

The Effect of Plant Population Density and Harvest Timing on Agronomic Fibre Yield  
and Quality Characteristics of Industrial Hemp (*Cannabis*), Cultivar Alyssa, Grown in  
the Parkland Region of Manitoba, Canada.

BY

ANNDREA MARIE HERMANN

A Thesis  
Submitted to the Faculty of Graduate Studies  
in Partial Fulfillment of the Requirements  
for the Degree of

MASTERS OF SCIENCE

Department of Plant Science  
University of Manitoba  
Winnipeg, Manitoba

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**Of**

**MASTER OF SCIENCE**

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## ABSTRACT

Hermann, Anndrea. M. M.Sc., The University of Manitoba, March 2008. The Effect of Plant Population Density and Harvest Timing on Agronomic Fibre Yield and Quality Characteristics of Industrial Hemp (*Cannabis*) cultivar Alyssa Grown in the Parkland Region of Manitoba, Canada. Major Professor: Dr. Martin H. Entz.

Hemp (*Cannabis sativa* L.) is a versatile plant that is grown for its premier grain and/or fibre characteristics. After 50 years of prohibition, Canadian hemp is being recognized and promoted worldwide. This has resulted in the increased need for Canadian based agronomic hemp research.

Split-plot replicated studies were conducted in 2006 and 2007 at five locations in the Parkland region of Manitoba, Canada to determine the effects of three targeted plant population densities (100, 200, and 300 plants m<sup>-2</sup>) and three harvest timings (hemp decimal codes 2102, 2306 and 2307) on hemp fibre yield and quality characteristics. Alyssa, a Manitoba bred cultivar, was used to determine the treatment effects on self-thinning, stem diameter, plant height, biomass, bast and hurd percentage along with the lignin, cellulose, hemicellulose and holocellulose content of bast and hurd in three 20 cm stalk sections.

The results from 5 site years indicated the targeted plant population density significantly influenced self-thinning, plant height and stem diameter in the bottom, middle and top sections of the plant. Due to inter-plant competition for available resources plant stands tended to be affected by increasing the population density, where final counts ranged from 50-280 plants m<sup>-2</sup>. Plant height (130-261 cm) and stem diameters (3-8 mm) in all sections were greatest at 100 plants m<sup>-2</sup>; thereafter decreases occurred.

Harvest timing significantly influenced self-thinning, biomass and the percentage of bast and hurd. Plant counts decreased with delayed harvest. Biomass yield tended to increase at the last harvest (3350-6967 kg ha<sup>-1</sup>) while the percentage of bast (7-29 %) and hurd (48-85 %) fibre tended to decrease as harvest was delayed. In general, bast fibre consisted of approximately 86% cellulose, 3% lignin, 7% hemicellulose and 93% holocellulose (combined cellulose and hemicellulose) while the hurd consisted of 56% cellulose, 16% lignin, 19% hemicellulose and 75% holocellulose at harvest 1 (technical maturity). Overall the Canadian cultivar, Alyssa was relatively stable across the imposed treatments.

## 1. INTRODUCTION

For at least 6,000 years hemp has been cultivated as a seed and fibre crop. Since 1997, cultivation, processing and thus marketing of industrial hemp has been allowed in Canada. Hemp is a viable grain, fibre or dual-purpose (grain and fibre) crop in the rotation. Developing best management practices for cultivating hemp in the different regions of Canada is an important step in facilitating a successful Canadian hemp industry. Very little peer-reviewed literature is available for hemp production in Canada. The objective of this thesis is to examine the effects of two management steps (targeted plant population density and harvest timing) on fibre yield and quality characteristics of the industrial hemp (*Cannabis*) cultivar, Alyssa. Five field experiments were conducted in the Parkland region of Manitoba, Canada during 2006 and 2007.

## 2. LITERATURE REVIEW

### 2.1 Plant Description

#### 2.1.1 Taxonomy

Industrial hemp is the common name for fibre and grain cultivars of *Cannabis sativa* L. (*C. sativa*). Hemp is an herbaceous annual that has been domestically cultivated for its bast and hurd fibres, whole and pressed seed, essential leaf oils and epidermal resins (Small and Marcus 2002; Booth 2003; per. comm. J. Lupien 2008).

In 1753, Carolus Linnaeus', a Swedish botanist, formally keyed the Cannabis plant as *Cannabis sativa* L. (Schultes 1970; Booth 2003). To date, a debate has existed over whether or not the Cannabis plant is monotypic, having one species with different varieties, or polytypic, having distinct subspecies (Booth 2003). In 1924, the Russian botanist D.E. Vanischewsky, studied feral wild Cannabis in the Volga River system of western Siberia and central Asia (Booth 2003). Vanischewsky supported the polytypic argument, claiming that in addition to *Cannabis sativa* and *Cannabis indica* there was a third distinct wild species: *Cannabis ruderalis* (Vavilov 1926; SLDP 2007).

Based on an extensive *Cannabis* cultivar review, Small and Conquist (1976) concluded that *C. sativa* was monotypic possessing two distinct subspecies. These subspecies were classified by the percentage of active cannabinoids (a category of molecules found only in *Cannabis*) (Clarke 1981), mainly delta<sup>9</sup>-tetrahydrocannabinol ( $\Delta^9$ -THC or THC) (the main psychoactive compound in *Cannabis*) (Clarke 1981), present in the dry weight of the upper part of the female inflorescence. Small and Conquist (1976) keyed the two subspecies: *C. sativa* subspecies *sativa*, with less than 0.3% THC

(3000 parts per million) and *C. sativa* subspecies *indica* (Lam), with more than 0.3% THC. Within the subspecies *sativa* are variety *sativa* and *spontanea*, and within the subspecies *indica*, are variety *indica* and *kafiristanica* (De Meijer 1994). Hilling and Mahlberg's (2004) examined the cannabinoid variations of 157 *Cannabis* accessions, in which their research findings supported the two-species theory, thus chemotaxonomically authenticating the *C. indica* sub-species biotype. Others have further categorized *Cannabis* based on geographical race and morphological and physiological characteristics (Bócsa and Karus 1998; McPartland et al. 2000). Nonetheless, the classification of *Cannabis* has been internationally controversial.

Taxonomically the genus *Cannabis* belongs to the Order Rosales; Family Cannabinaceae; Genus *Cannabis* (Bócsa and Karus 1998; Small and Marcus 2002). It was formally classified as belonging to the Urticaceae (Nettle) Family (Dewey 1901) and at one time was classified as part of the Moraceae (Mulberry) Family (Dempsey 1975; Frank 2005). Even though today, a general consensus has been reached regarding the taxonomic classification, the origin of *Cannabis* is truly unknown, due mainly to the *Cannabis* plants' ability to adapt to its environment, no matter the origin of its seed (McPartland et al. 2000).

### **2.1.2 Center of Origin**

The origin of *Cannabis* is decidedly unknown, because of its documented wide dispersion and cultivation across Eurasia early on in human history (Clarke 1999). Valilov (1926) suggested that the cultivation of hemp arose concurrently in different locations and that *Cannabis* likely originated in Central Asia. After an extensive cultural

and historical literature review, Clarke (1999) further supported Vavilov's 1926 theory, stating that Central Asia was the most likely centre of origin of *Cannabis*.

## **2.2. Historical review**

### **2.2.1 Major uses**

Historically, hemp has been one of the oldest multi-purpose plants used by man. As of 6,000 years ago, it was used for cordage, cloth and oil (Schultes 1970; Holmes 1982). Coarse hempen cloth has been found in some Europe's oldest human inhabited sites (Schultes 1970) and remains of hempen cloth have been found dating back 6 millennia (Small and Marcus 2002).

*Cannabis* has played an important role in human history. The Philosophical Transactions of the Royal Society, England, Scotland and Ireland (1665-1678), included hemp in its Enquiries Concerning Agriculture for arable land, as a kind of 'ufual' (useful) grain or seed. It has been documented that in 1606 the British began cultivating hemp in its North American colonies. Cultivation of hemp began in Virginia, USA, in 1611 and by the late 1700s hemp was being grown along the St. Lawrence (French: *Saint-Laurent*) River in Québec, Canada (Grinspoon 1977; Roffman 1982; Global Hemp 2001; Fair 2004; Hemphasis 2007).

Hemp production increased throughout North America during the 1840s and continued into the 1890s, primarily supplying cordage and sail cloth to the U.S. Navy. Since the late 1800s early 1900s, industrial hemp cultivation decreased throughout the western world. According to Holmes (1982) cultivation decreased in the Americas in the late 1800's because of the Civil War (*War Between States*) between the *Union* and the *Confederacy* this resulted in the loss of slave labour (which hemp cultivation depended

upon). In addition, the then bountiful southern fibre market declined and an increase in competition from foreign agri-fibre industries in the East occurred. A reduction in production also occurred due to the lack of access to mechanized fibre harvesting and decorticating equipment. A multitude of events laid hand to hemp's demise as a viable agricultural crop in the America's.

During the mid 1900s, the reduction and complete abandonment of hemp cultivation was also attributed in part to the increased innovation of synthetic chemicals these synthetic chemicals were replacing agri-based fibres. An American political uprising occurred during this time that lead to a reclassification of the *Cannabis* plant family, even industrial hemp, as an illegal drug and a controlled substance (per. comm. J. Lupien 2008). By the late 1900s hemp cultivation had almost completely disappeared in North America, except in Kentucky.

The Opium and Narcotics Control Act passed in 1938, made cultivation of *Cannabis* L. illegal in Canada (MAFRI 2006). Despite the ban, agronomic hemp research continued to be conducted by the Division of Economic Fibre Production, Dominion of Canada, Department of Agriculture until 1944. From the 1920s, 30s and 40s, the Dominion of Canada sourced hemp germplasm from Russia, France and the United States. This germplasm was evaluated for its agronomics and adaptability in the Eastern and Western Canadian environments.

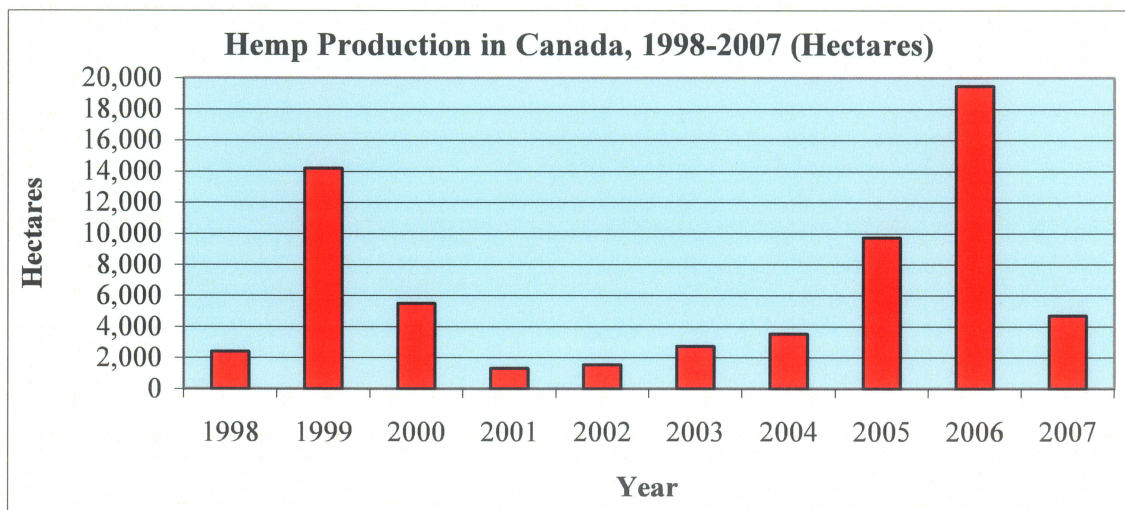
In 1994, the first hemp research plots were sown in southern Ontario and in 1997, after 50 years of hemp prohibition, the Canadian Controlled Drug and Substances Act was signed into law. This act thus provided the legislative authority and infrastructure to facilitate the commercial production of industrial hemp in Canada. On March 12<sup>th</sup>, 1998,



under the Industrial Hemp Regulations section, authorized by Health Canada's (HC) Office of Controlled Substances (OCS), Canada re-legalized industrial *Cannabis* for the purpose of agricultural production, and thus processing and manufacturing. This established the crucial regulatory support for industrial hemp fibre, grain and certified seed production in Canada (MAFRI 2006). Since 1997, the number of hectares licensed and thus seeded in Canada has varied (Figure 2.1).

Regulations set by Health Canada's; Office of Controlled Substances under the management of the Industrial Hemp Section requires licensing. These licenses facilitate all facets of the Canadian hemp industry including; research, breeding, certified seed production, cultivation, possession, sampling, testing, processing, distribution, and importing and exporting. In addition, strict guidelines set the percentage of delta-9-tetrahydrocannabinol (THC) that can be present when 50% of seeds set are resistant to compression at a maximum percentage of 0.3 % or 3000 parts per million. THC levels in hemp are monitored throughout the growing season, processing and final product. Monitoring of THC content is conducted because THC present in living *Cannabis* plant material is known to be influenced by growing conditions, such as environmental stress, pest pressures, temperature fluctuations, and nutrient deficiencies (Ranalli 1999; per. comm. K. Friesen 2006). In response to international THC concerns, the North American hemp food industries created a voluntary Hemp Test Pledge, under guidelines put forth by Vote Hemp, which requires pledging companies to commit to monitoring THC levels in processed hemp foods. THC levels in hemp oil is set at 5.0 parts per million and hemp nut is set at 1.5 parts per million (per. comm. G. Leson 2006).

Hemp industries have prospered in Canada and throughout Eurasia, the European Union and the United Kingdom. In the United States, The Drug Enforcement Agency (DEA) has actively enforced and discouraged hemp production (de Meijer 1995). In the United States, twenty-eight states have initiated state controlled hemp legislations, but only fifteen of those have passed to date. Only seven of those fifteen states have passed legislation facilitating research and production (VoteHemp.com). Currently two states, North Dakota and Vermont have legalized the cultivation of industrial hemp within the state. However, the DEA has not issued any federal hemp cultivation licenses to North Dakota farmers.



**Figure 2.1** Canadian national average licensed hemp hectares by year (1998-2007)  
(Source Health Canada)

The market demand for value added products and raw hemp materials are on a rise. This increased demand has been a direct result of the global greening movement (Ivanyi 2005) and because of the increased demand for enriched balanced foods produced in sustainable agricultural systems. Modernized technologies will have to be innovated



and practical in application for hemp to be a productive, sustainable and marketable agricultural crop. Hemp seed processing technologies such as cleaning, cold pressing, toasting, dehulling and milling were transferable and adaptable from other grain crop processes, such as canola crushing (per. comm. S. Crew 2005). Products currently produced from hemp include food, body care, fibre-based products and bio-energy feedstock. Modern uses for hemp are presented in Figure 2.2 (Toronto Hemp Company).

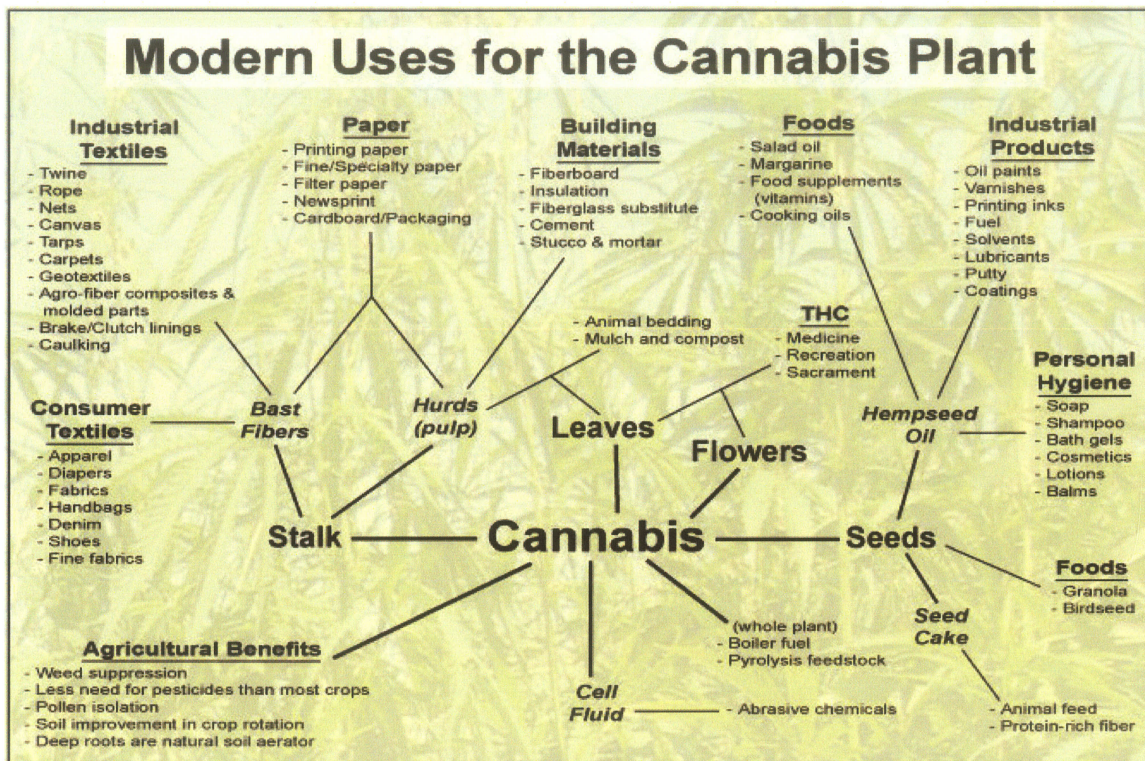


Figure 2.2 Modern uses of hemp (Sourced from the Toronto Hemp Company).

### 2.2.2 Food

Hemp seed can be eaten whole, toasted or dehulled; it can be pressed for its oil and seed cake. Products made from hemp include non dairy milk and cheese, protein powder, flour, bread, omega and essential fatty acids enriched oil, alternative pet foods, toasted snacks and nut (Roulac 1997). Some cultivars of hemp, such as Finola have been

determined to contain 75-90% polyunsaturated fatty acids (Omega 3, 6 and 9) and 9.7% saturated fatty acids present in its oil (PUFAs) (Callaway 2004; Callaway et al. 2005; Friesen 2006), making hemp oil a healthy choice. Hemp oil is not only important as a food product it has also been used as a wood preservative and as printer ink (Sacilik et al. 2003).

### **2.2.3 Fibre**

The quality characteristics of hemp fibre have been studied abroad to a greater extent than in North America. Quality research in Canada has been conducted on a limited basis at the University of Toronto, University of Queens and at the University of Saskatchewan (per comm. Dr. M. Sain, G. Scheifele and S. Panigrahi 2006). Hemp possesses three main fibre types: bast, tow and hurd. Renewable hemp fibres can be incorporated in to a wide range of commercial products such as composites, pressed boards, insulation, as a fibre-glass replacement, injection moulding, good-quality paper, non-woven's, plastics, hemp concrete (i.e. hempcrete), cordage and industrial and fine grade textiles. Sankari (2000) stated that even though quality parameters for non-woven hemp fibre products have not been determined, the attributes of the secondary (tow) fibres may be advantageous as a non-woven fibre fraction.

### **2.2.4 Energy**

There is an international trend towards employing renewable energy resources. One of these resources is cellulose, sequestered from the straw and stalk material of agricultural feedstock crops, such as hemp and maize (per. comm. D. Levin 2007). Hemp bast fibres are a good source of cellulose. These bast fibres consist of a relatively high

percentage of holocellulose (total cellulose) and low lignin making them a desirable feedstock alternative for bio-fuels (Kovas et al. 1992). Even though hemp stalks possess attributes that make it a strong bio-fuel feedstock candidate, some claim that it may not be as economical as other readily available feedstock sources such as switchgrass (*Panicum virgatum*) and maize (*Zea mays*) (Roulac 1997). Canadian hemp cultivars typically contain 30-35% oil which can be used in the making of high quality bio-diesel (per. comm. S. Crew 2006; per. comm. P. Bobbee 2007). However, for use in bio-diesel, hemp seed cultivars currently available do not have any economic advantage over the use of other seed oil crops such as rapeseed (*Brassica napus*) (Carus 2007). According to Carus (2007), hemp oil content exceeding 40% would be necessary to compete with other seed oil sources.

### **2.2.5 Body Care**

The market is no stranger to hemp oil body care products. These products range from designer cosmetics, body moisturizers, body and lip balms and soaps. Studies have determined that when specific fatty acids, like those found in hemp, are applied externally, skin ailments such as dry skin can be alleviated (Leson et al. 1999). Callaway et al. (2005) determined in a 20-week clinical trial comparing ingested hemp seed oil and olive oil that the hemp oil treatment significantly improved the blood plasma fatty acid profile and that qualities of both skin dryness and itchiness decreased. Subjects whom were randomly and blindly assigned to the hemp oil treatment stated that, after taking the treatment, their typical application of dermal medicine was reduced (Callaway et al. 2005).

### **2.2.6 Phytoremediation**

It has been suggested that *Cannabis* has the capability to take up and amass heavy metals in its roots and shoots. In an Italian study, Bona et al. (2007) investigated this theory, in which they concluded that fibre varieties of hemp are an excellent candidate for phytoremediation in soils contaminated with heavy metals, such as copper. Additional research should be conducted to evaluate this claim further in different contaminated environments.

## **2.3 Anatomy**

This thesis will focus on hemp fibre yield and quality characteristics as affected by targeted plant population density and harvest timing; however, the author felt it important to provide an extended plant anatomy and physiology section presented in Appendix I. A decimal code system for the development stages of *Cannabis* was developed by Mediavilla et al. (1998). This system formulated standard definitions and codes for the growth stages of *Cannabis sativa* L. plants (Appendix B).

### **2.3.1 Stalk**

The hemp stalk can reach heights of 1 to 5 meters (Hunt 1912; Bailey 1924; Hayward 1938; Stearn 1970; Dempsey 1975; Nykter 2006) and stem diameters of 0.5 - 3 cm (Scheifele et al. 1996; Bosca and Karus 1998) in 90 to 120 days, depending upon cultivar and environmental conditions.

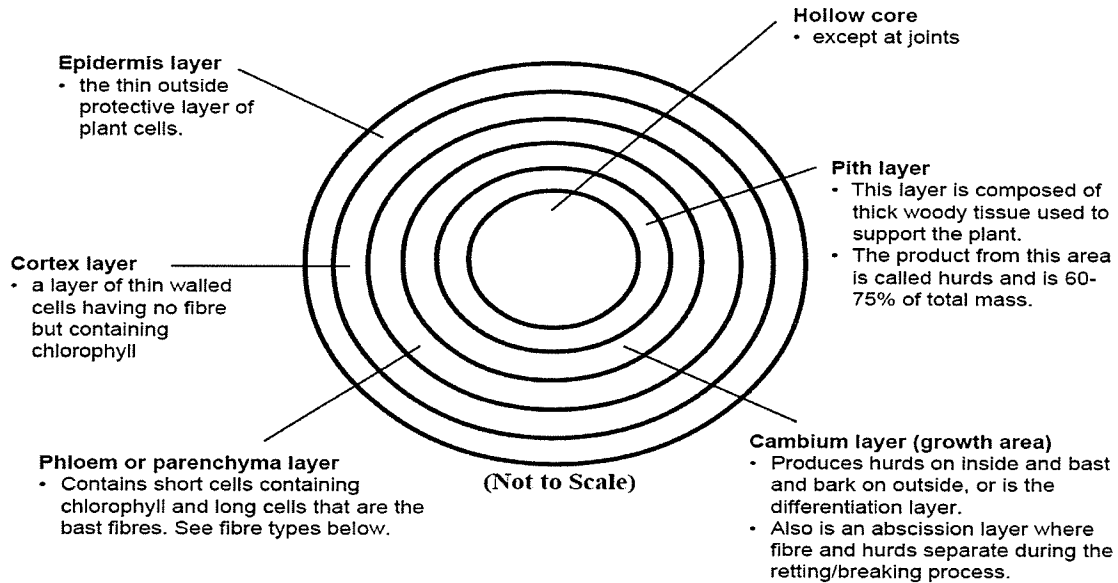
The exterior of the *Cannabis* stalk is covered with a thin protective pubescent (non-glandular trichomes) epidermis layer which allows for the stomatic regulation, thus ventilation and evaporation. The next layer inward contains a cortex of non-fibrous

chlorophyll (which gives the stalk its green color), then a layer of primary and secondary phloem and parenchyma fibre cells (bast and tow fibres), then a cambium growth layer and lastly the inner most portion, the primary xylem (woody hurd and pith). The anatomy of the *Cannabis* stem is depicted in Figure 2.3.1.

The stalk of the hemp plant consists of two main components, bast and hurd. Together, they comprise approximately 59-67% of the total above ground biomass (Scheifele et al. 1996). Bast fibres are considered to be “soft” fibres while the hurd is considered to be a “woody” or hard fibre. The first of these two components is the stem tissue outside the vascular cambium, commonly referred to as bark or bast. Bast fibres are comprised mainly of non-starch polysaccharides (Weightman and Kindred 2005). The bast fibre component contains heterogenous primary fibres that arise from the prodesmogen and secondary fibres which arise from the cambium (Kundu 1942; Hoffmann 1957: cited in van der Werf et al. 1994a, 1995c; Ranalli 1999). The second stem tissue component is located on the inside of the stem’s vascular cambium, commonly referred to as the hurd, core or shive (shiv). The hurd component is libriform in nature (i.e. a cell in the xylem that is very long and thin and has simple pits) (Esau 1965; Ranalli 1999) and typically measures 0.2 - 0.6 mm in length (Bosia 1976: cited in van der Werf et al. 1994a; Vignon et al. 1995).

The primary and secondary fibres within the stalk overlap in bundles containing 10 to 40 cells per bundle running from the root system to flowering tops (McPartland et al. 2000). These bundles are embedded in a ring of phloem, parenchyma and sclerenchyma cells that are located amid the epidermis and cambium (McPartland et al.

2000). These bundles contain chemical constituents such as cellulose, hemicelluloses and lignin.



**Figure 2.3.1** Cross section of a hemp stem (Oliver and Joynt 1999 p.3) (permission granted by the British Columbia Ministry of Agriculture and Food to reprint)

Cellulose is the principal structural component in green plants (Thomsen et al. 2005). Cellulose is a major quality component in agri-fibres and can be converted into mass produced products such as cellophane.

Lignin, which is indigestible by mammalian and animal enzymes, fills the spaces between the cells and is key for stalk strength. The fibre color changes from a dark to bright hue as the double bonded lignin structure is broken down by bacterial growth during the retting process or by the chemical or enzymatic preparations (Thomsen et al. 2005). The primary and secondary phloem layers amalgamate with the lignified primary xylem layer.



The primary xylem, otherwise referred to as hurd, is woody and thins to a pith which encircles the hollow hemp stalk (McPartland et al. 2000). The stalk consists of approximately 0.8% - 2.5% pectin, which is considered to be the main binder of the primary cell wall, middle lamella and of the cellulosic and non-cellulosic networks (Wang et al. 2003). Pectin helps to prevent the collapse of the structural and chemical constituent networks within green plants (Taiz and Zeiger 2002; Corriea and Roy 2005). Bast and hurd fibres contain both cellulosic and non-cellulosic properties (i.e. lignin) that differ in proportion. Thomsen et al. (2005) stated that while both bast and hurd fibres are cellulosic; a greater percentage of cellulose is located in the bast fibre while the hurd consist of a greater percentage of hemicellulose and lignin and less cellulose. Hemicellulose is made up of a group of tightly bonded heterogeneous polysaccharides located within the cell wall (Taiz and Zeiger 2002) that connects the cellulose and lignin. Glucose and xylose are the primary monosaccharides constitutes in hemp's hemicellulose.

Meijer et al. (1995) stated that hemp fibres could be used as a wood substitute in pulp and paper production. The hemicellulose in hemp is similar to that of Canadian grown aspen tree fibres which are readily extracted with an alkali solution (Corriea and Roy 2005). Correia et al. (1998) determined that the physical properties of hemp bast fibre cells were similar to softwood tree species; which are currently used in Canadian pulp and paper production.

Hemp bast fibres range from 0.5 - 10 cm in length; average lengths range from 1.5 - 5.5 cm with average thicknesses of 18-25  $\mu$  (micron) (Vignon et al. 1995; Bócsa and Karus 1998). The lengths of the hurd fibres typically do not exceed 0.5 mm (Bócsa and

Karus 1998). Increasing fibre lengths have been associated with increasing paper strength (Bócsa and Karus 1998).

The tensile strength of hemp bast fibres ranges from 593.72 to 1073.72 megapascal (MPa) (i.e. a unit of pressure or stress) depending upon the fibre separation method, i.e. untreated or alkalized treated (i.e sodium hydroxide (NaOH)) (Mwaikambo and Ansell 2006). Preliminary results from Manitoban grown hemp indicated that fibre from mature stalks produced stronger but stiffer and less elastic fibre from stalks cut early in the flowering stage (Moes and Empson 2000). Kamat et al. (2002) concluded that handsheet paper produced from unbleached pulp of hemp stalks harvested at 60- and 90-days after sowing produced insignificant differences in tensile strength (59 and 56 kilonewton/gram (kN/g) (i.e. an International System unit for force), respectively). Research conducted in Germany by Müssig and Martens (2003) further concluded that plant age in hemp did not significantly affect fibre strength.

In 1997 and 1999, United Kingdom researchers, Murphy-Bokern and Bruce (2005) collected and processed matured hemp stems into composites. They determined that even though composite strength was not affected by delayed harvest, composite stiffness was reduced at an approximate rate of 10% per delayed harvest week. In addition, the authors concluded that harvest date did not affect the stiffness of the hand-extracted hemp fibre bundles (Murphy-Bokern and Bruce 2005).

European fibre hemp stems have been determined to consist of high-cellulose low-lignin bark (bast-long fibre) and low-cellulose high-lignin core (hurd-short fibre) (van der Werf et al. 1994b). Since the greatest percentage of cellulose is located in the bark (bast), van der Werf et al. (1994b) stated that stem value primarily depends upon the

proportion of bast in the stem. Today, however, hurd value is on a rise due to its superior fibre qualities (i.e. absorbance, reduced dust, thermal and antibacterial properties) for animal bedding, building materials and bio-composites.

## **2.4 Hemp agronomic management**

Optimum agronomic practices are important for all crops. The development of best management practices (BMP) is therefore an important agronomic research goal. Best management practices are practical approaches to conserving resources while maximizing all areas of production. In Manitoba provincial BMPs for crops such as potatoes (*Solanum tuberosum*), barley (*Hordeum vulgare*) and wheat (*Triticum* spp.) provide recommendations on sanitation and storage, seed cleaning, selection and seed treatments, crop rotation and scouting. Best management practices are typically communicated via fact sheets and based on refereed scientific publications. Because of the lack of published hemp agronomy research, the Canadian agronomic BMP's for hemp have thus far been based upon the experience of producers, processors, agronomists and research conducted at provincial extension offices (Moes and Empson 2000; Baxter and Scheifele 2000; Friesen 2006).

### **2.4.1 Crop Rotation**

Crop rotation can improve or maintain soil fertility (Tilman 1998), reduce erosion (Carroll et al. 1997), reduce the build-up of pests and diseases (Abawi and Widmer 2000), reduce reliance on agricultural chemicals (Belay et al. 2004) and increase net profits (Zacharias and Grube 1984). Cropping hemp in rotation with other broadleaf crops, for instance, sunflower (*Helianthus annuus*), canola (*Brassica* spp.), buckwheat

(*Fagopyrum spp.*) and peas (*Pisum sativum*) should be avoided due to disease pressure, mainly *Sclerotinia sclerotiorum* (Hemp canker). Buckwheat in rotation with hemp can cause problems because its seeds are similar in size to the hemp seed making them difficult to separate out during cleaning. Ideal preceding crops for hemp include recently broken pastures, fields sown to perennial grasses and crops such as alfalfa (*Medicago sativa*), clover (*Trifolium spp.*) and other legumes (Friesen 2006). Even though alfalfa is known to be affected by *Sclerotinia trifoliorum* (Crown and stem rot), it has yet to pose an economic impact on the hemp production in Canada (per. comm. K. Friesen 2006). According to Friesen (2006) green manure crops (alfalfa breaking or annual legumes) are particularly good rotation crops in organic hemp production. However, due to contamination of flavour during seed processing rotating hemp with spice crops such as coriander (*Coriandrum sativum*) should be avoided (Friesen 2006). Companion cropping dioecious hemp cultivars (i.e. Finola) with crops such as black medic (*Medicago lupulina*), tall fescue (*Festuca arundinacea*) and Indianhead black lentils (*Lens culinaris*) has been successful (Friesen 2006). Crops that produce allelopathic compounds, such as fall rye (*Secale spp. L.*), when plowed under during the spring have been observed to completely prevent germination of spring planted hemp seed thus resulting in complete crop loss (per. comm. K. Friesen 2007). Leading Canadian hemp researchers, Gordon Scheifele and Peter Dragla from the University of Guelph, Ontario stated in a 1998 interview with Boyd and Kahar (1998) that even though hemp can and has been grown in monoculture for two to three years, it is not recommended. It was, however, suggested that in Southern Ontario hemp in conventional

production could be rotated with maize (*Zea mays*), soybeans (*Glycine max*) or cereal crops.

Based on interviews with producers, advisors and researchers a feasibility study conducted by Barron et al. (2003) investigated the integration of hemp into organic farming systems in the United Kingdom, France and Denmark. They concluded that hemp was a viable crop for rotation especially in organic systems, because it relies on minimal inputs (beyond fertility), it is competitive against weeds and it can improve soil conditions. Thus, concluding that integrating hemp into organic regimes would contribute to the local economy by creating diversity in the food and fibre industry sectors. No formal research has been conducted to determine the best management practices for hemp in organic or conventional crop rotation regimes in Canada.

#### **2.4.2 Canadian Industrial Hemp Varieties**

Access to adapted cultivars is vital to any crop production system. When the Canadian hemp breeding industry re-emerged in 1994-1997, cultivars were mostly imported from Ukraine: cultivars Zolo 11, USO 14, USO 31 and from Finland: cultivar Finola (Fin 314). These relatively well-adapted cultivars provided the basic germplasm for the present day Canadian hemp breeding programs.

Canadian bred cultivars now include Alyssa, Anka, Carmen, Crag, Deloris, Deni, ESTA-1, Heidrun, Jutta, Petera, UC-RGM and Yvonne (per. comm. K. Watson 2007; J. Baker 2007; G. Scheifele 2008). The germplasm for the current cultivars Crag and ESTA-1 were originally sourced from the broad based Vavilov Research Institute Gene Bank from across Russia and Romania (per. comm. G. Scheifele 2008). The cultivars Alyssa, Carmen, Deloris and Petera are maintained by the Parkland Industrial Hemp

Growers Co-op Ltd. breeding program. Anka, Carmen (both maintained by the Ontario Hemp Alliance), ESTA-1 (Advantage Seeds) and UC-RGM (Stonehedge BioResources) are maintained by Ontario breeders while Crag, USO 14 and 31, Finola and Zolo 11 are maintained by a Saskatchewan/Manitoban breeding project. Health Canada requires that all industrial hemp cultivars grown in Canada have to be approved for commercial cultivation and must be of pedigreed seed. List of Health Canada's approved hemp cultivars for 2007 are provided in Table 2.4.1. The Sengbusch Scale for monoecious intersex inflorescence types (Appendix A) provides a standard visual scale for maintaining monoecious hemp cultivars such as Anka, Jutta, Yvonne, USO 14 and 31.

**Table 2.4.1** Health Canada list of approved cultivars, country where maintained and THC testing exemption standing for the 2007 cropping season. (Adapted from Health Canada's 2007 list of approved hemp cultivars)

<b>Cultivar</b>	<b>Countries of Cultivar Maintenance</b>	<b>Thousand Kernel Weight (g)/1000 seeds</b>	<b><i>Industrial Hemp Regulation</i> subsection 16(1) cultivars exempt from THC testing indicated by **</b>
<i>Alyssa</i>	Canada	18	** in Manitoba only
<i>Anka</i>	Canada	18	** in Ontario only
<i>Carmagnola</i>	Italy		
<i>Carmen</i>	Canada	22	
<i>Crag</i>	Canada	16	**
<i>C S</i>	Italy		
<i>Deni</i>	Canada		
<i>ESTA-1</i>	Canada		
<i>Fasamo</i>	Germany	13	
<i>Fedrina 74</i>	France		
<i>Felina 34</i>	France	16	
<i>Ferimon</i>	France		
<i>Fibranova</i>	Italy		
<i>Fibriko</i>	Hungary		
<i>Fibrimon 24</i>	France		
<i>Fibrimon 56</i>	France		
<i>Finola</i>	Canada & Finland	12	
<i>Kompolti</i>	Hungary		
<i>Kompolti Hibrid TC</i>	Hungary		
<i>Kompolti Sargaszaru</i>	Hungary		
<i>Lovrin 110</i>	Romania		
<i>UC-RGM</i>	Canada	11	
<i>Uniko B</i>	Hungary		
<i>USO 14</i>	Canada & Ukraine	14	**
<i>USO 31</i>	Canada & Ukraine	16	**
<i>Zolotonosha 11</i>	Canada & Ukraine	16	** in Manitoba only
<i>Zolotonosha 15</i>	Canada & Ukraine		

### **2.4.3 Seeding**

#### **2.4.3.1 Seedbed**

Seed bed preparation and seed placement facilitates uniform germination, quick emergence and canopy closure (Benech-Arnold and Sánchez 2004). Hemp grows best when planted in to well drained, clean, moist but warm (6-10 °C) uniform medium textured soils, that are weed and chemical residue free (Friesen 2006). Cultivation of hemp in wet, heavy clay soils should be avoided (Baxter and Scheifele 2000). Hemp is not tolerant to wet soil conditions, especially during germination and emergence, cultivation in such environments will result in stunted chlorotic plants and/or total crop loss. Hutchinson (1938) reported a total hemp experimental plot loss due to heavy clay soils in the Ottawa region of Ontario, Canada. The suggested soil pH for hemp growth is above 6.0, with neutral to slightly alkaline soils (pH 7.0 - 7.5) being preferred (Bósca and Karus 1998; Baxter and Scheifele 2000). The effect of soil pH on hemp plant characters has not been examined to date.

van der Werf et al. (1995d) concluded that the germination of hemp seed requires a base temperature of 1-2°C. However, hemp seeds and seedlings have been reported as being frost tolerant (Scheifele et al. 1996; Friesen 2006). In the Netherlands, van der Werf et al. (1995a) noted a linear increase in leaf appearance and stem elongation at temperatures between 10°C and 28°C.



#### **2.4.3.2 Seeding depth**

For maximum yield, hemp seeds develop best when planted shallow at a depth of 2–3 cm (Scheifele et al. 1996). However, producers have had success with broadcasting the seed followed by an incorporating light tillage pass with heavy harrows (per. comm. K. Friesen 2008). The light tillage pass ensures good seed to soil contact.

#### **2.4.3.3 Row spacing**

The Institute of Bast Crops in the Ukraine, suggests a wide row spacing of 45-60 cm for dual-purpose (seed and fibre) production and 7.5-15 cm for fibre only hemp production (Holoborod'ko 1995). Studies conducted in Denmark determined that the total dry matter (stem and fibre) yields of hemp were maximized at 24 cm rather than at 48 cm row spacing (Deleuran and Flangmark 2005). On the other hand, Friesen (2006) suggested that for grain and or dual-purpose production in Canada, a row spacing greater than 20 cm seeding should be done in two passes, at half the targeted seeding rate in opposing angles. Baxter and Scheifele (2000) recommended row spacing of 15-18 cm for hemp grown for fibre and/or grain production. Field experiments conducted by Vera et al. (2006) determined that row spacing's of 18 and 36 cm did not significantly affect the seed weight, protein or oil content in two Canadian approved hemp grain cultivars, i.e. Finola and Fasamo.

#### **2.4.3.4 Sowing date**

Early sowing can be advantageous for increased water use efficiency (Eastman et al. 1999), increased yield due to a longer period for growth (Arvidsson et al. 2000) and increased weed control due to early canopy closure in hemp (Lisson and Mendham

2000a). Hemp requires 300-400 mm of equivalent rainfall, of which half (150-200 mm) of the amount is required during flowering and seed set (Baxter and Scheifele 2000). Hemp can overcome periods of water shortage by extending its tap and lateral roots to access water at deeper soil levels. However, prolonged periods of drought especially during flowering can cause reduced yields.

*Cannabis* is photoperiod sensitive crop (Clarke 1981). The shorting of the day length triggers flowering and seed set (Amaducci et al. 2008). Studies have been conducted to examine the effect of sowing date on hemp grain and fibre yields in the Netherlands and Australia and to a lesser extent in Canada. However, Gordon Scheifele (per. comm.), Ontario Hemp Alliance, stated that sowing hemp later in the season resulted in shorter, less competitive plant stands and reduced the average grain yield. In a Tasmanian study, Lisson and Mendham (2000a) examined hemp sowing dates between mid-September and mid-November. Their results showed that later sowing dates significantly reduced stem and bark (primary and secondary fibres and epidermis) yields due to a decline in thermal time and calendar days from sowing to flowering. This observation thus suggested that a long pre-flowering period is important for hemp fibre quality. The effect of sowing date and latitude has not been examined; however, agronomist Gordon Scheifele (per. comm.), has noted differences in days to flowering in Ontario verses Manitoba, especially in the grain-only cultivar Finola. Knowing the optimum sowing date for the cropping region is an important management tool so that a long pre-flowering vegetative period can be achieved. Unpublished data collected in Australia, suggested that subtropical hemp varieties should be sowed in October, thus maximizing the Australian long day photoperiod (Jobling and Warner 2000).

The optimum sowing date for hemp production in south-western Ontario was examined by Scheifele et al. (1996). In which, they found that early sowing was an important agronomic tool to maximize seed yield in hemp. Preliminary results provided by the Parkland Crop Diversification Foundation (PCDF) in Manitoba, showed that plant height (decreased by 7-12 cm) and total plant biomass decreased as sowing date was delayed from May 19th to June 14th (Stadnyk et al. 1999a, 2000). According to leading Canadian hemp agronomist K. Friesen (per. comm.), the average sowing date in Saskatchewan and Manitoba is May 25. In the Western Canadian Prairies, hemp crops sown in early June tend to be shorter in stature and have lower yields than those sown in late May (per. comm. K. Friesen 2008). Similar sowing date recommendations have been made for cereal crops such as oats (*Avena sativa*) in Manitoba (Hamill 2002).

#### **2.4.3.5 Crop Nutrition**

In Manitoba the nutrient utilization and partitioning of industrial hemp was examined by Heard et al. (2007). These preliminary results showed that the average nitrogen uptake into the whole crop was 202 kg ha<sup>-1</sup>, with 40 kg ha<sup>-1</sup> in the grain. The total phosphorus uptake was 47 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, with 18 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in the grain. Further, research conducted in Manitoba has examined nitrogen fertilization rates in hemp production of 63, 85, 113, 141 and 170 kg N ha<sup>-1</sup>, in which the best economic returns were achieved at the 85 kg N ha<sup>-1</sup> rate (Stadnyk et al. 1999b, 2000; Kostuik et al. 2001a; PCDF 2002, 2003). In the Parkland region of Saskatchewan, Vera et al. (2004) concluded that maximum seed yield for seed/grain type hemp cultivars was obtained at an application rate of 99-102 kg N ha<sup>-1</sup>.

Working in Ontario, Scheifele et al. (1996) determined that the nutrient uptake of the hemp grown for fibre-only production was 325 kg ha<sup>-1</sup> nitrogen, 92 kg ha<sup>-1</sup> phosphorus, 414 kg ha<sup>-1</sup> potassium, 54 kg ha<sup>-1</sup> magnesium and 389 kg ha<sup>-1</sup> calcium. It was further concluded that of the total plant nutrient uptake 69% nitrogen, 33% phosphorus, 53% potassium, 72% magnesium, and 72% calcium, was returned to the soil in an organic form in fibre-only production. It is thus necessary to combine soil and plant analysis to determine the proper nutrient requirements for cultivating hemp (Iványi 2005).

In a German study, Höpper and Menge-Hartmann (1995) examined two dual-purpose hemp cultivars (i.e. Felina 34 and Kompolti Hybrid TC) at two nitrogen rates (60 and 120 kg N ha<sup>-1</sup>). The overall yield (seed and fibre) was not significantly affected by either rate of nitrogen. In a Finnish study, Struik et al. (2000) examined the effect of three nitrogen levels (100, 160 and 220 kg N ha<sup>-1</sup>) on useable fibre yield and plant mortality. Results showed a steady increase in useable fibre yield with increasing nitrogen fertilizer rates while the number of plants that survived to harvest decreased with increasing nitrogen rates. Similar results were observed in an Italian study conducted over a 3 year time period (Amaducci et al. 2002a). In addition, Amaducci et al. (2002a) also observed increased plant loss with increasing rates of nitrogen.

Increasing nitrogen fertilizer rates is known to effect stem diameter and plant height in crops such as sunflowers (*Helianthus annuus*) and maize (*Zea mays*). In an Iranian study, Mojiri and Arzani (2003) determined that increasing nitrogen fertilizer from 0 to 75, 150 and 225 kg ha<sup>-1</sup> increased stem diameter and plant height of sunflowers. Working in Africa, Sétamou et al. (1995) noted positive effects on the stem diameter and

height of maize with increasing rates of nitrogen fertilizer (0, 60, 90 and 120 kg N ha<sup>-1</sup>). Schäfer (2005) observed that stem diameter and plant height in hemp increased as the nitrogen fertilization increased from 0, 60, 120 and 180 kg N ha<sup>-1</sup>. Forrest and Young (2006) stated that while it appeared that increased morphological growth occurred (i.e. height, diameter and number of internodes) with increased nitrogen fertilization, this did not result in increased yield.

Limited research has been conducted to examine the affects of phosphorus and sulphur on plant growth and yield. However, Stadnyk et al. (1999a) determined that hemp seeds are tolerant to seed placed and side banded phosphorus (P<sub>2</sub>O<sub>5</sub>) at rates of 0, 44, 67, 89 kg P ha<sup>-1</sup> and that amending soil sulphur is only beneficial where deficient. They further observed that the addition of phosphate increased seed yield where deficient. Vera et al. (2004) examined the effects of surface-broadcast ammonium nitrate (0, 40, 80 and 120 kg N ha<sup>-1</sup>) and seedrow placed monoammonium phosphate (0 and 20 kg P ha<sup>-1</sup>) fertilizer on two Canadian registered cultivars (Finola and Fasamo) on growth, seed yield and quality in the Parkland region of Saskatchewan. They concluded that increasing rates of surface-broadcast ammonium nitrate significantly increased plant height, biomass, seed yield and seed protein content while seedrow application of monoammonium phosphate increased plant loss, height, biomass and seed yield.

Recommended fertility rates in Canada are 84-112 kg N (nitrogen) ha<sup>-1</sup>, 56-78 kg P (phosphorus) ha<sup>-1</sup>, or 40-90 kg P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (potash) ha<sup>-1</sup> and 20-30 kg S (sulphur) ha<sup>-1</sup> where deficient (Friesen 2006; Baxter and Scheifele 2000).

#### 2.4.4 Pests of hemp

A *pest* can be classified as any organism which has destructive characteristics that are viewed by humans as detrimental. A pest problem occurs when non-beneficial populations exceed a threshold and impact crop development and quality in a negative manner, however not all pest are detrimental (COG 2005). The following list of pests, are known to affect hemp but are not known to be present in Canada was compiled under the Pest Risk Assessment (PRA) division of the Canadian Food Inspection Agency (CFIA) (CFIA 2006). Therefore, all hemp seed imported into Canada must be free of the following:

##### **Pests Associated with Hemp** (CFIA 2006)

*Pseudomonas syringae* pv. *cannabina* (bacteriosis of hemp)

*Xanthomonas campestris* pv. *cannabis* (leaf spot of hemp)

*Fusarium oxysporum* f. sp. *cannabis*

*Pseudoperonospora cannabina* (downy mildew of hemp)

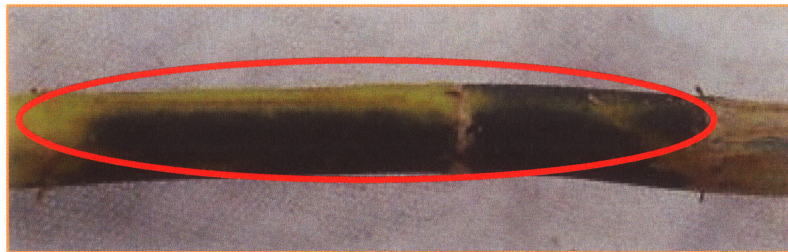
*Orobanche* spp. (broomrape)

##### 2.4.4.1 Disease

According to McPartland (1996) hemp can be affected by over 100 diseases. The major hemp diseases of interest in areas of agricultural cultivation are *Sclerotinia sclerotiorum* (hemp canker) and *Botrytis cinerea* (grey mould).

*Sclerotinia* is a soil-borne fungal pathogen that causes dark water spot like lesions (Figure 2.4.4.1) and ‘cankers’ (Figure 2.4.4.2) on the surface of the hemp stalk and branches (McPartland et al. 2000). These lesions are caused by internally located white mycelium, the vegetative part of the fungi (Hudyncia et al. 2000). Eventually, the

mycelium degrades the internal tissues resulting in pale stalks that contain hard black sclerotia bodies (Figure 2.4.4.3), which affects the flowering process and thus reduces yield (McPartland et al. 2000). At harvest the sclerotia bodies and diseased stalks are harvested or shredded and are spread over the field (per. comm. K. Watson 2007). The sclerotia bodies overwinter in the soil and infect subsequent susceptible crops. In order to successfully control sclerotinia over the long term, a 4 to 5-year crop rotation is suggested between susceptible crops such as canola, sunflowers, soybeans and peas (Bailey et al. 2003; SSCA 2007). Sclerotinia is of particular importance in Manitoba hemp production, because many other sclerotinia susceptible crops (mainly sunflowers and canola) are grown in rotation.



**Figure 2.4.4.1** *Sclerotinia sclerotiorum* lesions (A. S-Hermann 2004)



**Figure 2.4.4.2** *Sclerotinia sclerotiorum* external 'cankers' (A. S-Hermann 2004)





**Figure 2.4.4.3** *Sclerotinia sclerotiorum* internal sclerotia bodies and seed head damage. (Friesen and A. S-Hermann 2006)

Botrytis (*Botrytis cinerea*), or grey mould, is a fungal disease that attacks crops around the world, especially in wet and high humid environments. In hemp, grey mould attacks the flowering tops and stalks (McPartland et al. 2000). Grey mould has yet to have an economic impact on hemp production in western Canada. However, the Ontario Hemp Association reported incidences of botrytis like symptoms referred to as “head blight” in southern Ontario (McPartland et al. 2000; per. comm. G. Scheifele 2007). In the last few cropping seasons botrytis has become the greatest hemp disease threat in southern Ontario. According to Gordon Scheifele (per. comm. 2007) with the Ontario Hemp Alliance, botrytis has been seen in almost every hemp field in Southern Ontario at levels that have approached measurable crop loss in some cases.

Other diseases such as root rot, leaf spots, damping off and blight have been known to affect Cannabis production (McPartland et al. 2000). Most of these diseases can be managed with sustainable practices such as crop rotation, utilizing traps, barriers and by employing beneficial organisms (McPartland et al. 2000).



#### 2.4.4.2 Insects

Some herbivorous insects have been known to feed on the *Cannabis* plant leaf material (McPartland et al. 2000; Friesen 2006). Hemp research plots in south-east Queensland Australia, have been infested with *Nezara viridula*, commonly referred to as green vegetable bugs (Jobling and Warner 2000) or southern green stink bug (McPartland et al. 2000). Stink bugs have not been noted as a problematic insect in Manitoba as of yet, but according to Manitoba Agriculture, Food and Rural Initiatives in the summer of 1998 stink bugs were found in the medicinal crop fields and research plots of purple cone flower (*Echinacea*), valerian (*Valeriana officinalis*), milk thistle (Genus *Silybum*) and feverfew (Genus *Tanacetum*).

Hemp crops in the Canadian Western Prairies have been infested with Bertha Army Worms (*Mamestra configurata*) (Figure 2.4.4.4), Painted Lady Butterflies (*Vanessa cardui*) (Figure 2.4.4.5) and some species of grasshoppers (Figure 2.4.4.6) in varying degrees. The European corn borer (*Ostrinia nubilalis* (Hübner)) has affected hemp crops in Manitoba and in southern Ontario damaging the stalk (Figure 2.4.4.7). In 2007, for the first time, Aster Leafhoppers (*Macrostelus phytoplasma*) spread aster yellows, a viral-like phytoplasma, to some the hemp plant stands in the Parklnad region of Manitoba. The affected plants remained in an undifferentiated vegetative growth stage (Figure 2.4.4.8). Aster yellows affected plants could result in a total seed crop loss, but is it not known whether fibre quality would be affected. The Canola Council of Canada website states that there is no practical control measure for aster yellows for canola. To date, no studies been conducted to determine controls measures for aster yellow in hemp.

Preliminary research conducted by Scheifele et al. (1996) compared the effect of hemp versus soybean production on soybean cyst nematode (*Heterodera glycines*) populations in Southern Ontario (sampling area not indicated). The soybean cyst nematode larvae population decreased from 340 at seeding in the spring to 60 at harvest in the hemp testing area and increased from 340 at seeding in the spring to 2680 at harvest in the soybean testing area. Even though the preliminary results were positive showing hemp as a potential as a biological control measure for soybean nematodes, further research is needed to validate their findings.



**Figure 2.4.4.4** *Mamestra configurata* (Bertha Army Worm) (Friesen and S-Hermann 2006)



**Figure 2.4.4.5** *Vanessa cardui* (Painted Lady Butterflies) (Friesen and S-Hermann 2006)



**Figure 2.4.4.6** Grasshopper (S-Hermann 2007)

**European corn borer** (*Ostrinia nubilalis* (Hübner)) was identified in a field near Dauphin in 2007. The corn borer has been identified in the past as in this year in low numbers.

The borer is noticeable by a small pin hole in the stalk usually near a node. The worm then develops in the center of the plant and eats its way up the stem. Damage is usually noticed by plants that will be broken off somewhere up the stem, usually after a wind or rain storm.



**European corn borer in Hemp**

**Figure 2.4.4.7** European corn borer in hemp  
(Source Manitoba Agriculture and Food and Rural Initiative 2007- per. comm. Keith Watson)



**Figure 2.4.4.8** Undifferentiated vegetative growth caused by Aster Yellows (S-Hermann 2007)



#### 2.4.4.3 Weeds

In general, volunteer buckwheat (*Fagopyrum esculentum*), barley (*Hordeum vulgare*), wild oats (*Avena fatua*) and other cereals pose problems in hemp production. Buckwheat and barley are difficult to clean out while cereals cause cross contamination of gluten which affects marketing of gluten free products. Wild oats, on the other hand, have been observed to completely smother young hemp plants. However, with proper pre-seeding field preparation, a sufficient seeding rate, sufficient fertilization, good environmental conditions, germination and emergence hemp tends to suppress most weeds. Hemp naturally controls weeds by plant competition for sunlight. In Canada, no herbicides are fully registered for use in hemp seed cultivation. One post emergent grass herbicide, quizalofop-o-ethyl (+ oil concentrate (Sur-mix)), trademark Assure®II, has been granted a minimal usage permit in Ontario for non-food hemp fibre only production.

Hemp broomrape (*Orobanche ramosa* L.) is considered to be the worst parasitic plant in production of *Cannabis*. Broomrape infects the root system, starves the plant and only appears above the soil level for a short time to flower and set seed. Suggested control measures range from biological methods such as using geese to eat the broomrape, trap crops or fumigations (McPartland et al. 2000).

Hemp can become a weed and thus feral populations will arise if not maintained. Volunteer hemp can be controlled with application of broadleaf herbicide or by tillage. It is the responsibility of the producer or researcher to prevent and maintain volunteer hemp free production areas. This is a special and unique concern given the isolation restriction placed upon hemp production. Even though post-emergent pesticide application is prohibited for hemp production in Canada (except for the minor use permit in Ontario) it

is common practice for weeds to be controlled in conventional production regimes by a pre-emergent application of glyphosate; this also reduces volunteer hemp populations.

## **2.5 Harvesting Methods**

Harvesting methods for industrial hemp vary depending whether the cultivar is designated for seed only, fibre only or for dual-purpose production. The success of today's Canadian hemp grain production has been due mainly to farmer modification to conventional grain harvesters. The lack of knowledge on hemp fibre harvesting practices, i.e. timing, equipment, retting, baling, storage and lastly fibre processing, has limited the expansion of the Canadian hemp fibre industry. Methods for harvesting fibre currently differ between Canada and the EU. In the EU it is common for dual-purpose cultivars to be directly combined in which the combine cuts, threshes and cleans seed then binds and/or spreads the remaining stems. In Canada, on the other hand, harvest is typically a two-step process that includes combining the grain followed by swathing then baling the remaining fibre.

Hemp seed harvest in Canada has been successful with only limited combine modifications. Modifications for John Deere conventional combines and for Case IH axial flow combines have improved harvest of taller cultivars (see Appendix D). It is important that all sickles and guards are either new or sharpened and that the crop is cut a bit on the greener side. Friesen (2006) suggests that short stature hemp cultivars, like Finola, should be straight combined at 75-80% maturity at 14 to 16% seed moisture or swathed at ~85% maturity, windrowed and then combined. Swathing can cause seed yield loss due to shattering. Inclement weather after swathing increases the risk of

sprouting grain in the seed heads and/or moulding in the swath. Both can result in total crop loss (per. comm. K. Watson 2007).

During grain harvest, the straw chopper should be either completely removed or disabled, the cylinder/rotor speed should be set to low and the open concave distance from the cylinder/rotor should be large. The auger unloading speed should be slowed to reduce seed damage all the while watching for fibre wrapping on the feeder chain and other moving machine parts. To further reduce seed cracking and damage after combining, grain should be moved with large diameter augers at low speeds or with belt conveyor systems. Hemp grain moisture content for long-term storage should be below 9%. Hemp grain can be stored in a variety of bin types, although smooth sided inverted cone hopper bottom aeration bins tend to work best. To ensure proper storage, bins should be filled to below maximum bin capacity and the hemp grain should be flattened on top. If using natural air drying systems, the hemp grain should be turned 2 days after harvest then turned again in the spring and fall (Friesen 2006).

Harvesting and final processing of hemp biomass in the past has been problematic because conventional grain harvesting combines inability to process all of the above-ground plant material (Chen and Liu 2003). The decision to straight cut or swath is usually determined by the total plant height and crop biomass. Dual-purpose cultivars should be direct/straight combined and the remaining fibre stand should be mowed or cut at opposing angles (preventing fibre loss) immediately after grain harvest then allowed to dry or dew ret. Standard sickle-bar mowers, mower conditioners and hay swathers have been used to cut full-length stalks, though heavy duty sickle bar cutters tend to work best (Laprise 1998). Forage harvester units have been used to cut and chop the stalks into

desired lengths (Laprise 1998). Fibres have a tendency to wrap around rotating machine parts (Chen and Liu 2003). Caution must be taken to guard the under belly of the machine, to inspect equipment during cutting and baling and to prevent fibre wrapping, fire or equipment damage (pers. com. Keith Watson).

To facilitate field dew retting and fibre pickup (baling), hemp stalks should be cut at a swather width of 2.5-3 meters into a windrow of 0.9-1.2 meters wide (Laprise 1998). Although not required as of yet, turning the hemp windrow using a rotary rake delevels the plants, reduces total plant volume and reduces moisture content at baling time (Laprise 1998). Producers have found that both square and round balers equipped with a heavy duty pick up system can be successfully used to bale hemp but hard core round balers tend to perform better than soft core balers (Laprise 1998; Friesen 2006). To prevent fibre spoilage during storage, hemp fibre should be baled at 12% moisture (per. comm. C. Federowich 2005). Suggested hemp bale stacking orientation is provided in Figure 2.5.1 and Appendix C.



**Figure 2.5.1** Hemp bales (A. S-Hermann 2007)

## 2.6 Plant Population Density

The purpose of this study was to evaluate the effects of harvest timing and target plant population density on industrial hemp fibre yield and quality grown in the Parkland region of Manitoba.

Plant population density has been known to affect plant height, self-thinning, seed and fibre yield, stem diameter, light interception, canopy closure and plant development in hemp (van der Werf 1997; Weightman and Kindred 2005). The desired plant density for hemp is often based on the type of end use product. In the United Kingdom and Australia, Riddlestone et al. (2006) recommended a seeding rate of 55 kg ha<sup>-1</sup> for production of textile grade fibre; neither the average seed weight nor the target number of plants m<sup>-2</sup> was given.

The pedigree of hemp is as follows: breeder, foundation, registered and certified. As the pedigree (breeding level) of hemp seed increases the recommended targeted plant population density decreases. This decrease in density as pedigree increases allows for ease of crossing, rouging and maintenance practices. For instance, in the Ukraine, it is suggested for dual-purpose elite breeder seed production that the targeted seed density should be 60-90 seeds m<sup>-2</sup>, for foundation seed production 120-180 seeds m<sup>-2</sup> and for registered seed production 180-240 seeds m<sup>-2</sup>; no suggestions were provided for certified seed production (Holoborod'ko 1995). The Bast Institute in the Ukraine suggests a target plant density of at least 450-500 seeds m<sup>-2</sup> for fibre-only production.

Even though limited Canadian research is available, experienced agronomists suggest 100 viable seeds m<sup>-2</sup> for grain-only production and 300 viable seeds m<sup>-2</sup> for fibre-only production (per. comm. K. Friesen and K. Watson 2007).



### **2.6.1 Self-thinning**

Self-thinning occurs when the biological carrying capacity of a given area is reached. As a result plants within the area die off thus allowing the plant population to reach a sustainable population within the given area. A seeding rate which exceeds the maximum number of plants that a set area can sustain will expend valuable nutrients and carbon that would have otherwise be available to healthy plants (Loomis and Connor 1992). Self-thinning is typically viewed as a limitation to overall plant performance and is known to occur in all cultivated crops (Cade 2000). Density induced thinning can result from inter- and intra-specific plant competition for resources such as space, nutrients, moisture or light (Henderson et al. 2000). Competition can create a ‘size hierarchy’ in hemp that can cause suppression and death of smaller plants while increasing the overall variation in individual plant size (Ranalli 1999; Cade 2000; Amaducci et al. 2002b). Even though hemp is highly plastic and can be extremely productive in varying spatial zones, it is important to understand how final plant counts at harvest are affected by plant density. Thus, it is important to determine at what plant density yields are maximized while maximizing the total number of plants alive at harvest while minimizing overall production inputs, such as seed cost.

#### **2.6.1.1 Effect of plant density on plant self thinning**

Preliminary research conducted in Manitoba measured self-thinning on four Canadian grown dual-purpose cultivars, USO 14, USO 31, Alyssa, and Zolo at three targeted plant densities, 100, 200 and 300 plant m<sup>-2</sup> (PCDF 2003). Results showed that the average rate of self-thinning over all four cultivars was 17% with an 83% target-seeding survival rate. Self-thinning is known to occur in other plant species such as

mouse-ear hawkweed (*Hieracium pilosella*) (Bishop and Davy 1985), grain amaranth (*Amaranthus* spp.) (Myers 1996), sunflowers (*Helianthus annuus*) (Humston et al. 2005), spring wheat (*Triticum aestivum*) (YanJun 2005) and oats (*Avena sativa*) (Fransen 2005).

The goal in selecting a seeding rate or target plant density is to ensure that the plant population at emergence does not exceed the sustainable maximum density possible (van der Werf et al. 1995a). Optimal plant density of 90-100 plants m<sup>-2</sup> have been suggested by Amaducci et al. (2002a) and 50-120 plants m<sup>-2</sup> by van der Werf et al. (1995a). The tolerable rate of self-thinning within a given plant population has yet to be determined.

### **2.6.2 Effect of plant density on plant height**

Plant height in hemp is an important agronomic parameter because height has been shown to be related to fibre length and quality. Research conducted in Italy, Germany and the Netherlands has examined the effect of plant density on plant height in hemp. Previous hemp research conducted in Italy by Amaducci et al. (2002b) studied the relationship between plant height and plant density. They observed that plant height initially increased from 46 to 50 to 52 cm ( $P < 0.01$ ) as plant density increased from 45 to 90 to 180 plants m<sup>-2</sup>, thereafter the plant height decreased at harvest from 250 to 225 to 204 cm, respectively, as the plant density increased. Amaducci et al. (2002a) further determined that plant height decreased from approximately 230 to 170 cm as plant density increased from 50, 100, 150, 200 and 250 plants m<sup>-2</sup>. Plant density experiments conducted by Struik et al. (2000) in England, Italy and The Netherlands determined that plant height decreased from approximately 330 cm to 270 cm as target plant density increased from 30 to 90 to 270 plants m<sup>-2</sup>.

In an examination of 40 different hemp accessions, Schumann et al. (1999) observed that early in the hemp plant development, height and plant density were positively correlated, while at maturity, height and density were negatively correlated. The hemp crop plant heights were taken at 4 and 7 weeks after sowing, then again at technical maturity (i.e. initial start of seed maturity). It was determined that the number of initial plants and plant height at maturity was found to be negatively correlated ( $r^2 = -0.65$ ,  $P = 0.01$ ) for monoecious plant types and ( $r^2 = -0.45$ ,  $P = 0.01$ ) for dioecious plant types. In an Australian study, Lisson and Mendham (2000a) observed that among mature hemp plants stalk height reduced from 268 to 244 cm occurred as plant density increased from 50 to 300 plants  $m^{-2}$ . Heights of other crops such as grain amaranth (Henderson et al. 2000; Myers 1996) and Pima cotton (*Gossypium barbadense*) (Munk 2001) have also been determined to have reduced plant heights at high plant populations.

Researchers have attempted to understand the physiological basis for the plant density-plant height relationship. Amaducci et al. (2002b) stated that in the first phases of hemp plant development, competition for light causes the plant to initially grow taller. Ballaré et al. (1997) further explained that the initial increase in plant height is due to red to far-red light induced internodal elongation. The researchers concur that the initial increase in height is more than likely due to inter- and intra-plant competition for available spatial, nutrient and light resources.

### 2.6.3 Effect of plant density on stem diameter

Several European studies have documented the effects of plant population density on stem diameter in hemp. In most cases, stem diameter was inversely related to plant population densities ranging from 50 to 350 plants m<sup>-2</sup> (Scheifele et al. 1996; Schumann et al. 1999; Lisson and Mendham 2000a; Amaducci et al. 2002a and 2002b; Höppner et al. 2004; Svennerstedt and Svensson 2006; Schäfer and Honermeier 2006). Therefore, most studies agree that as plant population density increases, stem diameter decreases. Similar results have also been observed in sunflowers (Sloan et al. 2003), maize (Turgut et al. 2005), amaranth (Henderson et al. 2000; Myers 1996), cotton (*Gossypium barbadense*) (Munk 2001) and kenaf (*Hibiscus cannabinus*) (Massey 1973). Höppner et al. (2004) observed a significant difference in hemp stem diameter between harvest at initial start of seed set (technical maturity) for predominant fibre production at 221 plant m<sup>-2</sup> and harvest at seed maturity for predominant seed production at 116 plant m<sup>-2</sup> of 4.9 mm and 5.8 mm, respectively. Overall stem diameter decreased as the plant density increased.

Hennik (1994) examined the heritability of stem diameter to the parent-offspring relationships. The narrow sense heritability was found to be low, at 0.22, indicating that “selection based on stem diameter resulted in a positive correlated response to plant height.” Scheifele et al. (1996) found a 95 % correlation between hemp stem diameters and stem length. Sankari (2000) observed in dioecious hemp bio-types that as stem diameter increased bast fibre content decreased. They also found that the bast fibre content in monoecious bio-types was less sensitive to changes in stem diameter than dioecious bio-types. According to Amaducci et al. (2002b) stem diameter of hemp is a

function of density, further stating that smaller stem diameter is advantageous for decortication of bast and hurd fibres for textile applications.

#### **2.6.4 Effect of plant density on fibre yield**

Evidence in the literature suggests that plant density affects hemp fibre yield to some degree; however, in most cases, fibre yield is unaffected by plant density. Cromack (1998), Struik et al. (2000), Amaducci et al. (2002b) and Schäfer (2005) determined that stem total yield in European hemp cultivars was not significantly affected by increasing plant density. Three European cultivars (Futura 75, Fedora 17 and Felina 34) were examined in southern Sweden at two seeding rates (30 and 60 kg ha<sup>-1</sup>). The results showed similar biomass (9.6 – 11.6 kg ha<sup>-1</sup>) and fibre (2.2 – 2.7 kg ha<sup>-1</sup>) yields for each rate (Svennerstedt and Svensson 2006). Working in the United Kingdom, Bennett et al. (2006) determined that the percentage of total fibre per unit of land was greater at 300 seeds m<sup>-2</sup> (47.0%) than at 150 seeds m<sup>-2</sup> (51.4%). Bennett et al. (2006) further concluded that the percentage of bast (long) fibre was relatively unchanged from 13.4% at 150 seeds m<sup>-2</sup> to 13.2% at 300 seeds m<sup>-2</sup>. Deleuran and Flengmark (2005) concluded in a Danish study that total stem yield and total dry matter did not vary among by seeding rates of 8, 16, 32, 64 kg ha<sup>-1</sup>. The authors noted that bast fibre percentages, though not significantly different (31.8 and 31.6%), were greatest at seeding rates of 32 and 64 kg ha<sup>-1</sup>. Amaducci et al. (2002b) concluded that at lower plant densities of 30-40 plants m<sup>-2</sup>, hemp stem dry matter yield at final harvest in the Northern Italy averaged 16.8 and 17.8 t ha<sup>-1</sup>, respectively. In Australia, the stem dry matter yield of hemp was observed to be unaffected by plant densities between 80 and 400 plants m<sup>-2</sup> (RIRDC 1995).

In the United Kingdom, Cromack (1998) determined that French and Hungarian hemp cultivars (Fedora 19, Felina 34, Uniko B, Futura 77 and Komploti) produced similar average stem yields (9.57 and 10.38 t dry matter ha<sup>-1</sup>) at plant densities of 200 and 400 seed m<sup>-2</sup>, respectively. They did observe, however, that the bast fibre content increased from 1.3 to 3.5 t dry matter ha<sup>-1</sup> as plant density increased. van der Werf et al. (1995a) also observed that the percentage bark (bast) fibre as a part of the total stem fibre yield increased with increasing plant population density. In a German hemp study conducted by Schäfer (2005) the total plant yield consisted of the epidermis, primary fibres (bast) and xylem (hurds) decreased with increasing plant densities of 100 and 300 seeds m<sup>-2</sup>; however, yield data was not provided. These authors observed that the differentiating bast cell layers were smaller at higher plant densities; this can affect the overall fibre bundle size.

Preliminary research in southern Alberta, Canada concluded that hemp fibre cultivars (Beniko, Bialobrezski, Zolotonosha 11,13,15 and 24, USO 14 and 31, Kompolti, Unico, Fedora 19, Felina 34 and Futura 77) seeded at lower rates appeared to yield better than those seeded at the higher seeding rates (rates not published) (Blade et al. 1999). Vera et al. (2006) examined two oil seed cultivars (Finola and Fasamo) in the Parkland region of Saskatchewan, Canada. The authors concluded that increasing the seeding rate from 20 to 60 to 80 kg ha<sup>-1</sup>, with a target plant density at emergence of approximately 150-200, 500 and 750 plants m<sup>-2</sup>, based on average cultivar thousand kernel weight, respectively, increased the total crop biomass (2633, 2887 and 3323 kg ha<sup>-1</sup>, at each density respectively). They further concluded that increasing the sowing density increased grain yield and decreased the presence of weeds. The change in fibre

yield and biomass suggests that plant population density could be an essential crop management tool for specific-production of hurd and bast fibres (Cook and Scott 1998).

### **2.6.5 Effect of plant density on the bast and hurd chemical constitutes**

The effect of specific plant densities on the chemical composition of hemp has not been thoroughly examined and little detailed information was found in the literature. The percentage of cellulose and lignin are key parameters in determining stem quality, especially for the pulp and paper industry (de Meijer and van der Werf 1994). A literature review conducted by Ranalli (1999), concluded that the chemical composition of bast and hurd fibres change during the growing season but no reference was given to the effects of plant density.

van der Werf et al. (1994c) compared the effects of plant population densities ranging between 40 and 100 plants  $m^{-2}$  on the bast and hurd chemical constitutes of four European hemp cultivars (Fibrimon 56, Fédora 19, Kompolti Sárászárú and Kompolti Hybrid TC) in, the Netherlands. The mean cellulose content present in the hurd varied less (31.5% to 37.4%) than that of the bast fibre (53.2% to 74.3%). Changing the plant population density did not affect the lignin and hemicellulose content (20.8% and 17.8% in the hurd and 4.3% and 7.7% in the bast fibre, respectively). This suggests that cultivar, environmental conditions and harvest timing have a greater impact on the chemical profile of the hemp stalk than plant population density.

Kamat et al. (2002) analyzed the chemical profile of hemp grown in southern Ontario, at a seeding rate of 75 kg  $ha^{-1}$  (approx. 400 plants  $m^{-2}$ ). Kamat et al. (2002) found that whole hemp stems harvested at 60 and 90 days (technical maturity) after

sowing consisted on 44-45% cellulose, 11% lignin and 83-84% holocellulose. Correia et al. (2001) examined the chemical constituents of the bast and hurd of hemp grown for fibre in southern Ontario. Correia et al. (2001) found that the bast consisted of 60% cellulose, 10% lignin and 84% holocellulose and that the hurd consisted of 72% cellulose, 21% lignin and 72% holocellulose. Gutiérrez et al. (2006) analyzed hemp fibres grown in Spain for lignin content. The trial determined that lignin content accounted for 4.6% of the total bast fibre. The authors observed that the lignin content in the hemp samples were lower than that of other non-wood fibre crops (Gutiérrez et al. 2006), thus concluding that low lignified hemp fibre would be advantageous in pulp production.

## **2.7 Harvest Timing**

Harvest timing is an important parameter since based on the literature the fibre quality of hemp changes as the plant develops. Harvesting of fibre hemp typically involves cutting, retting and drying time which requires moisture and warmth then baling or stooking.

### **2.7.1 Effect of harvest timing on plant self-thinning**

Upon review of the literature no data was found which examined the effects of harvest timing on hemp plant self-thinning. Mediavilla et al. (2001) observed that the majority of hemp plants self-thinned during the first vegetative growth phase. For many crops, self-thinning is affected more by plant density rather than by harvest timing (Lonsdale 1990; Loomis and Connor 1992; Kikuzawa 1999). Westoby (1976) examined self-thinning in five cultivars of subterranean clover (*Trifolium subterraneum*) at 32, 51, 73, 100 and 141 days after sowing. Total plant loss due to self-thinning was determined



not to be significantly affected by delaying harvest. According to Kikuzawa (1999) larger individual plants within a given area will suppress the growth rate of smaller plants as the crop develops. Thus delaying harvest could increase plant loss of individual plants due to competition.

### **2.7.2 Effect of harvest timing on plant height**

The effect of harvest timing on plant height in hemp has not been thoroughly examined and little detailed information was found in the literature. Results from a German study suggested that plant height in hemp is influenced mostly by location, length of the vegetative period and plant density, but not harvest date (Hendrischke et al. 1998). Bennett et al. (2006) concurred, stating that hemp height was affected by plant densities of 150 and 300 seeds m<sup>-2</sup> but not by delaying harvest. Canadian agronomists have noted that maximum hemp stalk height is typically reached during the first week of August (developmental stage code 2102) in Manitoba and that at this time, hemp for fibre-only production should be harvested (per. comm. K. Watson 2006).

### **2.7.3 Effect of harvest timing on stem diameter**

The effect of harvest timing on stem diameter could be an important crop quality management tool when growing hemp for hurd and bast fibre-specific markets (Cook and Scott 1998). Mambelli and Grandi (1995) determined that basal stem diameter in kenaf (*Hibiscus cannabinus* L.) (an annual fibre crop with potential for pulp and paper) was not significantly affected by delaying harvest timing. Upon the limited data available, stem diameter in hemp seems to be a function of a plant density relationship rather than

harvest timing. No additional literature on the effect of harvest timing on stem diameter in hemp was found.

#### **2.7.4 Effect of harvest timing on fibre yield**

Yield and fibre quality can be affected by harvest timing. Amaducci et al. (2000) examined the stem dry matter yield of four annual fibre crops (hemp, kenaf, maize and sorghum) at three successive harvest dates (24 August, 22 September and 17 October) in the Northern Italy. They determined that the stem dry matter yield in hemp did not differ statistically among harvest dates (13.3, 13.1 and 12.2 t ha<sup>-1</sup>). Mediavilla et al. (2001) observed that hemp stem yield and fibre yield peaked at 'technical maturity'. Hemp fibre technical maturity has been determined to occur at the time of flowering of the male plants (Mediavilla et al. 2001). Hoffman (1961) suggested that the increased yield at technical maturity was due to an increased rate of lignin and production of shorter, thicker cell walled secondary fibres (hurd) which decrease the overall bark quality (cited in: Mediavilla et al. 2001; Bennett et al. 2006). In a German study, Schäfer (2005) determined that from the beginning to the end of the flowering stage fibre content decreased by 5%; however, from end of flowering to grain harvest the fibre content was unchanged. Agronomic experiments conducted in the United Kingdom by Bennett et al. (2006) determined that fibre yield decreased from 5486 to 3860 kg ha<sup>-1</sup> as harvest was delayed by 14 days.

Meijer et al. (1995) showed a decline in biomass yield in dual-purpose cultivars after flowering in the Netherlands and concluded that the reduction in biomass was a direct result of fat and protein synthesis in the seed and canopy senescence. Kamat et al. (2002) examined Canadian hemp harvested at 30, 60 and 90 days after planting. The

biomass of air-dried hemp plants increased from 200 to 5000 to 8000 kg ha<sup>-1</sup>, respectively with delayed harvest. They noted that biomass accumulation between 60 and 90 days was substantially less than between 30 and 60 days. van der Werf et al. (1994c) suggested sampling the stalk at 20 to 30% of the total stem height would give a “good approximation” of the percentage of bast to hurd fibre in the stem.

### **2.7.5 Effects of harvest date on the bast and hurd chemical constituents**

The effects of harvest date on the chemical constituents of the two main components of the hemp stalk, i.e. bast and hurd have been examined more so in Europe than in Canada. A literature review conducted by Ranalli (1999) concluded that the chemical composition of bast and hurd fibres change during the growing season. Amaducci et al. (2000) compared one European hemp cultivar (Futura 77) to three other fibre crops, i.e. kenaf, maize and sorghum, at three harvest times. They observed that in hemp the whole stalk percentage of cellulose increased from 56.5 % to 66.8 %, the percentage of lignin decreased from 12.7 % to 7.8 % and the percentage hemicellulose decreased from 17.8 % to 15.8 % as the harvest timing was delayed by 3 weeks (August 24, September 22 and October 17). Amaducci et al. (2000) noted that the decrease in percent of lignin did not concur with previous unpublished research findings. They proposed that the reduction in percentage of lignin as harvest was delayed was more than likely due to the loss of small and dead plants. In addition, Amaducci et al. (2002b) concluded that decreasing plant density from 270 to 180 to 90 to 45 to 30 plants m<sup>-2</sup> and postponing harvest time by approximately seven days resulted in the stem becoming richer in short and lignified secondary hemp bast fibres. In a comparison of three European cultivars, Struik et al. (2000) observed that cellulose yield in hemp stems

increased until the end of the growing season and at the same, lignin was increasing. Therefore, these observations support those of Amaducci et al. (2000) where lignification increased as the growing season progressed. Previously, van der Werf et al. (1994c) stated that harvest date affected the chemical composition of the bark (bast) fibre and to a lesser degree the core (hurd). They concluded that hemp stalks harvested at 'technical maturity' yielded a superior raw material for paper making. The mean cellulose content in the bark was 64.8% and in core was 34.5%. The mean hemicellulose content was 7.7% in the bark and 4.3% in the core while the lignin content was 17.8% in the bark and 20.8% in the core.

Toonen et al. (2004) examined three commercial European hemp cultivars, i.e. Chamaeleon, Felina 43 and Kompolti hybrid TC at a plant density of 120 plants m<sup>-2</sup> harvested in two week intervals, i.e. 40, 54, 68, 83, 96 and 110 days after sowing in the Netherlands. They concluded that the percentage of lignin present did not significantly increase with delayed harvest (10.12% at 42 days after sowing to 11.41% at 112 days after sowing) and that the percentage of cellulose and hemi-cellulose content increased significantly with delayed harvest.

Lignin content in wheat was determined to be low in young plants; however, during heading out lignin was observed to increase (Stone et al. 1951). To date, only one research project (i.e. Kamat et al. 2002) has been conducted in Canada examining the effects of plant age on the chemical constituents of whole hemp stems. Kamat et al. (2002) examined hemp stems harvested at 30, 60, 90 and 120 days after sowing in southern Ontario. The cellulose and holocellulose content in the stem fibre tended to increase between 30-60 days and was unchanged between 60-90 days. The percent of

lignin decreased from 14 % at 30 days to 13 % at 60 days to 11% at 90 and 120 days. Thus a decrease in the amount of lignin present in hemp stem occurred as harvest timing was delayed. Based on the low lignin and high cellulose content in hemp bast fibres, Kamat et al. (2002) concluded that hemp bast fibres would be a viable raw material for pulp and paper industries.

### **Objective**

The main objective of this thesis was to determine the effects of targeted plant population density and harvest timing on basic agronomic fibre yield and quality characteristics.

## **3. MATERIALS and METHODS**

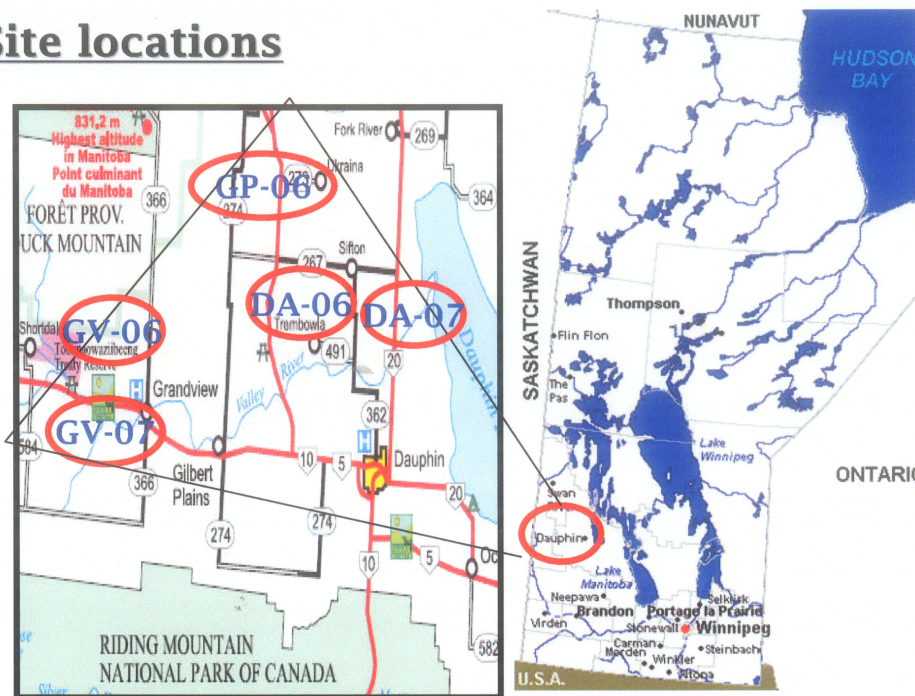
### **3.1 Field sites and general information**

Five field experiments were carried out in the non-saline black chernozemic loamy soils of the Parkland region of western Manitoba. Two sites were selected at Grandview and Dauphin in both 2006 and 2007 and one site at Gilbert Plains in 2006 (Figure 3.1.1). A sixth site was established at Gilbert Plains in 2007, but was lost due to flooding. The Health Canada-approved industrial hemp cultivar used in this study was Manitoba-bred Alyssa, a dual-purpose predominately female cultivar with a delta-9-tetrahydrocannabinol (THC) content below 0.15% and an average thousand kernel weight (TKW) of 18 grams (Watson 2005). Alyssa is characterized as being medium in height (150 to 180 cm), with medium branching, medium stem internodes and as having green

colored medium sized leaves with full seed maturity in 100-112 days after sowing (PCDF 2003). Tetra-hydrocannabinol (THC) percentage was determined to be <0.05% in all site years. The THC percentage was determined by Meatherall Consulting, a licensed lab located in Winnipeg, Manitoba. The analytical methods used to determine THC follow the Health Canada THC sampling and analytical guidelines.

Plots were maintained by Manitoba Agriculture Food and Rural Initiative (MAFRI) and by the Parkland Crop Diversification Foundation (PCDF). At each site, a complete randomized, split plot experimental design was employed with four blocks. Harvest timing was the main plot treatment and the targeted plant population density was the subplot treatment (Appendix E). An 18 m<sup>2</sup> buffer guard zone was planted between each main plot (harvest timing) treatment. The buffer zone was employed to decrease edge effects. Each plot was 9 rows wide at a 20 cm row spacing as suggested by Amaducci et al. (2002b). Subplots measured 2 x 9 m (18 m<sup>2</sup>) at sowing and 1.4 x 7 m (9.8 m<sup>2</sup>) at harvest.

## Site locations



**Figure 3.1.1** Industrial hemp fibre experimental plots located in the Parkland region of Manitoba in 2006 and 2007; Dauphin (DA)-06 and 07, Grandview (GV)-06 and 07, Gilbert Plains (GP)-06.

### 3.2 Crop and soil management

Fertilizer application was based on a soil test in 2006 only. The 2006 soil samples were taken at soil depths of 0-15 and 15-60 cm from four locations at each site. The samples from each depth were then combined and sent to Bodycote Testing Labs, Winnipeg, Manitoba, Canada. Method of extraction references provided by Bodycote Testing Labs for soil testing procedures are presented in Appendix J. The soil nutrient analyses determined the amount ( $\text{kg ha}^{-1}$ ) of nitrate (N), phosphorus (P), potassium (K), sulphate (S), pH and EC ( $\text{d/Sm}$ ) (Table 3.2.1). In 2006, fertilizer was seed placed at sowing time in all sites (Table 3.2.1). In both 2006 and 2007, all sites were treated with a post-planting pre-emergent application of glyphosate at a rate of  $1.2 \text{ L ha}^{-1}$ . In 2006, an

in crop post-emergent tank mix treatment of 0.89 L ha<sup>-1</sup> of clethodim and bromoxynil at 0.45 L ha<sup>-1</sup> was applied post-planting (Appendix F).

Soil testing was not conducted in the 2007 cropping season due to the initial isolation of the 2007 site locations, cooperator crop rotation and pre-seeding fertilizer application. The cooperating producer in Grandview-07 tilled the field in the fall of 2006, then harrowed it in the spring of 2007. Fertilizer was applied at time of spring wheat sowing at the Grandview-07 site. The subsequent wheat crop within the experimental site area was destroyed with an application of glyphosphate at a rate of 1.2 L ha<sup>-1</sup>. At the Dauphin-07, site fertilizer was applied by the cooperator in the fall (mid-September) of 2006 when the site was seeded to winter wheat.

Site soil classification, previous crop information, fertilizer application method and rate for all site years are recorded in Table 3.2.2. At all sites in 2007, a pre-planting application of 1.2 L ha<sup>-1</sup> of glyphosphate was applied. In addition, an in crop tank mix herbicide treatment of 0.22 L ha<sup>-1</sup> of Assure® II (quizalop-p-ethyl) and sure-mix adjuvant and 0.45 kg L<sup>-1</sup> of Pardner® (bromoxynil) was applied to eliminate the winter wheat crop and post-emergent weeds. It is the opinion of this author that application of any form of pesticide is not a dependent variable for optimal hemp production in Canada.



**Table 3.2.1** Site name, land location and soil tests used in the 2006 harvest timing x target plant density hemp study in the Parkland region of Manitoba.

Site	Soil test results						
	Sample depth (cm)	Extractable Nutrient (mg kg <sup>-1</sup> )					
		N	P	K	S	pH	EC(dS/m)
Gilbert Plains- 06	0-15	6	21	114	3	7.9	0.45
	15-60	5	<5	55	3	8.1	0.41
Grandview- 06	0-15	28	19	360	4	7.9	0.7
	15-60	17	6	254	3	6	0.72
Dauphin- 06	0-15	14	13	101	4	8.1	0.54
	15-60	6	6	62	4	8.3	0.52

N denotes Nitrate (NO<sub>3</sub>-N).

P denotes Phosphours (P).

K denotes Potassium (K).

S denotes Sulphate (S).

EC (dS/m) denotes electrical conductivity (salinity).

**Table 3.2.2** Site, land location, soil classification, previous crop and fertilizer applied in the 2006 and 2007 harvest timing and plant population density hemp experiments conducted in the Parkland region of Manitoba.

Site	Legal land location	Soil Classification Manitoba Soil Survey No. 9	Previous Crop	Fertilizer applied	
				Nitrogen kg ha <sup>-1</sup>	Phosphate kg ha <sup>-1</sup>
Gilbert Plains- 06	SW 27-27-22 W1	Meharry clay loam	Canola	51**	33*
Grandview- 06	NE 22-25-24 W1	Meharry clay loam deep phase	Fallow	100 (NH <sub>3</sub> )	33*
Grandview- 07	NW 11-26-23 W1	Plainview clay	Canola	78	33
Dauphin- 06	NW 26-26-19 W1	Lakeland loam	Canola	51**	33*
Dauphin- 07	NE 31-26-18 W1	Lakeland loam	Canola / Winter Wheat	89	39

NH<sub>3</sub> denotes fall applied anhydrous ammonia.

\* Seed placed fertilizer.

\*\* Broadcasted fertilizer.

Rates presented without notation indicates that fertilizer was applied by cooperators prior to seeding.

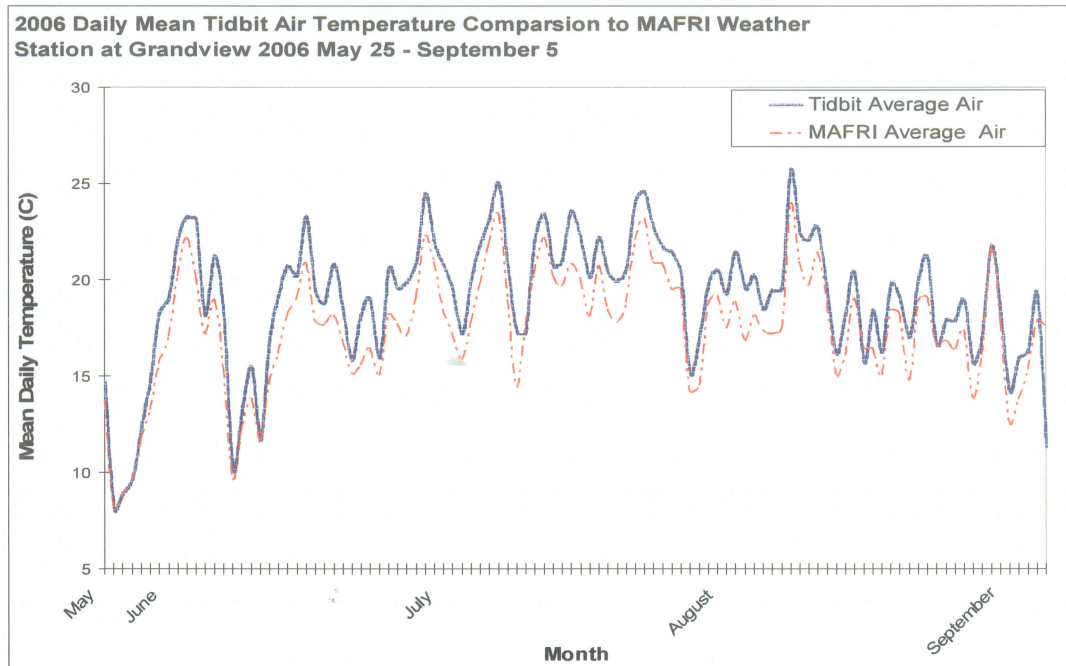
Soil classification sourced from Manitoba Soil Survey's No. 9 and 21 (Mills and Smith 1981 and Ehrlich et al. 1959).

### 3.3 Environmental conditions

Monthly mean rainfall (mm) and air temperature (C°) were measured at each field site between May and September. The monthly mean rainfall (mm) and air temperature (C°) from each site and the 30 year regional averages are presented in Table 3.3.1. Air temperatures were collected by suspending an Onset StowAway Tidbit Temperature Logger within a Styrofoam™ cup from a post in the ground (Figure 3.3.1). This system for air temperature data collection was compared to a local weather station before being employed. The selected method for air temperature measurement recorded a slightly higher temperature than that of the MAFRI weather station located one mile from the experimental site (Figure 3.3.2). Monthly precipitation was collected by mounting an Onset Hobo Data Logging Rain Gauge opposite the suspended Tidbit (Figure 3.3.3). The mean rainfall (mm) in 2006 was extremely dry and hot in comparison to the 30-year average while 2007 was closer to the 30-year average.



**Figure 3.3.1** Air temperature data collection mode. A StowAway Tidbit Temp Logger by Onset suspended from within a Styrofoam™ cup (A. S-Hermann 2006).



**Figure 3.3.2** Daily mean air stowaway tidbit data collector compared to MAFRI weather station located 1 mile from Grandview site in 2006.



**Figure 3.3.3** Precipitation and air temperature data collection equipment and placement. The Hobo data logging rain gauge was mounted opposite the suspended air temperature collection system. (A. S-Hermann 2006).

**Table 3.3.1** Monthly mean precipitation and air temperature for the 2006, 2007 growing season at the five experimental sites in the Parkland region of Manitoba compared to the regional 30 year average.

	Monthly growing season precipitation (mm)			Mean monthly Temperature (°C)		
	2006	2007	30-yr average (1971-2000)	2006	2007	30-yr average (1971-2000)
<i>-----Gilbert Plains-----</i>						
May	5		48	21		10
June	3		83	19		15
July	2		78	19		18
August	3		73	18		16
September	0		59	17		10
Total	13		341			
<i>-----Grandview-----</i>						
May	10	11		12	12	
June	7	84		19	17	
July	3	40		21	22	
August	4	32		19	17	
September	2	2		15	18	
Total	26	169				
<i>-----Dauphin-----</i>						
May	14	12	51	14	15	11
June	15	83	86	20	18	15
July	17	42	75	21	22	18
August	32	20	60	21	18	17
September	0	4	66	17	19	11
Total	78	161	338			

Precipitation and temperature data collected from weather data recorder at each location. 30 year average (1971-2000) sourced from Environment Canada climate normals. 30 year averages were not available for the Grandview area.

### 3.4 Treatments

The main plot treatment was harvest timing based on developmental stage, using the Mediavilla et al. (1998) *Cannabis* decimal code. The first harvest (H1) coincided with developmental stage 2102, when 50% of the male staminate flowers were open and dehiscing. Harvest 1 (H1) is considered to be the technical maturity stage for fibre hemp, which in this case roughly coincided with maximum height. The second harvest (H2) coincided with developmental stage 2306, when 50% of the mature seed was resistant to compression. The final harvest (H3) coincided with decimal code 2307, when 95% of the mature seed present was hard and the bract was separated from the seed.

The sub plot treatment was a targeted plant population density of 100, 200 and 300 plants m<sup>-2</sup> after hand thinning. All sites in 2006 were seeded at one and one half times the targeted plant density rate on May 25<sup>th</sup> and two times the targeted rate in 2007 on May 29<sup>th</sup> and 30<sup>th</sup>. Seeding was conducted using a Fabro cone plot seeder (Figure 3.4.1) and the seed was placed at a seeding depth of 1.5 cm (Figure 3.4.2).

Plant population data was collected from a one m<sup>-2</sup> area in each plot. These areas were delineated by string markers that were placed in the middle of each subplot. Mambelli and Grandi (1995) delineating the sampling area by markers in previous industrial hemp research. This area contained the innermost five rows and the length of the area was one meter. Plant populations were determined immediately after full emergence within each sampling area in each subplot. The delineated area within each subplot was hand thinned to the targeted plant population density 5-12 days after full plant emergence. In both the initial and post-thinning, plants counts were taken from each of the delineated meter square areas. Ticked meter square sticks of 20, 40 and 60



ticks per meter of length for 100, 200 and 300 plant densities, respectively were employed as a guide for the hand thinning process (Figure 3.4.3).

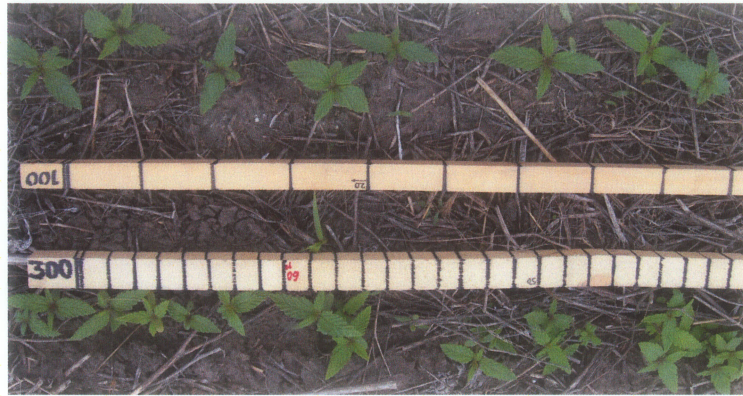
Average plant density after hand thinning for each targeted plant density is presented in Table 3.4.1. Hand thinning to achieve certain targeted plant population densities has been used previously in hemp research (Mambelli and Grandi 1995; Cook and Scott 1998; Lisson and Mendham 2000; Stuik et al. 2000; Amaducci et al. 2000b and 2002a; Toonen et al. 2004). The delineated areas were also used to monitor plant self-thinning and to mark the hand harvest area for biomass yield ( $\text{kg ha}^{-1}$ ), stem diameter (mm), percentage of fibre (bast and hurd) and chemical constituents. The advantage of hand harvesting is that total useable biomass yield is measured and losses due to machinery are avoided.



**Figure 3.4.1** Fabro cone plot seeder (A S-Hermann 2006).



**Figure 3.4.2** Hemp seeding depth of 1.5 cm (A S-Hermann 2006).



**Figure 3.4.3** Meter sticks used to for guide hand thinning of hemp plants within each subplot (A S-Hermann 2006).

**Table 3.4.1** Average hemp plant counts after hand thinning per delineated m<sup>-2</sup> by target plant density at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location				
	Gilbert Plains	Grandview		Dauphin	
	2006	2006	2007	2006	2007
	-----Average plant count after hand thinning-----				
Target (plants m <sup>-2</sup> )					
100	86	93	99	96	96
200	178	188	191	187	190
300	260	276	296	286	293

### 3.5 Post-establishment sampling methods

The 2006 harvests occurred on August 9<sup>th</sup> (harvest timing 1), August 23<sup>rd</sup> (harvest timing 2) and September 5<sup>th</sup> (harvest timing 3) at all sites. The 2007 harvests occurred on August 9<sup>th</sup> (harvest timing 1), August 23<sup>rd</sup> (harvest timing 2) and on September 6<sup>th</sup>



(harvest timing 3) at all sites. At each harvest, all above ground plant material, apart from senesced leaf matter, was hand harvested from within the designated  $m^{-2}$  area from each sub plot. All plants present in the designated  $m^{-2}$  areas were hand cut three cm above the soil level from each subplot at each of the main harvest timings (Figure 3.5.1).

Plant height was measured on each harvest date; a 3 meter stick was used. Five random plants heights were taken in each sub plot for height assessment (Figure 3.5.2).

As introduced in section 3.4 the number of plants that were present in each designated  $m^{-2}$  were counted at the beginning of the growing season after hand thinning and recounted at the time of hand harvesting. All plants present in the delineated meter square were counted. The amount of self thinning during the growing season was calculated by subtracting the initial plant count  $m^{-2}$  (after hand thinning) from the final plant count  $m^{-2}$  at each harvest timing. Average plant counts after hand thinning for each targeted plant density is presented in Table 3.4.1.

All one  $m^{-2}$  hand harvested samples were divided into two parts approximately equaling a half  $m^{-2}$  each. One half of the  $m^{-2}$  was reserved for the biomass ( $kg\ ha^{-1}$ ) sample. The biomass sample was weighed wet (g) in the field then air dried and stripped of all leaf matter and branches, bagged and oven dried at  $65^{\circ}C$  for at least four days or until the moisture content by weight was unchanged. Since the biomass sample was taken from only an approximate 0.5 meter square area, the following formulas were used to determine stalk biomass per  $m^{-2}$ .

1. 
$$\frac{\text{Field wet weight of the one half } m^{-2} \text{ biomass sample (g)}}{\text{Total field wet weight of the entire } m^{-2} \text{ area}}$$
$$= \text{Proportion of area sampled (} m^{-2} \text{)}$$

2. 
$$\frac{\text{Oven dried weight of the one half m}^{-2} \text{ biomass sample (g)}}{\text{Proportion of area sampled (m}^{-2}\text{)}} = \text{The amount of dry biomass (g m}^{-2}\text{)}$$
3. 
$$\text{Amount of dry biomass (g m}^{-2}\text{)} \times 10 = \text{The amount of dry biomass (kg ha}^{-1}\text{)}$$

The wet weight (g) of the approximated half meter square area was divided by the total wet weight from the entire meter square. This provided the proportion of area sampled (m<sup>-2</sup>). The oven dried weight (g) was then divided by the proportion of area sampled, thus giving the amount of dry biomass per meter square. The g m<sup>-2</sup> was then multiplied by 10, thus converting g m<sup>-2</sup> to kg ha<sup>-1</sup>. This complicated adjustment to yield estimates could have been avoided if the meter square sampling area that had been delineated had been further delineated into two exact half meter square areas.

The remaining approximate half meter square sample was air dried, defoliated and all branches were removed. Following this, the sample was cut and bound into 20 cm sections at three different heights from the soil level: 25-45 cm (bottom); 65-85 cm (middle); and 105-125 cm (top) (Figure 3.5.3 and 3.5.4) (Keller et al. 2001; Kuroda et al. 2005). The cut and bound sections were sent to Biolin Inc., Saskatoon, SK for determination of stem diameter and fibre (bast and hurd) percentage. Five stem diameter measurements (mm) were acquired by electronic calipers from each bundle. Measurements were taken for each stalk section (bottom, middle and top) to determine the average stem diameter for each treatment. This sampling method for determination of

stem diameter has been previously used in other hemp research experiments (Sankari 2000; Schäfer 2005; Schäfer and Honermeier 2006).

Fibre analysis was conducted as follows. Water retting was used to remove the fibre binding pectins (Figure 3.5.5 and 3.5.6) and manual decortication was used to extract the bast and hurd (including the epidermis) fibre. As employed by de Meijer and van der Werf (1994) the contents of bast and hurd of each section were expressed as a percentage of the section dry weight. The detailed methods used by Biolin Inc. are reported in Appendix G.

Of the five site years, only the samples from the Gilbert Plains-06 site were subjected to further fibre analysis. Sectioned stalk samples from harvest 1 (decimal code 2102) and harvest 3 (decimal code 2307) for all sub plots (100, 200 and 300 plant m<sup>-2</sup>) were tested for the chemical percentage of cellulose, hemicellulose, holocellulose and lignin. The water retted, manually decorticated and separated (bast and hurd) sectioned samples processed by Biolin. Inc. was used for this chemical determination. This work was conducted at the University of Manitoba, Animal Science Department Nutrition Laboratory on the returned decorticated samples from Biolin Inc. The samples were ground in a Techeor Mill equipped with a 1 mm round whole screen (Figure 3.5.7). The bast and hurd samples were ground separately for each main and sub plot treatment (Figure 3.5.8). After each section from each sub plot was ground, the three sections for each of the partitioned fibres were combined and reground into one sample, one sample for hurd and another for bast. Combining the ground sectioned samples aided in determining an approximate total chemical constitute profile of the bast and hurd separately from the entire stalk from each main and sub plot treatment.

ANKOM fibre analysis methods for determining Neutral Detergent Fibre (%NDF), Acid Detergent Fibre (%ADF), Acid Detergent Lignin (%ADL) and dry matter were used to determine the percentage of the chemical constituents present in bast and hurd separately. These ANKOM methods are described in detail in Appendix H 1-4 (AH.1; %NDF, AH.2; %ADF, AH.3; %ADL and AH.4 dry matter). These samples were analyzed with the assistance of the University of Manitoba Animal Science Nutrition Lab.

As presented in Toonen et al. (2004) the %ADL is considered to be the lignin part of the fibre based on dry matter. The % of ADF subtracted from the % of NDF is considered to be the % of hemicellulose in the fibre based on dry matter. The % of ADF subtracted from the % of lignin is considered to be the % of cellulose in the fibre based on dry matter. The % cellulose added to the % hemicellulose is considered to be the total amount of cellulose, holocellulose based on dry matter (Toonen et al. 2004). Calculations used in the present study are as follows:

**1. Neutral Detergent Fibre (%NDF) Calculation:**

Calculate percent NDF on a dry matter basis.

Bag Correction: Initial blank weight minus the final blank bag weight. A net loss is entered as a negative number a net gain is entered as a positive number.

$$\%NDF = \frac{(\text{Final Weight} - \text{Bag Weight} - \text{Bag Correction}) \times 100}{\text{Sample Weight on a dry matter basis}}$$

**2. Acid Detergent Fibre (%ADF) Calculation:**

Calculate percent ADF on a dry matter basis.

Bag Correction: Initial blank weight minus the final blank bag weight. A net loss is entered as a negative number a net gain is entered as a positive number.

$$\% \text{ ADF} = \frac{(\text{Final Weight} - \text{Bag Weight} - \text{Bag Correction})}{\text{Sample Weight on a dry matter basis}} \times 100$$

**3. Blank Correction Calculation:**

Calculate blank correction by dividing the weight loss upon ignition by the original blank bag weight.

W1=Bag Tare Weight

W2=Blank bag wt after acid digestion

W3=Weight loss upon ignition (W2 - ash wt)

$$\text{Blank correction (C1)} = (W3/W1)$$

**4. Calculation for Determining % Lignin:**

Calculate the % lignin by subtracting the blank correction (C1) from the weight loss upon ignition and dividing that number by the dry mattered sample weight and multiplying by 100.

W4=Sample weight expressed on a dry matter basis

W5=Sample and bag wt after acid digestion

W6=Weight loss upon ignition (W5 - ash wt)

$$\% \text{ Lignin} = [(W6 - C1)/W4] * 100$$

**5. Calculation for Determining % Hemicellulose:**

To determine hemicellulose acid Detergent fiber (ADF) is subtracted from neutral Detergent fiber (NDF).

$$\%NDF(DM) - \%ADF(DM) = \% \text{ Hemicellulose}(DM)$$

**6. Calculation for Determining % Cellulose:**

To determine cellulose Lignin is subtracted from acid Detergent fiber.

$$\% ADF(DM) - \%Lignin (DM) = \%Cellulose(DM)$$

**7. Calculation for % Dry matter:**

$$\frac{(W3 - W1)}{(W2)} \times 100 = \% DM$$



**Figure 3.5.1** Hand harvesting of hemp (A S-Hermann 2006)





**Figure 3.5.2** Height at harvest method (A S-Hermann 2007)



**Figure 3.5.3** Cutting of hemp stalk into three 20 cm sections. (A.S-Hermann 2006).



**Figure 3.5.4** Hemp stalk cut into three 20 cm sections (top, middle and bottom) (A S-Hermann 2006).





**Figure 3.5.5** Hemp stalk section in water retting tub (A S-Hermann 2006).



**Figure 3.5.6** Hemp bast fibre separating from hurd during water retting process (A S-Hermann 2006).



**Figure 3.5.7** Techeor Mill equipped with a 1 mm round whole screen (A S-Hermann 2007).





**Figure 3.5.8** Separated hemp bast and hurd were ground separately from each main and split treatment (A S-Hermann 2007).

### 3.6 Statistical analysis

Analyses of variance was performed on all measured variables using the Proc Mixed, Type 3, REML and CORR procedure in the Statistical Analysis System (SAS) software (SAS 2004; per. comm. Dr. G. Crow 2006-2008). Each site was analyzed separately by year due to annual variation in crop from year to year. Block was considered random and all other effects were considered fixed. Type 3 and Proc Mixed REML outputs were used to determine equal variances. Proc Univariate was used to determine normality by Shaprio-Wilk, Kolmogorov-Smirnov, Cramer-von Mises and Anderson-Darling test. Treatment means were compared using least square means (LSM) test at the  $P < 0.05$  level. When observed consistently over site years significant interaction effects are presented separately (Hamill 2002). When a significant interaction between plant density and harvest timing occurred the significant ANOVA factors for each treatment were nulled due to the treatment interaction.

## 4. RESULTS and DISCUSSION

### 4.1 Hand thinned populations

The targeted plant population densities in the present study were acquired by hand thinning plants after emergence. The practice of over seeding followed by hand thinning has been used previously in experiments involving the estimation of physiological and yield parameters in agricultural crops (Boerma 1977; Angadi and Entz 2002). In some crops such as chilies (*Capsicum spp.*), hand thinning helps to solve problems associated with low germination rates and post-emergence pests, diseases and environmental conditions (Lillywhite 2004).

Hand thinning to acquire specific plant populations has been employed in previous hemp field experiments (Struik 2000; Lisson and Mendham 2000a; Amaducci et al. 2002a; Toonen 2004). In these experiments, targeted plant populations ranged from 30 to 300 plants m<sup>-2</sup> after hand thinning.

Initial plant densities after hand thinning in the present study ranged from 86 to 296 plants m<sup>-2</sup>. Hand thinned plant population for the three subplot treatments are given in Table 4.1.1. In general, hand plant populations averaged across all treatments and sites were within 10% of the desired targeted plant population densities. A significant harvest timing x targeted plant population density interaction occurred at Grandview-07 and Dauphin-07 (Table 4.1.3).

**Table 4.1.1** Average hemp plant density (plants m<sup>-2</sup>) after hand thinning at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location				
	Gilbert Plains	Grandview		Dauphin	
	2006	2006	2007	2006	2007
	-----Average plant count after hand thinning-----				
Target (plants m <sup>-2</sup> )					
100	86	93	99	96	96
200	178	188	191	187	190
300	260	276	296	286	293

#### 4.1.2 Self-thinning

The difference in the plant population density after hand thinning in June and harvest in September was considered as the number of ‘self-thinned’ plants. In this study, hemp plant counts at harvest ranged from approximately 50 to 280 plants m<sup>-2</sup> while the initial hand thinned populations ranged from 86 to 296 plants m<sup>-2</sup>. Therefore, self-thinning ranged from approximately 1-120 plants m<sup>-2</sup> (1 – 40 %). Plant counts observed by Lisson and Mendham (2000a) in response to self-thinning were similar to that of the present study.

##### 4.1.2.1 Effect of harvest timing

Harvest timing did not significantly affect self-thinning (Table 4.1.2). Averaged across all site years, growing season plant losses averaged 17 plants m<sup>-2</sup> at harvest 1, 21 plants m<sup>-2</sup> at harvest 2 and 35 plants m<sup>-2</sup> at harvest 3. The present observations indicated

that plant losses due to self-thinning in the Canadian hemp cultivar, Alyssa, tend to increase over time.

In the present the number of hemp plants that died over time due to self-thinning tended to increase as harvest timing was delayed. Self-thinning is caused by individual plant competition effects for available resources such as light (van der Werf 1997). Van der Werf (1997) concluded that hemp plant losses increased with planting densities up to 270 plant m<sup>-2</sup>, and this loss was directly related to a decline of the radiation-use efficiency (RUE).

Self-thinning is not isolated to hemp. Self-thinning commonly occurs in most plant communities (Loomis and Connor 1992). Westoby (1976) and Lonsdale (1990) also determined greater self-thinning in dicots (such as subterranean clover (*Trifolium subterraneum*)) as harvest was delayed due to competition. After an extensive literature review Weller (1987) explained that in even-aged herbaceous dicots (such as rapeseed (*Brassica napus*), sunflower (*Helianthus annuus*) and alfalfa (*Medicago sativa*)) plant populations thin to a greater extent than herbaceous monocots (such as wheat (*Triticum*) and meadow fescue (*Festuca pratensis*)) with time. To the contrary, however, Longdale and Watkinson (1983) determined that the herbaceous monocot, meadow fescue (*Festuca pratensis*) thinned to a greater extent than the herbaceous dicots, corncockle (*Agrostemma githago* L.) or chicory (endive) (*Cichorium endivium* L.). van der Werf (1994a) concluded that the rate of self-thinning in three European hemp cultivars (Kompolti hybrid TC, Kompolti Hyper Elite and Kozuhara) was greater than that of other herbaceous dicots (such as rapeseed (*Brassica napus*), sunflower (*Helianthus annuus*) and alfalfa (*Medicago sativa*)) (Weller 1987) at the same growth rate.

Westoby (1976) determined that two of five clover (*Trifolium*) species suffered extensive die-off over time with increasing planting densities (plant counts not provided). Research conducted by Mediavilla et al. (2001) suggested that the majority of self-thinning in Swiss hemp crops occurred during the first phase of vegetative growth. These researchers concurred that increased plant loss was due to individual plant competition for spatial resources. These findings are similar to that of self-thinning counts in European hemp cultivars.

**Table 4.1.2** The effect of harvest timing and target plant population density on the total number of hemp plants self-thinned at all sites in the Parkland region of Manitoba 2006 and 2007.

Treatment	Location					
	Gilbert Plains		Grandview		Dauphin	
	2006	2006	2007	2006	2007	
-----Total number of plants self-thinned (m <sup>-2</sup> )-----						
Harvest timing 1	11	12	25	10	25	
Harvest timing 2	3	19	25	-1	61	
Harvest timing 3	11	33	63	10	59	
LSD ( $\alpha = 0.05$ )	15 <sub>NS</sub>	27 <sub>NS</sub>	16	16 <sub>NS</sub>	24	
Target (plants m <sup>-2</sup> )						
100	2b	3a	10	-2b	21	
200	6ba	20a	35	13a	44	
300	17a	41b	69	7ab	79	
LSD ( $\alpha = 0.05$ )	12*	19*	14	14*	16	
ANOVA	df	<i>P&gt;F</i>				
Harvest timing	2	0.4967	0.1564	0.001	0.091	0.0272
Plant density	2	0.0471*	0.0009*	<0.0001	0.0289*	<0.0001
Harvest*Density	8	0.8189	0.3445	0.0004*	0.1648	0.0271*

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant differences

Self-thinning plant losses were determined by subtracting plant counts after hand thinning from plant counts at harvest.

When a significant interaction between plant density and harvest timing occurred the significant ANOVA factors for each treatment were nulled due to the treatment interaction.

#### **4.1.2.3 Effect of the targeted plant population density**

A significant target plant population density effect on the number of plants self-thinned was observed at Gilbert Plains-06, Grandview-06 and Dauphin-06 (Table 4.1.2). Plant stands tended to decrease with increased planting densities. Across all site years, plant populations decreased by an average of 17 plants  $m^{-2}$  between 100 to 200 plants  $m^{-2}$  targeted plant population density and by an average of 19 plants  $m^{-2}$  between 200 to 300 plants  $m^{-2}$  targeted plant population density. It was observed across all site-years that as the targeted plant population density increased from 100 to 300 plants  $m^{-2}$  plant populations decreased by an average of 36 plants  $m^{-2}$ .

A significant harvest timing x target plant population density for hemp plant loss was observed at Grandview-07 and Dauphin-07 (Table 4.1.3). At both locations, the amount of self-thinning due to increased plant population was greater for harvest date 3 compared to harvest date 1. It was thus observed that self-thinning varied with population density and harvest timing.

**Table 4.1.3** The interaction effect of harvest timing and target plant population density on the total number of hemp plants self-thinned in Grandview-07 and Dauphin-07 sites.

Treatment		Location	
Harvest timing	Target (plants m <sup>-2</sup> )	Grandview	Dauphin
		Year	
		2007	
Total number of plants self-thinned (m <sup>-2</sup> )			
Harvest timing 1	100	17	8
	200	25	34
	300	31	32
Harvest timing 2	100	1	29
	200	19	46
	300	55	106
Harvest timing 3	100	10	26
	200	59	57
	300	120	98
	LSD ( $\alpha = 0.05$ )	33	40
ANOVA		<i>P&gt;F</i>	
	df		
Harvest timing	2	0.001	0.0272
Plant density	2	<0.0001	<0.0001
Harvest*Density	8	0.0004*	0.0271*

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant differences

Self-thinning plant losses were determined by subtracting plant counts after hand thinning from plant counts at harvest.



#### 4.1.2.4 Conclusions

Overall, the number of hemp plants that self-thinned, presumably due to intra-plant competition for available resources, generally increased as the targeted plant population density increased and as harvest was delayed. It was observed that the majority of thinning occurred at the targeted plant density of 300 plants  $m^{-2}$  and between harvest 2 and harvest 3. Thus, self-thinning in hemp tends to increase as plant density increases and as harvest timing is delayed. Self-thinning continued to occur after technical maturity. Thus, harvesting at technical maturity (harvest 1; decimal code 2102) would allow for collection of the majority of available plants in the field than at a later harvest date.

Self-thinning trends observed in the present study concurred in previous hemp field experiments (van der Werf 1999; Lisson and Mendham 2000a, 2002b; Amaducci et al. 2002a). From these studies, it appears that if hemp is planted at the upper plant populations of 300 plants  $m^{-2}$  the crop compensates through competition to the available environmental resources (light, soil moisture and nutrients) by reducing the total number of plants to a more sustainable population ( $\sim 250$  plants  $m^{-2}$ ).

This work has established that the Canadian hemp cultivar, Alyssa, is susceptible to self-thinning during the growing season and that plant density at harvest is a function of the initial plant density and harvest time. Density induced plant loss during the growing season can be prevented by assuring that the initial plant density does not exceed the maximum density capacity of a given area. Therefore, based on a professional review of the overall trends, the Canadian hemp cultivar, Alyssa, can be planted at a targeted

plant population of 180-250 plants m<sup>-2</sup> (50-70 kg ha<sup>-1</sup>) for optimal plant stand at harvest. These conclusions are similar to suggested plant densities in the European hemp industry.

## **4.2 Plant height**

In the present study, hemp plant heights ranged from 130 to 261 cm. Previous studies with the cultivar Alyssa reported average plant heights of 109 to 235 cm (PCDF 2004; Kostuik 2006). Depending on cultivar and location, maximum hemp plant heights in the Parkland region of Manitoba are typically reached by the first to second week of August. This timing is approximately 70 days after sowing which coincides with the present study's first harvest timing, decimal code 2102. In the present study, hemp plant height was significantly affected by the targeted plant population density at two site year but not by harvest timing. No interaction between plant density and harvest date occurred at any site year.

### **4.2.1 Effect of harvest timing**

Delaying harvest timing had no influence on hemp plant heights at any site year. However, Dauphin-07 approached significance at  $P=0.674$  ( $P < 0.10$ ). Plant height averaged 156 cm across all harvest timings. Plant height was relatively unchanged as harvest timing was delayed (Table 4.2.1). In the present study, harvest did not begin until hemp decimal code 2102 (technical maturity). Therefore, it appeared that the hemp crop had already maximized its height potential by this plant development stage. Previous hemp studies in the United Kingdom and Germany showed that hemp plant heights were not affected by harvest timing between hemp decimal codes of 2102 to 2307 (present studies Harvest 1 and Harvest 3, respectively) (Hendrischke et al. 1998; Bennett et al.

2006). Thus, Hendrichke et al. (1998) concluded that plant heights in hemp are influenced to a greater extent by location, vegetative interval, available resources and plant density than by harvest timing.

**Table 4.2.1** The effect of harvest timing and target plant population density on hemp plant height (cm) at all sites in the Parkland region of Manitoba in 2006 and 2007.

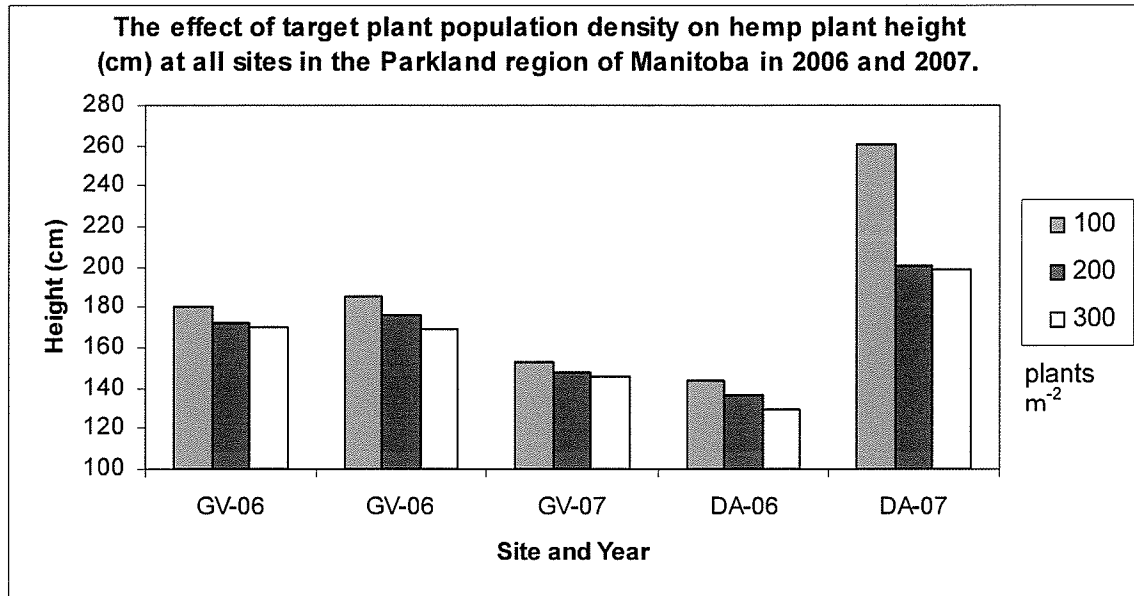
Treatment	Location					
	Gilbert Plains	Grandview		Dauphin		
	Year					
	2006	2006	2007	2006	2007	
	-----Height (cm)-----					
Harvest timing 1	161	176	149	143	146	
Harvest timing 2	173	178	150	138	149	
Harvest timing 3	179	176	149	131	150	
LSD ( $\alpha = 0.05$ )	10.5 NS	7.8 NS	11.4 NS	30.0 NS	9.6 NS	
Target (plants m <sup>-2</sup> )						
100	180	185 <i>a</i>	153	143	261 <i>a</i>	
200	172	176 <i>b</i>	148	139	201 <i>b</i>	
300	170	169 <i>c</i>	147	130	199 <i>b</i>	
LSD ( $\alpha = 0.05$ )	9 NS	6.4*	6.7 NS	13.6 NS	7.8*	
ANOVA	df	<i>P</i> > <i>F</i>				
Harvest timing	2	0.1635	0.6812	0.9798	0.6444	0.0674
Plant density	2	0.0656	0.0002*	0.1434	0.1365	<0.0001*
Harvest*Density	8	0.8313	0.9737	0.769	0.6835	0.3363

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

#### **4.2.2 Effect of the targeted plant population density**

A significant target plant population density effect on plant height was observed at Grandview-06 and Dauphin-07 but not at Gilbert Plains-06, Dauphin-06 or Grandview-07 (Table 4.2.1). At Grandview-06, hemp plant heights decreased by 9 and 7 cm ( $P < 0.05$ ) as target plant population density increased from 100 to 200 and 200 to 300 plants  $m^{-2}$ , respectively. At Dauphin-07, heights decreased by 62 and 60 cm ( $P < 0.05$ ) as target plant population density increased from 100 to 300 and from 100 to 200 plants  $m^{-2}$ , respectively. The effect of density on plant height was nearly significant ( $P < 0.10$ ) at Gilbert Plains-06, where  $P = 0.0656$ . Previous hemp experiments have determined that the relationship between plant height and plant density was initially positive early in the plant's life, but as the hemp plants mature, increasing the plant density resulted in shorter plants (Schurmann et al. 1999; Amaducci et al. 2002b). In the opinion of the author if the sample size was increased the effect of density on plant height would be pronounced more. Figure 4.2.2 depicts the overall trend of the effect of plant density on plant height in the cultivar Alyssa.



**Figure 4.2.2** The average hemp plant height by plant density and site-year in the Parkland region of Manitoba in 2006 and 2007.

#### 4.2.3 Conclusions

In the present study, hemp plants grown at lower plant population densities were taller than plants grown at higher plant population densities. The present findings concur with several hemp studies conducted in Europe which observed greater plant heights at maturity with lower plant densities (Schumann et al. 1999; Stuik et al. 2000; Lisson and Mendham 2000a; Amaducci et al. 2002b; Bennett et al. 2006). Lisson and Mendham (2000a) suggested that the inverse relationship between height and plant density was due to an increase in crop competition effects. At higher plant population densities, greater competition for available resources, such as light (Ballaré 2006) and space (Schafer and Honermeier 2006) results in shorter plants. At higher plant densities available assimilate resources are limited depressing stalk elongation and therefore at some point upward growth ceases and overall plant height is reduced. Results of the study suggest that hemp is a density-dependent plant with respect to plant height (Loomis and Connor 1992;

Lockwood 2006) and that plant height in the cultivar Alyssa, tended to decrease as plant density increase.

### **4.3 Stem diameter**

Stem diameter in crops such as maize (*Zea mays*), sunflowers (*Helianthus annuus*) and kenaf (*Hibiscus cannabinus* L.) are known to be directly affected by inter-plant spacing. Even though Canada does not have an industry wide grading system for fibre hemp, stem diameter may become an important quality classification marker for hemp fibre production in the future. In Hungary for instance, grading of raw hemp fibre stalks is based upon seven different quality classifications. One of these classifications is based on the maximum thickness of 85% of the hemp stems in the grading sample. The stem diameter classification for quality is as follows: grade I (10 mm), grade II (12 mm) and grade III (14 mm) (Bócsa and Karus 1998). As suggested by Ranalli (1999) the quantity of hurd of the hemp stalks in the present study was proportional to stem diameter. Stem diameter and hurd content were found to be significantly ( $P < 0.05$ ) correlated ( $r^2 = 0.49$ ) across all stalk sections.

Hemp stem diameters in Canada and abroad range from 4-26 mm depending upon plant spacing and cultivar (Scheifele et al. 1996; Bócsa and Karus 1998). In the present study stem diameter was measured separately in each of the three 20 cm stalk sections where stem diameters ranged from 1.5-7.6 mm. The bottom section represents the stem from 25 to 45 cm above the soil surface. The middle and top sections represent 65 to 85 cm and 105 to 125 cm above the soil level, respectively. Data from each treatment effect

on stalk section i.e. top, middle and bottom will be presented and discussed separately. Stem diameter data was not collected at Gilbert Plains-06.

#### **4.3.1 Effect of harvest timing on stem diameter**

The main plot treatment, harvest timing, did not have a significant ( $P > 0.05$ ) effect on average stem diameter of any of the three stem sections in this study (Table 4.3.1, 4.3.2 and 4.3.4). Although not statically different ( $P < 0.05$ ) at Dauphin-07 the affect of harvest timing approached significance at  $P=0.0966$  ( $P < 0.10$ ), where the top stem diameter increased as harvest was delayed. The lack of significant timing effects was most likely due to the fact that stem diameter had reached its maximum width by the first harvest timing. These results are similar to a study conducted in Italy by Mambelli and Grandi (1995), where the basal stem diameter of kenaf (an annual fibre crop) was not significantly affected by delaying harvest timing. No literature was found which examined the effects of harvest timing on the stem diameter of hemp.

#### **4.3.2 Effect of the targeted plant population density on stem diameter**

Stem diameters in all stalk sections; i.e., bottom, middle and top, were significantly ( $P < 0.05$ ) decreased by high targeted plant population densities at Dauphin-06, Grandview-06 and Grandview-07 (Table 4.3.1, 4.3.2 and 4.3.4). However, at Grandview-06, the targeted plant density did not significantly ( $P > 0.05$ ) affect the middle stem diameter. Dauphin-07 was significantly affected by the treatment interaction (Table 4.3.3).

### 4.3.3 Bottom stalk section

At all site years, the bottom stem diameter significantly decreased as the targeted plant population density increased (Table 4.3.1). In general, the largest decrease in stem diameter occurred between 100 and 200 plants  $m^{-2}$ . The stem diameter of the bottom section never differed ( $P > 0.05$ ) as the targeted plant population increased from 200 to 300 plants  $m^{-2}$ .

At Grandview-06, the bottom stem diameter decreased by 0.8 mm ( $P < 0.05$ ) as the targeted plant population density increased from 100 to 200 plants  $m^{-2}$ . As the targeted plant population density increased from 100 to 300 plants  $m^{-2}$  the bottom stem diameter decreased by 1.2 mm. At Dauphin-06, the bottom stem diameter decreased by 0.6 mm as the targeted plant population density increased from 100 to 200 plants  $m^{-2}$ . As the targeted plant population density increased from 100 to 300 plants  $m^{-2}$  the bottom stem diameter decreased by 0.9 mm. At Grandview-07, the bottom stem diameter decreased ( $P < 0.05$ ) by 0.6 mm as the targeted plant population density increased from 100 to 300 plants  $m^{-2}$ . However, no significant difference in stem diameter ( $P > 0.05$ ) was observed at Grandview-07 as the targeted plant population density increased from 100 to 200 plants  $m^{-2}$ . At Dauphin-07, the bottom stem diameter decreased by 0.6 mm as the plant population increased from 100 to 200 plants  $m^{-2}$ .



**Table 4.3.1** The effect of harvest timing and target plant population density on stem diameter (mm) in the bottom hemp section (25-45 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location				
	Gilbert Plains	Grandview		Dauphin	
	2006	2006	2007	2006	2007
	Bottom Section -----Stem Diameter (mm)-----				
Harvest timing 1		4.2	4.6	3.0	6.9
Harvest timing 2		4.6	4.3	3.2	6.8
Harvest timing 3		4.6	4.2	2.8	7.3
LSD ( $\alpha = 0.05$ )		0.8 NS	0.8 NS	0.7 NS	0.8 NS
Target (plants m <sup>-2</sup> )					
100		5.1 <i>a</i>	4.7 <i>a</i>	3.5 <i>a</i>	7.5 <i>a</i>
200		4.3 <i>b</i>	4.4 <i>ab</i>	2.9 <i>b</i>	6.9 <i>b</i>
300		3.9 <i>b</i>	4.1 <i>b</i>	2.6 <i>b</i>	6.5 <i>b</i>
LSD ( $\alpha = 0.05$ )		0.5*	0.4*	0.5*	0.5*
ANOVA	df	$P > F$			
Harvest timing	2	0.3764	0.5442	0.4971	0.3434
Plant density	2	0.0014*	0.0341*	0.0032*	0.0017*
Harvest*Density	8	0.3657	0.547	0.6329	0.2406

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

Stem diameter data was not collected from Gilbert Plains 2006.

#### 4.3.4 Middle stalk section

A significant target plant population density effect on the middle section stem diameter was observed at Dauphin-06 only (Table 4.3.2). In all cases, stem diameter decreased ( $P < 0.05$ ) as the targeted plant population density increased. At Dauphin-06, the middle stem diameter was unchanged as the targeted plant population density increased from 100 to 200 plants  $m^{-2}$ . However, as the targeted plant population density increased from 100 to 300 plants  $m^{-2}$ , middle stem diameter decreased by 0.6 mm ( $P < 0.05$ ). Although not statically different ( $P < 0.05$ ) at Grandview-07 the affect of the target plant population density approached significance at  $P=0.0518$ , where the middle stem diameter decreased as density increased. Thus, the middle section stem diameter was affected to a greater extent by increasing the targeted plant population from 100 to 200 plants  $m^{-2}$ , than by an increase in density from 200 to 300 plants  $m^{-2}$ .

A significant harvest timing x target plant population density interaction effect on the middle section stem diameter occurred at Dauphin-07 (Table 4.3.3). The stem diameter decline effect at higher targeted plant population density was greatest at harvest 3 compared to the two earlier harvests.

**Table 4.3.2** The effect of harvest timing and target plant population density on stem diameter (mm) in the middle hemp section (65-85 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Gilbert Plains		Grandview		Dauphin	
	2006		2006	2007	2006	2007
Middle Section -----Stem Diameter (mm)-----						
Harvest timing 1			4.3	4.3	2.7	6.1
Harvest timing 2			4.2	3.7	2.8	6.3
Harvest timing 3			4.0	3.8	2.3	7.1
LSD ( $\alpha = 0.05$ )			0.7 <sub>NS</sub>	0.7 <sub>NS</sub>	0.6 <sub>NS</sub>	0.8
Target (plants m <sup>-2</sup> )						
100			4.3	4.3	2.9 <sub>a</sub>	7.6
200			3.9	3.6	2.8 <sub>a</sub>	6.0
300			4.0	3.8	2.3 <sub>b</sub>	5.7
LSD ( $\alpha = 0.05$ )			0.4 <sub>NS</sub>	0.5 <sub>NS</sub>	0.4*	0.5
<hr/>						
ANOVA	df		$P > F$			
Harvest timing	2		0.7371	0.1	0.4487	0.071
Plant density	2		0.1193	0.0518	0.0089*	<0.0001
Harvest*Density	8		0.1883	0.9971	0.8196	0.035*

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

Stem diameter data was not collected from Gilbert Plains 2006.

When a significant interaction between plant density and harvest timing occurred the significant ANOVA factors for each treatment were nulled due to the treatment interaction.

**Table 4.3.3** The interaction effect of harvest timing and target plant population density on the middle section (at 65-85 cm) stem diameter (mm) at the Dauphin-07 site.

Treatment		Location
Harvest timing	Target (plants m <sup>-2</sup> )	Dauphin
		Year
		2007
		Middle Section --Stem diameter (mm)--
Harvest timing 1	100	7.1
	200	5.8
	300	5.3
Harvest timing 2	100	6.9
	200	5.7
	300	6.1
Harvest timing 3	100	8.8
	200	6.6
	300	5.8
LSD ( $\alpha = 0.05$ )		1.3
ANOVA		$P > F$
Harvest timing	2	0.071
Plant density	2	<0.0001
Harvest*Density	8	0.035*

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

#### **4.3.5 Top stalk section**

At all site years, target plant population density significantly decreased the top stem diameter (Table 4.3.4). At both locations in 2006 and at Dauphin-07, the significant density effect on the top stem diameter was observed between 100 to 200 plants  $\text{m}^{-2}$ , with no further decrease ( $P < 0.05$ ) in stem diameter as the targeted plant population density increased to 300 plants  $\text{m}^{-2}$ . However, at Grandview-07, the greatest effect of plant density on the top stem diameter occurred between 200 and 300 plants  $\text{m}^{-2}$ . Overall, the greatest and most frequent decrease in top stem diameter resulted from increasing the plant population increased from 100 to 200 plants  $\text{m}^{-2}$ .

**Table 4.3.4** The effect of harvest timing and target plant population density on stem diameter (mm) in the top hemp section (105-125 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location				
	Gilbert Plains	Grandview		Dauphin	
	2006	2006	2007	2006	2007
Top Section -----Stem Diameter (mm)-----					
Harvest timing 1		3.5	3.9	2.1	5.4
Harvest timing 2		3.8	3.1	2.1	5.7
Harvest timing 3		3.5	3.4	1.8	6.3
LSD ( $\alpha = 0.05$ )		0.5 NS	0.7 NS	0.8 NS	0.8 NS
Target (plants m <sup>-2</sup> )					
100		4.1 <i>a</i>	3.8 <i>a</i>	2.5 <i>a</i>	6.7 <i>a</i>
200		3.4 <i>b</i>	3.7 <i>a</i>	1.9 <i>b</i>	5.5 <i>b</i>
300		3.3 <i>b</i>	2.9 <i>b</i>	1.5 <i>b</i>	5.0 <i>b</i>
LSD ( $\alpha = 0.05$ )		0.4*	0.6*	0.5*	0.6*
<hr/>					
ANOVA	df	<i>P</i> > <i>F</i>			
Harvest timing	2	0.3815	0.1153	0.6929	0.0966
Plant density	2	0.0009*	0.0129*	0.0033*	<0.0001*
Harvest*Density	8	0.9123	0.9536	0.2808	0.727

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

Stem diameter data was not collected from Gilbert Plains 2006.

#### 4.3.6 Conclusions

At all site years, harvest timing did not have a significant affect on the average stem diameter ( $P > 0.05$ ) of the hemp stalk. Therefore, the maximum stem diameter in this trial was achieved prior to harvest timing 1 (i.e., technical maturity).

Plant population density did, however, strongly influence average hemp stem diameter. It was observed that decreasing the area per plant by increasing the targeted plant population density reduced the average stem diameter in all stalk sections. The greatest reduction in stem diameter in the present study was observed as the targeted plant population increased from 100 to 200 plants  $m^{-2}$ . Thus stem diameter was maximized at a targeted population of 100 plants  $m^{-2}$ . Averaged across all site years stem diameter in the bottom and top section were found to be positively ( $P < 0.05$ ) correlated ( $r = 0.83$ ) with plant height and negatively correlated ( $r = 0.38$ ) with plant density at harvest. The correlations further support the relationship findings between planting densities, stem diameters and crop height at harvest.

European (Schumann et al. 1999; Lisson and Mendham 2000a; Amaducci et al. 2002a, 2002b; Höppner et al. 2004; Svennerstedt and Svensson 2006; Schäfer and Honermeier 2006) and Canadian (Scheifele et al. 1996) researchers also determined that stem diameter in hemp was inversely related to plant population density ranging from 50 to 350 plants  $m^{-2}$ . Similar to the present study, von Wettberg and Weiner (2003) found that the stem diameter of rapeseed (*Brassica napus*) decreased with decreasing distance to other individual plants.

Depending upon end use, stem diameters ranging from 2 to 14 mm are considered to be ideal for hemp fibre production (Bósca and Karus 1998; Olsen 2004; Lewin 2007).

Therefore, hemp stem diameters observed in the present study are within the range of high quality fibre hemp. For paper pulping, for example, quantity of hurd depends upon stem diameter (Ranalli 1999). As the stem diameter decreases the quantity of hurd tends to increase in comparison to that of the bast fibre (Ranalli 1999). Even though no general hemp fibre grading standards exist in Canada for stem diameter, in some countries such as Hungary, stem diameter is used as one of the criteria for hemp fibre quality for cordage production. In the Hungarian hemp grading system a sample the maximum stem thickness of 85% of the hemp stem diameters fall into three grades, i.e., I, II and III with diameters of 10, 12 and 14 mm, respectively (Bósca and Karus 1998). Smaller stem diameters in hemp are advantageous for decortication of bast and hurd fibres. These decorticated fibres are used for high grade textiles where fineness of the bast fibres are important.

In conclusion, hemp stem diameters observed in the present study appear to be a function of plant density rather than harvest timing. These findings are supported by those of Amaducci et al. (2002b). Thus, as the spatial area between individual plants increases, stem diameters also increase.

#### **4.4 Fibre yield components**

##### **4.4.1 Biomass**

Biomass yield, in the present study, represents total oven dried stalk, stripped of all inflorescences, branches and leaf material. As in previous studies, the present study's biomass yields assumes 100% harvest, as there was no allowance made for harvest losses from equipment and handling (per. comm. J. Kostuik 2006-2008).



In Canada, biomass yields of dual-purpose industrial hemp cultivars average between 762 and 1,524 kg ha<sup>-1</sup> of dry stalk biomass. These averages, dependent upon cultivar, represent swathed stubble after grain harvest (MAFRI 2006; per. comm. K. Watson 2007). In Canada, biomass yields for hemp grown for fibre-only production average between 1,000 and 6,000 kg ha<sup>-1</sup> (MAFRI 2006; per. comm. K. Watson 2006). Biomass yields in the present study represent hemp grown for fibre-only production. Yields from the 2006 and 2007 experimental studies ranged from 3,599 to 6,967 kg ha<sup>-1</sup>, similar to biomass yields were observed by Kostuik (2006) for the cultivar Alyssa.

#### **4.4.1.1 Effects of harvest timing**

Biomass was not significantly ( $P > 0.05$ ) affected by harvest timing at any site year (Table 4.4.1.1). Across all site years, however, biomass on average yielded 4,442 kg ha<sup>-1</sup> at harvest 1, 4,838 kg ha<sup>-1</sup> at harvest 2 and 4,971 kg ha<sup>-1</sup> at harvest 3. Although not statically different ( $P < 0.05$ ) at Dauphin-07 and Grandview-07 the affect of harvest timing was nearly significance ( $P < 0.05-0.10$ ) at  $P = 0.0656$  and  $P = 0.0644$ , respectively, where the biomass tended to increased as harvest was delayed. Even though not significant, the data suggest that in the selected environments harvesting after harvest 1 (technical maturity) could result in continued dry matter accumulation beyond technical maturity. Yield gains in hemp as proposed by Dewey (1916) continue because the weight of the hurd is about five times that of the bast fibre. Thus the increase in biomass as harvest is delayed is therefore related to an increase in percentage of hurd fibre present in the stalk.

#### 4.4.1.2 Effects of targeted plant population density

Above ground biomass was significantly affected by target plant population density at Grandview-06 only (Table 4.4.1.1). At Grandview-06, biomass was unchanged as plant density increased from 100 and 200 and from 200 to 300 plants  $m^{-2}$ . However, an increase in biomass ( $P < 0.05$ ) did occur as the planting density increased from 100 to 300 plants  $m^{-2}$ . On average, biomass yielded 4,580  $kg\ ha^{-1}$  at 100 plants  $m^{-2}$ , 4,834  $kg\ ha^{-1}$  at 200 plants  $m^{-2}$  and 4,808  $kg\ ha^{-1}$  at 300 plants  $m^{-2}$ . Biomass and plant density were not significantly ( $P > 0.05$ ) correlated ( $r = 0.03\%$ ). Results of the present study do not generally support those of Scheifele et al. (1996). Scheifele et al. (1996) observed that at lower plant densities hemp should result in greater biomass yields due to the thicker and longer stems. The present observations suggest, therefore, that the biomass of hemp follows the law of constant yield. Constant yields occur when the targeted plant population density is adequately high and resources become limited, the effects of competition results in a constant biomass due to a relative decrease in the size of individual plants (Caldwell and Shifflett 1992). The law of constant yield is explained further in section 4.4.1.4.

**Table 4.4.1.1** The effect of harvest timing and target plant population density on hemp biomass ( $\text{kg ha}^{-1}$ ) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location					
	Gilbert Plains	Grandview		Dauphin		
	Year					
	2006	2006	2007	2006	2007	
	-----Biomass ( $\text{kg ha}^{-1}$ )-----					
Harvest timing 1	4949	4296	3350	3867	5750	
Harvest timing 2	5307	4929	4069	3874	6011	
Harvest timing 3	5445	4744	3902	3797	6967	
LSD ( $\alpha = 0.05$ )	1179 <sub>NS</sub>	982 <sub>NS</sub>	615 <sub>NS</sub>	1431 <sub>NS</sub>	1041 <sub>NS</sub>	
Target ( $\text{plants m}^{-2}$ )						
100	5295	4186 <i>b</i>	3599	3685	6135	
200	5216	4710 <i>ab</i>	3783	3964	6497	
300	5191	5074 <i>a</i>	3789	3889	6097	
LSD ( $\alpha = 0.05$ )	739 <sub>NS</sub>	595*	354 <sub>NS</sub>	731 <sub>NS</sub>	711 <sub>NS</sub>	
ANOVA	df	$P > F$				
Harvest timing	2	0.5971	0.2574	0.0644	0.9894	0.0656
Plant density	2	0.9536	0.0217*	0.2577	0.7129	0.5340
Harvest*Density	8	0.1156	0.1526	0.2274	0.6617	0.4875

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

#### 4.4.1.3 Conclusions

Biomass was unaffected as harvest was delayed (Table 4.4.1.1). Biomass yield increases beyond technical maturity were not observed at any of the five site years. The current findings are different to those of a previous Canadian hemp study where biomass was observed to increase as harvest was delayed from 30 to 60 to 90 day after sowing (Kamat et al. 2002). The increase observed by Kamat et al. (2002) in biomass yield from initial harvest (decimal code 2102) to seed maturity (decimal code 2307) has been determined to be related to the production of highly lignified shorter and thicker cell walled secondary hurd fibres (Hoffman 1961 cited in: Mediavilla et al. 2001; Bennett et al. 2006). Results of the present study are similar to Bennett et al. (2006) who observed that the stem dry matter yield (biomass) of European hemp cultivars did not differ statistically with delayed harvest from approximated decimal code 2301 to 2305. As in our study, they determined that the yield was maximized at technical maturity. Meijer et al. (1995) determined that biomass yield in dual-purpose hemp cultivars, such as Alyssa, actually decreased after flowering. They suggested that biomass dropped with delaying harvest, due to canopy senescence and fat and protein synthesis in the seed (Ranalli 1999).

Biomass remained mostly unchanged with increases in the target plant population density (Table 4.4.1.1). It was observed that similar biomass yield at low and high planting densities observed in this study were the result of crop compensation. This form of compensation for resources follows the law of final constant yield (Lockwood 2006; Kira et al. 1953). This widely accepted law states that beyond a certain plant density, the effects of competition for limiting resources will result in a constant biomass due to a

relative decrease in the size of the individual plants within the stand (Caldwell and Shifflett 1992; Lockwood 2006). Eventually, the population reaches a level where any increase in the mass of some individual plants at lower planting densities is offset by increased plant losses and a reduction in individual stem size at higher plant densities (Hay and Walker 1989). This conclusion is supported by the fact that in the present study as the targeted plant population density increased the number of plants lost due to self-thinning increased and stem diameter decreased. The biomass thus plateaus at the minimum or at a modest plant density (Milthorpe and Moorby 1974). The law of constant yield explains why biomass was relatively unaffected by increasing plant density.

#### **4.4.2 Bast fibre**

Hemp bast fibres are considered to be the world longest and strongest natural fibres. Bast fibres can be used in a wide range of fibre based products from non-wovens to fine textiles. In addition, hemp bast fibres are easier to extract than other herbaceous annuals such as ramie (*Boehmeria nivea*) bast fibres (Needham and Kuhn 1994).

In the present study, bast fibre percentages ranged from 22-25% in the bottom stalk section, 18-29% in the middle stalk section and 7-30 % in the top stalk section. The percentage of bast fibre in the hemp stalk is approximated to be 10-40% (Lloyd and Seber 1996). Limited literature is currently available on the effects of harvest timing on the percentage of bast fibre allocated in the hemp stalk. However, some literature was found pertaining to bast fibre percentage and plant population density (van der Werf et al. 1995a; Cromack 1998; Bennett et al. 2006).

#### **4.4.2.1 Effect of harvest timing**

#### **4.4.2.2 Bottom stalk section**

Harvest timing significantly affected the percentage of bast fibre present in the bottom section at the Grandview-07 site only (Table 4.4.2.1). It is generally presumed that maximum bast fibre percentage is achieved at technical maturity. At Grandview-07, the percentage of bast fibre in the bottom section averaged 26% at harvest 1, 22% at harvest 2 and 25% at harvest 3. Even though not significant at  $P < 0.05$ , the percentage of bast fibre approached significance ( $P < 0.10$ ), at Grandview-06 ( $P = 0.0827$ ), where the percentage of bast fibre in the bottom section slightly increased (23 to 25%) as harvest was delayed (Table 4.4.2.1).

#### **4.4.2.3 Middle stalk section**

The percentage of bast fibre in the middle section averaged 24% at harvest 1, 24% at harvest 2 and 26% at harvest 3 across all site years. Harvest timing significantly affected the percentage of bast fibre present in the middle section at the Gilbert Plains-06 and Grandview-06 but not at Dauphin-06, Dauphin-07 or Grandview-07 (Table 4.4.2.2).

At Gilbert Plains-06, the percentage of bast fibre located in the middle stalk section increased ( $P < 0.05$ ) by approximately 2% as harvest timing was delayed from harvest 1 to 2, and 3% as harvest timing was delayed from harvest 1 to harvest 3. However, the percentage of bast fibre in the middle section was not significantly different as harvest timing was delayed from harvest 2 to harvest 3. At Grandview-06, the percentage of bast fibre located in the middle stalk section was only significantly affected

by delaying harvest from harvest 1 to harvest 2 where bast fibre percentage increase by 3%.

#### **4.4.2.4 Top stalk section**

Harvest timing significantly affected the percentage of bast fibre present in the top section at the Gilbert Plains-06, Grandivew-06 and Grandview-07 but not at Dauphin-06 or Dauphin-07 (Table 4.4.2.3). At both Gilbert Plains-06 and Grandview-06, the percentage of bast fibre located in the top stalk section increased ( $P < 0.05$ ) by approximately 5% as harvest was delayed from harvest 1 to 2. The percentage of bast fibre increased ( $P < 0.05$ ) by approximately 8% as harvest was delayed from harvest 1 to 3. However, the percentage of bast fibre was unaffected ( $P < 0.05$ ) by delaying harvest timing from harvest 2 to harvest 3.

At Grandview-07, the percentage of bast fibre located in the top section increased by 5% ( $P < 0.05$ ) as harvest was delayed from harvest 1 to harvest 3. An increase in bast fibre of 7% ( $P < 0.05$ ) occurred as harvest was delayed from harvest 2 to harvest 3. However, the percentage of bast fibre was unaffected by delaying harvest timing from harvest 1 to harvest 2.

Even though not significant at  $P < 0.05$ , the percentage of bast fibre approached significance ( $P < 0.10$ ), at Dauphin-06 and -07 ( $P = 0.0803$  and  $0.0752$ , respectively), the percentage of bast fibre in the top section increased slightly as harvest was delayed (Table 4.4.2.3).

#### **4.4.2.5 Effect of targeted plant population density**

#### **4.4.2.6 Bottom stalk section**

The percentage of bast fibre in the bottom stalk section was significantly affected by increasing the targeted plant population density at Dauphin-07 only (Table 4.4.2.1). The percentage of bast fibre in the bottom section increased ( $P < 0.05$ ) by 2% as plant density increased from 100 to 200 plants  $m^{-2}$ . However, as the targeted plant population increased from 200 to 300 or from 100 to 300 plants  $m^{-2}$  the percentage of bast fibre was not significantly affected.



**Table 4.4.2.1** The effect of harvest timing and target plant population density on the percentage of bast fibre in the bottom hemp section (25-45 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location					
	Gilbert Plains	Grandview		Dauphin		
	Year					
	2006	2006	2007	2006	2007	
	Bottom Section ----- Bast fibre (%) -----					
Harvest timing 1	24	23	26 <i>a</i>	23	24	
Harvest timing 2	24	25	22 <i>b</i>	23	23	
Harvest timing 3	24	25	25 <i>a</i>	25	25	
LSD ( $\alpha = 0.05$ )	1.4 NS	1.8 NS	2.4*	3 NS	2.9 NS	
Target (plants m <sup>-2</sup> )						
100	24	25	24	23	23 <i>b</i>	
200	24	25	24	22	25 <i>a</i>	
300	24	24	25	24	24 <i>ab</i>	
LSD ( $\alpha = 0.05$ )	0.6 NS	1.5 NS	1.2 NS	2.6 NS	1.6*	
ANOVA	df	<i>P &gt; F</i>				
Harvest timing	2	0.5817	0.0827	0.0125*	0.3721	0.2393
Plant density	2	0.5976	0.4185	0.3578	0.5995	0.0473*
Harvest*Density	8	0.0738	0.8344	0.5024	0.8399	0.1134

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

The percentage of bast fibre has been rounded up to the nearest whole number.

#### 4.4.2.7 Middle stalk section

The percentage of bast fibre ( $P > 0.05$ ) present in the middle stalk section was not significantly affected by increasing the target plant population density in any site year (Table 4.4.2.2).

**Table 4.4.2.2** The effect of harvest timing and target plant population density on the percentage of bast fibre in the middle hemp section (65-85 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location					
	Gilbert Plains	Grandview		Dauphin		
		Year		Year		
	2006	2006	2007	2006	2007	
	Middle Section -----Bast fibre (%) -----					
Harvest timing 1	23 <i>b</i>	23 <i>b</i>	26	20	28	
Harvest timing 2	25 <i>a</i>	26 <i>a</i>	23	20	26	
Harvest timing 3	26 <i>a</i>	25 <i>ab</i>	24	24	29	
LSD ( $\alpha = 0.05$ )	1.8*	3.3*	3.3 NS	6.5 NS	3.6 NS	
Target (plants m <sup>-2</sup> )						
100	24	23	23	22	27	
200	24	25	25	22	28	
300	25	25	25	18	28	
LSD ( $\alpha = 0.05$ )	1.04 NS	2.9 NS	2.8 NS	4.2 NS	1.9 NS	
ANOVA	df	$P > F$				
Harvest timing	2	0.0054*	0.0436*	0.1682	0.2114	0.2024
Plant density	2	0.4143	0.5805	0.3615	0.096	0.4037
Harvest*Density	8	0.4315	0.3171	0.7515	0.2775	0.7022

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

The percentage of bast fibre has been rounded up to the nearest whole number.

#### 4.4.2.8 Top stalk section

The percentage of bast fibre present in the top stalk section was significantly affected by increasing the target plant population density at Grandview-06 and Dauphin-06 only (Table 4.4.2.3). At Grandview-06, bast fibre decreased ( $P < 0.05$ ) by 5% as the targeted plant population density was increased from 100 to 200 plants  $m^{-2}$ . Bast fibre decreased ( $P < 0.05$ ) by 6% as the targeted plant population density increased from 100 to 300 plants  $m^{-2}$  (Table 4.4.2.3). There was no significant change in the bast fibre percentage in the top section as the targeted plant density increased from 200 to 300 plants  $m^{-2}$ . At Dauphin-06, the percentage of bast fibre decreased ( $P < 0.05$ ) by 8% as the target density increased from 100 to 200 plants  $m^{-2}$ . However, the percentage of bast fibre was not significantly different amongst the other targeted plant densities.

**Table 4.4.2.3** The effect of harvest timing and target plant population density on the percentage of bast fibre in the top hemp section (105-125 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location					
	Gilbert Plains	Grandview		Dauphin		
	Year					
	2006	2006	2007	2006	2007	
	Top Section -----Bast fibre (%) -----					
Harvest timing 1	16 <i>b</i>	12 <i>b</i>	16 <i>b</i>	7	28	
Harvest timing 2	20 <i>a</i>	18 <i>a</i>	14 <i>b</i>	16	26	
Harvest timing 3	24 <i>a</i>	21 <i>a</i>	21 <i>a</i>	9	30	
LSD ( $\alpha = 0.05$ )	3.4*	5.2*	4.9*	8.3 <sub>NS</sub>	3.6 <sub>NS</sub>	
Target (plants m <sup>-2</sup> )						
100	20	21 <i>a</i>	19	15 <i>a</i>	27	
200	20	16 <i>b</i>	17	11 <i>ab</i>	28	
300	19	15 <i>b</i>	16	7 <i>b</i>	28	
LSD ( $\alpha = 0.05$ )	2.7 <sub>NS</sub>	4.5*	4.2 <sub>NS</sub>	4.8*	2.2 <sub>NS</sub>	
ANOVA	df	$P > F$				
Harvest timing	2	0.0029*	0.0163*	0.0243*	0.0803	0.0752
Plant density	2	0.4568	0.0435*	0.416	0.0255*	0.5567
Harvest*Density	8	0.6303	0.5848	0.8845	0.1278	0.6554

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

The percentage of bast fibre has been rounded up to the nearest whole number.

#### 4.4.2.9 Conclusions

Harvest timing significantly affected the percentage of bast fibre in the bottom stalk section at one site-year, in the middle stalk section at two site-years and in the top stalk section at three site-years. The results of the present study suggest a tendency for an slight increase in the percentage of bast fibre with delaying harvest. This increase presumably occurred because of continued upward plant growth throughout time, especially in the top section. Thus, harvesting at technical maturity (i.e. harvest 1) tended to result in optimal bast fibre percentage. The targeted plant population density did not significantly affect the percentage of bast fibre in the middle section. However, plant density significantly affected the percentage of bast fibre in bottom stalk section at two site years and in the top stalk section at one site-year. Therefore, the percentage of bast fibre remained relatively stable with increasing plant densities.

The effects of plant density on bast fibre were similar to that of a previous hemp study conducted by Bennett et al. (2006) in the United Kingdom where the percentage of bast fibre in the total stalk was relatively unchanged (13.4 to 13.2%) as plant density increased from 150 to 300 seeds m<sup>-2</sup>. Conversely, Cromack (1998) and van der Werf et al. (1995a) noted that the percentage bast fibre at the mid-point tended to increase with increasing plant population densities in hemp.

Overall, the results from the present study show that the percentage of bast fibre present in the three stalk sections i.e. top, middle and bottom was relatively unchanged as harvest timing was delayed and as the target plant population density was increased.

In order to decrease the amount of samples used to determine fibre content van der Werf et al. (1994c) suggested that the stalk be analyzed at the mid-point (at

approximately 20 to 30% of the total stem height). This would thus provide a general percentage of fibre of the overall stalk yield without the need for multiple sampling points. The data from the present study supports this recommendation.

#### **4.4.3 Hurd fibre**

The available literature provided little information on the effects of harvest timing and target plant population density on the percentage of hemp hurd fibre. However, it has been suggested that the quantity of hurd present in the hemp stalks is proportional to stem diameter (Ranalli 1999). This suggests that stem diameter and hurd content should be positively correlated. In the present study the correlation between stem diameter the hurd fibre content in each corresponding section was significant ( $P < 0.05$ ) at  $r^2$  values of 0.58 in bottom section, 0.20 in the middle section and 0.33 the top section. The percentage of hurd fibre was greater in 2007 than in 2006, due to location, crop variation and environmental conditions.

##### **4.4.3.1 Effect of harvest timing**

##### **4.4.3.2 Bottom stalk section**

Harvest timing significantly affected the percentage of hurd fibre present in the bottom section at Gilbert Plains-06, Grandview-07 and Dauphin-07 but not at Grandview-06 or Dauphin-06 (Table 4.4.3.1). The percentage of hurd fibre in the bottom section across all site years, as determined in SAS programming, averaged 63% at harvest 1, 60% at harvest 2 and 61% at harvest 3. The percentage of hurd fibre in the

bottom stalk section was not statistically different as harvest timing was delayed from harvest 2 to harvest 3 in all site years (Table 4.4.3.1).

At Gilbert Plains-06, the percentage of hurd fibre in the bottom stalk section increased ( $P < 0.05$ ) by 2% as harvest was delayed from harvest 1 to 2. The percentage of hurd increased by 3% ( $P < 0.05$ ) as harvest was delayed from harvest 1 to 3. At Grandview-07 and Dauphin-07, opposite results were produced in the bottom where the percentage of hurd fibre decreased by an average of 7.5% as harvest was delayed from both harvest 1 to 2 and from harvest 1 to 3. The results are inconclusive since in one site year an increase occurred and in the other two a decrease occurred. It appears, therefore, that hurd fibre content in this study was maximized between harvest 1 (technical maturity) and harvest timing 2.

**Table 4.4.3.1** The effect of harvest timing and target plant population density on the percentage of hurd fibre in the bottom hemp section (25-45 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location				
	Gilbert Plains	Grandview		Dauphin	
	Year				
	2006	2006	2007	2006	2007
	Bottom Section -----Hurd fibre (%) -----				
Harvest timing 1	54 <i>b</i>	58	73 <i>a</i>	59	73 <i>a</i>
Harvest timing 2	56 <i>a</i>	57	63 <i>b</i>	59	67 <i>b</i>
Harvest timing 3	57 <i>a</i>	58	65 <i>b</i>	56	67 <i>b</i>
LSD ( $\alpha = 0.05$ )	1.9*	2.1 NS	2.8*	7.5 NS	2.7*
Target (plants m <sup>-2</sup> )					
100	56	58	69 <i>a</i>	56	67
200	56	58	67 <i>b</i>	60	69
300	55	58	65 <i>b</i>	57	68
LSD ( $\alpha = 0.05$ )	1.7 NS	1.6 NS	1.7*	6.4 NS	2.3 NS

ANOVA	df	$P > F$				
Harvest timing	2	0.0411*	0.2712	0.0005*	0.6406	0.0026*
Plant density	2	0.3916	0.9675	0.0017*	0.2899	0.3267
Harvest*Density	8	0.1245	0.9378	0.5898	0.3585	0.4368

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

The percentage of hurd fibre has been rounded up to the nearest whole number.



#### 4.4.3.3 Middle stalk section

The percentage of hurd fibre in the middle section across all site years averaged 60% at harvest 1, 56% at harvest 2 and 57% at harvest 3. Harvest timing significantly affected the percentage of hurd fibre present in the middle section at Gilbert Plains-06 but not at Grandview-06, Dauphin-06 or Grandview-07 (Table 4.4.3.2). At Gilbert Plains-06, the percentage of hurd fibre located in the middle section increased by approximately 2% ( $P < 0.05$ ) as harvest was delayed from harvest 1 to 2 and from harvest 1 to 3 (Table 4.4.3.2). Therefore, hurd fibre was increased by delayed harvest at one site. However, the percentage of hurd fibre approached significance at Grandview-07 ( $P < 0.10$ ), where the hurd content decreased as harvest was delayed (Table 4.4.3.2).

At Dauphin-07, a significant harvest timing x target plant population density affect was observed in the percentage of hurd in the middle section (Table 4.4.3.4). The interaction was caused by a decrease in the hurd fibre percentage with increase plant population densities at harvest timing 2, but little change occurred at harvest timing 1 or 3 (Table 4.4.3.4). The reason for the interaction at Dauphin-07 is not clear.

**Table 4.4.3.2** The effect of harvest timing and target plant population density on the percentage of hurd fibre in the middle hemp section (65-85 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location					
	Gilbert Plains	Grandview		Dauphin		
	Year					
	2006	2006	2007	2006	2007	
	Middle Section -----Hurd fibre (%) -----					
Harvest timing 1	52 <i>b</i>	52	71	55	71	
Harvest timing 2	54 <i>a</i>	52	59	51	64	
Harvest timing 3	54 <i>a</i>	52	63	52	65	
LSD ( $\alpha = 0.05$ )	1.6*	5 NS	9.9 NS	7.5 NS	2.6	
Target (plants m <sup>-2</sup> )						
100	53	53	65	50	67	
200	53	51	66	54	67	
300	52	51	62	53	66	
LSD ( $\alpha = 0.05$ )	1.3 NS	4.3 NS	5.1 NS	6.4 NS	1.8	
ANOVA	df	<i>P</i> > <i>F</i>				
Harvest timing	2	0.0358*	0.8581	0.0631	0.378	0.0021
Plant density	2	0.0869	0.6896	0.1609	0.4221	0.3882
Harvest*Density	8	0.1421	0.8303	0.6491	0.8759	0.0388*

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

The percentage of hurd fibre has been rounded up to the nearest whole number.

When a significant interaction between plant density and harvest timing occurred the significant ANOVA factors for each treatment were nulled due to the treatment interaction.

#### 4.4.3.4 Top stalk section

Harvest timing significantly affected the percentage of hurd fibre present in the top section at Grandview-07 and Dauphin-07 sites only (Table 4.4.3.4). In both site years the percentage of hurd fibre was affected only by delaying harvest timing from harvest 1 to 2.

At Grandview-07, the percentage of hurd fibre in the top section decreased ( $P < 0.05$ ) by 12% as harvest was delayed from harvest 1 to 2. A decrease in percentage hurd of 18% was also observed as harvest timing was delayed from harvest 1 to harvest 3. At Dauphin-07, the percentage of hurd fibre located in the top section decreased by approximately 7% as harvest was delayed from harvest 1 to 2, and decreased by 8% as harvest timing was delayed from harvest 1 to harvest 3 (Table 4.4.3.3). The percentage of hurd fibre in the top section approached significance at Gilbert Plains-06 ( $P < 0.10$ ), where the hurd content increased (50-54%) as harvest was delayed (Table 4.4.3.2).

At Dauphin-06, a significant harvest timing x target plant population density affect was observed in the percentage of hurd in the top section (Table 4.4.3.4). The interaction was caused by a decrease of 1% in hurd fibre with increasing plant population density at harvest timing 1, an increase of 5% at harvest timing 2; a decrease occurred at harvest 3 from plant density 100 to 200 plants  $m^{-2}$  while an increase occurred at harvest 3 between plant densities 100 and 300 plants  $m^{-2}$ . The reasons for this interaction are not clear.

**Table 4.4.3.3** The effect of harvest timing and target plant population density on the percentage of hurd fibre in the top hemp section (105-125 cm from ground level) at all sites in the Parkland region of Manitoba in 2006 and 2007.

Treatment	Location				
	Gilbert Plains	Grandview		Dauphin	
	Year				
	2006	2006	2007	2006	2007
	Top Section -----Hurd fibre (%) -----				
Harvest timing 1	48	50	85 <i>a</i>	53	72 <i>a</i>
Harvest timing 2	49	52	67 <i>b</i>	50	65 <i>b</i>
Harvest timing 3	50	54	73 <i>b</i>	51	64 <i>b</i>
LSD ( $\alpha = 0.05$ )	2.6 NS	9.7 NS	8.4*	7.5 NS	4.5*
Target (plants m <sup>-2</sup> )					
100	49	53	77	52	69
200	50	51	77	48	67
300	48	51	74	54	67
LSD ( $\alpha = 0.05$ )	2.06 NS	8.1 NS	7.1 NS	4.4 NS	2.5 NS

ANOVA	df	<i>P</i> > <i>F</i>				
Harvest timing	2	0.0657	0.1288	0.0055*	0.6494	0.0276*
Plant density	2	0.1196	0.8588	0.5514	0.0498	0.5188
Harvest*Density	8	0.1227	0.6319	0.2804	0.0372*	0.2275

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference. The percentage of hurd fibre has been rounded up to the nearest whole number.

When a significant interaction between plant density and harvest timing occurred the significant ANOVA factors for each treatment were nulled due to the treatment interaction.

**Table 4.4.3.4** The interaction effect of harvest timing and target plant population density on the percentage hurd fibre in the top section (105-125 cm from ground level) at Dauphin-06 and in the middle section (65-85 cm from ground level) at Dauphin-07.

Treatment		Location	
Harvest timing	Target (plants m <sup>-2</sup> )	Dauphin	
		Year	
		2006	2007
		Section	
		Top	Middle
-----Hurd fibre (%) -----			
Harvest timing 1	100	53	71
	200	53	72
	300	52	70
Harvest timing 2	100	48	67
	200	49	64
	300	54	62
Harvest timing 3	100	55	64
	200	43	64
	300	56	66
	LSD ( $\alpha = 0.05$ )	13.8*	4.6*

ANOVA	df	$P > F$	
Harvest timing	2	0.0498	0.0021
Plant density	2	0.6494	0.3882
Harvest*Density	8	0.0372*	0.0388*

NS, \* Not significant or significant at  $P \leq 0.05$ .

a-c Means followed by the same letter in a column for each year are not significantly different at  $P \leq 0.05$  (LSD). Absence of letters indicates there was no significant difference.

The percentage of hurd fibre has been rounded up to the nearest whole number.

#### **4.4.3.5 Effect of targeted plant population density**

#### **4.4.3.6 Bottom stalk section**

The percentage of hurd fibre ( $P < 0.05$ ) in the bottom section across all site years averaged 61% at 100 plants  $m^{-2}$ , 62% at 200 plants  $m^{-2}$  and 61% at 300 plants  $m^{-2}$ . At Grandview-07, the targeted plant population density significantly affected the percentage of hurd fibre present in the bottom section (Table 4.4.3.1). The percentage of hurd increased ( $P < 0.05$ ) by 2% as the plant population increased from 100 to 200 plants  $m^{-2}$ . However, hurd fibre decreased as the plant population increase from 100 to 300 plants  $m^{-2}$ . Very little change in hurd fibre percentage was observed with a change in the plant population density.

#### **4.4.3.7 Middle stalk section**

The percentage of hurd fibre in the middle section across all site years averaged 58% at 100 plants  $m^{-2}$ , 58% at 200 plants  $m^{-2}$  and 57% at 300 plants  $m^{-2}$ . No significant effects on the percentage of hurd fibre ( $P > 0.05$ ) present in the middle section were observed (Table 4.4.3.2).

#### **4.4.3.8 Top stalk section**

The targeted plant population density did not significantly affect the percentage of hurd fibre present in the top section at any site year (Table 4.4.3.4).

#### **4.4.3.9 Conclusions**

Overall, the results from the present study show that the percentage of hurd fibre present in the three stalk sections, i.e. top, middle and bottom, tends to decrease as harvest timing is delayed and was relatively unchanged as the target plant population density increased. However, in the majority of cases no significant difference was observed.

In the present study the treatments affected the section stalk variables differently. Fibre determination in a standard location along the stalk could potentially be an important quality parameter for specialized fibre applications (Mediavilla et al. 2001). Currently processors do not have a desired percentage hurd fibre. Desired hurd yield will be dependent upon the end user and product. Applications such as paper making, animal bedding and hempcrete would require higher percentages of hurd. Therefore, bast fibre would essentially become a by-product (opportunity) in Canada without a bast fibre market. Of the above ground total mass of hemp plant the stem consist of approximately 67% of the total (Scheifele et al. 1996). Of the 67% that is stem material, approximately 15 to 30% is bast fibre with the remaining being hurd fibre (Dewey 1916).

The present study suggests that hurd fibre yield is typically maximized at harvest 1 (technical maturity) at lower plant densities. The present findings, however, do not reflect other quality parameters such as fibre strength or length (Bócsa and Karus 1998)

## **4.5 Chemical constituents**

The results from this portion of the study are from one site-year only and should be considered as preliminary, only.

### **4.5.1 Bast fibre profile**

The chemical profile of hemp bast fibre consists mainly of cellulose, lignin, hemicellulose and holocellulose. Holocellulose is the combined total of cellulose and hemicellulose. The percentage of cellulose and lignin in hemp bast fibre ranges from 53-68% and 4-12%, respectively, while the percentage of hemicellulose and holocellulose ranges from 7-17% and 68-85%, respectively, depending upon cultivar and harvest timing (van der Werf et al. 1994c; Amaducci et al. 2000; Correia et al. 2001; Kamat et al. 2002; Gutiérrez et al. 2006). In the present study the bast fibres consisted of approximately 86% cellulose, 3% lignin, 7% hemicellulose and 93% holocellulose. Hemp bast fibre chemical profiling in the current study was conducted at only one of the site years, at Gilbert Plains-06. Samples were collected from harvest 1 and harvest 3 at all targeted plant populations densities (100, 200 and 300 plants m<sup>-2</sup>).

The results from the present study indicated no significant effect of harvest timing on the percentage of cellulose, lignin, hemicellulose or holocellulose ( $P > 0.05$ ) within the hemp bast fibres (Table 4.5.1.1). Thus in the present study the percentage of cellulose, lignin, hemicellulose and holocellulose in the hemp bast fibre had plateaued prior to harvest 1 at technical maturity.

Two European studies showed that cellulose increased while lignin and hemicellulose decreased as harvest timing was delayed beyond technical maturity (Amaducci et al. 2000; Stuik et al. 2000). Toonen et al. (2004), on the other hand,



observed a significant ( $P < 0.001$ ) increase in cellulose and hemicellulose and no change in lignin as the hemp harvest was delayed by two weeks from 26 days after sowing.

The percentage of holocellulose in the bast was significantly affected by the targeted plant population density while lignin, hemicellulose and cellulose were not (Table 4.5.1.1). The percentage of holocellulose present in the bast fibre decreased significantly, by 3.3% ( $P < 0.05$ ) as the targeted plant population density increased from 100 to 200 plants  $m^{-2}$ . Non-significant differences occurred between populations of 200 to 300 plants  $m^{-2}$  and 100 to 300 plants  $m^{-2}$ . Thus, the cultivar Alyssa, was not affected by targeted plant population increases except for in holocellulose.

Research conducted on hemp by Kamat et al. (2002) and Corriera et al. (2002) in Ontario, Canada also revealed no difference in means between the selected chemical components (holocellulose, lignin and cellulose) across a range of target plant densities.

#### **4.5.1.1 Conclusions**

The results from this portion of the study should be used only as a base for future research and should be considered as preliminary. The results show that the chemical constituents in the bast fibre appeared to have reached their maximum level prior to harvest timing 1 (at technical maturity). Plant densities effects on the chemical constitute of the bast fibre were not strong, and the one significant effect of plant density (i.e., holocellulose) was not conclusive.

In this study the bast cellulose content was observed to be greater (86%) than that of the European average (53-68%). This may be due to differences in cultivar, fibre separation or analytical method. The present study employed the ANKOM chemical fibre analysis as presented in Toonen et al. (2004), unlike most of the European studies that

employed the TAPPI standards (de Meijer and van der Werf 1994; van der Werf et al. 1994c,1995a; Correria et al. 1998; Sankari 2000; Correria et al. 2001). Toonen et al. (2004) stated that the ADL, NDL and ADF (ANKOM method) was not as discriminative in chemical compounds as others testing methods i.e Klason lignin. In this study the bast and hurd fibres were separated by means of water retting however unlike in the majority of European experiment fibres were separated using a NaOH (Sodium Hydroxide) method. No literature was found comparing the direct effect of the method of fibre separation on them chemical profile, however Banik et al. (2003) suggested that water retting may effect the chemical fibre profile.

**Table 4.5.1.1** The effect of harvest timing and target plant population density on the percentage of lignin, hemicellulose, cellulose and holocellulose in hemp bast fibre at Gilbert Plains 2006.

Treatment	Location				
	Gilbert Plains				
	Year				
	2006				
-----Chemical constituent (%) bast fibre-----					
	Lignin	Hemicellulose	Cellulose	Holocellulose	
Harvest timing 1	3.2	6.7	86.8	93.5	
Harvest timing 3	2.3	7.5	86	93.5	
LSD ( $\alpha = 0.05$ )	1.9 NS	2.7 NS	2.4 NS	4.1 NS	
Target (plants m <sup>-2</sup> )					
100	2.3	8	87.2	95.2 <i>a</i>	
200	3.4	6.8	85.1	91.9 <i>b</i>	
300	2.5	6.5	86.9	93.4 <i>ab</i>	
LSD ( $\alpha = 0.05$ )	1.6 NS	2.3 NS	2 NS	2.5*	
ANOVA	df	<i>P &gt; F</i>			
Harvest timing	1	0.2013	0.4237	0.3612	0.9756
Plant density	2	0.4019	0.3609	0.0957	0.0434*
Harvest*Density	2	0.6998	0.1551	0.753	0.079

NS, \* Not significant or significant at  $P < 0.05$

a-c Means followed by the same letter in a column for each year are not significantly different at  $P < 0.05$  (LSD). Absence of letters indicates there was no significant difference.

#### **4.5.2 Hurd fibre profile**

The chemical profile of hemp hurd fibre consists mainly of cellulose, lignin, hemicellulose and holocellulose but in different proportions compared to that of the bast fibres. The percentage of cellulose and lignin in hemp hurd fibre ranges from 31-37 % and 18-21%, respectively, while the percentage of hemicellulose ranges from 15-19%, depending upon cultivar and harvest timing (Bócsa and Karus 1998; Franck 2005). No information was available in the literature on the percentage of holocellulose in hemp hurd fibres.

In the present study the hurd fibre consisted of approximately 56% cellulose, 16% lignin, 19% hemicellulose and 75% holocellulose at technical maturity (harvest 1). These results are similar to those reported by van der Werf et al. (1994c) where hemp hurd fibre consisted of 17.8% hemicellulose and 20.8% lignin. However, the hurd cellulose percentage in the present study was much greater than the estimated 35-37% observed by van der Werf et al. (1994c) and Bócsa and Karus (1998), but were similar to the average cellulose 50-70% estimated by Young (1991) and Rothenberg (2001).

The hemp hurd fibre chemical profiling in the current study was conducted at only one of the site years, Gilbert Plains-06. Samples were analyzed from harvest timing 1 and harvest timing 3 from each of the targeted plant populations. The percentage of cellulose, lignin, hemicellulose or holocellulose in the hemp hurd fibre was not significantly affected by either harvest timing or target plant population density (Table 4.5.2.1). Therefore, the percentage of cellulose, lignin, hemicellulose and holocellulose in the hemp hurd fibre appeared to have plateaued prior to harvest 1 and was not significantly changed thereafter.

No previous studies have examined the effects of a target plant population density affect on the chemical component profile of hemp hurd fibres.

A significant harvest timing x target plant population density effect was observed for the percent holocellulose in the hurd fibre (Table 4.5.2.2). At harvest timing 1, the percentage of holocellulose ( $P < 0.05$ ) increased significantly with increasing target plant density while at harvest 3 the percentage of hurd fibre holocellulose initially increased then decreased. Although the reason for this increase then decrease was not fully obvious the holocellulose content may have decayed over time as the plant population increased and harvest was delayed. Since both cellulose and hemicellulose content decreased, although not significant ( $P > 0.05$ ) when examined separately, the holocellulose (combined cellulose and hemicellulose) interacted with harvest and density. As proposed by Banik et al. (2003) the chemical profile of hemp may be affected by the water retting process, the method used in the present study to separate the fibres. However this proposition requires further testing.

#### **4.5.2.2 Conclusions**

The percentage of cellulose, lignin, hemicellulose or holocellulose in the hemp hurd fibre was not significantly affected by harvest timing or by the change in the target plant population density. It is thus assumed that the chemical components were maximized by harvest timing 1 (technical maturity). In this study the hurd cellulose content was observed to be greater (56%) than that of the European average (31-37%). This may be due to differences in cultivar or analytical methods. The results from this

portion of the study, however, are from only one site year and should be considered as preliminary.

**Table 4.5.2.1** The effect of harvest timing and target plant population density on the percentage of lignin, hemicellulose, cellulose and holocellulose in hemp hurd fibre at Gilbert Plains 2006.

Treatment	Location				
	Gilbert Plains				
	Year				
	2006				
-----Chemical constituent (%) hurd fibre-----					
	Lignin	Hemicellulose	Cellulose	Holocellulose	
Harvest timing 1	16.4	19.4	56.1	75.5	
Harvest timing 3	17	20.3	56	76.4	
LSD ( $\alpha = 0.05$ )	1.4 NS	5.7 NS	3.0 NS	4.1	
Target (plants m <sup>-2</sup> )					
100	17.4	17.3	57.1	74.4	
200	16.1	22.2	54.6	76.8	
300	16.6	20.1	56.5	76.6	
LSD ( $\alpha = 0.05$ )	1.2 NS	4.8 NS	2.5 NS	3.4	
ANOVA	df	<i>P &gt; F</i>			
Harvest timing	1	0.2696	0.6925	0.9805	0.5514
Plant density	2	0.1437	0.1493	0.1337	0.2875
Harvest*Density	2	0.6693	0.0562	0.868	0.012*

NS, \* Not significant or significant at  $P < 0.05$

a-c Means followed by the same letter in a column for each year are not significantly

**Table 4.5.2.2** The effect of the interaction between harvest timing and target plant population density on the percentage of holocellulose in the hurd fibre at Gilbert Plains 2006.

Treatment		Location
Harvest timing	Target (plants m <sup>-2</sup> )	Gilbert Plains
		Year
		2006
		Chemical constituent (%) hurd fibre
		Holocellulose
Harvest timing 1	100	72.7
	200	74.3
	300	79.5
Harvest timing 3	100	76.1
	200	79.4
	300	73.7
	LSD ( $\alpha = 0.05$ )	6.8
ANOVA	df	<i>P</i> > <i>F</i>
Harvest timing	2	0.5514
Plant density	2	0.2875
Harvest*Density	8	0.012*

NS, \* Not significant or significant at  $P < 0.05$

## 5. GENERAL DISCUSSION

This thesis reports on the first agronomic industrial hemp (*Cannabis*) study to be conducted at The University of Manitoba since the late 1920s.

The present study determined that increased self-thinning was associated with increasing the targeted plant population density and delaying the harvest timing. Plant counts were affected by self-thinning due to inter-plant competition for available resources in three site-years. The mean plant height and stem diameter tended to decrease with increasing population densities. Overcrowding in hemp restricted the overall potential growth and thus the final plant size. However, plant height and stem diameter were not affected by harvest timing. This suggests that plant density influences self-thinning, plant height and stem diameter to a greater extent than delaying harvest beyond technical maturity. It is concluded that the segregation of the plants into 'higher' and 'lower' plants population densities is an essential factor responsible for the size differentiation formation of stem diameter and plant height. The targeted plant density would therefore differ depending upon the end use for the hemp.

Even though, biomass was affected at one site year by increasing the targeted plant population density the correlation was weak and not significant. The weak correlation between biomass and plant density at the one significant site and the non-significance determined at the other four sites supports the law of constant yield theory. The law of constant yield states that beyond a threshold density a consistent or stable yield will occur at varying planting densities throughout time due to competition affects (Kira et. al. 1953). This stable yield is caused by an increase in overall plant size at lower plant populations and an increase in plant losses at higher plant population densities.



Thus, any potential increase in biomass through an increase in the number of individual plants is exactly matched by the decline in dry weight per plant, because plants are forced to share a finite amount of resources as time progresses (Azam-Ali 2002). Any gains in biomass in hemp would therefore have to be obtained using other approaches such as increased soil fertility or development of higher yielding cultivars.

Harvest timing did not impact biomass at technical maturity. Gains in biomass beyond technical maturity did not surpass expected average fibre yields.

The percentage of bast and hurd fibre tended increased with delayed harvest while increasing plant population tended to decrease the percentage of bast and hurd fibre. However, the data demonstrated that in most cases the percentage of bast and hurd fibre is relatively stable across treatments. Thus, in order to produce a high quantity of hemp fibre, planting density should be low (e.g. 200 plants m<sup>-2</sup>) and harvest should be late. However, beyond technical maturity gains may not be of any benefit because the percentage of fibre is relatively unchanged and as the crop matures, the fibres become more rigid and mechanical wrapping increases.

The percentage of bast (7-30%) and hurd (48-85%) were similar to that of European hemp cultivars. This project showed that as stem diameter increased, the percentage of hurd tended to increase also. For this reason low planting density increases the quantity of hurd at harvest. This, however, is only a qualitative parameter that does not reflect other fibre quality parameters not examined in this study.

Chemical constituents within the hurd fibre were not affected by the harvest timing or by the targeted plant population density at the one site-year where these parameters were measured. These preliminary results suggest that the chemical

constituents in the hemp cultivar Alyssa were maximized at technical maturity and were not dependent upon plant density.

Hemp grown for fibre only applications should be harvested at technical maturity and grown at a density that is sufficient to suppress weeds without increased competition and thus plant loss. In this study it is thus recommended that for optimal fibre production hemp should be harvested at technical maturity (50% of the male staminate flowers are open and dehiscencing; decimal code 2102) approximately 70-80 days after sowing and planted at a targeted density of 200 plants m<sup>-2</sup>.

In the current study the Canadian industrial hemp cultivar, Alyssa, appears to be relatively stable across a wide range of agronomic parameters. Thus, the production of the cultivar, Alyssa, for fibre-only production would be a viable crop option in Canadian cropping rotation.

The present study did not examine other testable fibre quality characteristics. The samples produced from this study are in storage and could be used to determine other quality parameters such as tensile strength, absorbance, burning point, fibre length, stiffness, cell formation, luster, shear strength, fineness, thermal, acoustic or anti-bacterial properties, pectin content, composite applications or ultra violet radiation resistance. Further examination is required to determine nutrient uptake, water-use efficiency (WUE), phytoremediation, radiation-use efficiency (RUE) and photosynthetically active radiation (PAR). In addition, since Alyssa is a dual-purpose cultivar aspects of seed quality and yield could be examined in future studies.

## 6. SUMMARY and CONCLUSIONS

Canada has become a global leader in hemp grain production. However, agronomic information on hemp fibre production in Canada is lacking. This study evaluated the effect of harvest timing and targeted plant population density on the yield and quality of one hemp cultivar, Alyssa. Summary of overall trends are presented in Figure 6.1.

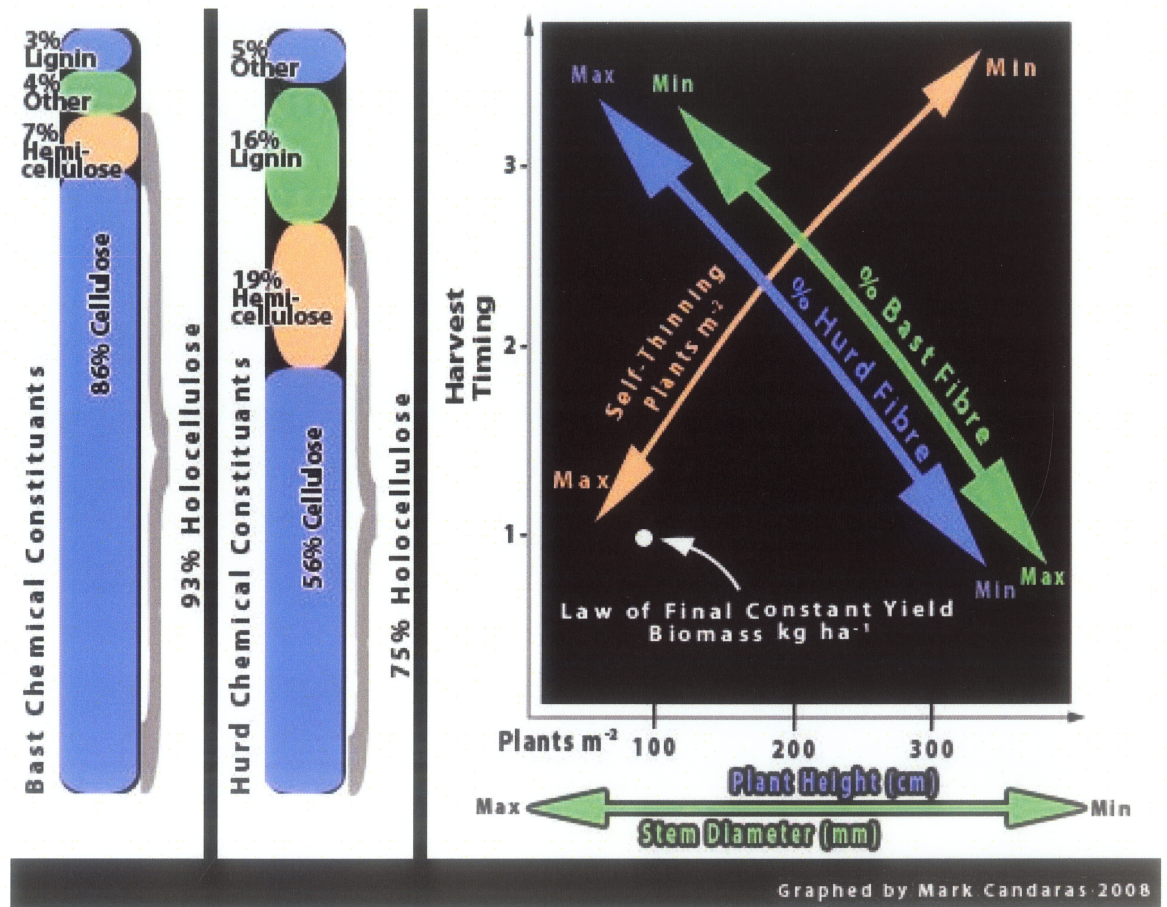


Figure 6.1 Graphical depiction of overall treatment trends from the present study. (Photoshop per. comm. Mark Candaras 2008.)

The following points outline the findings of the present study.

- Plant population density and delaying harvest beyond technical maturity did not provide any substantial yield or quality benefits.
- Harvesting hemp after technical maturity increased plant losses.
- Plant height and stem diameter was maximized by technical maturity in all plant populations.
- Biomass was relatively unaffected by increasing plant population density or by delaying harvest beyond technical maturity.
- The percentage of bast fibre tended to be stable with delaying harvest.
- The percentage of hurd fibre tended to be consistent as harvest was delayed.
- The percentages of bast and hurd fibre were unaffected by increasing the plant density.
- The chemical constituents of the bast and hurd fibres were not affected plant density or by harvest timing at the single site-year where measured.
- A greater percentage of cellulose and hemicellulose was located in the bast fibre while the hurd consisted of a greater percentage of lignin and hemicellulose.

The present study has determined that no substantial agronomic benefit in yield or quality was observed beyond technical maturity at harvest 1 (hemp decimal code 2102). Thus, the quality and yield of the industrial hemp cultivar, Alyssa, grown for fibre-only production in the Parkland region of Manitoba was generally insensitive to harvest timing and planting density and showed to be relatively stable across environments.

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## **8. APPENDIES**

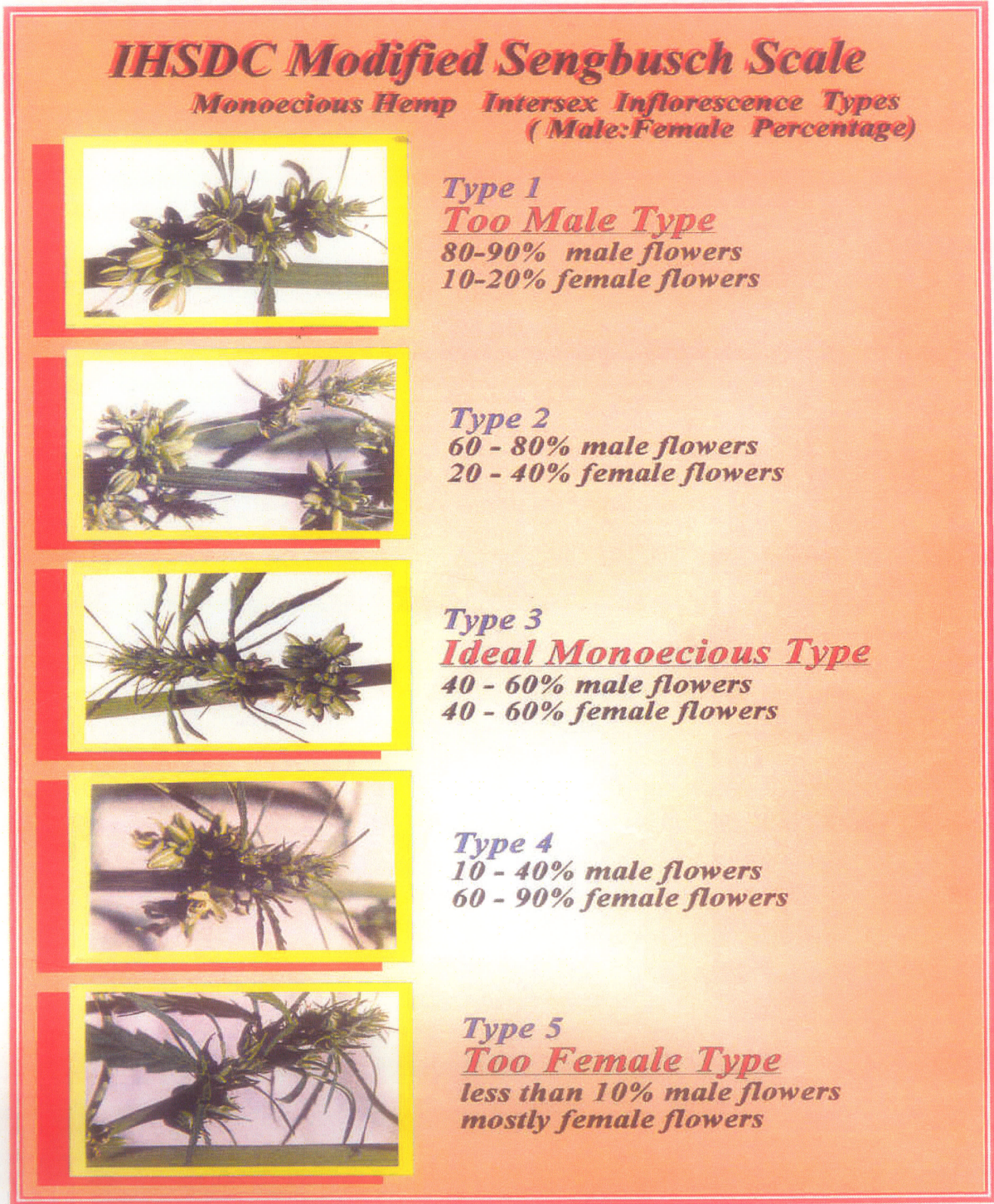


Appendix A IHSDC Modified Sengbusch Scale

Figure A.1

IHSDC modified Sengbusch Scale for monoecious intersex inflorescence types.

Sourced from the late per. comm. Peter Dragula M.Sc. (2002)



## Appendix B Developmental Stages of Hemp

Decimal code for dioecious hemp plants table adapted from (Mediavilla et al. 1998)

Developmental stages of hemp have been determined by Mediavilla, V., Jonquera, M., Schmid-Slembrouck, I. and Soldati, A. 1998. Decimal code for growth stages of hemp (*Cannabis sativa* L.). J. Int. Hemp Assoc. 5 (2), 67-72

For complete *Cannabis sativa* L. decimal codes for all developmental stages please see (Mediavilla et al. 1998).

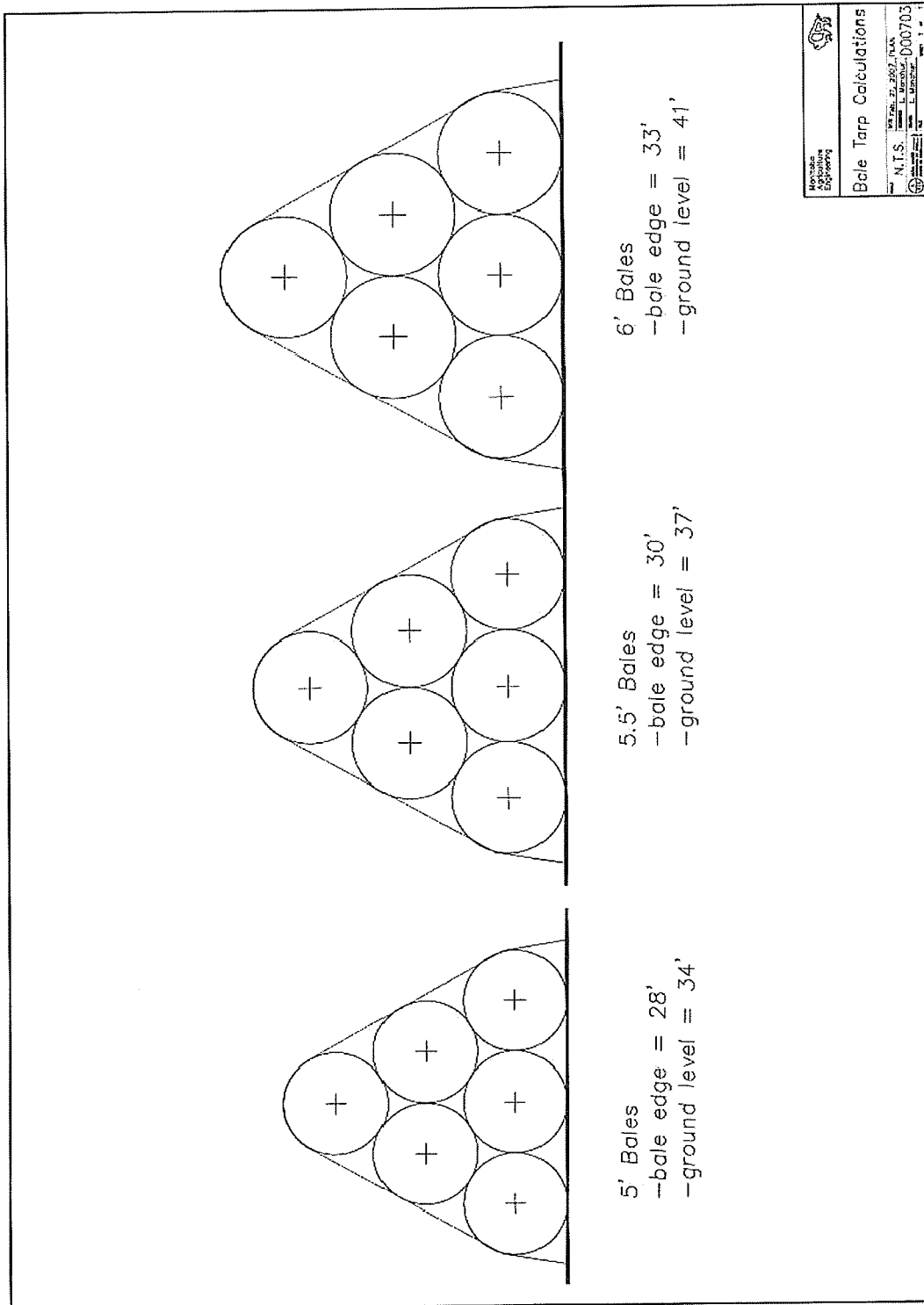
**Table B.1** Definitions and codes for the growth stages of *Cannabis sativa* L.

(Adapted from Mediavilla et al. 1998).

Code	Definition	
<b>1. Germination and emergence</b>		
0000	dry seed	
0001	radicale apparent	
0002	emergence of hypocotyl	
0003	unfolded cotyledons	
<b>2. Vegetative stage refers to main stem. Leaves are considered as unfolded when leaflets are at least one cm long.</b>		
1002	1st leaf pair	1 leaflet
1004	2nd leaf pair	3 leaflet
1006	3rd leaf pair	5 leaflet
1008	4th leaf pair	7 leaflet
1010	5th leaf pair	:
	:	:
10xx	11th leaf pair	xx = 2 (n <sup>th</sup> leaf pair)
<b>3. Flowering and seed formation refers to the main stem including branches</b>		
2000	GV point	
2001	Flower primordia	Indistinguishable sexual structures
<b>4. Male plants</b>		
2100	Flowers forms	First closed staminate flowers
2101	Flowering begins	First opened staminate flowers
2102	Full flowering	50% opened staminate flowers
2103	Flowering ends	95% of staminate flowers open or withered
<b>5. Female plants</b>		
2200	Flowers form	First pistillate flowers: Bract with no styles
2201	Flowering begins	Styles on first female flowers
2202	Full flowering	50% of bracts formed
2203	Seeds start maturing	First seeds hard
2204	Seeds mature	50% of seeds resistant to compression
2205	End of seed maturity	95% of seeds hard or shattered
<b>6. Senescence</b>		
3001	Leaves dry	Leaves dry
3002	Stem dry	Leaves dropped
3003	Stem decomposition	Bast fibres are free

## Appendix C Bale Stacking

Figure C.1 Bale tarp calculations (Sourced from MAFRI permission granted to reprint)





## Appendix D Combine Modifications & Settings

(Reprinted in entirety with permission from Hemp Oil Canada Inc.  
([www.hempoilcan.com](http://www.hempoilcan.com)) and Friesen 2006)

### 1) JD Conventional Combines (i.e. 7720 / 8820 / 9400 & 9600 series)

#### General Settings

Cylinder speed ~ 400 to 700 RPM      Cylinder setting ½ to 2”  
Fan speed 650 ~ 800 RPM (little less than wheat)  
Sieve / Shoe: 3/16 ~ ¼ (similar to wheat)

#### Some examples of setting used by producers in 2005 for JD combines

Variety	H2O	Model	Modified	Cyl. RPM	Concave	Fan	Sieve
USO 31	25%	8820	No – Note 1	600	½”	650	1/4 inch
Finola	15%	9600	No – Note 2	350	1.5-2	650	
USO 31	20%	7720	No – Note 3	750	½-3/4	800	3/16
Finola	13%	6620	No – Note 4				
Finola	13%	8820	No – Note 5	900		800	

All above were straight cut

*Note 1 – producer manufactured a push bar to flatten stubble under header to minimize wrapping under combine on header and drive shafts – crop 10' tall! Got wrapping on every moving part of combine!*

*Note 2 – chopper disabled / 4 ft crop / minimal wrapping on feeder chain shaft*

*Note 3 – put plastic pipe over hex shaft to minimize wrapping. Removed chopper*

*Note 4 – wrapping on upper and lower feeder chain shafts, and return auger shaft above cylinder*

*Note 5 – wrapping on top of feeder chain shaft*

#### Advice

Use hook or carpet knife to frequently remove any fibre build-up.  
Try to obtain smooth uniform flow – the fibre will wrap on irregularities  
Clean hopper at end of the day  
Check machine after every hopper  
Unload at half speed

#### Possible Modifications

- 1) Narrow intake on header 6 inches either side
- 2) Replace feeder chain with baler canvas – see Belt modification for instructions
- 3) Extend hydraulics by about 1 foot to be able to raise header higher
- 4) Shield any exposed bearings
- 5) ABS pipe over front drive shafts
- 6) Tie in cables close to machine ( because fibre can catch on anything)
- 7) Mac Don draper header works better than auger type headers
- 8) Drop knives on chopper
- 9) Rubber / Canvas Belt Modification  
– prevents fibre wrapping on feeder chain shaft

## Courtesy of:

SYDOR FARM EQUIPMENT  
HWYS 5 & 10  
DAUPHIN MB R7N2T9  
Canada  
204-638-6443  
Contact: Tim Cruickshanks

## PARTS LIST

144 3/8 nylon loc nuts  
17 3/8 metal loc nuts  
64 3/8 flange nuts  
15 3/8 heavy flat washers 24H1314  
64 3/8 x 1 round head bolts  
155 3/8 x 1 hex head bolts  
15 7/16 jam nuts  
2 3/8 x 1 1/4 hex head bolts  
4 3/8 x 1 1/2 hex head bolts  
8 ski doo sliders  
60" x 175" 2ply semi rough belting  
557 feeder chain made to fit 9600 combine length  
39 spring steel slats [flat bar]

## ASSEMBLY INSTRUCTIONS

ASSEMBLE SPRING STEEL SLATS WITH 3/8X1 AND NYLOC NUTS TORQUE 45LBS.  
ON THE LAST 2 SLATS ON CHAIN INSTALL WITH 3/8 METAL LOC NUTS IN ALL HOLES. ON  
THE CENTER CONNECTOR SLAT INSTALL 3 METAL LOC NUTS ON THE CENTER 3 HOLES  
ONLY. WELD ALL METAL LOC NUTS TO SLATS. THEN WELD LAST 2 SLATS TO FEEDER  
CHAIN. REMOVE ALL BUT THE OUTSIDE BOLTS THAT HOLD THE METAL LOC NUTS IN  
PLACE.

ON THE CENTER CONNECTOR SLAT DIE GRIND THE CHAIN MOUNTING HOLES ALONG TO  
THE OUTSIDE ABOUT 1/4" TO ALLOW FOR SOME ADJUSTMENT ON THE FINAL ASSEMBLY.

FOR THE BELTING SQUARE OFF ENDS FOR A 175"LENGTH. MARK A LINE 2 1/2" FROM EACH  
END FOR MOUNTING HOLES. USE 3 SLATS TO LOCATE THE MOUNTING HOLES, MARK AND  
PUNCH OUT HOLES WITH 3/4" BELT PUNCH. THIS WILL RESULT IN THE BELT BEING 170"  
FROM CENTER OF HOLES TO CENTER OF HOLES TOTAL LENGTH.

FROM CENTER OF MOUNTING HOLES ON ONE END..

MARK A LINE AT

21 1/4"

63 3/4"

106 1/4"

148 3/4"

LINE UP FULL SLIDER/SLATS MARK HOLES AND PUNCH. FOR THE 1/2 SLIDER SLATS [4  
FULL LENGTH SLATS CUT IN HALF GIVING 8 SHORT SLIDER/SLATS] MEASURE 14 1/4"  
FROM FULL SLAT LINE AND MARK HOLES. PUNCH HOLES TO 3/8" FOR MOUNTING SLATS.  
THE LONG SLATS SHOULD BE 4 1/8" FROM THE EDGE AND THE SHORT SLATS SHOULD BE  
17 1/8" FROM THE EDGE. SEQUENCE OF SLIDER/SLATS SHOULD BE  
1 SHORT , 1 LONG, 2 SHORT, 1 LONG, 2 SHORT, 1 LONG, 2 SHORT, 1 LONG, 1 SHORT

3/8 FLANGE BOLTS WILL SLIDE INTO SLAT GROOVE AND INSTALL SLATS ON BELT WITH 3/8 X 1 ROUND HEAD BOLTS AND FLAT WASHERS.

FOR FINAL ASSEMBLY

4 3/8 X 1 1/2" BOLTS

2 3/8 X 1 1/4" BOLTS

9 3/8 X 1" BOLTS

15 7/16" JAM NUTS

15 3/8" HEAVEY FLAT WASHERS

THE JAM NUTS ARE USED AS SPACERS ON THE BOLTS FOR THE BELT CONNECTION.

TIM CRUICKSHANKS

## **IH Axial Flow Combines (ie 1400 to 2100 series)**

### **General Settings**

Rotor speed ~ 350 - 400 when 17 – 25% moisture (speed up as it gets drier)

Rotor setting ~ 3 – 5 (close to corn setting – fairly open)

Fan speed 700

Shoe: top 1/2 bottom 1/8

Concaves like canola, open as it gets drier

### **Some examples of setting used by producers in 2005**

<b>Variety</b>	<b>H2O</b>	<b>Model</b>	<b>Modified</b>	<b>Rotor RPM</b>	<b>Concave</b>	<b>Fan</b>	<b>Sieve</b>
Crag	21%	1688	Yes -Note 1	500	4	800	1/2 inch
Finola	12-18%	1680	No - Note 2	425	4-5	800	3/8~1/2 & 1/8 inch
Finola	17%	2188	No – Note 3	400	5	700	1/8
Finola	11~18%	1480	None	450	3-4	700	1/2

All above were straight cut

*Note 1 – 1688 modified with rotor kit from Joe F – field on irrigation in AB – yield 30 bu /acre, crop 6.5 ft tall. MacDon draper header.*

*Consistent wrapping on rock trap beater, and some near front bearing on rotor*

*Note 2 – IH 1010 header 3 ft organic crop dryland yield low*

*Note 3 – Fibre wrapping on rock trap – will try to narrow opening next year*

### **Advice**

Use hook or carpet knife to frequently remove any fibre build up.

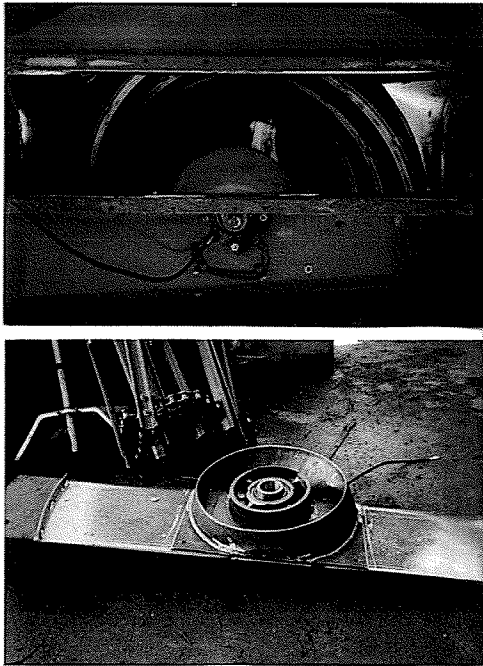
Try to obtain smooth uniform flow – the fibre will wrap on irregularities.

Clean hopper at end of the day.

Unload at half speed.

## Possible Modifications

- 1) New knife and new guards
- 2) Narrow intake on header 6 - 8 inches either side
- 3) Put matching sprockets on feeder and beater – ie corn sprockets (Ask Dauphin CIH dealer Miller Farm Equipment as per below.)
  - a. Strip beater bar, grind off bolts and any imperfections.
  - b. Or, Reverse beater bars – so “L” faces inward.
- 4) Extend hydraulics by about 1 foot to be able to raise header higher for taller varieties
- 5) Shield any exposed bearings
- 6) Puckboard underneath combine to reduce fibre catching
- 7) Tie in cables close to machine ( because fibre can catch on anything)
- 1) Drop knives out of chopper, or drop chopper.



For IH Axial Flow combines there is a kit (designed by long time hemp grower Joe Fedorowich) which greatly increases harvesting efficiency for hemp crops. This kit fits over the front on the rotor, replaces the elephant ears, bearing, and adds a paddle, smoothing out feeding, reducing pounding and fibre wrapping and also reduces overall maintenance costs. The kit costs approximately \$2750+, with parts exchange, and possibly some other upgrades may be required. Contact Warren Cowling at 1-204-638-5558 at Miller Farm Equipment in Dauphin MB for more details.

### Some examples of setting used by producers in 2005 for other combines

Variety	H20	Model	Modifications	Cyl. RPM	Concave	Fan	Sieve
USO 31	25%	860MF	Some -Note 1	800	½ open	low	front ½ open, back 0 clearance
Finola	12%	CR 960 NH	No -Note 2	800	1.1/4"		
Finola	9~12%	NH TR95	No-Note 3	720	9-10	580	Chaffer 3/8-1/2 Sieve 1/8-1/4
Finola	14%	TX-66 & CX-880	Yes-Note 4	650	3/4"	480	Shoe 1/4"

**NOTE 1:** retimed feeder house paddles / fibre wrapping on feeder house & cylinder / no chopper / height 8 ft

**NOTE 2:** some wrapping on rear beater / checking each hr / as grain dried from 12.5% to 11.5% slowed from 3\mph to 1 mph! Advise starting at 16% - sharp cutting bar. No modifications

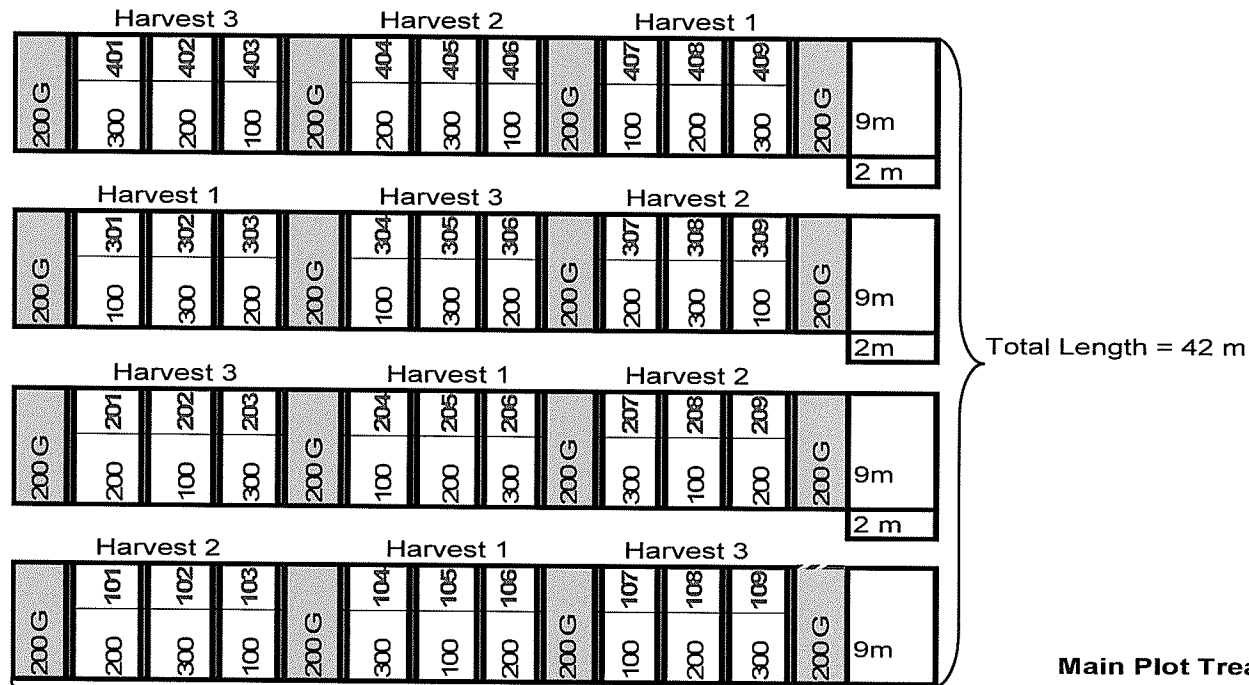
**NOTE 3: Finola SWATHED.** Best window 10.5 to 12.5% - up to 18% OK. Minor wrapping on Redekop Straw Chaff, and also on feeder chain shafts when moisture below 10%.

**NOTE 4:** Installed canvas belt modification on feeder chain for TX-66

All above were straight cut except NH TR 95/Note 3 which was swathed Finola

## Appendix E Sample split plot design

2006-2007 Hemp Fibre Trial Location Plot Plan



Total width = 29.66 m

Seeded Plot width = 2 m  
length = 9 m  
Area = 18 m<sup>-2</sup>

Row spacing = .2m  
Space between plots = .305 m

Total Area = 1245.72 m<sup>-2</sup> = 0.1246 ha

**Main Plot Treatment:** Harvest Timing/Stage

Harvest timing 1: Decimal code 2102

Harvest timing 2: Decimal code 2306

Harvest timing 3: Decimal code 2307

**Sub Plot Treatment:** Plant Density

Target Plant Population Density after hand-thinning

1) 100 m<sup>-2</sup>

2) 200 m<sup>-2</sup>

3) 300 m<sup>-2</sup>

## Appendix F Herbicide Mode of Action

**Table F.1** Herbicide groups based on mode of action, formulation, site of uptake and symptoms (MAFRI 2006b).

Herbicide group	Formulation	Mode of action	Site of uptake	Symptoms	
				Broadleaf weeds	Grass weeds
1	Assure II	Systemic	Foliar	Tolerant	Growth reduced and yellowing at growing point in 1-3 weeks
1	Centurion	Systemic	Foliar	Tolerant Leaves yellow in 2-4 days and death in 1-2 weeks	Growth reduced and yellowing at growing point in 1-3 weeks
6	Pardner	Contact	Foliar		Leaf burn possible
9	Roundup	Systemic	Foliar	Wilting and yellowing in 7-10 days	Wilting and yellowing in 7-10 days

This table was adapted from Table 2 in the 2006 Guide to Crop Production for weeds, plant diseases and insects.

## Appendix G Biolin Inc. Methods

Each sample was weighed and measured for number of stalks per half m<sup>-2</sup>, average diameter of stalks, manual fibre content, long line fibre yield, tow fibre yield, and hurd & epidermis percentage.

A sub-sample bundle of straw 4 cm in diameter and 19.0 cm was bound with two elastic bands and tagged with a plastic-coated label.

Each sub sample was then retted separately in a tub for 7 to 10 days in water at 36° C & 6.8 pH. The sample was determined to be optimally retted using a Fried Shake Test.

The Fried Shake test involves placing fifteen pieces of retting straw, each approximately 10.0 cm in length, each from a different bundle, in fifteen test tubes half full of boiling water with one piece of straw in each test tube. The test tubes were stoppered and placed in a machine designed by Biolin Research to violently shake test tubes for fifteen seconds. After shaking, the straw in each test tube was visually scored for loose fibres on a whole number scale from “0” to “3” with “0” representing no loose fibres and “3” representing total loosening of all bast fibres on the stem. When the average score was above 2.8 and there were no “0” scores and at least twelve test tubes had a score of “3”, the bundles in the batch were considered fully retted. The bundles were then taken out of the tank, rinsed in tap water and set in drying racks, next to heater fans, for approximately four days to dry. The fibre was then extracted from each dried, retted, sub-sample using a reciprocating blade-type breaker/decorticator unit.

The sample was processed using a blade decortication unit, this unit separated the hurd from the fibre. Fibre separates from the shive as the epidermis is consumed in a retting process, decortication is the process of 'breaking' the shive and allowing it to 'fall' from the fibre, if the sample is not retted enough the fibre will not separate from the shive with ease.

Each decorticated sample was then combed to separate the remaining hurd from the fibre and to also divide the fibres into long line quality and tow quality fibres. On some samples the tow was hand picked using a magnifying lens with a light and tweezers due to that with tub retting multiple samples not all samples will be optimally retted.

All the measurements were entered into an excel sheet to calculate the desired measurements and the coefficient of variation to determine the variability of the measurements among the similar samples.

## Appendix H 1-4 ANKOM fibre analysis methods

AH.1 Neutral Detergent Fibre (%NDF)

AH.2 Acid Detergent Fibre (%ADF)

AH.3 Acid Detergent Lignin (%ADL)

AH.4 Dry matter

(Permission granted to reprint by The Dept. of Animal Science, Nutrition Lab at the University of Manitoba)

### AH.1 Neutral Detergent Fibre (%NDF)

#### Method for Determining Neutral Detergeant Fiber (NDF) Using the ANKOM Fiber Analyze

##### Reagents:

1. Sodium laurel sulphate (SDS)
2. Disodium ethylenediamine (EDTA)
3. Disodium hydrogen phosphate, anhydrous
4. Sodium borate, decahydrate
5. Ethylene glycol monoethyl ether (cellosolve)
6. Sodium sulfite- $\text{Na}_2\text{SO}_3$  anhydrous
7. Acetone
8. Alpha-amylase-Heat-stable bacterial alpha-amylase:  
Activity =340,000 Modified Wolgemuth Units/ml

##### Solutions:

Neutral Detergeant Buffer-1 litre

EDTA	18.61 grams
Sodium borate	6.81 grams
Sodium laurel sulphate	30.00 grams
Cellosolve	10 mls
Sodium Phosphate	4.56 grams

1. Weigh the EDTA and borate into a beaker and add distilled water. Warm to dissolve.
2. Weigh the SDS into a separate beaker and add the cellosolve and some distilled water. Warm to dissolve.
3. When both solutions are dissolved mix them together.
4. Add the sodium phosphate to the solution. Stir to dissolve
5. Allow the solution to cool to room temperature and bring to volume.
6. Check the pH. It should be between 6.9 and 7.1. Adjust if necessary.



**Equipment:**

1. Digestion Apparatus-ANKOM Fiber Analyzer
2. ANKOM F57 filter bags
3. Heat sealer-Requires high enough temperature to melt and seal polymer in filter bags
4. Desiccator

**Safety Precautions:**

1. Acetone is highly flammable. Avoid inhaling or contact with skin.
2. Sodium Lauryl sulfate will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.
3. When running the digestion unit always open the valve and exhaust the hot solution before opening the lid. The solution in the vessel is under pressure and opening the lid first could result in severe burns for the operator. Make sure that the exhaust hose is securely positioned for safe disposal of the solution.

**Procedure**

1. Number and weigh the filter bags. When numbering the bag always use either a wax pencil or a soft lead pencil. Marking pens and ink pens will wash off during digestion. Weigh 2 blank bags to be included in digestion to determine the blank bag correction. New blanks are required only when a new solution is prepared or when using a new batch of bags.
2. Weigh 0.5 g (+ or - 0.5 g) of air dried sample, ground to pass through a 1-mm screen, directly into a filter bag. Be careful not to spill sample on the outside of the bag.
3. Seal the bags closed within 1 cm from the open edge using the heat sealer.
4. Spread the sample uniformly inside the filter bag by shaking gently and lightly flicking the bag to eliminate clumping.
5. Place the bags in the bag suspender three to a tray. A maximum of 24 bags may be processed at a time. All nine baskets are used for every run. Stack the trays on the center post rotating each one so that the small plastic bubbles on the underside of the tray fit into the small indentations on the top of the lower tray. The weight is placed on top of the empty ninth tray. If blanks are run place one on the bottom tray and one on the eighth tray.
6. Add 2 liters of NDF solution into the digestion vessel. If processing less than 20 bags add 100 ml of solution per bag. Never use less than 1500 mls. Add 20 grams (0.5g/50 ml of soln) of sodium sulfite to the solution in the vessel. If the samples have starch in them add 4.0 ml of alpha- amylase.
7. Place the bag suspender into the digestion vessel. Turn agitate and heat on. Close and tighten the lid of the digestion vessel. Digest for 60 minutes from the time the lid is close.

8. Turn agitator and heat off after the 60 minutes are finished. Open the valve and exhaust the hot solution into a sink. Make sure to have cold water running to dilute the chemicals in the pipes. Follow the instructions listed under safety precautions very carefully.
9. When the solution has been exhausted close the valve and open the lid. Add 2 liters of hot distilled H<sub>2</sub>O and 4 mls of amylase if it is required. The amylase is used for the first two rinses only. Lower the lid but do not tighten. Agitate for 4 minutes. Do not turn the heat on. Exhaust the water and repeat the rinse for a total of four rinses.
10. Remove the bag suspender from the vessel. Take the bags and gently squeeze the excess water out. The easiest way to do this is to place them flat in a beaker and use a smaller beaker to gently press on them.
11. Add enough acetone to the beaker to cover the bags. Allow the bags to soak for approximately 3 minutes. Lightly press out the excess acetone using a smaller beaker.
12. Remove the bags from the beaker and spread out on a tray. Allow to air dry for a short period of time and then complete drying in an oven at 100 C for at least 4 hours. The bags are usually dried overnight.
13. Remove the bags from the oven and place in a dessicator until cooled to ambient temperature. Weigh the bags. This is the final weight.

#### **Calculations:**

Calculate percent NDF on a dry matter basis

Bag Correction-Initial blank weight minus the final blank bag weight.

A net loss is entered as a negative number a net gain is entered as a positive number.

$$\%NDF = \frac{(\text{Final Weight} - \text{Bag Weight} - \text{Bag Correction}) \times 100}{\text{Sample Weight on a dry matter basis}}$$

#### **References:**

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## AH.2 Acid Detergent Fibre (%ADF)

### Method for Determining Acid Detergent Fiber (ADF) Using the ANKOM Fiber Analyzer

#### Reagents:

1. Suphuric Acid reagent grade (H<sub>2</sub>SO<sub>4</sub>)
2. Hexadecyltrimethylammoniumbromide technical grade-CTAB
3. Tris (Hydroxymethyl) Aminomethane-akalimetric standard
4. Acetone

#### Solutions:

Acid Detergeant Buffer	1 Litre
Sulfuric Acid	27.9 ml
CTAB	20.0 gm

1. Dilute the sulfuric acid to 1N with distilled water. Bring to volume.
2. Standardize using 2 gm of THAM in approximately 35 mls of distilled water using mixed indicator. Adjust acid to 1N with water if it is too strong or sulfuric acid if it is too weak.
3. Add CTAB and stir to dissolve.

#### Equipment:

1. Digestion Apparatus-ANKOM Fiber Analyzer
2. ANKOM F57 filter bags
3. Heat sealer-Requires high enough temperature to melt and seal polymer in filter bags
4. Desiccator

#### Safety Precautions:

1. Acetone is highly flammable. Avoid inhaling or contact with skin.
2. When running the digestion unit always open the valve and exhaust the hot solution before opening the lid. The solution in the vessel is under pressure and opening the lid first could result in severe burns for the operator. Make sure that the exhaust hose is securely positioned for safe disposal of the solution.

## Procedure

1. Number and weigh the filter bags. When numbering the bag always use either a wax pencil or a soft lead pencil. Marking pens and ink pens will wash off during digestion. Weigh 2 blank bags to be included in digestion to determine the blank bag correction. New blanks are required only when a new solution is prepared or when using a new batch of bags.
2. Weigh 0.5 g (+ or - 0.5 g) of air dried sample, ground to pass through a 1-mm screen, directly into a filter bag. Be careful not to spill sample on the outside of the bag.
3. Seal the bags closed within 1 cm from the open edge using the heat sealer.
4. Spread the sample uniformly inside the filter bag by shaking gently and lightly flicking the bag to eliminate clumping.
5. Place the bags in the bag suspender three to a tray. A maximum of 24 bags may be processed at a time. All nine baskets are used for every run. Stack the trays on the center post rotating each one so that the small plastic bubbles on the underside of the tray fit into the small indentations on the top of the lower tray. The weight is placed on top of the empty ninth tray. If blanks are run place one on the bottom tray and one on the eighth tray.
6. Add 2 liters of ADF solution into the digestion vessel. If processing less than 20 bags add 100 ml of solution per bag. Never use less than 1500 mls.
7. Place the bag suspender into the digestion vessel. Turn agitate and heat on. Close and tighten the lid of the digestion vessel. Digest for 75 minutes from the time the lid is closed.
8. Turn agitator and heat off after the 75 minutes are finished. Open the valve and exhaust the hot solution into a sink. Make sure to have cold water running to dilute the chemicals in the pipes. Follow the instructions listed under safety precautions very carefully.
9. When the solution has been exhausted close the valve and open the lid. Add 2 liters of hot distilled H<sub>2</sub>O. Lower the lid but do not tighten. Agitate for 4 minutes. Do not turn the heat on. Exhaust the water and repeat the rinse for a total of four rinses.
10. Remove the bag suspender from the vessel. Take the bags and gently squeeze the excess water out. The easiest way to do this is to place them flat in a beaker and use a smaller beaker to gently press on them.
11. Add enough acetone to the beaker to cover the bags. Allow the bags to soak for approximately 3 minutes. Lightly press out the excess acetone.
12. Remove the bags from the beaker and spread out on a tray. Allow to air dry for a short period of time and then complete drying in an oven at 100 C for at least 4 hours. The bags are usually dried overnight.
13. Remove the bags from the oven and place in a dessicator until cooled to ambient temperature. Weigh the bags. This is the final weight.

### Calculations:

Calculate percent ADF on a dry matter basis

Bag Correction-Initial blank weight minus the final blank bag weight

A net loss is entered as a negative number a net gain is entered as a positive number.

$$\% \text{ ADF} = \frac{(\text{Final Weight} - \text{Bag Weight} - \text{Bag Correction}) \times 100}{\text{Sample Weight on a dry matter basis}}$$

### References:

1. Association of Official Analytical Chemists. 1990. Official methods of analysis, 15 ed. AOAC. Arlington, VA.
2. Komarek, A. R. 1993. An Improved Filtering Technique for the Analysis of Neutral Detergeant Fiber and Acid Detergeant Fiber Utilizing the Filter Bag Technique ANKOM Company, Publication #101, 1993

## **AH.3 Acid Detergent Lignin (%ADL)**

### **Method for Determining Acid Detergent Lignin (ADL) Using the ANKOM Fiber Analyzer**

#### **Reagents**

1. Sulfuric acid
2. Acetone

#### **Solutions**

Sulfuric acid 72% by weight

Mix manually by standardizing reagent grade H<sub>2</sub>SO<sub>4</sub> to specific gravity 1.634 at 20 C or 24.00N. Add 1200 g H<sub>2</sub>SO<sub>4</sub> to 440 ml H<sub>2</sub>O in 1 L volumetric flask with cooling. Standardize to 1634 g/L by removing solution and adding H<sub>2</sub>O or H<sub>2</sub>SO<sub>4</sub> as required.

#### **Safety Precautions:**

1. Acetone is highly flammable. Avoid inhaling or contact with skin.
2. Sulfuric is extremely corrosive. Use a fume hood and rubber gloves when preparing solutions or processing samples. Always add acid to water. If acid contacts skin wash with copious amounts of water.

#### **Equipment and Supplies**

1. Digestion apparatus-ANKOM fiber analyzer
2. Heat Sealer
3. Desiccator
4. Glass beakers-2 L and 1 L in size
5. ANKOM F57 filter bags
6. Glass ashing tubes

#### **Sample Preparation**

Prepare the samples and blank by performing Acid Detergent Fiber (ADF) determinations on them using the ANKOM fiber analyzer and the F57 bags.

## Lignin Procedure

1. Place 24 ADF bags into a 2 L beaker and add approximately 250 mls of 72% H<sub>2</sub>SO<sub>4</sub> to cover the bags. The bags must be completely dry and at ambient temperature before adding the acid. If moisture is present the bags will turn black.
2. Place a 1 L beaker inside the 2 L beaker to keep the bags submerged. Agitate the bags at the start and at 30 minute intervals by pushing the 2L beaker up and down approximately 30 times.
3. After 3 hours pour off the H<sub>2</sub>SO<sub>4</sub> and rinse with hot (90-100 C) to remove the acid. Repeat rinses until the pH is neutral. Pour acetone over the bags and let them soak for 3 minutes. Pour off the acetone and squeeze the bags gently using a beaker. Allow the bags to air dry.
4. Dry the bags in an oven at 105 C for 4 hours of overnight.
5. Remove the bags from the oven and place in a Desiccator. Cool to ambient temperature and weigh.
6. Place each bag in a preweighed ashing tube and ash at 525 C for 12 hours. Cool and weigh.

### Calculations:

#### Blank Correction

Calculate blank correction by dividing the weight loss upon ignition by the original blank bag weight

W1=Bag Tare Weight

W2=Blank bag wt after acid digestion

W3=Weight loss upon ignition (W2 - ash wt)

$$\text{Blank correction (C1)} = (W3/W1)$$

### Lignin Calculation:

Calculate the % lignin by subtracting the blank correction (C1) from the weight loss upon ignition and dividing that number by the dry mattered sample weight and multiplying by 100.

W4=Sample weight expressed on a dry matter basis

W5=Sample and bag wt after acid digestion

W6=Weight loss upon ignition (W5 - ash wt))

$$\% \text{ Lignin} = [(W6 - C1)/W4]*100$$

### **Calculation for Determining % Hemicellulose:**

To determine hemicellulose acid Detergent fiber (ADF) is subtracted from neutral Detergent fiber (NDF).

$$\% \text{NDF(DM)} - \% \text{ADF(DM)} = \% \text{Hemicellulose(DM)}$$

### **Calculation for Determining % Cellulose:**

To determine cellulose Lignin is subtracted from acid Detergent fiber.

$$\% \text{ADF(DM)} - \% \text{Lignin (DM)} = \% \text{Cellulose(DM)}$$

### **References:**

1. Association of Official Analytical Chemists. 1990. Official methods of analysis, 15 ed. AOAC. Arlington, VA.
2. Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral Detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.



## AH.4 Dry matter

### Final or Lab Dry Matter Determination in Feeds

#### Reference Method

Based on AOAC Method 934.01 Moisture in Feed, 15<sup>th</sup> edition 1990

#### Introduction

Final or lab dry matter determination is necessary to compare values of individual components in feedstuffs that vary in moisture content. The dried sample material can only be used for ashing and fat analysis.

#### Scope

This method is applicable to all plant material and liquid and solid manure.

#### Principle

Moisture is evaporated from the sample using heat. The amount of moisture removed is determined gravimetrically.

#### Sample Requirements

Sample container: plastic vial or bag

Storage conditions: room temperature

Sample pre-treatment: oven dried at 60°C Wiley or Tecator™ mill ground to 1 mm

#### Equipment

Forced Air Digital Oven at 104° C  $\pm$  2°

Balance capable of measuring to the 4<sup>th</sup> decimal place (0.0001 g)

Desiccator

#### Supplies

Aluminum dish with lid (50 mm dia. X 15 mm deep)

#### Safety

Precautions for electrical equipment and hot dry matter tins.

## Special Precautions

Adhere to proper temperature control and timing.

Desiccator must contain active desiccant. When the indicator turns from blue to purple it is time to regenerate the desiccant by placing it in a 200° C oven for 6 hours or until chips return to blue color.

## Test Procedure

1. Weigh dry matter tins to the fourth decimal place (.0001g). Record the weight (W1).
2. Weigh 1 to 2 grams of sample into the tins and record the sample weight (W2). If fat analysis using the VELP unit is going to be done use at least 5 grams of sample material in the dry matter tins.
3. Prewarm the oven to 104° C for an hour. Prewarming is not necessary if the samples will be in the oven overnight.
4. Place the tins in the drying oven. The lids should be open and resting on top of the tin to allow the moisture out.
5. The samples should be dried for 5 hours or overnight.
6. Close the lids on the tins and remove them from the oven. Place them in a Desiccator to cool.
7. Weigh tins and record the weight (w3).
8. Clean the tins by discarding the sample into a garbage bag and wiping them with a Kim Wipe™ or a paper towel.

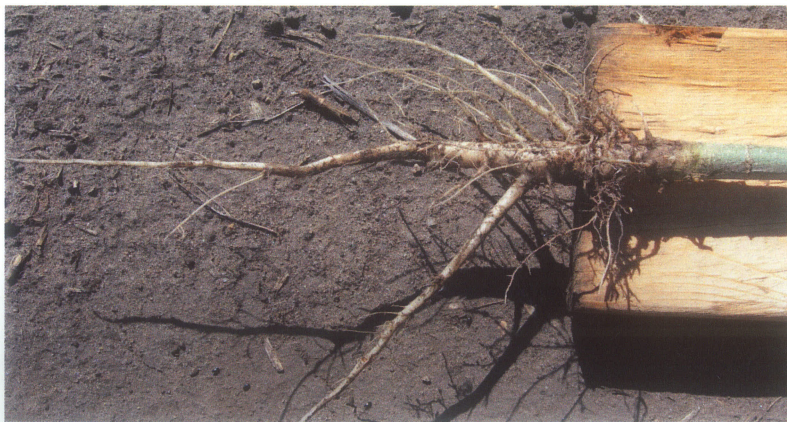
### Dry matter Calculations:

$$\left( \frac{W3 - W1}{W2} \right) \times 100 = \% \text{ DM}$$

## Appendix I Extended hemp anatomy

### AI.1 Root system

The cannabis root tip consist of the root cap, meristematic, elongation and maturation zones which emerge from the germinated achene (seed) typically 3 to 7 days after spring planting (Clarke 1981). Secondary roots form laterally from the downward growth of the primary taproot system (Figure AI.1). Root growth is known to be controlled by naturally occurring plant hormones i.e. *auxins*, *cytokinins* and *gibberellins*. Depths of the primary and secondary root growth are dependent upon the soils physical and chemical characteristics, the cultural agronomic practices employed and the vegetative period. A well-developed taproot can penetrate depths of 2.0-2.5 meters in well-cultivated soils that have a permeable subsoil while secondary roots may latterly extend 60-80 cm (Dempsey 1975; Bosca 1998). The root mass of fibre hemp contributes 8-15% of the plants entire mass which supplies the above ground biomass nutrients (Bosca 1998; Scheifele et al. 1996). The Cannabis root system has been extolled for preventing soil erosion while increasing water infiltration and soil aeration.



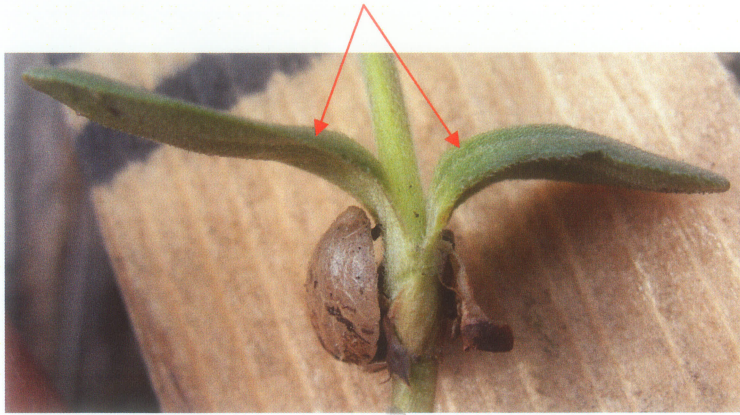
**Figure AI.1** Hemp tap and secondary roots (A. S-Hermann 2006)

## AI.2 Leaf characteristics

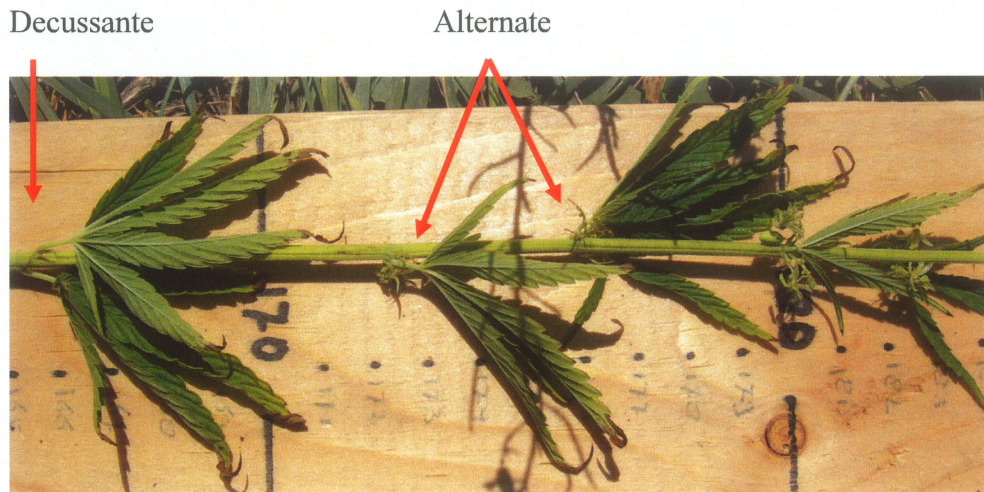
Plants in the Cannabaceae family are dicots (Hayward 1938) (Figure AF.2.1), which share common leaf characteristics that differ slightly amongst varieties. During the pre-floral vegetative stage the usual leaf phyllotaxy is decussate, in which the leaves along the stem appear to be opposite, having two leaves per node and during the floral generative stage leaves are usually alternate, in which one leaf appears at each node along the stalk, resulting in a staggered affect (Clarke 1981; Schumann et al. 1999) (Figure AI.2.2). Schaffer (1926) determined that the phyllotaxy transformation was caused by a change in the photoperiodicity (Schaffner 1926). Heslop-Harrison (1956) greenhouse experiments determined that the phyllotaxy of hemp plants under a short-day photoperiod treatment (16 hour dark) transitioned at the eighth or ninth node while plants that remained in a long-day treatment (21-22 hours light) no phyllotaxy transition occurred before the 18<sup>th</sup> node. Lisson et al. (2000d) noted that there was slower rate of node production as well as a reduced leaf area per node as plant density increased. Female plants produce thicker vegetative leaves than that of male plants (Tibeau 1936).

Figure AI.2.3 shows the compound palmate leaf type of the hemp plant. The serrated palmate pubescent leaflets are attached to the node by a petiole (2 to 8 cm in length (Holmes 1982)) and have a net pinnate venation. The number of leaflets increase with the age of the plant and vary in number from 5 to 11 at maturity (Hayward 1938; Clarke 1981; Bocsa and Karus 1998) and comprise approximately 20.0% of the total above ground biomass (Scheifele et al. 1996).





**Figure AI.2.1** Dicotyledonous June 9, 2006 (A. S-Hermann 2006).



**Figure AI.2.2** Leaf phyllotaxy change from decussate to alternate July 18<sup>th</sup>, 2006. (A. S-Hermann 2006).



**Figure AI.2.3** The compound palmate leaf type of the hemp plant July 18<sup>th</sup>, 2006. (A. S-Hermann 2006).

### AI.3 Flower structure

In angiosperms (flowering plants) the reproductive structures are located in the flowers. *Cannabis* flowers are conspicuous and imperfect, possessing separate male (staminate) or female (pistillate carpellate) reproductive structures and within the Cannabaceae family, reproductive structures can be found in both dioecy and monoecy states. In which the reproductive structures are indistinguishable during the formation of the undifferentiated primordium.

Dioecy is the production of pistillate and staminate flowers on different plants: true dioecy promotes cross pollination (Poehlman and Sleper 1995) (Figure AI.3.1). True unisexual gynoeocious (female) pistillated structures produce axil paired flowers that posses paired stipule subtending bracts which rest aside a glandular trichome covered pistillate calyx (floral sheath) and posses an indeterminate raceme type inflorescence (Clarke 1981). Enclosed within the calyx is the placenta, ovule and the ovary with two extended fused style and stigma. True dioecious male plants tend to be taller than pure female plants and die off after dehiscence (pollen release).



Figure AI.3.1 Dioecious plants. (Friesen 2006)



Unlike the pistillated flowers, the androecious (male) staminate floral buds have indeterminate panicle paired sac development. Within the simple non-glandular trichome calyxes (approx. 5 millimeters long (Bosca and Karus 1998)), that vary in shades of yellowish green, enclose five filament attached suspended stamens which contain anthers (pollen sacs) that hold the microsporangia (pollen grains). Cannabis pollen grains vary in size from 25 to 30 microns ( $\mu$ ) (Small 1972; Small and Cronquist 1976; Clarke 1981). Pollination can occur by both means of anemophily (wind) (Small 1973, 1976; Schumann et al. 1999) and may potentially be pollinated entomophily (insect) (Figure AI.3.2).



**Figure AI. 3.2** Honey Bee collecting pollen. (A. S- Hermann 2007)

It is known that feral Cannabis populations will emerge, if allowed to escape from controlled agricultural practice. These populations have become well established, due in part to long-distance genetic interchange and inter and intra-crossing (Small 1972, Small and Cronquist 1976). Bosca and Karus (1998) concluded that pollen can travel as far as 12 km and reach altitudes of 20-30 meters. 18<sup>th</sup> century, low THC feral hemp populations in the Upper Canada region of southern Ontario have been genetically re-captured and are being re-introduced as pre-extinction fibre cultivars. de Meijer (1995) concluded that there is a “considerable mutual genetic relatedness among modern

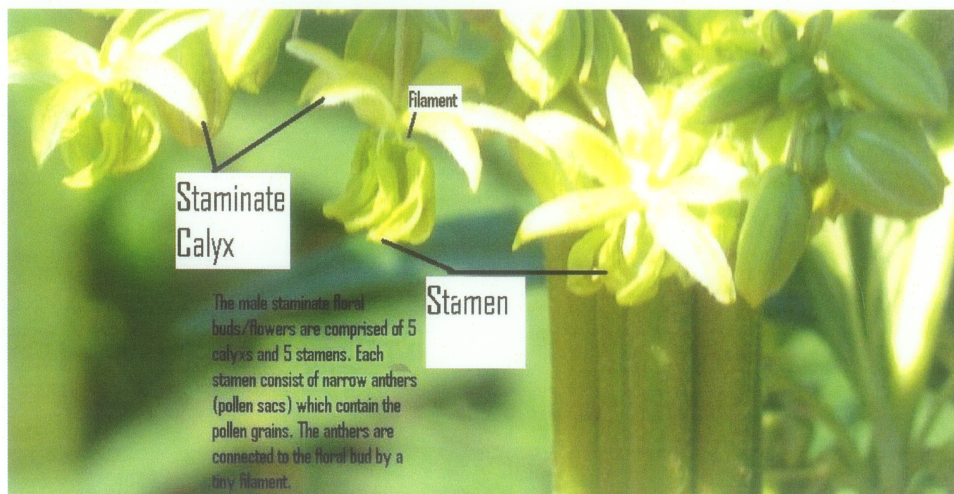
European and West Asian cultivars.” Hillig (2005) strongly suggested that germplasm from feral, local and wild Cannabis landraces should be collected and maintained before foreign or hybrid strains are introduced to a region, thus preventing lose of adapted parental germplasm.

Monoecious plants are characterizes by the staminate and pistillate reproductive structures being located on the same plant stalk (Figure AI 3.3; AI 3.4). The above flowering structural description holds true for monoecy hemp plants as well except that structures are present on one plant. Within the Canadian hemp breeding industry the standard guide for determining the degree of monoecy is the IHSDC Modified Sengbusch Scale for Monoecious Hemp of Intersex Inflorescence types (Type1-5) (Appendix A). This scale rates the degree of monoecy by the percentage of male and female flowers present on one stalk. It has been noted that hemp will ‘revert’ to its natural dioecious state if not properly maintained and even though monoecious varieties of cannabis can be found, it is not the natural state (van der Werf et al. 1994c) that must be maintained by strict rouging breeding procedures (Schumann et al. 1999). Breeding programs assess and maintain the degree of monoecy and diocey, chemical phenotypes and plant characters upon the varieties classification.



**Figure AI.3.3** Monoecious plant: staminate and pistillate (A. S- Hermann 2007)





**Figure AI 3.4** Staminate details (A. S- Hermann 2006)

#### **AI.4 THC (delta<sup>9</sup>-tetrahydrocannabinol) regulations**

The dominate cannabinoid phenotype of interest in hemp is delta-9-tetrahydrocannabinol (THC). THC is concentrated in the trichomes, calyxes and bracts of the flowering parts (Kim and Mahlberg 1997). In Canada, the level or percentage of THC is determined in ordinance with Health Canada’s Industrial Hemp Technical Manual ([http://www.hc-sc.gc.ca/dhp-mps/pubs/precurs/hemp-indus-chanvre/tech-man/index\\_e.html](http://www.hc-sc.gc.ca/dhp-mps/pubs/precurs/hemp-indus-chanvre/tech-man/index_e.html)). The regulatory methods for sampling THC vary slightly between countries for example: Canada requires a random sample of 60 plant tops no matter the size of area planted, in the EU, regulation number (VO (EG) 1177/2000), requires a random sample size of 50 plant tops (Mechtler et al. 1999) and regulations in the United Kingdom require THC analysis to be conducted on at least 20% of the total area of hemp production for each cultivar which must be comprised of at least 20% of the registered producer fields (Weightman and Kindred 2005). Since 1988, the allowable THC content for industrial hemp cultivated in the Ukraine has been 0.1% of dry weight plant material (Holoborod’ko 17/03/2005), even lower than that of Canada’s 0.3% limit. Mechtler et al.

(1999) determined that the presence of the psychoactive component, THC, did not have an adverse affect on fibre.

### **AI.5 Seed (achene) structure**

The cannabis seed is not a true seed at all, it is actually an achene (nut) fruit. For the purpose of this report the achene fruit of Cannabis will be referred to as a seed (Figure AI.5.1). The hemp seed is prized for its nutritional balance of omega-6 to omega-3 fatty acids, its relatively high contents of gamma-linolenic acid (GLA, 18:3 omega -6) and stericonic acid (18:4 omega-3) which have been determined to be 4 and 2 percent, respectively, in the Finland bred Canadian grown cultivar, Finola (Fin-314) (Leson et al. 1999).

Hemp is day length sensitive, in that the seed maturity occurs as day length shortens. Harvested hemp grain must be dried to at least 9% for storage and is by weight 25 - 35% oil, 20-30% carbohydrates, 10-15 % insoluble fibre and the seed cake (after cold pressing) contains approximately 24-40% protein and is rich in minerals (Saciclik et al. 2003). Wang et al. (2008) compared hemp protein isolates (HPI) to soy protein isolates (SPI) in which the results determined that HPI was a good source of readily digestible human consumption suitable protein than that of SPI. The hemp seed has a mottled pericarp (fruit husk), is orbicular (egg or oval) shaped and ranges in size from 2 to 6 mm long and 2 to 4 mm in diameter (van der Werf 1994a; Bósca and Karus 1998). The thousand kernel weight (TKW) varies from 2 to 70 grams, depending on cultivar.

Canadian Researchers' in 1934-1936 examined total and line fibre yield, from 'large and small' sized hemp planting seed. The total fibre yield was greater from 'large seed' plots (6,267 lb ac<sup>-1</sup>) than that of the 'small seed' (5,920 lb ac<sup>-1</sup>) and the 'small seed'

plots yielded more line fibre per acre (583 lb ac<sup>-1</sup>) than that of the 'large seed' plots (557 lb ac<sup>-1</sup>). They concluded that the difference in the 'large and small seed' plots total fibre and line yield, was more than likely due to a thicker stand of plants in the 'small seed' plots (Hutchinson 1938).

The seed contains two cotyledons, a rootlet and a thin undeveloped starch containing endosperm (Bósca and Karus 1998). It is pertinent that the handling (i.e. seeding, harvesting, cleaning, storage, and processing) of the seed/grain be done with great care; cracking of the seed hull will result in rancidity and increased peroxide levels, which will cause delivery refusal. A decimal code for the development stages of hemp was determined by Mediavilla et al. (1998) which formulated standard definitions and codes for the growth stages of *Cannabis sativa* L. plants. (Appendix B).

The goal of certified seed production is to breed and maintain cultivars that are uniform and that possess predetermined quality characters i.e. oil, nut, protein, nutritional and fatty acid profiles.

The effects of varying plant densities ranging from 75-350 seed m<sup>-2</sup> of Canadian certified cultivars on seed yield was examined by McGregor (1998), Stadnyk (1999, 2000), Kostuik (2001), PCDF (2002, 2003)(Table 5.1.4.1). Overall, the authors determined that increase plant density had little to no effect on seed yield.

Methods for determining fibre yield vary depending upon researcher. Methods of fibre separation are examined further in the Harvesting section of this document.



**Figure AI.5.1** Hemp seeds A.S-Hermann 2006

Table 5.1.4.1 Effects of plant density on seed yield of four Canadian hemp cultivars.  
(Adapted from McGregor 1998, Stadnyk 1999, 2000, Kostuik 2001, PCDF 2002, 2003).

Cultivar	Treatment (seed m <sup>-2</sup> )	Year					
		1998	1999	2000	2001	2002	2003
		<u>Seed yield (lbs/ac)</u>					
USO 14	75	602	415				
	100	565	371	620	551	367	526
	125	621	319				
	150			585			
	200					322	503
	300				454	339	468
	350			503			
USO 31	75	438					
	100	564		583	577	306	495
	125	552					
	150			511			
	200					339	394
	300					331	486
	350			459			
Fin 314 (Finola)	75		392				
	100		470	459	205		
	125		365				
	150			376			
Anka	100				530	525	
	200					271	
	300				549	556	
Alyssa	100						696
	200						671
	300						504

## Appendix J Method of extraction for soil testing

Provided by Bodycote Testing Labs, Winnipeg, Manitoba Canada  
<http://www.bodycotetesting.com/category.aspx?catid=11268>

P/K Extraction Ashworth, J. and K. Mrazek. *Comm. Soil Sci. Pl. Anal.*, 1995. 26:731-739, *Modified Kelowna Soil Test*.

P analysis based on American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 20<sup>th</sup> edition, 1998. APHA 4500-P D, *Stannous Chloride Method*.

K analysis based on Alberta Research Council. *Methods Manual for Chemical Analysis of Water and Wastes*, 1996. Method 19403 565, *Potassium, Dissolved (Automated Flame Photometry Method)*.

From Carter et al, *Soil Sampling and Methods of Analysis*, 2008:

Olsen P Extraction based on Chapter 8, *Sodium Bicarbonate-Extractable Phosphorus*.

Analysis based on American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 21<sup>st</sup> edition, 2005. APHA 4500-P D, *Stannous Chloride Method*.

Boron Extraction and analysis based on 9.2.2 and 9.4.4 respectively.

Organic Matter Based on 21.3.1, Dichromate Redox Colorimetric Method, and A. Walkley and I. Armstrong Black (1934). *An Examination of Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method*, *Soil Sci.* (37:29-37).

From Carter et al, *Soil Sampling and Methods of Analysis*, 1993:

N/S Extraction based on 9.2 Determination of Soil Solution Sulfate, followed by APHA 3120 B, *Inductively Coupled Plasma (ICP) Method for S*, and APHA 4500-F NO<sub>3</sub><sup>-</sup>, *Automated Cadmium Reduction Method for nitrate-N*.

Ca, Mg, Na Extraction based on 5.2 Ammonium Acetate Extraction followed by APHA 3120 B as above. Base saturation is calculated from the total exchangeable cations (Ca, Mg, Na and K).

Cu, Fe, Mn, Zn Chapter 11.3 DTPA Extraction followed by APHA 3120 B as above.

pH/EC Based on J.A. McKeague. *Manual on Soil Sampling and Methods of Analysis*, 1978. Method 4.12, *1:2 Soil:Water Ratio*.

CEC McKeague, J.A. 1978. *Manual on Soil Sampling and Methods of Analysis*, 1978. Method 3.32, *CEC and Exchangeable Cations by NH<sub>4</sub>OAc at pH 7*.