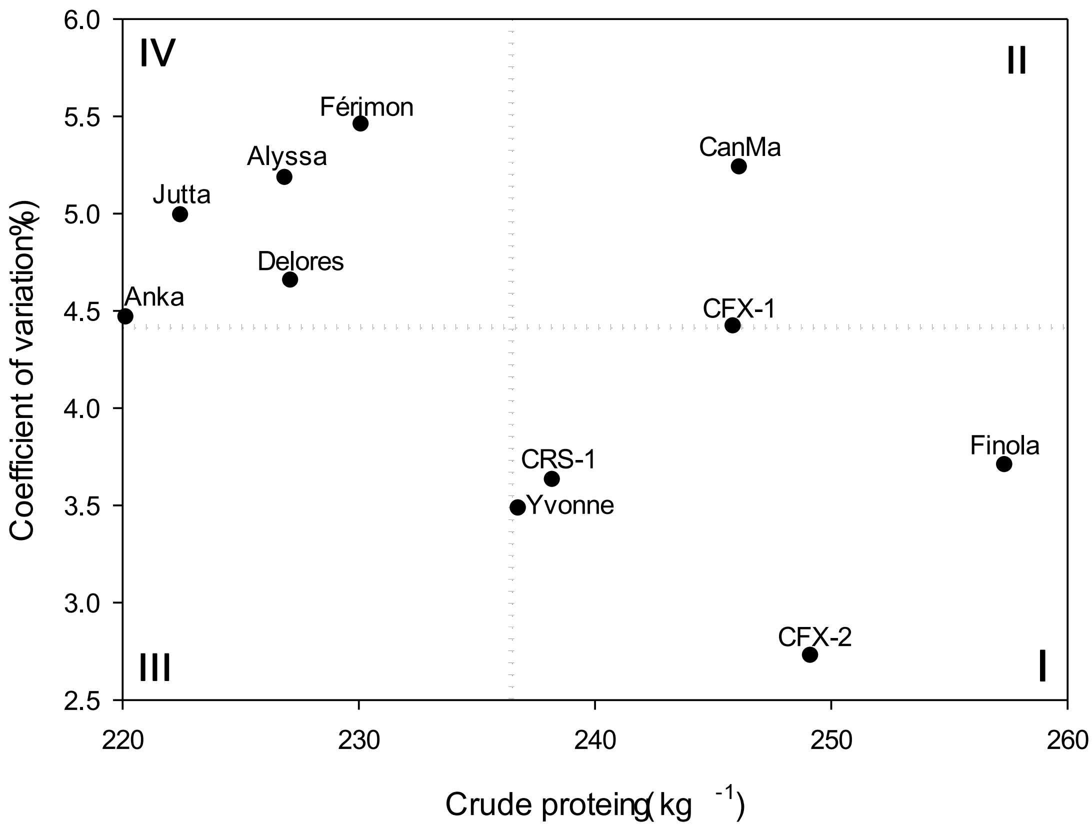

Figure 3.3 Average industrial hemp crude protein concentrations and coefficients of variation of 11 genotypes grown in 7 environments.

Table 3.1 Monthly precipitation and average temperature at six sites in eastern Canada from May to September.

	SAB† 2012	**QC 2012**	**SAB 2013**	**SMB 2013**	**QC 2013**	**LAP 2013**
			mm			
May	93.5	121.9	62.3	99.4	259.6	166.7
June	54.8	179.1	145	180.6	112.3	76.7
July	85.5	93.8	78.3	63.4	125.4	48.8
August	49.2	66.1	84	122.2	92.5	120.7
September	95.6	71.3	80.5	64.2	66	65.9
Total	378.6	532.2	450.1	529.8	655.8	478.8
			C°			
May	15.6	13	14.9	14.4	12.5	11.1
June	19.4	17.4	17.5	17.2	14.6	13.7
July	21.8	19.9	21.4	21.4	20	19.4
August	21.3	19.6	19.6	19.3	17.9	17.3
September	15.4	13.6	14.7	14.1	13	12.5
Average	18.7	17.5	17.6	17.3	16.3	14.8

† Abbreviations of the weather station names: SAB. Ste-Anne-de-Bellevue 1; QC. Québec/Jean Lesage Intl.; SMB. L'assomption; LAP. La Pocatière.

Table 3.2 Analysis of variance of agronomical parameters and seed and fiber characteristics of 10 industrial hemp genotypes grown in 3 different environments in Québec Canada in 2012.

Source of variation (p-value)	**Seed yield**	**Fiber Yield**	**Harvest Index**	**Height**	**Density**	**Maturity**	**Crude protein**	**Cellulose**	**Hemicellulose**	**Lignin**
	kg ha^{-1}		%	cm	plants m^2	days from seeding to harvest	g kg^{-1}	g kg^{-1}		
Genotype (G)	0.2802	0.0994	0.0217	<.0001	0.0086	0.0105	<.0001	0.0368	0.0073	0.3656
Environment (E)	<.0001	0.0002	0.03375	0.0002	0.0975	<.0001	0.0154	0.0009	0.4263	0.0567
G × E	<.0001	<.0001	0.0008	<.0001	<.0001	<.0001	0.7706	0.0226	0.1107	0.0741
Average (n=90)	1173	2705	33	93	131	90	241	569	123	93
Maximum	2768	7917	64	178	456	106	283	601	150	109
Minimum	200	233	17	25	22	69	208	510	110	73

Table 3.3 Analysis of variance of agronomical parameters and seed and fiber characteristics of 10 industrial hemp genotypes grown in 4 different environments in Québec in 2013.

Source of variation (p-value)	Seed yield	Fiber Yield	Harvest Index	Height	Density	Maturity	Crude protein	Cellulose	Hemicellulose	Lignin
	kg ha^{-1}		%	cm	plants m^2	days from seeding to harvest	g kg^{-1}	g kg^{-1}		
Genotype (G)	<.0001	<.0001	0.0031	<.0001	0.0004	0.0128	0.0546	0.082	0.361	0.4168
Environment (E)	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	0.0002	0.0004	<.0001	0.7005
G × E	0.3705	<.0001	<.0001	0.1696	<.0001	<.0001	<.0001	0.0676	0.055	0.0751
Average (n=120)	1398	3451	31	115	102	98	233	558	123	93
Maximum	3181	8837	79	336	239	110	270	622	146	129
Minimum	47	172	6	44	3	85	201	433	99	78

Table 3.4. Seed yield of 11 industrial hemp genotypes grown in 7 different environments in Québec.

	Environment														Average of 7 environments	
Genotype	**SAB-a 2012**	**Rank**	**SAB-b 2012**	**Rank**	**QC 2012**	**Rank**	**SAB 2013**	**Rank**	**SMB 2013**	**Rank**	**QC 2013**	**Rank**	**LAP 2013**	**Rank**	**Seed yield**	**Rank**
	kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}	
Alyssa	1301	4	2205	1	844	1	255	10	2314	3	670	7	1709	7	1328	6
Anka	1431	3	1437	10	655	6	926	4	2632	2	800	4	1898	4	1397	3
CanMa	932	6	1729	7	663	5	926	5	2151	6	1166	1	2134	2	1386	4
CFX-1	1809	1	1892	3	404	9	625	7	2057	7	809	3	1822	5	1345	5
CFX-2	795	8	1888	4	586	7	736	6	2213	4	652	8	1542	8	1202	9
CRS-1	915	7	1489	9	726	4	1163	2	2169	5	601	9	1800	6	1266	7
Delores	676	9	2092	2	793	2	544	8	2024	9	754	6	1539	9	1203	8
Finola	608	10	1642	8	237	10	496	9	1844	10	513	10	1166	10	930	11
Jutta	1484	2	1838	5	759	3	970	3	2030	8	792	5	1980	3	1408	2
Yvonne/Férimon†	1005	5	1824	6	536	8	1363	1	2825	1	989	2	2334	1	1122/1878	10/1
Average	1096		1804		620		801		2226		775		1792		1315	
LSD (0.05)	348.3		548.2		236.7		349.8		882.1		339.6		392.9			

† Yvonne used in 2012 was replaced by Férimon in 2013 due to inconsistent seed supply.

Table 3.5 Fiber yield of 11 industrial hemp genotypes grown in 7 different environments in Québec.

	Environment														Average of 7 environments	
	SAB-a		SAB-b		QC		SAB		SMB		QC		LAP			
Genotype	2012	Rank	2012	Rank	2012	Rank	2013	Rank	2013	Rank	2013	Rank	2013	Rank	Fiber yield	Rank
	kg ha-1		kg ha-1		kg ha-1		kg ha-1		kg ha-1		kg ha-1		kg ha-1			
Alyssa	2981	4	4739	3	1769	4	649	10	6509	3	3088	7	3631	2	3338	5
Anka	4770	2	4640	4	1970	2	4283	3	7122	2	5549	2	3806	1	4591	2
CanMa	2185	7	3539	7	1013	7	2633	5	4492	6	3486	5	1257	6	2658	7
CFX-1	5198	1	3669	6	707	8	1818	9	3345	8	2543	8	1174	7	2636	8
CFX-2	2054	8	2523	9	696	9	2149	7	2999	9	2409	9	460	10	1898	10
CRS-1	1822	9	1922	10	1052	6	3433	4	4373	7	3134	6	973	8	2387	9
Delores	2521	5	7416	1	1119	5	1888	8	5258	5	3933	4	2038	5	3453	4
Finola	1136	10	2886	8	461	10	2187	6	2179	10	2188	10	609	9	1664	11
Jutta	3712	3	3693	5	2120	1	4541	2	6481	4	5473	3	2652	4	4096	3
Yvonne/Férimon†	2306	6	4750	2	1770	3	5566	1	8164	1	6484	1	3094	3	2942/ 5827	6/1
Average	2869		3978		1268		2915		5092		3829		1969		3226	
LSD (0.05)	1054.5		2094.5		398.4		1306.2		1184.3		698.6		933.2			

† Yvonne used in 2012 was replaced by Férimon in 2013 due to inconsistent seed supply.

Table 3.6 Crude protein (CP) concentration of 11 industrial hemp genotypes grown in 7 different environments in Québec.

	Environment														Average of 7 environments	
Genotype	SAB-a 2012	Rank	SAB-b 2012	Rank	QC 2012	Rank	SAB 2013	Rank	SMB 2013	Rank	QC 2013	Rank	LAP 2013	Rank	CP conc.	Rank
	g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}	
Alyssa	227	7	224	9	235.0	9	236.3	3	218	7	241	8	207	9	228	8
Anka	214	10	220	10	234.0	10	216.5	10	211	10	233	9	211	8	222	11
CanMa	245	2	249	3	264.0	3	232.1	5	241	4	262	2	232	5	246	3
CFX-1	240	4	248	4	266.0	2	230.3	6	243	2	248	5	245	3	244	4
CFX-2	244	3	251	2	261.0	4	241.5	2	243	3	252	3	251	2	252	2
CRS-1	235	5	244	5	250.0	5	234.1	4	225	5	245	6	234	4	238	6
Delores	225	8	230	8	238.0	7	227.5	7	214	8	241	7	214	7	227	9
Finola	251	1	261	1	271.0	1	246.7	1	251	1	268	1	252	1	257	1
Jutta	219	9	230	7	238.0	8	219.6	9	213	9	231	10	206	10	224	10
Yvonne/Férimon†	232	6	232	6	246.0	6	225.8	8	223	6	249	4	223	6	240/230	5/7
Average	233		239		250		231		228		247		228		237	
LSD (0.05)	12.8		9.2		9.6		12.0		10.2		14.7		20.8			

† Yvonne used in 2012 was replaced by Férimon in 2013 due to inconsistent seed supply.

Table 3.7 Cellulose concentration of industrial hemp stems from 11 genotypes grown in 7 environments in Québec.

	Environment														Average of 7 environments	
Genotype	SAB-a 2012	Rank	SAB-b 2012	Rank	QC 2012	Rank	SAB 2013	Rank	SMB 2013	Rank	QC 2013	Rank	LAP 2013	Rank	[Cellulose]	Rank
	g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}		g kg^{-1}	
Alyssa	568	9	570	7	573	1	597	2	499	10	586	7	536	2	561	7
Anka	578	6	580	2	562	5	597	1	548	3	608	1	549	1	575	2
CanMa	583	4	572	6	557	7	591	5	531	8	592	6	498	10	561	9
CFX-1	573	7	574	3	537	9	590	6	537	5	575	9	503	9	556	10
CFX-2	581	5	573	4	556	8	580	9	526	9	579	8	534	4	561	8
CRS-1	591	1	582	1	559	6	589	7	542	4	595	4	517	8	568	5
Delores	570	8	570	8	567	3	592	4	534	6	593	5	535	3	566	6
Finola	N/A	N/A	559	10	519	10	N/A	N/A	534	7	534	10	522	7	534	11
Jutta	585	3	569	9	564	4	585	8	551	1	603	3	526	6	569	4
Yvonne/Férimon†	590	2	572	5	570	2	593	3	549	2	607	2	532	5	578/570	1/3
Average	579		572		555		590		534		585		524		561	
LSD (0.05)	21.4		18.5		21.5		NS		NS		NS		NS			

† Yvonne used in 2012 was replaced by Férimon in 2013 due to inconsistent seed supply.

CONNECTING TEXT FOR CHAPTER 4

The previous chapter characterized eleven industrial hemp cultivars in terms of their seed and fiber yield and quality. The study demonstrated the potential of industrial hemp production under Québec growing conditions, and highlighted better suited cultivars for dual-harvest of seeds and fibers.

The following study evaluated the effect of N, P and K fertilization levels on industrial hemp production in Québec. It examined agronomic parameters such as seed and fiber yields, as well as seed crude protein concentrations and stem fiber cellulose, hemicellulose and lignin concentrations in response to the various fertilization levels.

Chapter 4 was written as a manuscript which will serve as a basis for eventual submission to a publication. The manuscript was co-authored by the candidate, Dr. Philippe Seguin, thesis co-supervisor, and Dr. Jean-Benoît Charron, thesis co-supervisor, both from the Department of Plant Science, McGill University. All authors contributed to editing the manuscript. Laboratory analyses were conducted by the candidate under the supervision of Dr. Philippe Seguin, Dr. Arif Mustafa from the Department of Animal Science, McGill University, and Dr. Gaëtan Tremblay from Agriculture and Agri-Food Canada.

CHAPTER 4

EFFECTS OF N, P AND K FERTILIZATION LEVELS ON INDUSTRIAL HEMP PRODUCTION IN QUÉBEC

4.1 Abstract

Industrial hemp is gaining popularity in Canada and it has a great potential as a multipurpose crop. The lack of adapted agronomical guidelines in eastern Canada represents however a limiting factor for hemp production. This study assessed seed and fiber yield and quality of the cultivars CRS-1 and Anka, following various N, P, K fertilization rates (0, 50, 100, 150 and 200 kg N or K ha^{-1}; 0, 25, 50, 75, 100 kg P ha^{-1}). The experiment was conducted in 4 environments in the province of Québec. A positive linear response of both seed and fiber yield, and seed crude protein concentration following an increase in nitrogen fertilization was observed in all environments. Cultivar × environment interaction was observed due to a difference in term of magnitude of the response of the two cultivars evaluated. Significant N fertilization main effects were significant for cellulose and hemicellulose, but the overall response was minimal. Phosphorus and potassium fertilization had no effect on agronomical yields and seed quality. Rates of 50, 75 and 100 kg P ha^{-1} were associated with higher cellulose concentrations in hemp stems.

4.2 Introduction

The growing interest for industrial hemp in Canada is locally reflected by a steady increase in production area and demand for production licences. Production area in Canada has increased from 4000 to approximately 27000 ha between 2008 and 2013 (Anum Laate, 2012; CHTA, 2014). Hemp seeds are highly nutritive with high concentrations of protein, fiber and oil, while hemp fibers versatility has been demonstrated for being suited for use in a large variety of products, including fabrics, paper, wall insulator panels, concrete, car body and animal bedding (AAFC, 2007; House et al., 2010; Chen, He et al., 2010; Serecon Management Consulting Inc., 2012). Although industrial hemp production in Canada has been re-authorized more than 15 years ago, recommendations regarding best field management practices remain scarce, especially for Eastern Canada, most research and production occurring in Western Canada (AAFC, 2007).

A positive response of hemp to nitrogen fertilization has been reported in Western Canada. Increasing nitrogen fertilization rates positively impacted hemp seed yield, fiber yield and plant height. Optimal nitrogen fertilization rates were reported to be between 100 and 150 kg N ha^{-1} (Vera et al., 2004; 2010). Although phosphorus application was reported to increase plant height under certain growing conditions, its effect on industrial hemp seed and fiber yield is inconsistent and often not significant (Vera et al., 2004;

2010). Very few trial testing potassium fertilization have been conducted and no effect of potassium on hemp yield was reported (Finnan and Burke, 2012).

Despite the growing interest generated by hemp production in the province of Québec, agronomical recommendations for eastern Canada remain scarce. There is thus a need to establish optimal agronomical practices for hemp production grown in this area in order to optimize seed and fiber yields. The present study reported on the effects of nitrogen, phosphorus and potassium fertilization on seed and fiber yields of two industrial hemp cultivars, CRS-1 and Anka, in multiple environments in the province of Québec.

4.3 Material and methods

4.3.1 Experimental sites and treatments

Experiments were conducted in two to four environments in Québec, Canada, in 2012 and 2013. The total number of environment depended on the element evaluated; nitrogen, phosphorus, and potassium experiments being conducted separately. The sites were located in Sainte-Anne-de-Bellevue (SAB: 45° 26' N, 73° 55' W; 2950 CHUs), Lanoraie (LAN: 45° 58' N, 73° 12' W; 2850 CHUs), and Saint-Augustin-de-Desmaures (QC: 46°43' N, 71°30' W; 2400 CHUs). The nitrogen experiment was conducted at SAB and LAN in 2012 and at SAB in 2013, the phosphorus experiment in SAB, LAN and QC in 2013, and the potassium experiment in SAB in 2012 and 2013. The soil type was a Chicot light sandy loam (Gleysol Melanic Brunisol) at SAB in 2012 and 2013, a sand

(Brunisol) in LAN in 2012 and 2013, and a sandy loam (Gleysol orthic) for the nitrogen trial but a loam (Gleysol) for the phosphorus one in QC.

In each experiment, two cultivars approved in Canada were evaluated: CRS-1 and Anka. Seeding was done at a rate of 43 kg ha^{-1} of pure live seeds. At SAB and QC, plots were seeded at a 1 cm depth using a cereal seeder with an 18 cm row spacing. LAN, plots were seeded using a precision garden seeder. Each plot was manually fertilized prior to seeding. Fertilization rates of 0, 50, 100, 150 or 200 kg ha^{-1} were used for nitrogen (27.5 – 0 – 0) and potassium (0 – 0 – 60) fertilization trials, and rates of 0, 25, 50, 75 and 100 kg ha^{-1} were used for phosphorus (0 – 46 – 0) fertilization trials. Fertilizer was manually applied and incorporated the same day using rakes. Apart from the specific fertilization treatments, additional phosphorus was added in the potassium trials and potassium in the phosphorus ones based on results of soil tests conducted at each site following general recommendations for hemp production in Ontario (Baxter and Scheifele, 2000). Similarly, nitrogen was added at a rate of 110 kg ha^{-1} to plots from the phosphorus and potassium trials. Weeding was done manually throughout the growing season.

4.3.2 Data collection

Plant density was determined at the 4 leaves stage by counting plants along 1 meter, in two non-border rows per plot. The average height of 10 plants per plot was measured at harvest. Plants were harvested at maturity (late August to early September) and the area harvested was approximately 3 m², the exact area depending on the site. Only the middle rows of plots were harvested to avoid potential border effects. At harvest, stems were manually cut approximately 2 cm from the ground. Following harvest, whole plants were dried at 45ºC for 48-72 hours. Whole plants were then threshed using a stationary combine to separate seeds from the rest of the total biomass. Total dry matter (DM) fiber yield was calculated by subtracting seed yield from total biomass.

4.3.3 Chemical analyses

4.3.3.1 Seed preparation

Seeds from all environments were ground to a fine powder (approximately to 1 mm) using a standard coffee mill (Smartgrind™ CBG100S, Black & Decker, Middleton, WI), followed by the use of a pestle and mortar.

4.3.3.1.1 Crude protein concentration

Crude protein concentration of a randomly selected set of samples was first quantified following elemental nitrogen analysis by combustion (Truspec Nitrogen Determinator System, Leco Corp., St. Joseph, MI) and using a nitrogen to protein conversion factor of 6.25. All the samples were further scanned by near infrared reflectance spectroscopy (NIRS) using a NIRSystem 6500 monochromator (Foss, Silver Spring, MD) in the range from 400 to 2500 nm. Calibration equations were developed using a modified least squares regression method of the WinISI III software (Infrasoft

International LLC, State College, PA) with the use of the previously determined chemical values and their respective spectrum. The prediction equation for the crude protein concentration was successfully validated with a ratio of prediction to deviation (RPD) of 3.70.

4.3.3.2 Fiber composition

For each plot, a composite sample of hemp stems was ground through a 1 mm screen using a Wiley mill (standard model 3; Arthur H. Thomas Co., Philadelphia, PA). All samples were first scanned by near infrared reflectance spectroscopy (NIRS). From the resulting spectra, 74 samples were selected for chemical analysis to provide reference values to the software for the development of a calibration equation. Neutral detergent fiber (NDF) (Van Soest et al., 1991), acid detergent fiber (ADF) and acid detergent lignin (ADL) (AOAC, 1990) concentrations, were quantified in the 74 samples using an Ankom Fiber Analyzer (Ankom Technology Corp., Fairport, NY). Moisture content of samples was determined with the oven method at 105°C (AOAC, 1990). The NDF, ADF and ADL concentrations of the entire population were then predicted using the developed calibration equation. NIRS predictions for NDF and ADF concentrations were considered moderately successful and moderately useful with a RPD of 2.33 and 2.17 respectively. However, no prediction for ADL concentration was possible due to a RPD of 0.99 (data not shown). In addition, cellulose concentrations were estimated using NIRS with a RPD of 2.43. Hemicellulose and lignin concentration were determined by calculation.

4.3.4 Statistical analyses

The experimental design for all experiments was a randomized complete block design with split-plot restriction and three replicates. The cultivars CRS-1 and Anka were assigned to main plots and fertilization levels to sub-plots. Environments and replicates were considered random effects, whereas cultivars and fertilization rates were considered fixed effects. The general linear model (GLM) procedure in SAS (SAS Institute, 2008) was used for data analysis and comparisons between means was made using the least significant difference (LSD). The trends related to fertilization treatments (linear and quadratic) were estimated using orthogonal contrasts. Statistical significance was assumed at $P \leq 0.05$.

4.4 Results and discussion

Regarding nitrogen fertilization, environment × fertilization interactions were observed for many parameters including seed yield, fiber yield and crude protein concentration (Table 4.1). These interactions reflect a different magnitude of the plants response at each site. A positive trend was nonetheless observed across the four environments following nitrogen fertilizer application. More specifically, nitrogen fertilization had a significant effect on seed yield, fiber yield, height, 1000-seeds weight and crude protein concentration. A quadratic positive response ($P < 0.05$) was observed for four of these parameters following nitrogen fertilization, but no plateau was observed. Therefore, it was not possible to determine the optimal nitrogen fertilization rate to maximize agronomical yield or seed quality. Possible reasons that would explain the absence of a plateau in the observed response of hemp to N could be an inefficient plant nutrient use, or too low fertilizer rates. Indeed, each treatment was applied and incorporated in a single fertilizer dose before seeding, which could have led to nitrogen leaching. The positive effect of nitrogen on agronomical parameters as well as crude protein concentration was in accordance with the literature (Vera et al., 2004; 2010; Mihoc et al., 2013). Seed yields ranged from 166 to 2707 kg ha^{-1}.The highest average seed yield was observed in SAB in 2012 with 1808 kg ha^{-1} (Table 4.1). Average seed yield across all environments was 935 kg ha^{-1}, which is similar to the average seed yield in Canada which usually range between 800 and 1000 kg ha^{-1} (AAFC, 2007; Beaulieu and Doucet, 2013). Fiber yield following nitrogen fertilization ranged from 422 to 8041 kg ha^{-1}, with the highest average of 4734 reported at SAB in 2012 (Table 4.1). Average fiber yield across sites was 3112 kg ha^{-1} which is below the expected fiber yield of 6000 kg ha^{-1} generally reported in western Canada (AAFC, 2007). No specific fertilization rate was determined as no plateau was reached for the selected rates applied. Crude protein concentration ranged from 207 to 265 g kg^{-1}, with the highest average of 234 g kg^{-1} reported in SAB in

2012 (Table 4.1). Average crude protein concentration across sites was 229 g kg^{-1}, which is close to the values generally reported in the literature (House et al., 2010; Mihoc et al. 2013).

The effect of the environment was significant for all the parameters presented, except for the crude protein (Table 4.1). Cultivar main effect was significant for fiber yield, crude protein and other agronomical parameters, but was not significant for seed yield. Furthermore, there was no environment × cultivar interaction for the major variables of interest (Table 4.1). Cultivar × fertilization interactions were observed for fiber yield and crude protein. These interactions were explained by a difference in the magnitude of the response of the two cultivars. Both cultivars showed an increase in agronomical yields and crude protein, but Anka had generally a greater response of the seed and fiber yield than CRS-1 to nitrogen fertilization, while CRS-1 had a higher crude protein concentration than Anka (data not shown). For that reason, Anka and CRS-1 means were combined to present the general trend across each environment (Figure 4.1, 4.2 and 4.3).

Environment main effect was significant for cellulose, hemicellulose and lignin concentration, whereas cultivar main effect was only significant for lignin concentration (Table 4.1). An E × C interaction was observed regarding lignin concentration but no clear trend was observed when comparing cultivars across sites (data not shown). Nitrogen fertilization main effect was statistically significant for cellulose and hemicellulose, however, only very minimal variations of these fiber concentrations were observable. It was previously stated that nitrogen did not have an apparent impact on stem composition (Thomsen et al., 2005). Cultivar × fertilization interactions were observed for hemicellulose concentration (Table 4.1). The cultivar Anka showed a constant and gradual increase in hemicellulose as nitrogen fertilizer dose increased, whereas CRS-1 associated response was erratic. Finally, environment × fertilization interactions were significant for cellulose and lignin (Table 4.1). Cellulose concentrations decreased at 150 and 200 kg N ha^{-1} at SAB in 2012 and 2013, while no consistent trend was revealed at LAN and QC. In addition, while lignin concentrations responded positively to increasing N fertilization levels at SAB in 2012 and 2013, an opposite response was observed in LAN and QC.

Phosphorus fertilization had no effect on the agronomical parameters studied (Table 4.2). Environment × cultivars (E × C) interaction was significant for the fiber yield but reflected differences of magnitude in the response of the two cultivars evaluated. Anka overall had the greatest fiber yield in all environments and yields varied depending on the growing environment (data not shown). Cultivar × fertilization (C × F) interaction was significant for the plant height variable (Table 4.2). Variations were due to the degree of response of each cultivar, and to their inconsistent response associated with the various fertilizer levels (data not shown). Anka plants height was greater than CRS-1 plants height at each fertilization level, but plant response to P fertilization was inconsistent. Therefore, it was not possible to relate phosphorus fertilization level to plants height. Inconsistent hemp response following phosphorus fertilization has already been reported (Vera et al., 2010). It was previously stated that phosphorus effect varied upon growing conditions (Vera et al., 2004).

There was no phosphorus fertilization main effect for crude protein concentration. This lack of response has been previously reported (Vera et al., 2004; Izsáki and Ivány, 2008). There was also a three-way interaction (E × C × F) interaction for crude protein concentration (Table 4.2) which reflected that in one environment (i.e., QC), CRS-1 tended to respond positively to an increase in phosphorus fertilization while Anka responded inversely. The response of the two cultivars to P was inconsistent in the other environments (data not shown).

Regarding fiber composition, the environment main effect was significant for cellulose, hemicellulose and lignin concentration. Furthermore, the cultivar main effect was only significant for lignin concentration, reflecting that CRS-1 lignin concentration was slightly greater than for Anka (Table 4.1). The E × C interaction was also significant for lignin which could be due to the lower values observed for both cultivars in LAN. A phosphorus fertilization main effect was observed only for cellulose concentration (Table 4.2). Although variations were not substantial, rates of 50, 75 and 100 kg P_2O_5 ha^{-1} increased cellulose concentration compared to rates on 0 and 25 kg P_2O_5 ha^{-1} (data not shown). Finally, the E × C × F interaction was significant for hemicellulose (Table 4.2).

Hemicellulose concentration was greater in LAN in 2013, and variations between both cultivars and fertilization rates were inconsistent (data not shown).

Potassium fertilization had no effect on the agronomical parameters studied (Table 4.3). These results are in accordance with the previous studies that reported an absence of potassium effect on hemp total biomass and seed yield (Finnan and Burke, 2012). An environment × cultivar interaction was observed for fiber yield and reflected a difference in the magnitude of the response of both cultivars. Anka had the greatest fiber yield in all environments and yields depended upon the growing environment (data not shown). A significant E × C × F was observed for plant height (Table 4.3). This interaction was mostly due to a notably great response of both cultivars to the specific rate of 200 kg K_2O ha^{-1} in SAB in 2012. However, no variation in height was observed between fertilization rates in the other environment where it was tested (data not shown). No effect of potassium fertilization on crude protein concentration was observed. It was previously stated that phosphorus and potassium did not have an effect on crude protein concentration, but were required for proper nitrogen plant uptake (Mihoc et al., 2013). Scientific information related to potassium effect on hemp seed quality remains limited.

Environment main effect was significant for cellulose and hemicellulose. Fertilization main effect was significant for cellulose, but the essence of potassium fertilization effect on cellulose was nevertheless unclear (data not shown). Indeed, cellulose concentrations at higher potassium fertilizer rates were greater than concentrations for the controls, except at 100 kg K_2O ha^{-1} for which cellulose concentration was below the control. Environment × fertilization interactions were observed for hemicellulose and lignin concentrations. Concentrations varied across sites, and the effect of potassium rates was too inconsistent to determine a trend. Lastly, E × C × F interaction was significant for hemicellulose, due to inconsistent response to fertilization of the cultivars in different environments (data not shown).

4.5 Conclusion

A positive response of both seed and fiber yield, and seed crude protein concentration following an increase in nitrogen fertilization was observed in all environments. A cultivar × environment interaction was observed for these variables due to a difference in term of magnitude of the response of the two cultivars. Anka consistently had higher seed and fiber yields than CRS-1, while CRS-1 had a higher crude protein concentration than Anka. No plateau was observed for the yield responses. Therefore optimal nitrogen fertilization rate could not be determined. A nitrogen fertilization main effect was statistically significant for cellulose and hemicellulose, but the observed differences between fertilization treatments were biologically insignificant. Phosphorus and potassium fertilization had no effect on seed and fiber yields and seed crude protein. However, rates of 50, 75 and 100 kg P ha^{-1} were associated with higher cellulose concentrations in hemp stems. Cultivar × fertilization interactions were observed for plant height following phosphorus fertilization principally due to a difference in the degree of response of cultivars. However no clear trend could be established. Environment × cultivar × fertilization interactions were observed for a few parameters following phosphorus and potassium trials, but variations were inconsistent and no clear effect of P and K fertilization could be identified.

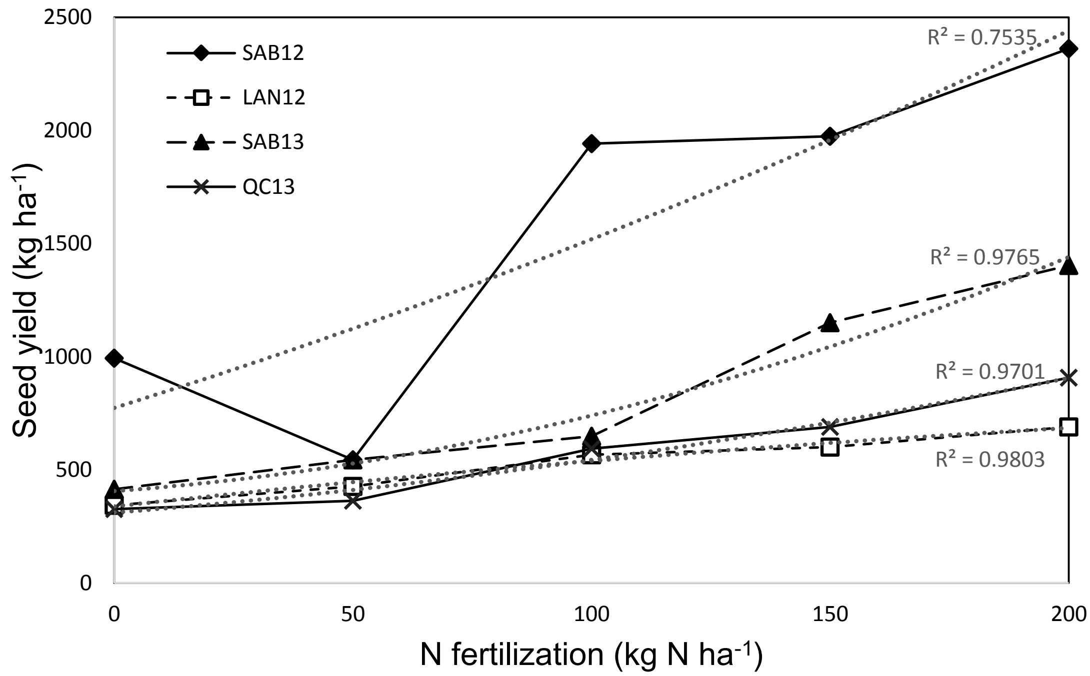

Figure 4.1 Average seed yield of CRS-1 and Anka at different N fertilization rates across 4 environments in Québec. Linear and quadratic regressions were significant ($P < 0.0001$).

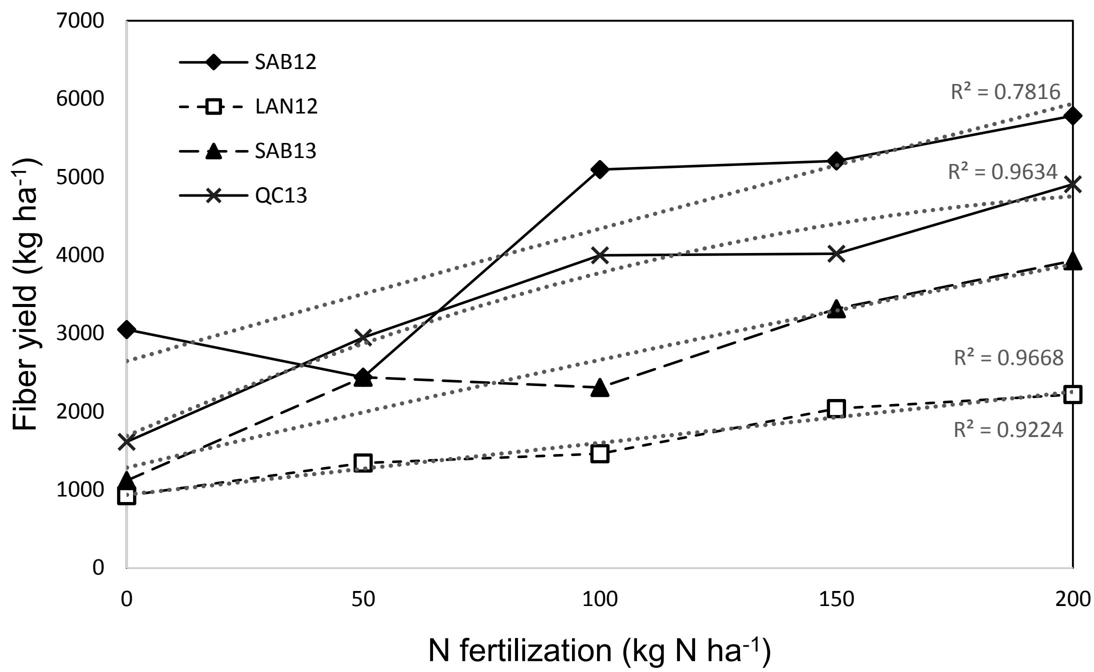

Figure 4.2 Average fiber yield of CRS-1 and Anka at different N fertilization rates across 4 environments in Québec. Linear and quadratic regressions were significant (*P* < 0.0001).

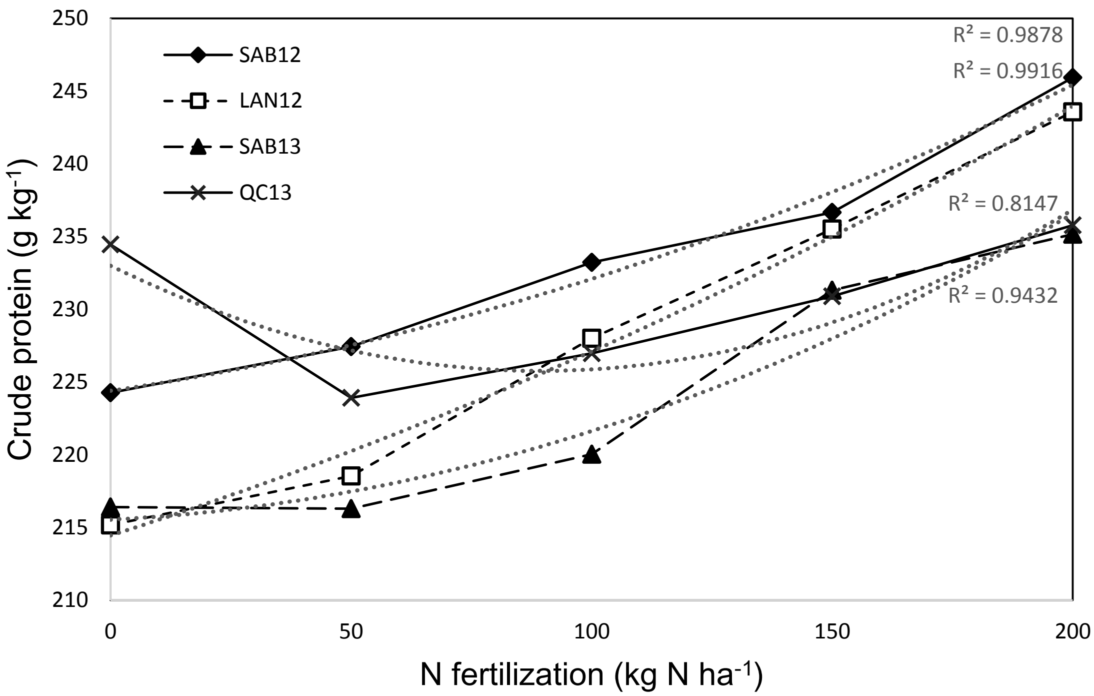

Figure 4.3 Average crude protein concentration of CRS-1 and Anka at different N fertilization rates across 4 environments in Québec. Linear and quadratic regressions were significant ($P < 0.0001$).

Table 4.1 Analysis of variance of agronomical variables of 2 industrial hemp cultivars fertilized with different levels of nitrogen and grown in 4 environments in Québec.

Treatments	Seed yield	Fiber yield	Harvest index	Height	Density	Crude protein	Cellulose	Hemicellulose	Lignin
	—— kg ha^{-1} ——		%	cm	plants m^{2}	—————— g kg^{-1} ——————			
Environment (E)	<.0001	0.0002	0.0007	<.0001	<.0001	0.2142	<.0001	<.0001	0.0085
Cultivar (C)	0.2005	0.0006	0.0003	0.0006	0.0070	<.0001	0.5952	0.7520	0.0349
E × C	0.3600	0.2010	0.1340	0.1828	0.0031	0.2622	0.4750	0.2357	0.0093
Fertilization (F)	<.0001	<.0001	0.1911	<.0001	0.1038	<.0001	0.0291	0.0081	0.2940
Linear	<.0001	<.0001	0.0903	<.0001	0.8162	0.0130	0.1521	0.0024	0.1888
Quadratic	<.0001	<.0001	0.3967	<.0001	0.0322	<.0001	0.9194	0.0283	0.6525
Cubic	0.0008	0.0528	0.1391	0.4532	0.8202	<.0001	0.0038	0.8779	0.0841
C × F	0.2310	0.0019	0.9815	0.2155	0.6550	0.0025	0.9703	0.0122	0.9980
E × F	<.0001	0.0339	0.5144	0.0308	0.0008	0.0016	0.0136	0.3965	0.0230
E × C × F	0.1753	0.6835	0.6255	0.6489	0.4650	0.8866	0.9106	0.2058	0.7564
Average	935	3112	24	111	138	229	718	842	105
Maximum	2707	8041	63	190	356	265	584	700	82
Minimum	166	422	5	31	19	207	671	791	93

Table 4.2 Analysis of variance of agronomical variables of 2 industrial hemp cultivars fertilized with different levels of phosphorus and grown in in 3 environments in Québec.

Treatments	**Seed yield**	**Fiber yield**	**Harvest index**	**Height**	**Density**	**Crude protein**	**Cellulose**	**Hemicellulose**	**Lignin**
	—— $kg\ ha^{-1}$ ——		%	cm	plants m^2	—— $g\ kg^{-1}$ ——			
Environment (E)	0.0098	0.0003	<.0001	<.0001	0.0077	0.2411	0.0224	0.0003	0.0398
Cultivar (C)	0.5852	0.0013	<.0001	0.0011	0.0119	0.0196	0.2182	0.1173	0.0300
E × C	0.7915	0.0276	0.0209	0.1592	0.2019	0.3869	0.5256	0.2235	0.0676
Fertilization (F)	0.8550	0.7800	0.9385	0.7600	0.5593	0.7537	0.0102	0.6128	0.3600
Linear	0.7101	0.7888	0.9327	0.9713	0.2872	0.6497	0.1799	0.3955	0.4684
Quadratic	0.8442	0.9817	0.5214	0.6276	0.3113	0.7838	0.7617	0.9282	0.8639
Cubic	0.4935	0.5010	0.7838	0.2536	0.4235	0.2129	0.4702	0.7695	0.2675
C × F	0.6701	0.4623	0.5842	0.0170	0.8562	0.5762	0.9091	0.4461	0.7787
E × F	0.4449	0.8815	0.8699	0.1562	0.9568	0.2183	0.2935	0.2255	0.9460
E × C × F	0.8396	0.4576	0.9033	0.2965	0.7097	0.0040	0.8746	0.0292	0.8391
Average	751	3029	22	114	17	231	132	590	84
Maximum	1795	7661	41	204	20	249	164	621	112
Minimum	240	473	7	43	14	206	99	535	58

Table 4.3 Analysis of variance of agronomical variables of 2 industrial hemp cultivars fertilized with different levels of potassium and grown in 2 environments in Québec.

Treatments	Seed yield	Fiber yield	Harvest index	Height	Density	Crude protein	Cellulose	Hemicellulose	Lignin
	kg ha^{-1}		%	cm	plants m^2	g kg^{-1}			
Environment (E)	0.2323	0.0003	0.0054	<.0001	0.0221	0.9931	0.0009	0.0769	0.9631
Cultivar (C)	0.0701	0.0001	0.0005	0.0005	0.8417	0.0103	0.8763	0.5066	0.8403
E × C	0.2197	0.0271	0.2455	0.5354	0.9540	0.2084	0.9585	0.6056	0.5334
Fertilization (F)	0.3430	0.1818	0.7273	0.5833	0.2600	0.9604	0.0427	0.7728	0.1655
Linear	0.1824	0.2604	0.9523	0.2519	0.1425	0.8781	0.042	0.3898	0.6691
Quadratic	0.2676	0.0542	0.3156	0.6690	0.9695	0.8229	0.4755	0.6904	0.1088
Cubic	0.3043	0.4984	0.3883	0.2592	0.8819	0.4702	0.2413	0.5179	0.0549
C × F	0.6053	0.9143	0.3278	0.4535	0.0365	0.6355	0.3419	0.505	0.2829
E × F	0.2295	0.5416	0.1998	0.3368	0.2151	0.5882	0.7689	0.0169	0.0354
E × C × F	0.3001	0.1349	0.8306	0.0204	0.0387	0.7086	0.777	0.0331	0.1856
Average	1081	3758	26	135	130	221	111	590	94
Maximum	1758	8870	48	197	356	242	124	616	108
Minimum	453	488	12	65	22	206	86	561	81

CHAPTER 5

CONCLUSIONS AND SUMMARY

Industrial hemp is multi-purpose crop for which there is a growing interest in Canada. Principally grown in western Canada and the prairies, agronomic information on this emergent crop is limited in Eastern Canada and especially in Québec. The aim of the present study was therefore to characterize industrial hemp cultivars and establish agricultural guidelines adapted to the province of Québec.

The first objective was to assess the adaptability of a range of industrial hemp cultivars in Eastern Canada. Hemp demonstrated a good potential under Québec growing conditions. Seed yields were often above the Canadian average, but fiber yield was on average below the average fiber yield in Canada. Cultivars performance greatly varied across experimental sites. Nonetheless, many cultivars stood out from other in terms of agronomic yields and their superior stability across environments. Férimon, Anka, Jutta and CanMa produced greater and more consistent seed yields compared to the other cultivars evaluated, and Férimon, Anka and Jutta also distinguished themselves in regards of their fiber yields. These three specific cultivars were then identified to be better suited for dual purpose cultivars. Finola, CFX-1, CFX-2, CRS-1 and Yvonne had a superior and consistent crude protein concentration across the seven environments. However, these cultivars had lower agronomic yields that varied across environments.

The second objective focused on identifying optimal guidelines related to N, P and K fertilization. No significant effects of potassium and phosphorus fertilization were observed for the parameters of interest. On the other hand, nitrogen fertilization effect was considerable for plant height, seed and fiber yield, as well as crude protein concentration. It was also found significant for cellulose and hemicellulose stem concentrations, although response to nitrogen was biologically limited.

Furthermore, this study demonstrated the potential of near infra-red spectrometry to predict seed crude protein concentration and stem cellulose, NDF and ADF concentrations. This technology was nonetheless limited when it came to predict stem hemicellulose and lignin concentrations.

CHAPTER 6

RECOMMENDATION FOR FUTURE RESEARCH

The results presented in this study highlighted the necessity for further research on industrial hemp in Eastern Canada. Further experiments focusing on the characterization of industrial hemp cultivars should be replicated at the regional scale in order to identify cultivars best suited to specific regions or environmental conditions. A special attention should be given to the cultivars Anka, Jutta, and Férimon that demonstrated a good potential to serve as dual purpose cultivars in many environments.

Additional fertilization trials would be necessary to determine optimal N-P-K fertilization rates. Split application experimentation could be a solution to ensure fertilizer use efficiency in nitrogen fertilization trials. Higher fertilization levels should also be evaluated given that a plateau was not reached in the response to nitrogen we observed. Studies should also take into consideration the contribution from the previous crop as well as plant nutrient uptake to establish precise fertilization guidelines.

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APPENDICES

Table A.1 Health Canada list of approved hemp cultivars for 2012 and 2013 growing seasons.

Cultivar	**Country of Origin**
Alyssa	Canada
Anka	Canada
Canda	Canada
CanMa	Canada
Carmagnola	Italy
Carmen	Canada
CFX-1	Canada
CFX-2	Canada
Crag	Canada
CRS-1	Canada
C S	Italy
Delores	Canada
Deni	Canada
ESTA-1	Canada
Fasamo	Germany
Fedrina 74	France
Felina 34	France
Férimon	France

Fibranova	Italy
Fibriko	Hongary
Fibrimon 24	France
Fibrimon 56	France
Finola	Canada (Finland)
Joey	Canada
Jutta	Canada
Kompolti	Hongary
Kompolti Hibrid TC	Hongary
Kompolti Sargaszaru	Hongary
Lovrin 110	Romania
Petera	Canada
Silesia	Canada
UC-RGM	Canada
Uniko B	Hongary
USO 14	Canada (Ukraine)
USO 31	Canada (Ukraine)
X-59 (Hemp Nut)	Canada
Yvonne	Canada
Zolotonosha 11	Canada (Ukraine)
Zolotonosha 15	Canada (Ukraine)

(Beaulieu, 2013)

Table A.2 Country where maintained, sex, maturity and use of 11 hemp cultivars produced in Canada.

Cultivar	Country where maintained	Sexual type	Maturity	Use
Alyssa	Canada	Monoecious	Late-maturing	Dual purpose
Anka	Canada	Monoecious	Late-maturing	Dual purpose
CanMa	Canada	Dioecious	Early-maturing	Grain
Jutta	Canada	Monoecious	Late-maturing	Dual purpose
Yvonne	Canada	Monoecious	Late-maturing	Dual purpose
Férimon	France	Monoecious	Early-maturing	Dual purpose
CRS-1	Canada	Dioecious	Early-maturing	Grain
CFX-1	Canada	Dioecious	Early-maturing	Grain
CFX-2	Canada	Dioecious	Early-maturing	Grain
Finola	Canada (developed in Finland)	Dioecious	Early-maturing	Grain
Delores	Canada	Monoecious	Early-maturing	Dual purpose

(Girouard et al., 1999; Beaulieu and Doucet, 2013)

Table A.3 Industrial hemp licence No. delivered to McGill and Laval Universities in 2012 and 2013.

McGill University 2012	**Licence No. 12-C0142-R-02** R1-011, R1-003 and R1-004 Phytotorium, Raymond Building, 21 111 Lakeshore Rd., Ste-Anne-de-Bellevue, QC. Possession, production, sending, transportation, and delivery.	**Licence No. 12-C0142-R-01** Emile A Lods Agronomy Centre Field 8, 20 965, ch. Ste-Marie, Ste-Anne-de-Bellevue, QC. Possession, production, sending, transportation, and delivery.
McGill University 2013	**Licence No. 13-C0142-R-01** R1011, R1003, R1004, R2003, R1015, Raymond Building, 21 111 Lakeshore Rd., Ste-Anne-de-Bellevue, QC. Possession, production, sending, transportation and delivery.	**Licence No. 13-C0142-R-02** Emile A Lods Agronomy Centre: Field 8, 20 965, ch. Ste-Marie, Ste-Anne-de Bellevue, QC. Possession, production, sending, transportation and delivery.
Laval University 2012	**Licence n° 12-V0049-R-01** Field C23, Route 138 St-Augustin-de-Desmaures, QC. Possession, production, sending, transportation and delivery.	
Laval University 2013	**Licence n° 13-V0049-R-02** Field C23, Board 40G St-Augustin-de-Desmaures, QC. Possession, production, sending, transportation and delivery (for THC analysis only).	