NOTE TO USERS

THE EFFECTS OF ORGANIC AND INORGANIC NITROGEN FERTILIZER ON THE MORPHOLOGY AND ANATOMY OF INDUSTRIAL FIBRE HEMP (*CANNABIS SATIVA* L.) GROWN IN NORTHERN BRITISH COLUMBIA, CANADA

by

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Abstract

The effect of organic and inorganic nitrogen fertilizer on the morphology and anatomy of *Cannabis sativa* var. *fédrina* was investigated in both a greenhouse and field setting in Northern British Columbia. Plots (90 stems/m^2) treated with 0, 75, 150 or 300 kg N/ha of inorganic nitrogen or fishmeal, bloodmeal or sea star organic fertilizer were also replicated with 90 kg inorganic P_2O_5/ha application. The application of 150 and/or 300 kg N/ha of any nitrogen fertilizer type benefited field-grown plant morphology, secondary phloem fibre and xylem development, while greenhouse-grown plant morphology, secondary phloem fibre and xylem were positively influenced by 90 kg P_2O_5/ha. Primary phloem fibre characteristics of both greenhouse and field-grown plants were benefited by the absence of either nitrogen or phosphorus fertilizer. This study determined that organic can be used in place of inorganic nitrogen fertilizer for the production of a majority of fibre characteristics of *C. sativa* var. *fédrina*.

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1 Introduction and Objectives

The potential of crop cultivation, including industrial fibre hemp (*Cannabis sativa* L.), for community economic development was investigated by Dave Ryan and the Gitsegukla Economic Corporation of Gitsegukla, British Columbia. Dr. Jane Young, from the Biology Program at UNBC, was asked to assist Mr. Ryan in his endeavours by offering scientific data on fertilizer, specifically organic, requirements for productive *C. sativa* fibre crops in Northern British Columbia. Another goal of this project was to test locally derived organic fertilizers as the Gitsegukla Economic Corporation anticipated future production of a fish head meal organic fertilizer, which would also minimize waste in their commercial fishing (Ryan *pers. comm.* 1999). With such production still under development, it was requested that Alaskan Fish Meal (white cod bonemeal) organic fertilizer (Alaska Fish Fertilizer Company, Renton, Washington) be tested as a nitrogen source.

The village of Masset, Queen Charlotte Islands, British Columbia, supplied a sea star meal fertilizer, which has been traditionally used on local gardens (Forai *pers. comm.* 2000). Sea star is a by-catch product of the commercial fishing industry, and a joint investigation with the Department of Fisheries and Oceans (DFO) into the feasibility of a commercial sea star industry was initiated (Forai *pers. comm.* 2000). A third organic fertilizer, Blood Meal, was purchased through Gaia Green Products Ltd., Grand Forks, British Columbia. All three organic fertilizers were compared with inorganic nitrogen fertilizer (Evergro Products, Inc., Delta, British Columbia) in this study.

To assist Mr. Ryan in meeting the goal, the objective of this thesis was to assess the effect of nitrogen source and level on morphological and anatomical characteristics of *C. sativa* var. *fédrina* grown in both field and greenhouse settings. Statistical significance of between treatment effects of three levels of three organic nitrogen fertilizers, one inorganic

nitrogen fertilizer and a control were determined for plant height, diameter, weight, cell dimensions and areas and volumes of the three fibre types: primary phloem, secondary phloem and xylem. The anatomical component focused on primary phloem fibre, as it is valued for its length, flexibility, high cellulose content and lower quantity of more easily removed lignin (Kundu 1941, Van der Werf *et al.* 1994b, 1995b, Bocsa and Karus 1998, Correia 1999, Keller *et al.* 2001, Mediavilla *et al.* 2001).

The field site for this project was at Gitsegukla and ran adjacent to the Skeena River, on a field not cultivated for almost 100 years. The greenhouse site was located at the I.K. Barber Enhanced Forestry Laboratory, UNBC, Prince George, British Columbia. The greenhouse was used as a "controlled" site to minimize the effect of other factors and to assess potential influence of different nitrogen fertilizers.

Previous research has focused on the effect of nitrogen level on *C. sativa* cultivation (Jordan *et al.* 1946, Hessler 1947, Ruzsanyi 1970, Ritz 1972, Basso and Ruggiero 1976, Haralanov and Babayashev 1976, Van der Werf *et al.* 1994a, 1995a, 1995c, Höppner and Menge-Hartmann 1995, Meijer *et al.* 1995, Van der Werf and Van den Berg 1995, Ivonyi *et al.* 1997, Cromack 1998, Scheifele 1999, Amaducci *et al.* 2000, BCMAF 2000, Lisson and Mendham 2000, Sankari 2000, Struik *et al.* 2000, Keller *et al.* 2001, Mediavilla *et al.* 2001), but not on potential differences between those of organic and inorganic fertilizers.

2 Literature Review

2.1 History

Cannabis and *Humulus* are the only two genera of the Cannabaceae family (Schultes 1970, Stearn 1970). Industrial hemp and marijuana share the same genus and species, *Cannabis sativa* L., only differing in their variety, which is primarily determined by the

content of THC (δ (delta)-9-tetrahydrocannabinol) (Health Canada 1998, British Columbia Ministry of Agriculture and Food (BCMAF) 1999). Canadian regulations require that industrial hemp contain less than 0.3% THC, while marijuana varieties have been known to contain 8-30% THC (Health Canada 1998, BCMAF 1999).

The word *Cannabis* has roots in Sanskrit ancient vernacular and although Caspar Baughin first used the name *Cannabis sativa* in 1623, Linnaeus created the *Cannabis* genus in 1735 (Schultes 1970). Despite the fact that numerous species names have occurred, i.e., *C. chinensis* (Delile), *C. erretica* (Sieve.), *C. foetens* (Gilib.), *C. indica* (Lam.), *C. lupulus* (Scop.), *C. macrosperma* (Stokes), *C. americana* (Pharm. Ex Wehmer.), *C. generalis* (E.H.L. Krause), *C. gigantea* (Crevost), *C. ruderalis* (Janischevskii), and a hybrid, *C.*X. *interstitia* (Sojak), it is now generally agreed that *Cannabis* is a monotypic genus with one species. There are no true morphological or botanical varieties within the species, but instead many ecotypes and cultivated races exist (Schultes 1970). This point is often ignored as the term 'variety' is commonly used in the *Cannabis* literature.

C. sativa is not only considered to be one of the oldest crops used for fibre, but one of the first crops to be cultivated by humans (Schultes 1970, Dempsey 1975). Generally considered an "Old World Plant" and Asiatic in origin, it has been found in tombs dating back to 8000 BC (Schultes 1970, BCMAF 1999). In China, there is evidence of its use as a food grain and fibre source 6500 years ago and it is documented in Chinese writings as early as 2700 BC (Blade *et al.* 1998, BCMAF 1999). Around 1500 BC, Scythian invaders from Asia introduced *C. sativa* to Western Europe (Schultes 1970). The first reports of its cultivation in the New World occurred in Chile in 1545 (Blade *et al.* 1998). Introduction into North America was around the year 1632, by pilgrims of New England (Schultes 1970). *C.*

sativa has been found growing wild from Iran to southern Siberia and may be one of the most widely distributed plants, now spread throughout most of the temperate and tropic regions of the world (Haney and Bazzaz 1970, BCMAF 1999).

Over 50,000 items can be made from *C. sativa* fibres and seeds (BCMAF 1999). Fibres have been used to produce fabric, paper, rope, sails, fishing nets, oakum, upholstery and carpet (Ramey, Jr. 1980, Van der Werf *et al.* 1996, Fraanje 1997). Until the early 19th century, paper was made out of rags (worn-out cloths) which were solely *C. sativa* and *Linum* (flax) [occasionally *Gossypium* (cotton)], therefore, almost all paper in history was made of the fibres of these two plants (Van Roekel 1994). Sail-powdered navies of the world were reliant upon the use of fibrous crops such as *C. sativa*, *Corchorus* (jute) and *Linum*, and farmers of England and American colonies were legislated to dedicate a portion of their land to the production of *C. sativa* (Vignon and Garcia-Jaldon 1996, Blade *et al.* 1998).

Introduction of *C. sativa* to Canada occurred in Nova Scotia (Francis 1996). The popularity of *C. sativa* farming in Eastern and Central Canada rose in the 18th and 19th centuries (Blade *et al.* 1998). From 1923 to 1942, the Canadian Department of Agriculture tested agronomic management, processing and crop improvement techniques in ~30 locations across Canada (Blade *et al.* 1998). High production costs and the increased use of motorized boats, synthetic fibres and fibre crops grown in the tropics lead to a decline in *C. sativa* cultivation (Ramey, Jr. 1980, Vignon and Garcia-Jaldon 1996, BCMAF 1999). In 1938, cultivation was made illegal under the Canadian Opium and Narcotics Act, although limited production was granted during World War II (BCMAF 1999). In 1994, a license was granted for a low-THC *C. sativa* research plot in southern Ontario. On March 12, 1998, after a 60 year Canadian ban, it became legal to grow 23 varieties of industrial *C. sativa* under licenses granted by Health Canada (BCMAF 1999).

2.2 Morphology

2.2.1 Root

C. sativa has a tap root system that includes numerous lateral roots with horizontal and vertical extensions (Kundu 1941, Dempsey 1975, Bocsa and Karus 1998). Rooting depth can be influenced by soil characteristics, groundwater level, cultivation technique, sex and variety (Bocsa and Karus 1998). In well-cultivated, permeable soils with high mineral content subsoil, taproots can reach depths of 2-2.5 m. In heavy humus or marshy soil, taproots reach 30-40 cm (Dempsey 1975, Bocsa and Karus 1998). Under ideal conditions, lateral roots can extend as far as 60-80 cm (Bocsa and Karus 1998).

2.2.2 Stem

C. sativa is an annual dicot (Kundu 1941, Stearn 1970, Dempsey 1975). When young, the succulent stem lignifies rapidly becoming erect, branched, rigid and woody (Kundu 1941, Dempsey 1975, Fraanje 1997, Bocsa and Karus 1998). Under optimal conditions, seeds germinate within 3-7 days and growth to the fourth internode occurs in 2-3 weeks (Clarke 1999, Scheifele 1999). *C. sativa* can grow 4-10 cm a day and as high as 5 m in its ~110-115 day vegetative period (Dempsey 1975, Bocsa and Karus 1998, Clarke 1999, Scheifele 1999). Male plants grow 10-15% taller than females, with smaller diameters and fewer branches (Bocsa and Karus 1998). *C. sativa* can have 6-11 internodes, as long as 20-50 cm varying in length along the stem (Kundu 1941, Dempsey 1975, Mediavilla *et al.* 1998). Stem diameter can range from 3.6-60 mm (Jordan *et al.* 1946, Dempsey 1975, Scheifele 1999). Large-diameter stems are more suitable for seed than fibre production while 4-5 mm diameters are optimal for fibre production (Jordan *et al.* 1946, Dempsey 1975, Scheifele 1999).

C. sativa stems are obtusely hexagonal, grooved or furrowed, and hollow in most

varieties (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998). They are covered by minute, non-erect, upward-curved glandular hairs (Stearn 1970). The stem provides 65-70% of the total mass of a fully-grown *C. sativa* plant (Bocsa and Karus 1998). Leaves appear on the stem, but are shed off in the lower portion as the plant matures (Dempsey 1975).

2.2.3 Foliage

C. sativa plants have epigeous cotyledons at the first node, followed by 7-12 leaf pairs (Kundu 1941, Mediavilla *et al.* 1998). Leaf pairs are arranged in decussate (opposite) phyllotaxy until photoperiod change prompts a transition to alternate arrangement and subsequent onset of flowering (Kundu 1941, Dempsey 1975, Mediavilla *et al.* 1998, Clarke 1999, Lisson and Mendham 2000).

Leaves are palmately compound, most commonly with 5-9 leaflets, but their rangecan be from 3-11 (Stearn 1970, Dempsey 1975). The number of leaflets is determined by variety, position of leaves on the stem and plant age (Bocsa and Karus 1998). The narrow-lanceolate leaflets have a wedge-shaped base, a coarse, saw-toothed edge and a long pointed tip (Stearn 1970). They vary in length from 5-15 cm and 1-2 cm in width (Stearn 1970, Dempsey 1975). There is an average of 4-14 teeth per edge, with sharp points towards the tip of each leaflet. Oblique leaf veins occur from leaflet midrib to teeth tips (Stearn 1970). Leaflets are rough and dark green on the adaxial surface, but somewhat lighter on the abaxial surface (Dempsey 1975). Numerous hairs cover the surface of the leaflets creating a soft texture (Stearn 1970, Bocsa and Karus 1998). Leaf glandular hairs excrete minimal amounts of THC in their resin (Bocsa and Karus 1998).

C. sativa laminas are attached to the stem by stiff, 3-15 cm long petioles with persistent pointed stipules at their base (Kundu 1941, Stearn 1970, Dempsey 1975, Bocsa and

Karus 1998). Internode length, and size and complexity of leaves, increase until the midpoint of the stem where the longest internode (~18 cm) and the largest leaves are situated (Kundu 1941).

In the vegetative phase, leaf mass can comprise ~24-25% of the total plant mass but by the end of this phase can be reduced to ~8-14% due to wind and natural attrition. Irregularly shaped or coloured leaves commonly develop from fused leaflets, dual-leaf formation or chlorophyll defects (Bocsa and Karus 1998).

2.2.4 Floral Characteristics

C. sativa is sexually dimorphic (Bocsa and Karus 1998). Originally dioecious, it is also possible to breed monoecious varieties (Bocsa and Karus 1998, Van der Werf *et al.* 1994a). Experimental modification to decrease daily exposure to light can increase the occurrence of monoecious plants while prolonged exposure decreases the proportion of flowering plants, prevents male and monoecious flowering and reduces flowering in female plants (Dempsey 1975, Van der Werf *et al.* 1994a). Although monoecious *C. sativa* yields fewer flowers and less pollen, the characteristics of monoecious flowers are similar to those of female dioecious plants (Bocsa and Karus 1998).

It is claimed that male and female plants are morphologically indistinguishable before flowering (Van der Werf *et al.* 1994a). However, in dioecious *C. sativa*, the male (staminate) plants are taller and narrower, with fewer leaves than female (pistillate) plants, which are shorter, stockier and house a broad crown of leaves associated with the terminal inflorescence (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998). Male plants have a 4-6 week shorter vegetative period than females and die after shedding the pollen, while female plants survive until the fruits are mature (Kundu 1941, Dempsey 1975, Bocsa and Karus 1998). Unlike most

dioecious crops, which have a 1:1 sex ratio, in *C. sativa* crops female plants predominate slightly with 53% female and 42% male plants (Bocsa and Karus 1998). The number of male and female flowers is relatively constant under normal conditions within a single variety, but may vary between geological races, varieties or environmental conditions (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998). Plant mass for both the male and female plants are uniform in a fibre crop at regular harvest time, although delayed harvesting results in lower weight for males (Bocsa and Karus 1998).

2.2.4.1 Staminate

Male flowers can reach 18-30 cm in length as long, loose, multi-branched, clustered panicles that develop in the axils of leaves either singly, in pairs, or in groups (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998, Clarke 1999). Each inflorescence branch supports three apetalous florets, one median and two laterals, on bracts or stipules (Dempsey 1975). Florets have a deeply parted, simple calyx with five yellow-green or red lobes (~5 mm long) that house five stamens with anthers suspended from long threadlike filaments (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998). Anthers have an elongated prism shape prior to maturation and turn light yellow after maturation. As fibre crops are planted densely to decrease branching, flowers are restricted to the terminal end of the stem resulting in fewer flowers per plant than a crop with wide spacing (Bocsa and Karus 1998).

C. sativa is anemophilous (wind pollinated) with dry, floury dense clouds that travel as far as 12 km and as high as 20-30 m (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998, Clarke 1999). It produces more pollen than any other cultivated plant (BCMAF 1999). At anthesis, a single male plant can release up to 30-40 g of pollen from terminal pores of the anther (Dempsey 1975, Bocsa and Karus 1998, Mediavilla *et al.* 1998). The white, papillate

or smooth, circular-oblate pollen grains are 25-30 μm in diameter with 2-4 circular germ-pores (Stearn 1970, Dempsey 1975).

2.2.4.2 Pistillate

Female flowers form a dense, club-shaped or erect raceme inflorescence, in close aggregate pairs on stipules at leaf axils (Stearn 1970, Dempsey 1975, Clarke 1999). A papery, scabrous, green, single-leaf calyx or bract, creates a tubular sheath around the ovary (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998, Mediavilla *et al.* 1998). The thin membranous bract excretes resin from the short-stalked or stalkless circular glands of its slender trichomes (hairs) (Stearn 1970, Clarke 1999). The bract surrounds two thin (3-8 mm) pistils, with fused style and dual-forked stigma, and the ovary, which houses one seed (Bocsa and Karus 1998, Mediavilla *et al.* 1998). With pollination, stigmas turn from white to red, but if pollination is deficient or does not occur, pistils can turn bright white and reach 10-20 mm (Bocsa and Karus 1998).

Although the fruit of *C. sativa* is technically an achene, it has also been called a nut and is most commonly referred to as a 'seed'. Bracts surround and imprint a mottle on the hard, thin, net-veined pericarp, which is light brown to dark grey, smooth and somewhat compressed, orbicular-oval or ellipsoid in shape (Stearn 1970, Dempsey 1975, Bocsa and Karus 1998, Clarke 1999).

The single, oil-rich seed contains a strongly curved embryo with two cotyledons packed together along one side and the thin radicle along the other side of the starchy endosperm (Stearn 1970, Bocsa and Karus 1998). On average, *C. sativa* fruits are 2-6 mm long, 2-4 mm wide and 1-4 mm in diameter weighing 4.7-23.7 mg (Stearn 1970, Bocsa and

Karus 1998, Clarke 1999, Oomah *et al.* 2002). Thousand seed weights average 20 g, with a range from 3-60 g and monoecious seed averages of 16 g, dioecious averages of 21 g and fibre varieties ranging from 14-23 g (Dempsey 1975, Clarke 1999). Individual seeds mature in 3-5 weeks (Mediavilla *et al.* 1998).

C. sativa seeds contain 25-35% oil, 20-30% carbohydrates, 20-25% protein and 10-15% dietary fibre (Deferne and Pate 1996). They are high in essential fatty acids (37%) and carotene, low in saturated fats and have a favourable balance of 3:1 Ω (omega)-6 to Ω-3 fatty acids (Deferne and Pate 1996, BCMAF 1999, Leizer *et al.* 2000). There is a range from ~2.2:1 to a 3:1 ratio of linoleic to α-linolenic acid (BCMAF 1999, Leizer *et al.* 2000). Seeds have <1 mg/g condensed tannin (catechin), <3 mg/g inositolpentaphosphate and <15 mg/g inositolhexaphosphate (phytic acid) (Matthäus 1997). They also contain phosphorus, potassium, calcium, magnesium, sulphur, iron, zinc, terpenes, cannabinoids, phenolics (including methyl salicylate), β-sitosterol which has hypocholesterolemic properties and tocopherols which have antioxidant and anticancer properties (Deferne and Pate 1996, Leizer *et al.* 2000).

The seed is the only part of *C. sativa* that has been used for food. Whole seeds are used in soups or ground into cakes, animal feed, non-dairy cheese, milk and ice cream (Roulac 1997, BCMAF 1999). There are no known negative effects, or toxicity reports, of the consumption of seed oil or its cannabinoids (Leizer *et al.*, 2000).

The seed oil can also be used in the manufacturing of fuel, paint, cosmetics, soaps, detergents, hydraulic oils, lubricants, biofuels, pharmaceuticals, leather and textiles (Matthäus 1997, BCMAF 1999). The UV-B and UV-C absorbance capacity may make it a potential source for broad-spectrum UV protection (Oomah *et al.* 2002). Residues arising

from manufacturing processes of *C. sativa* seed oil can also be used in animal feeds (Matthäus 1997).

2.3 Chemicals

THC (δ -9-tetrahydrocannabinol) is produced by glandular trichomes and accumulates in specialized non-cellular, intra-wall secretory cavities (Kim and Mahlberg 1997). It is secreted by epidermal resin glands, which are associated with the glandular hairs of the leaf, stem and female flowers (Stearn 1970, Blade *et al.* 1998, Bocsa and Karus 1998, BCMAF 1999, Clarke 1999). It has been suggested that THC offers the plant UV-B protection, protection from animals, or may work in conjunction with other chemicals to act as a means of plant recognition to other organisms in the environment (Stearn 1970, Kim and Mahlberg 1997, Blade *et al.* 1998).

Within a particular variety of *C. sativa*, the THC content is largely dependent upon environmental conditions (Bocsa and Karus 1998). At a lower latitude (46° N) or elevation (200-250 m), the same variety may produce higher levels of THC than at a higher latitude (55° N) or elevation (500-600 m). There are also differences in the THC levels of individual geographic races. Central Russian geographical races have intermediate levels of THC, while Asiatic races have the highest concentrations (Bocsa and Karus 1998).

Oils and other chemicals are also found in *C. sativa*. Cannabinol occurs in leaves, flowers and tips of plants (Dempsey 1975). Although concentrations are similar between male and female plants, the increased foliage of female plants results in higher cannabinol yields (Argurell 1970). Cannabinol is composed of >95% cannabidiolic acid and <3% δ -tetrahydrocannabinolic acid (Argurell 1970). Cannabidiolic acid appears at early growth

stages, increases through plant development and is known to have antibiotic activity by the eighth week of growth (Krejci 1970). Cannabinoids, and their associated terpenes, may play roles in antidessication, antimicrobial, antifeedant and UV-B pigmentation (Pate 1994). There are 58 monoterpenes, 38 sesquiterpenes, simple ketones and esters that have also been isolated in different *C. sativa* preparations (Ross and ElSohly 1996).

2.4 Anatomy

As in other 'woody' annual dicotyledons, a *C. sativa* stem is composed of epidermis, cortex, primary phloem, secondary phloem, vascular cambium, secondary xylem, primary xylem, and pith, which becomes hollow as the plant matures (Kundu 1941, Mauseth 1988, Raven *et al.* 1999).

Cortex can be composed of parenchyma, collenchyma and/or sclerenchyma cells (Mauseth 1988, Raven *et al.* 1999). Parenchyma cells have a thin primary wall. They provide plant organs with mechanical strength derived from osmotic-induced turgor pressure. Parenchyma cells are also involved in synthesis, structure, transport, secretion and meristematic functions. Collenchyma cells have thick, cellulose rich, primary walls with plasticity that offers strength during organ elongation and can lignify at its completion. They are found in dicots, and occur in the margins and ribs of stems, petioles and leaves, often directly beneath the epidermis but also in strands or cylinders within the cortex (Mauseth 1988, Raven *et al.* 1999). There are no examples of use of collenchyma exclusively in fibre production which relies on high tensile strength (McDougall *et al.* 1993).

Sclerenchyma cells have continuous graduation in size, shape and function; therefore, classification of sclerenchyma is divided between conducting and non-conducting sclerenchyma (Mauseth 1988, Raven *et al.* 1999). Conducting sclerenchyma are tracheids

and vessel elements of the xylem. The secondary walls of each are high in lignin and also lack protoplasm at maturity, which makes the cells conducive to water conduction. Non-conducting sclerenchyma can be divided into either fibres or sclereids, i.e., xylary (xylem) or extraxylary sclerenchyma (Mauseth 1988, Raven *et al.* 1999). A 'fibre cell' is an individual sclerenchyma cell, a 'fibre bundle' is a collection of such cells and the term, 'fibre', is a mix of cells and bundles (Hobson *et al.* 2001).

2.4.1 Vascular Tissue Differentiation Factors

Plant growth regulators (PGRs) (phytohormones) believed to be involved in vascular tissue and fibre differentiation include, but are not exclusive to, auxin, gibberellin and cytokinin (Aloni 1979, Saks *et al.* 1984, Aloni 1995). Auxin and gibberellin are commonly associated with vegetative portions of the stem such as the developing buds and leaves, while cytokinin is found in the roots (Saks *et al.* 1984, Taiz and Zeiger 1991, Aloni 1995, Raven *et al.* 1999).

Auxin is the limiting and controlling factor for phloem and xylem differentiation (Aloni 1995). Phloem differentiation can occur at low auxin levels, and in the absence of xylem differentiation, however, xylem differentiation can only occur with higher auxin levels. The highest ratio of phloem/xylem occurs under optimal cytokinin levels. In the early stages of vascular differentiation, cytokinin involvement requires the presence of auxin while in later stages, differentiation can occur in the absence of cytokinin. The presence of cytokinin increases tissue sensitivity to auxin (Aloni 1995).

Auxin is the main inducing and limiting factor in primary phloem fibre differentiation (Aloni 1979). Gibberellin is involved, however, unlike auxin, it cannot act alone and requires the presence of auxin to be involved. Auxin/gibberellin ratios determine the development and

size of primary phloem fibres and stem elongation. A high auxin ratio results in rapid fibre differentiation, thick secondary cell wall development and inhibits stem elongation, while a high gibberellin ratio enhances fibre, internode and stem elongation (Aloni 1979).

Cytokinin is a limiting and controlling factor for fibre (particularly xylem) differentiation, and increases secondary xylem lignification. In the early stages of secondary xylem fibre differentiation, cytokinin can play either a promotional or inhibitory role depending on the physiological state of the plant (Saks *et al.* 1984).

Cannabis research involving PGRs has found gibberellin to be involved in lateral branch suppression, poor development of root systems and in increased internode and stem elongation, fresh and dry weights, bast/core ratios, number of phloem fibres, phloem fibre diameters and lengths (up to 10x), lignification (Atal 1961) and plant sex differentiation (Atal 1961, Mohan Ram and Jaiswal 1972, Khryanin and Milyaeva 1977).

2.4.2 Phloem

Primary and secondary phloem, composed of parenchyma, sieve tubes, companion cells and fibres, are located to the outside of the vascular cambium (Mauseth 1988, Raven *et al.* 1999). Primary phloem originates from the outer cells of the procambium and secondary phloem originates from the vascular cambium (Raven *et al.* 1999). In *C. sativa*, tissues to the outside of the vascular cambium are commonly referred to as the bark (Van der Werf *et al.* 1995b, Keller *et al.* 2001) or bast (Ramey Jr. 1980, Bocsa and Karus 1998).

C. sativa bast can be 8.7-50.4% of the stem, with 40-68% phloem fibre composed of 8.4-89% primary phloem fibre and 0-45% secondary phloem fibre (de Meijer 1994, 1995, Van der Werf *et al.* 1994b, Correia 1999, Scheifele 1999, Kamat 2000, Sankari 2000, Mediavilla *et al.* 2001). It contains 60-74% cellulose, 2.9-11% lignin and 10-18%

hemicellulose (Ramey Jr. 1980, Bocsa and Karus 1998, Correia 1999, De Jong *et al.* 1999).

C. sativa stem quality is primarily determined by phloem fibre content (Van der Werf *et al.* 1994b, McDougall *et al.* 1993, Mediavilla *et al.* 2001). Phloem fibres function in plant mechanical support, herbivore and sap-sucking insect protection, and are a major component of commercial fibres (Mauseth 1988). High phloem fibre yield, with low secondary phloem fibre content is optimal for both textiles and paper production (Van der Werf *et al.* 1994b, Mediavilla *et al.* 2001). Increase in phloem fibre content reflects greater increase of secondary rather than primary phloem fibre (de Meijer 1994).

2.4.2.1 Primary Phloem Fibre

During the vegetative stage of *C. sativa*, the first fibres to be formed are primary phloem fibres (Mediavilla *et al.* 2001). This development occurs during the phase of rapid stem elongation (Sankari 2000). Up to 40 individual thick-walled, multinucleated (7-21) fibres are arranged in bundles of 100-300 mm average lengths (Kundu 1941, McDougall *et al.* 1993, Meijer *et al.* 1995, Vignon *et al.* 1996, De Jong *et al.* 1999, Keller *et al.* 2001). Fibres are bound by middle lamella into round, oval, elliptical, rectangular or outward-tapered pyramid-wedge shapes (Kundu 1941, Vignon *et al.* 1996, Bocsa and Karus 1998). Fibre bundles are separated by pectin and hemicellulose-rich cortex parenchyma cells (Vignon and Garcia-Jaldon 1996, Garcia-Jaldon 1998).

Primary phloem fibres are pointed at both ends and reach their final length and double their cross-sectional area when the internode completes its extension (Kundu 1941). Final cell lengths of 0.5-25 cm can be 100-1000 times the diameter (13-78 μm), with 14 μm thick walls (Kundu 1941, Ramey Jr. 1980, Van der Werf *et al.* 1994b, Bocsa and Karus 1998, Correia 1999, De Jong *et al.* 1999, Kamat 2000, Sankari 2000). Individual cells complete

elongation before secondary cell wall development becomes apparent by the third, fourth, or eighth internode and continues throughout the lifespan of the plant (Kundu 1941, Ramey Jr. 1980, Mediavilla *et al.* 2001). Thickening of the wall can reduce the lumen to less than 10% of the cross-sectional area of the cell (McDougall *et al.* 1993). The original circular shape is lost when fibre cells are compressed during secondary growth of the stem (Bocsa and Karus 1998). Pits occur between fibres, but not between fibres and parenchyma (Kundu 1941, McDougall *et al.* 1993).

Primary phloem fibre length slightly decreases and diameter increases with plant age (Kamat 2000). Reduction in fibre length is due to increased interwoven lignification and decreased turgor pressure (Kamat 2000). As the plant matures, lignification increases tensile strength of the fibres, but reduces break and torque resistance and elasticity (Bocsa and Karus 1998). Most cells die after differentiating, but do not collapse upon drying unlike other fibres such as those of *Gossypium* (Ramey Jr. 1980, McDougall *et al.* 1993).

2.4.2.2 Secondary Phloem Fibre

Uninucleate secondary phloem fibres form tangential bands of 10-40 cells (McDougall *et al.* 1993). Fibres average 0.2-2 cm in length with 22 μm diameter (Kundu 1941, McDougall *et al.* 1993, Van der Werf *et al.* 1994b, Sankari 2000). Filling of secondary phloem fibre cell walls occurs at the time of flower induction (Mediavilla *et al.* 2001). After phyllotaxy change, an abrupt increase in secondary phloem fibre is apparent in female plants (Mediavilla *et al.* 2001). The proportion of secondary phloem fibre within the phloem decreases up the stem, but increases with stem diameter and weight (Van der Werf *et al.* 1994b).

2.4.3 Xylem

The tissues between the vascular cambium and the hollow core (part of pith) are primary and secondary xylem and the remaining pith. Primary xylem originates from the inner cells of the procambium and secondary xylem originates from the vascular cambium (Mauseth 1988, Raven *et al.* 1999). In *C. sativa*, they are often referred to together as the hurd or woody core (Dewey and Merrill 1916, Van der Werf *et al.* 1995b, Bocsa and Karus 1998, De Jong *et al.* 1999).

Xylem is composed of parenchyma cells, vessel elements, fibre-tracheids and fibre. Although a graduation of all cell types and their intermediates exist, there are two main xylem fibre types: libriform fibres, with thicker cell walls and reduced pits, and fibre-tracheids, an intermediate form (Mauseth 1988, Raven *et al.* 1999).

Xylem comprises the largest portion of a *C. sativa* stem (49.6-80%), and its area increases with plant age (de Meijer 1994, 1995, Vignon *et al.* 1996, Correia 1999, Cromack 1998, Scheifele 1999, Kamat 2000). As they are less flexible than phloem fibres, the aggregation of xylem fibres supplies the stem with vertical strength (Bocsa and Karus 1998). *C. sativa* xylem contains 34-40% cellulose, 20-25% lignin and 20-35% hemicellulose (Bocsa and Karus 1998, De Jong *et al.* 1999).

Xylem fibre cells can be 0.2-1.0 mm long with 0.1-3 μm wall widths and 10-41 μm diameters (de Meijer 1994, Van der Werf *et al.* 1995c, Vignon *et al.* 1996, Bocsa and Karus 1998, Correia 1999, De Jong *et al.* 1999, Kamat 2000). Fibre lengths can reach 27.5 times their diameter (De Jong *et al.* 1999). Male plant fibres can be 20 μm shorter and 2.5 μm wider, and fibre varieties tend to have wider fibres than non-fibre varieties (de Meijer 1994). As with phloem fibres, xylem fibre diameters increase and lengths decrease with plant age

and the subsequent increased lignification and decreased turgor pressure (Kamat 2000).

2.5 Growth Requirements

One of the main obstacles for hemp cultivation is adequate crop establishment (Struik *et al.* 2000). *C. sativa*'s sensitivity to poorly structured or compact soils is particularly apparent on heavy clay soil types which experience increased crop variability and decreased growth (BCMAF 1999, Scheifele 1999, Struik *et al.* 2000). Well-drained loam or loess soils, with favorable water balance, permeability and nutrient accumulation are optimal. *C. sativa* requires a pH between 5.8-7.5, with > pH 6.0 recommended, and pH 7.0-7.5 preferred (Dempsey 1975, Bocsa and Karus 1998, BCMAF 1999). The same variety grown on pH 6.6 soil can be 30-45 cm higher than grown on pH 6.0 soil (Scheifele 1999). Crops are improved if grown on a <5% slope of south facing lowland (Bocsa and Karus 1998).

Until seedlings reach 2-3 weeks old, they are both drought tolerant and sensitive to high soil moisture (Seale *et al.* 1957, BCMAF 1999, Scheifele 1999). Low moisture levels can result in mass reduction and hastened maturity, while excess moisture causes death or plant stunting (BCMAF 1999, Scheifele 1999). *C. sativa* plants prefer semi-humid conditions with 250-700 mm of water during the full duration of growth or 125 mm per month (Dempsey 1975, Bocsa and Karus 1998, BCMAF 1999). Irrigation can increase total biomass and stem dry matter. Water requirements for this species are lower than both *Zea* (maize) and *Hibiscus* (kenaf) (Amaducci *et al.* 2000).

Seed germination can begin at 1-2°C soil temperatures, but requires 6-12°C soil temperatures to germinate within 8-12 days (Bocsa and Karus 1998, BCMAF 2000). Lower than optimal temperatures may result in delayed or poor emergence (25-50%) (Mediavilla *et*

al. 1998, BCMAF 2000). The optimal air temperature for growth is 13-25°C with rapid daily growth of 4-6 cm achieved upon daily averages of 16°C (Dempsey 1975, Bocsa and Karus 1998). Seedlings and mature plants can endure frost to -5°C (BCMAF 1999).

C. sativa is a short-day plant, requiring shorter daylengths (<14 hours) to enter its reproductive phase (Dempsey 1975, Van der Werf *et al.* 1994a, Bocsa and Karus 1998, Lisson *et al.* 2000).

2.6 Cultivation Techniques

2.6.1 Stem Density

Increased crop density results in a greater risk of self-thinning (Van der Werf *et al.* 1995a). In an even-aged monoculture crop, size inequality increases over time until the onset of self-thinning which results in decreased inequality (Weiner and Thomas 1986). Plant competition is usually 'one-sided' as a result of competition for light. However, size inequality also results from the effects of differences in age, genetics, pests, disease and environmental conditions (Weiner and Thomas 1986).

For fibre, *C. sativa* can be cultivated at densities between 50-750 stems/m^2 (Jakobey 1965, Dempsey 1975). Lower densities (30-90 stems/m^2) are capable of maintaining their density through to harvest, while higher planting densities (180-300 stems/m^2) are more susceptible to self-thinning (Van der Werf *et al.* 1995c, Lisson and Mendham 2000, Struik *et al.* 2000). Higher plant densities result in delayed flowering, thin stems, lower height and weight, produce lower crop yields and increased susceptibility to disease (Meijer *et al.* 1995, Van der Werf *et al.* 1995c, Van der Werf 1997, Lisson and Mendham 2000, Sankari 2000, Struik *et al.* 2000).

2.6.2 Nutrients

The highest nutrient demand occurs during the rapid growth phase and can increase until technical maturation (full flowering) when only the female plants continue to absorb nutrients until their seeds mature (Bocsa and Karus 1998). *C. sativa* removes more nutrients per hectare than *Gossypium*, *Linum*, *Triticum* (wheat), *Zea*, *Secale* (rye) or *Avena* (oats) (Dempsey 1975). The amount of available nutrients in the soil and the application of fertilizers can increase *C. sativa* stem lengths by 50-60% (Bocsa and Karus 1998).

2.6.3 Pests and Disease

Either biotic sources such as genetic disposition, fungi, nematodes, parasitic plants, bacteria and viruses, or abiotic sources such as environmental stress, nutrient deficiency or pollutants cause *C. sativa* disease or injury (McPartland 1996a). Different diseases affect different varieties, growth stages and plants in different geographic locations or climates. Fungi cause more problems for *C. sativa* than bacteria. The borer species are the most important insect pests, and mites are the most important non-insect pests (McPartland 1996a,b). Although ~100 diseases and ~300 pests, mainly insects, have been identified for *C. sativa*, very few are considered serious and rarely cause economic crop loss (McPartland 1996a,b, Bocsa and Karus 1998). Considered pest-tolerant, *C. sativa* has fewer pests than many other crops and there are no specific pesticides or herbicides for this species (McPartland 1996b, BCMAF 1999).

Uniform and healthy *C. sativa* crops can suppress and be virtually free of weeds requiring little or no use for herbicides (Höppner and Menge-Hartmann 1995, Van der Werf *et al.* 1995b, Van der Werf *et al.* 1995c, Van der Werf and Van den Berg 1995, Bocsa and Karus 1998, BCMAF 1999, Scheifele 1999). One of the most important weed or parasitic

plants of *C. sativa* is broomrape (*Orobanche ramosa*) (Bocsa and Karus 1998).

Important *C. sativa* diseases include grey mold (*Botrytis cinerea*), hemp canker or white mold (*Sclerotinia sclerotiorum*), pythium disease (*Pythium debaryanum*) and hemp rust (*Melampsora cannabina*). The most common pests of this plant are the hemp borer (*Grapholita delineana*), European corn borer (*Ostrinia nubilalis*), hemp flea (*Psylliodes attenuata*), hemp greenfly (*Phorodon cannabis*), Bertha armyworm (*Mamestra configurata*), lygus plant bug, cutworm, stinkbug, grasshopper, root knot nematode, caterpillar, beetle, leafminer, slug, rodent and bird (McPartland 1996b, Bocsa and Karus 1998).

There is evidence that *C. sativa* may offer moderate efficacy as a repellent crop or botanical pesticide, although ironically, some of the pests it controls are also those of the plant itself (McPartland 1997). It has also been found to be effective in suppressing soil borne pathogens such as nematodes (*Meloidogyne chitwoodi*), fungus (*Verticillium dahliae*) and the survival structures (microsclerotia) of *Verticillium dahliae* (Kok *et al.* 1994). When THC is isolated, it has proven pesticide abilities against bacteria and fungi, but its efficacy on insects is uncertain. It is believed that cannabinoids play a small role and that it is actually the combination of the effects of the ~400 chemicals in *C. sativa* that provide the pesticide capability (McPartland 1997).

2.7 Fibre Cultivation

C. sativa fibres are used to manufacture textiles and in the pulp and paper industry to strengthen paper for bank notes, cigarette paper, religious books, filter paper, coffee filters, tea bags, specialty non-wovens, insulating paper (electrical condensators), greaseproof papers, security papers and various specialty art papers (Van Roekel 1994, Van der Werf *et al.* 1996, Vignon and Garcia-Jaldon 1996, Johnson 1999, Mediavilla *et al.* 2001).

For textile and paper production, the stronger, longer fibres of the phloem, primary phloem in particular, are more valuable than xylem fibres (Van der Werf *et al.* 1994b, Bocsa and Karus 1998, Mediavilla *et al.* 2001). In paper manufacturing, fibre length is positively correlated with paper strength (Van der Werf *et al.* 1994b). However, xylem fibres have also been used to produce printing, writing and copying paper, in addition to fuel, barnyard litter, stable bedding and a sawdust substitute (Dewey and Merrill 1916, De Jong *et al.* 1999).

New *C. sativa* fibres can be added to enhance old paper for reuse on a high quality level. Fibres used in newspapers can also be reused to produce paper wool insulation, which can be recycled further into new material, composted or incinerated with other feedstock (Fraanje 1997).

2.7.1 Cellulose

The cellulose level of raw material is positively correlated with chemical pulp yield (Van der Werf *et al.* 1994b). Cellulose influences pulp viscosity, and high viscosity indicates strong pulp due to its higher degree of polymerization and reduced susceptibility to acid hydrolysis (Correia 1999).

Cellulose production increases throughout the life of the plant, but after the lumen of the cells are filled, encrustation of lignin in cell walls occurs and decreases cellulose quality (Struik *et al.* 2000). *C. sativa* phloem fibres contain more cellulose (64.8-79%) than xylem fibres (34.5%) (Van der Werf *et al.* 1995b, Keller *et al.* 2001). The low cellulose levels of the xylem may restrict it to mechanical pulping (Bosia 1975).

In *C. sativa,* the highest cellulose levels occur in upper stem regions and in male plants with dioecious varieties having higher levels than monoecious varieties (Bedetti *et al.* 1979, Keller *et al.* 2001). Nitrogen has been found to have a negligible effect on stem

cellulose content (Struik *et al.* 2000). However, harvest after flowering yields more favourable cellulose content, as raw material for paper manufacturing (Van der Werf *et al.* 1994b).

2.7.2 Lignin

Lignin develops in the fibre cell wall at the completion of cell elongation (Keller *et al.* 2001). Higher lignin levels are often found in weaker fibres (Hessler 1947). It is difficult to enzymatically or chemically digest lignin, which makes its removal difficult to do in an environmentally friendly manner (Van der Werf *et al.* 1995b, Keller *et al.* 2001). Harvest scheduling for fibre should aim for low lignin levels, and the low lignin containing portions of the stems should be maximized (Keller *et al.* 2001).

Phloem fibre lignin decreases over the growing season resulting in a lower quantity (4.3%) of more easily removed lignin than from xylem fibres (20.8%) (Van der Werf *et al.* 1994b, Correia 1999). Lignin levels in primary phloem fibres are lower than those of secondary phloem fibres (Kundu 1941, Mediavilla *et al.* 2001). Due to the low lignin levels, mechanically and chemically pulped phloem fibre can be characterized as wood free and opportunities for unbleached or non-chlorine bleached pulp production are higher than for wood pulp (Van der Werf *et al.* 1995b, Van Roekel *et al.* 1995).

2.7.3 Plant Variety (Ecotype/Cultivated Race)

Variety is one of the main factors affecting the proportion of phloem fibre in *C. sativa* stems (de Meijer 1994, Van der Werf *et al.* 1996, Cromack 1998, Sankari 2000). It affects the proportion of bast, but not the proportion of core, or the bast hemicellulose or lignin content, nor is it attributed to bast content decrease with increased stem dry weight (Van der Werf *et*

al. 1994a,b).

Dioecious male plants are more important for fibre production, however, for phloem fibre in particular, neither dioecious nor monoecious varieties are superior over the other (Horkay and Bocsa 1996, Bocsa and Karus 1998, Sankari 2000, Mediavilla *et al.* 2001).

Optimal harvest date is later for late flowering varieties (Van der Werf *et al.* 1996). In such varieties, stem growth continues longer, resulting in greater heights (de Meijer and Keizer 1994, Van der Werf *et al.* 1996). Stem height is correlated with stem yield (de Meijer and Keizer 1994), therefore, late- or non-flowering varieties are suggested for fibre cultivation (Van der Werf *et al.* 1994a).

2.7.4 Photoperiod

Photoperiod extension does not affect phyllotaxy change, however, at longer photoperiods flowering will be delayed in females and prevented in male and monoecious plants (Van der Werf *et al.* 1994a, Lisson *et al.* 2000). Decreased allocation of dry matter to floral parts is apparent by the shorter (~8 times) inflorescence of plants grown in extended daylengths (Van der Werf *et al.* 1994a).

Prolonged daylength increases growth rates between flowering and harvest (Van der Werf *et al.* 1994a). The prevention of flowering results in increased stem yield, however, extended daylengths decrease the quality of the stem content (Van der Werf *et al.* 1994a). Similarly, the total amount of light during the vegetative period influences fibre quality more than quantity (Bocsa and Karus 1998).

2.7.5 Plant Sex

Stems of male plants accumulate primary phloem earlier and in higher quantities.

However, female stems are stronger due to thicker, stronger phloem fibres and a higher proportion of secondary phloem fibre which develops earlier than in male stems (Bocsa and Karus 1998, Mediavilla *et al.* 2001).

2.7.6 Stem Density

Plant density is positively related to primary phloem content and fibre fineness, but inversely related to stem diameter, height, weight, crop yield and flowering date (Jakobey 1965, Van der Werf *et al.* 1995a,c, Van der Werf 1997, Cromack 1998, Correia 1999, Lisson and Mendham 2000, Sankari 2000, Struik *et al.* 2000). Although it is claimed that there is no correlation between total phloem fibre content and stem diameter, primary phloem content is more affected by stem diameter of dioecious varieties than monoecious varieties (Van der Werf *et al.* 1994b, Sankari 2000). Smaller diameter stems (4-5 mm) are particularly suitable for fibre production (Jordan *et al.* 1946, Dempsey 1975, Scheifele 1999). It is also claimed that total phloem content is inversely related to stem dry weight, yet there is no correlation between total phloem fibre content and stem dry weight (Van der Werf *et al.* 1994a,b).

Plant density continues to improve stem quality beyond the point of stem yield increase. Therefore, the optimal plant density for phloem fibre production is higher than the lowest plant density that offers the maximum stem dry matter yield (Van der Werf *et al.* 1995c).

Densities of 90, >100 or 110 stems/m^2 produce the most optimal *C. sativa* stem yields for phloem fibre, while higher densities such as 270 stems/m^2 reduce the proportion of phloem in the stem and increase the proportion of non-fibre components (Van der Werf *et al.* 1995b, c, Cromack 1998, Lisson and Mendham 2000). Xylem libriform fibre lengths increase with densities up to 90 stems/m^2, but diameters are not affected by plant density (Van der

Werf *et al.* 1995c).

2.7.7 Stem Height

The main factors affecting the proportion of stem dry matter are flowering date, plant density and sexual orientation of the crop (Van der Werf *et al.* 1996). Plants can add mass by increasing in height, radius or volume of already occupied stem space (Van der Werf *et al.* 1995a). Stem height is negatively related to plant density, but positively related to stem yield (de Meijer and Keizer 1994, Struik *et al.* 2000). Taller plants tend to be later flowering varieties, male plants and earlier planted stems (de Meijer and Keizer 1994, Bocsa and Karus 1998, Van der Werf and Van den Berg 1995, BCMAF 2000). Variation in stem height and weight is greater in female plants (Van der Werf and Van den Berg 1995).

2.7.8 Nutrients

2.7.8.1 Nitrogen

Nitrogen is a component of nucleoside phosphates and amino acids and, therefore, is involved in the formation of nucleic acids and proteins of plant cells. As it is associated with many plant cell components, a characteristic deficiency symptom is stunted plant growth (Taiz and Zeiger 1991). In *C. sativa*, nitrogen absorption increases continuously throughout the vegetative period until the onset of flowering with daily nitrogen uptake of 3-4 kg N/ha and a maximum uptake point of 142-256 kg N/ha incorporated into the plant. Increases in nitrogen uptake occur when nitrogen supply increases (Ivonyi *et al.* 1997, Hendrischke *et al.* 1998). During field retting, plant material is easily mineralized with 16% dry matter and 40% nitrogen (67 kg N/ha) lost and, therefore, concerns of nitrate leaching can exist (Hendrischke *et al.* 1998).

It is claimed that nitrogen is the most important nutrient for *C. sativa* growth and stem yield, and that compared to its absence, and up to a certain level, nitrogen increases plant height, and both stem and fibre yield (Jordan *et al.* 1946, Hessler 1947, Ivonyi *et al.* 1997, Bocsa and Karus 1998, BCMAF 2000, Struik *et al.* 2000). Stem yield increase due to nitrogen occurs regardless of phosphorus or potassium level applied, yet the application of nitrogen fertilizer results in lower requirements of these elements (Ivonyi *et al.* 1997).

On low nitrogen soils, nitrogen deficiency may be apparent and the application of nitrogen fertilizer may result in a significant stem yield and diameter increase which may not occur on fertile soils (Jordan *et al.* 1946). Nitrogen fertile soils can produce adequate yields, and even slightly increased yields compared to fertilizer treatments (Jordan *et al.* 1946, Struik *et al.* 2000). On these soils, nitrogen fertilizer addition presents either a limited or moderate effect, or no significant yield or height increases (Jordan *et al.* 1946, Hendrischke *et al.* 1998, Struik *et al.* 2000).

High levels of soil or nitrogen fertilizer can result in no yield increase and even small yield decreases (Hessler 1947, Hendrischke *et al.* 1998, Struik *et al.* 2000). It may increase stem diameter beyond that favourable for fibre, and create greater variability in stem diameter, height and weight (Jordan *et al.* 1946, Van der Werf and Van den Berg 1995, Scheifele 1999). Leafier, succulent growth, increased stem breakage, higher proportions of female to male stems and enhanced self-thinning (due to higher competition for light than for nitrogen) can also occur with high nitrogen (Jordan *et al.* 1946, Van der Werf *et al.* 1995a, Van der Werf and Van den Berg 1995, Struik *et al.* 2000). Increased nitrogen levels create large stems with thin phloem sections of low fibre quantity and quality, as the fibres are weaker and coarser (Jordan *et al.* 1946, Hessler 1947, Bocsa and Karus 1998). The detrimental effect of even moderately excessive nitrogen levels on fibre quality is also known

in *Linum* research (Jordan *et al.* 1946).

For *C. sativa* fibre cultivation, nitrogen has been applied at levels ranging from 3-220 kg N/ha (Jordan *et al.* 1946, Hessler 1947, Ruzsanyi 1970, Ritz 1972, Basso and Ruggiero 1976, Haralanov and Babayashev 1976, Van der Werf *et al.* 1994a, 1995a, 1995c, Höppner and Menge-Hartmann 1995, Meijer *et al.* 1995, Van der Werf and Van den Berg 1995, Ivonyi *et al.* 1997, Cromack 1998, Scheifele 1999, Amaducci *et al.* 2000, BCMAF 2000, Lisson and Mendham 2000, Sankari 2000, Struik *et al.* 2000, Keller *et al.* 2001, Mediavilla *et al.* 2001). Significant differences between nitrogen level treatment effects that have been found include greater fibre yields with 56 or 112 kg N/ha treatment than in the absence of a nitrogen fertilizer application, with 112 kg N/ha producing the highest yield (Jordan *et al.* 1946). Compared to 0 kg N/ha, treatment with 113 kg N/ha in conjunction with 56 kg P_2O_5/ha results in stem yield increase with the additional combination of 52 kg K_2O/ha resulting in the greatest (20%) increase (Ruzsanyi 1970). Comparing 60 and 120 kg N/ha has also resulted in no stem yield increase (BCMAF 2000), however, the application of 80 or 160 compared to 0 kg N/ha has presented a stem yield increase (Ivonyi *et al.* 1997). The application of 240 kg N/ha did not increase stem yield beyond that achieved by 80 or 160 kg N/ha treatment (Ivonyi *et al.* 1997).

Compared to 80 kg N/ha, plants grown with 200 kg N/ha experienced increased stem yield, radial growth, biomass (Van der Werf *et al.* 1995a) and variability in height and weight (Van der Werf and Van den Berg 1995). However, 80 kg N/ha has caused increased bast content (Van der Werf and Van den Berg 1995), stem height, proportion of stem in the dry matter, self-thinning rate (Van der Werf *et al.* 1995a) and ratio of male plants (Van der Werf and Van den Berg 1995). There were also fewer flowering plants and nodes on the stems (Van der Werf and Van den Berg 1995).

Based on several *C. sativa* fertilization studies over three years and locations, it was found that stem yield was greater with increasing nitrogen level treatment from 100, 160 to 220 kg N/ha, however, the effect was only slight and it was concluded that relatively low levels of nitrogen are adequate for *C. sativa* fibre crops (Struik *et al.* 2000). This is complemented by the suggestion that the nitrogen requirements of *C. sativa* fibre crops are not as high as expected and that not only can its application levels be decreased, from 140 to 100 kg N/ha, but also that the phosphorus and potassium levels should be increased (Scheifele 1999).

When soil nitrogen level needs are supplemented with fertilizer, either liquid or solid form is acceptable, as the form of fertilizer has no significant effect on stem yield (Basso and Ruggiero 1975). It has been claimed that starter fertilizer does not significantly affect stem height, nor does the time of nitrogen fertilizer application significantly affect the percentage of fibre achieved (Ritz 1972, Scheifele 1999). However, it is also claimed that nitrogen fertilizer is more effective if applied at or just after sowing followed by one or two applications, for example, at emergence and at the point of the third leaf pair formation (Haralanov and Babayashev 1976, Van der Werf *et al.* 1995c, Mediavilla *et al.* 1998). Barn manure treatment has been found to be less effective than synthetic fertilizers for *C. sativa* production. Increased moisture improves fertilizer effectiveness and subsequent stem yield (Ruzsanyi 1970). Inadequate precipitation inhibits nitrogen fertilizer effectiveness (BCMAF 2000).

2.7.8.2 Phosphorus

Phosphorus is a component of metabolic nucleotides, i.e., DNA and RNA. As sugar-phosphate, it is involved in respiration, photosynthesis and phospholipid cell membrane

composition (Taiz and Zeiger 1991). Until flowering, *C. sativa* requires phosphorus to aid in nitrogen-use efficiency, the development, elasticity and tensile strength of fibre cells, bundles and total fibre yield (Bocsa and Karus 1998). Daily uptake of 0.25-0.64 kg P_2O_5/ha is constant throughout the growth of the plant with a maximum uptake point of 52-67 kg P_2O_5/ha incorporated into the plant (Ivonyi *et al.* 1997). Phosphorus has been applied to *C. sativa* fibre crops at levels ranging from 18-121.5 kg P_2O_5/ha (Jordan *et al.* 1946, Hessler 1947, Ruzsanyi 1970, Haralanov and Babayashev 1976, Meijer *et al.* 1995, Ivonyi *et al.* 1997, Cromack 1998, Scheifele 1999, BCMAF 2000, Lisson and Mendham 2000, Sankari 2000).

It is claimed that *C. sativa* is likely able to absorb the small quantity of phosphorus it needs from the soil and studies have found that, on low phosphorus soils, the application of phosphorus fertilizer alone can result in decreased stem height and weight (Ruzsanyi 1970, Ivonyi *et al.* 1997). It is also claimed that on low nitrogen soils, phosphorus application can significantly increase fibre yield and that phosphorus requirements are lower when nitrogen fertilizer is applied (Jordan *et al.* 1946, Ivonyi *et al.* 1997).

Studies comparing phosphorus levels have found that compared to 0 kg P_2O_5/ha, treatment with 33 kg P_2O_5/ha produces greater fibre yields (Jordan *et al.* 1946) and 100 kg P_2O_5/ha increases stem yield, but higher levels do not (Ivonyi *et al.* 1997). Compared to 113 or 169 kg P_2O_5/ha, treatment with 56 kg P_2O_5/ha in combination with 113 kg N/ha increased stem yield, with the greatest yield occurring with the additional combination of 52 kg K_2O/ha (Ruzsanyi 1970). Similar outcomes have been found when the increase in application from 30 to 60 kg P_2O_5/ha only resulted in increased yield when combined with 50 kg K_2O/ha (BCMAF 2000).

2.7.8.3 Potassium

Potassium is involved in plant cell osmotic potential regulation and activation of respiration and photosynthesis enzymes (Taiz and Zeiger 1991). The potassium uptake of *C. sativa* plants increases from germination through to harvest, with the highest uptake occurring during fibre development when potassium plays a more important role in fibre quality than phosphorus (Ivonyi *et al.* 1997, Bocsa and Karus 1998). At the most intensive point, daily uptake is 3-6 kg K_2O/ha with a maximum uptake point of 223-358 kg K_2O/ha incorporated into the plant (Ivonyi *et al.* 1997). Potassium has been applied to *C. sativa* fibre crops at levels ranging from 32.5-300 kg K_2O/ha (Jordan *et al.* 1946, Hessler 1947, Ruzsanyi 1970, Haralanov and Babayashev 1976, Meijer *et al.* 1995, Cromack 1998, Scheifele 1999, BCMAF 2000, Lisson and Mendham 2000, Sankari 2000).

As with phosphorus, the absence of nitrogen fertilizer application can result in increased potassium requirements (Ivonyi *et al.* 1997). Although no difference has been found between the effects of 0 and 22 kg K_2O/ha treatment on fibre yield (Jordan *et al.* 1946), when 52 kg K_2O/ha is combined with 56 kg P_2O_5/ha and 113 kg N/ha, stem yield is greater than with the same combination with 139 kg K_2O/ha level substitution (Ruzsanyi 1970). However, it is also believed that higher than required potassium levels can increase stem yield (Ivonyi *et al.* 1997), particularly when the nitrogen and phosphorus levels are equal (BCMAF 2000).

2.7.8.4 Other Elements

Although the role of trace elements in *C. sativa* growth is limited, it is known that sulphur, calcium (CaO) and magnesium (MgO) are important for particular crop rotations (Bocsa and Karus 1998, Scheifele 1999).

2.7.9 Harvest

Stem and fibre development increase rapidly until phyllotaxy change when stem elongation slows and fibre yield decreases (Van der Werf *et al.* 1994a, Mediavilla *et al.* 1998, 2001, Keller *et al.* 2001). It is possible for elongation to continue past flowering, in which case earlier flowering results in more length acquired during the floral phase, while later flowering plants extend more before flowering (de Meijer and Keizer 1994). The ideal time to harvest for fibre is when dioecious male plants flower (Seale *et al.* 1957, Jakobey 1965, Bocsa and Karus 1998, Mediavilla *et al.* 1998, 2001). At this point, primary phloem cells predominate and low lignin levels are advantageous for fibre separation (Keller *et al.* 2001, Mediavilla *et al.* 2001). Fibre quantity may be lower, but quality is higher than at later harvests (Jakobey 1965). The maximum fibre yields achieved at the end of male flowering and peak of female flowering are due to increased production of secondary phloem fibre (Mediavilla *et al.* 2001).

The effect of harvest date on the proportion of bast in the stem is dependent upon plant variety and density (Van der Werf *et al.* 1994b). Harvest date affects the chemical composition of the bast more than the core (Van der Werf *et al.* 1994b). Delayed harvest results in increased lignification of fibre, increased production of shorter, higher lignin containing secondary phloem fibre, and difficult manual separation of fibres (Bocsa and Karus 1998, Struik *et al.* 2000, Keller *et al.* 2001, Mediavilla *et al.* 2001). High phloem fibre yield, with low secondary phloem fibre content, is optimal for both textiles and paper production (Van der Werf *et al.* 1994b, Mediavilla *et al.* 2001). An increased proportion of secondary phloem fibres can result in a reduced total phloem fibre length and a subsequent reduction in value for paper production (Van der Werf *et al.* 1994b).

Fibre strength does not vary in different locations along the stem, nor does it decrease

with delayed harvest, but the longest fibres are found at the longest internodes (Kundu 1941, Keller *et al.* 2001). It is possible for individual fibres to diverge at incoming leaf trace bundles and continue into the next internode (Kundu 1941). The top portion of a *C. sativa* stem has weaker fibres and a lower phloem fibre content (Hessler 1947, Cappelletto *et al.* 2001). Phloem fibre in the top 1/3 of the stem is more difficult to separate, therefore, the lower 2/3 of the stem is more valuable for fibre production and the top portion can be removed and discarded at harvest (Van der Werf *et al.* 1994b, Mediavilla *et al.* 2001). Variation in stem diameter, height and weight are undesirable for harvesting equipment, mechanical defoliation and bast and core separation (Van der Werf and Van den Berg 1995).

2.7.9.1 Fibre Removal

Phloem fibres are removed from other stem tissues by retting (Ramey Jr. 1980, Vignon and Garcia-Jaldon 1996, Kamat 2000, Hobson *et al.* 2001, Keller *et al.* 2001). In field retting, water and microorganisms (fungi) secrete a wide spectrum of enzymes that decompose the vascular cambium, tannins, pigments, sugars, gums and pectins (Vignon and Garcia-Jaldon 1996, Johnson 1999, Kamat 2000, Cappelletto *et al.* 2001). Chemical bonds between fibres and surrounding tissue are cleanly separated with little damage to the fibres (Ramey Jr. 1980, Hobson *et al.* 2001). Cellulose has resistance to decomposition by bacterial retting and as a result, dew-retted fibre contains ~70% cellulose and 30% encrustant material that varies with variety, growing condition and method of retting (Hessler 1947, Bocsa and Karus 1998).

As retting is time consuming, can be costly and carry uncertainty, other mechanical techniques are being tested (Vignon *et al.* 1996, Hobson *et al.* 2001, Keller *et al.* 2001). Fibre yield, length, distribution and strength are similar for retted and unretted stems (Hobson *et al.*

2001). However, over-retted fibres are weaker (Hessler 1947). Due to less degradation by microorganisms, cellulosic fibres endure less damage using mechanical fibre removal (Vignon *et al.* 1996). Unretted fibre is coarser, with 4% impurities, while retted fibre has only 2%. The light colour and low fungal contamination of unretted fibre is considered a marketing advantage. Unretted fibre results in a less variable product which may be more cost effective (Hobson *et al.* 2001).

2.8 Present Study

2.8.1 Stem Sampling Location and Technique

Previous research has performed fibre measurements at locations such as 5 cm (Kamat 2000) or 30 cm from the root base (Correia *et al.* 1998), or at 20-30% of the stem height for the best approximation of the primary and secondary phloem content (Van der Werf *et al.* 1994b). When a stem is divided into ten even segments, the third segment has been used for xylem and phloem analysis (de Meijer and Van der Werf 1994) and is best suited for secondary phloem fibre assessment (Van der Werf *et al.* 1994b).

Specific phloem fibre measurements have been determined using cross-sectional images (Kamat 2000) and processes of TBO (toluidine blue O) (Mediavilla *et al.* 2001) and carmino green of Mirande solution staining of cross-sections, and with gold-palladium coated samples (Vignon *et al.* 1996). However, various traditional wood chemistry and manual dissection techniques have more commonly been used to determine mass fractions of stem components (Jordan *et al.* 1946, Hessler 1947, de Meijer 1994, Van der Werf *et al.* 1994a, 1994b, de Meijer 1995, Cromack 1998, Scheifele 1999, Lisson and Mendham 2000, Sankari 2000, Cappelletto *et al.* 2001).

2.8.2 *Fédrina*

Fédrina is one of more than 33 different "varieties" of *C. sativa*, which range in origin from France, Hungary, Poland, Italy and the former USSR (BCMAF 1999, Ranalli 1999). Some varieties are specialized for seed production while others are for fibre. Bred by M. Arnoux and J.P. Mathieu, *Fédrina* 74 is derived from *Fibrimon* 24, a monoecious crossbred variety with high fibre content, and the dioecious *Fibridia*. *Fédrina* 74 is a late-maturing hybrid variety within the Central and Northern ecotypes category (de Meijer 1995, Bocsa and Karus 1998).

Originally a monoecious variety, after genetic drift, it now has 15-30% males and a substantial number of true females within a crop (de Meijer 1995, Van der Werf *et al.* 1994a). In France, it is grown for use in the paper industry and as a dual-purpose crop for fibre and seed. Although the fibre quality is considered mediocre, it has sufficient stem/fibre yield and is a variety recommended for areas with medium soil fertility and poor, rainy weather (de Meijer 1995, Bocsa and Karus 1998). French varieties typically require a photoperiod of 14-15.5 hours to enter their reproductive phase, and flower in August after stem growth slows and ceases. They should be harvested in early September (Van der Werf *et al.* 1995c, Van der Werf *et al.* 1996, Struik *et al.* 2000).

Initial densities of at least 90 *Fédrina* stems/m^2 provide adequate weed suppression (Van der Werf *et al.* 1994a) and self-thinning rates are lower with emergence rates of 86-114 stems/m^2 than 186-823 stems/m^2 (Meijer *et al.* 1995). With a planting density of 140 stems/m^2, 179 cm heights, 7.4 mm diameters (at base of stem) and 893 g/m^2 stem yields can be achieved (Lisson and Mendham 2000), while planting densities of 160 *Fédrina* stems/m^2 result in ~80 stems/m^2 at harvest with 194-228 cm heights and 6.7-7.1 mm diameters (Cappelletto *et al.* 2001). With a harvest density of 175 *Fédrina* stems/m^2, 165-181 cm

heights and 4.09-7.8 t/ha dry matter yield can result (Scheifele 1999).

In *Fédrina*, the proportion of stem in the aboveground dry matter can reach 78-84% (Meijer *et al.* 1995, Van der Werf *et al.* 1996) and remain constant through to harvest (Meijer *et al.* 1995), unaffected by flowering or seed filling (Van der Werf *et al.* 1996). However, it has been stated that the bast content is either unaffected by flowering or seed filling (Meijer *et al.* 1995) or that it is higher at flowering than when seeds are ripe (Van der Werf *et al.* 1994b). As a variety, *Fédrina* has a greater bast content decrease with increased stem dry weight (Van der Werf *et al.* 1994a).

Fédrina phloem comprises 22.6% (de Meijer 1995), 20.9-27.5% (Cappelletto *et al.* 2001) and 32.8% (Lisson and Mendham 2000) stem mass fractions, with 18.9% primary phloem fibre and 3.6% secondary phloem fibre (de Meijer 1995). Xylem equates to 60.0% (de Meijer 1995) and 61.3-67.1% (Cappelletto *et al.* 2001) stem mass fractions. Both phloem and xylem percentages are lower in the top 1/4 of *Fédrina* stems than the middle and base sections (Cappelletto *et al.* 2001). The hemi-cellulose (14.7-15.6%) and α-cellulose (65.0-70.5%) levels are highest mid-stem, and the greatest lignin level (3.5-8.9%) is found in the top region of the stem (Cappelletto *et al.* 2001).

In *Fédrina,* extended daylength causes an increased number of nodes with alternate phyllotaxy and proportion of stem in the aboveground dry matter, but reduced bast content and inflorescence yield (Van der Werf *et al.* 1994a). The prevention of flowering by 24 hr daylengths can increase stem dry matter to 89%, increase leaf matter and reduce inflorescence dry matter (Van der Werf *et al.* 1996).

2.8.3 Organic Agriculture

Pollution and the increasing cost of chemical fertilizers (especially nitrogen) have contributed to greater use of organic materials in crop production (Sharma and Mittra 1991). Organic fertilizers originate from waste and residue of plant and animal life and contain mineral nutrients in the form of complex organic molecules, while chemical fertilizers contain inorganic salts (Taiz and Zeiger 1991). It has been proposed that organic farming practices have lower nitrogen input although not necessarily lower subsequent nitrate leaching, an important effect of agricultural practice on nitrogen loading in natural water systems (Kirchmann and Berström 2001). There is a belief that organically grown crops are of higher quality and are healthier for human consumption than those grown with chemical fertilizers (Taiz and Zeiger 1991).

Organic farming practices avoid the use of synthetic fertilizers, pesticides, growth regulators and livestock feed additives and instead rely on natural, cultural and biological controls, crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes, mechanical cultivation and mineral-bearing rocks to maintain soil fertility and crop productivity. Practices are employed that measure short-term viability against long-term environmental sustainability by working with natural processes and cycles to conserve resources, minimize waste and environmental damage (Hill and MacRae 1992).

Developing countries traditionally used organic materials to maintain and improve productivity and fertility on agricultural lands until the 1950s when chemical fertilizer use increased (Parr and Colacicco 1987). They were relatively inexpensive, easily available, less bulky, and easier to store, transport and apply and often produced dramatic yield improvement. Increased chemical fertilizer use and the failure to maintain effective soil conservation practices have resulted in excessive soil erosion, nutrient run off losses and a

decrease in stable soil organic matter levels. In turn, this has led to extensive degradation, decreased crop use efficiency of applied chemical fertilizers and declined productivity of agricultural soils in many developing countries (Parr and Colacicco 1987). Organic fertilizers have been known to cause positive effects on the accumulation of soil organic matter in reclaimed soils, particularly within the first few years (Delschen 1999).

Studies have shown that plants derive equal amounts of nitrogen from soil, organic and inorganic sources (Azam *et al.* 1985). Soil mineral nitrogen is neither constant in amount nor location, but is increased by mineralization of organic nitrogen and decreased by denitrification and immobilization, when nitrate components move downward by leaching (Addiscott and Darby 1991).

Most organic sources slowly release nutrients, and compared to inorganic sources, have a greater residual effect on soil fertility (Parr and Colacicco 1987). Organic nitrogen is transported in greater amounts to the roots than the shoots, which may explain why, after an initial nutrient flush, organic fertilizer components are released more slowly, provide a more continuous supply, leach less and create a residual effect which influences yield responses from succeeding crops. When organic material is applied in combination with that of inorganic, there is ~ 30% less nitrogen loss and crop yield is often higher than when either is applied alone, which suggests that organic materials can increase the efficacy of inorganic fertilizers (Azam *et al.* 1985, Parr and Colacicco 1987). Inorganic fertilizer is released and utilized faster, with more inorganic nitrogen transported to the shoot, indicating that this form is more mobile than organic nitrogen (Azam *et al.* 1985). It often has higher macro- and micronutrient content than organic sources (Parr and Colacicco 1987).

A Canadian organic agriculture movement emerged in the 1950s and both the interest and practice of various forms of alternative agriculture in Canada are in a state of exponential

growth. Consumer demand for organically farmed products has been driven by increased awareness of correlations between food and health, lifestyles and degradation of the environment, and the depressed state of the Canadian farm economy (Hill and MacRae 1992).

3 Materials and Methods

The effect of four nitrogen fertilizers on the morphology and anatomy of *Cannabis sativa* var. *fédrina* was studied in two experiments using randomized complete block designs in both a greenhouse setting at I.K. Barber Enhanced Forestry Laboratory, UNBC, Prince George, British Columbia (53°53' N 122°48' W) and field site at Gitsegukla, British Columbia (55°11' N 127°46' W). In each study area, twenty treatments were replicated in four blocks for a total of 80 plots of plants grown at a density of 90 stems/m^2.

3.1 Greenhouse Study

On July 4 (Day 1), 2000, *C. sativa* var. *fédrina* seeds were hand-planted in individual wooden boxes in the greenhouse (Figure 1). The over-planted (~144 stems/m^2) boxes averaged 82% germination. The 0.0625 m^2 sample plots and surrounding 0.125 m buffers (un-sampled) of all 4 blocks were thinned to 90 stems/m^2 on Day 9 (July 12, 2000). Therefore, each plot contained 6 sample plants, resulting in 24 sample plants for each of the twenty treatments and a total of 480 greenhouse grown plants sampled.

Phyllotaxy change became apparent by Day 44 (August 16, 2000) and flowering by Day 52 (August 24, 2000). Plots were harvested by hand from Days 61 to 64 (September 2 to 5, 2000).

Figure 1. UNBC greenhouse design of *Cannabis sativa* var. *fédrina* grown at 90 stems/m^2. Twenty treatments were replicated over 4 blocks with 24 sample plants per treatment for total of 480 plants sampled.

3.1.1 Environmental Data

Greenhouse temperature (21°C day, 18°C night) was controlled by a Greystone 4-20ma Room Temperature Sensor (Greystone Energy Systems Inc., Moncton, New Brunswick). Relative humidity data was collected by a Siemens Room Relative Humidity Transmitter (Siemens Building Technologies, Inc., Brampton, Ontario). Sunrise and sunset data was obtained for Prince George, BC (53°55' N 122°45' W) through the National Research Council of Canada (Appendix A). Plants were watered daily with tap water.

3.1.2 Soil Characteristics

The greenhouse soil was 3:1 sand and potting soil with a pH of 6.55. Nitrogen, phosphorus and potassium levels were determined (as per Kalra and Maynard 1991) to be 8

kg N/ha, <1 kg P/ha and 126 kg K/ha, respectively.

3.1.3 Fertilization

Greenhouse soil nitrogen and phosphorus levels were considered negligible. Potassium levels met those of previous *C. sativa* research (see literature review); therefore, no potassium fertilizer was added.

Powder or granular forms of inorganic nitrogen fertilizer (ammonium sulphate; 21:0:0; Evergro Products, Inc., Delta, British Columbia) and the two organic fertilizers, Sea Star fertilizer (5:0:0; Masset, British Columbia) and Alaskan Fish Meal (8:5:1; Renton, Washington) were applied to the soil at 75, 150 or 300 kg N/ha (Table 1). Each treatment was repeated with the addition of a granular form of inorganic phosphorus fertilizer (treble superphosphate; 0:45:0; Evergro Products, Inc., Delta, British Columbia) at 90 kg P_2O_5/ha. Control treatment (0 kg N/ha) was also repeated with the addition of 90 kg P_2O_5/ha.

One third of each nitrogen fertilizer treatment was applied one week (Day 16; July 19, 2000) after ~90% germination and two thirds at one month after germination (Day 37; August 9, 2000). Phosphorus fertilizer was applied to the soil two weeks (Day 25; July 28, 2000) after germination. No herbicides or pesticides were used.

3.2 Field Study

On July 6 (Day 1), 2000, *C. sativa* var. *fédrina* was planted with a tractor disc seed drill in north-south running rows at 18 cm row spacing (Figure 2). Due to a high germination rate, the 1m^2 plots and surrounding 1m buffers were thinned from ~400-500 stems/m^2 to 90 stems/m^2 (Days 15 to 17; July 20 to 22, 2000). Each plot (including buffer) was separated from adjacent plots by a 1m wide area without seed. Plots contained 10 sample plants;

therefore, 40 plants were sampled from each of the twenty treatments for a total of 800 plants.

Phyllotaxy change became apparent by Day 46 (August 20, 2000) and flowering by Day 53 (August 27, 2000). Plots were harvested by hand from Days 66 to 68 (September 9 to 11, 2000).

Figure 2. Gitsegukla field design of *Cannabis sativa* var. *fédrina* grown at 90 stems/m^2. Twenty treatments were replicated over 4 blocks with 40 sample plants per treatment for a total of 800 plants sampled.

3.2.1 Environmental Data

Daily temperature, precipitation, sunrise and sunset data were obtained through Environment Canada (Appendix B). Temperature and precipitation data were averaged between those of Murder Creek (55°31' N 127°28' W) and Suskwa Valley, British Columbia (55°17' N 127°10' W) and daylength data were recorded in Smithers, British Columbia (54°49' N 127°11' W).

3.2.2 Soil Characteristics

Field soil was determined (as per Kalra and Maynard 1991) to be loam in texture with nitrogen, phosphorus and potassium levels of 2 kg N/ha, <1 kg P/ha and 263 kg K/ha, respectively. It had a pH of 6.06.

3.2.3 Fertilization

Field soil nitrogen and phosphorus levels were considered negligible. Potassium levels met those of previous *C. sativa* research (see literature review); therefore, no potassium fertilizer was added.

Powder or granular forms of inorganic nitrogen fertilizer (ammonium sulphate; 21:0:0) and the two organic fertilizers, Blood Meal (15:0:0; Gaia Green Products Ltd., Grand Forks, British Columbia) and Alaskan Fish Meal were applied to the soil at 75, 150 or 300 kg N/ha (Table 1). Each treatment was repeated with the addition of a granular form of inorganic phosphorus fertilizer (treble superphosphate) at 90 kg P_2O_5/ha. Control treatment (0 kg N/ha) was also repeated with the addition of 90 kg P_2O_5/ha.

One third of each nitrogen fertilizer treatment was applied one week (Day 18; July 23, 2000) after ~90% germination and two thirds at one month after germination (Day 38; August 12, 2000). Phosphorus fertilizer was applied two weeks after germination (Day 25; July 30, 2000). No herbicides or pesticides were used.

Table 1. Treatment regime for *Cannabis sativa* var. *fédrina* fertilizer trials in the UNBC greenhouse and Gitsegukla field.

Greenhouse	Nitrogen Fertilizer Type
0 kg N/ha	
0 kg N/ha + 90 kg P_2O_5/ha	
75 kg N/ha	Sea Star Fishmeal Inorganic
75 kg N/ha + 90 kg P_2O_5/ha	Sea Star Fishmeal Inorganic
150 kg N/ha	Sea Star Fishmeal Inorganic
150 kg N/ha + 90 kg P_2O_5/ha	Sea Star Fishmeal Inorganic
300 kg N/ha	Sea Star Fishmeal Inorganic
300 kg N/ha + 90 kg P_2O_5/ha	Sea Star Fishmeal Inorganic

Field	Nitrogen Fertilizer Type
0 kg N/ha	
0 kg N/ha + 90 kg P_2O_5/ha	
75 kg N/ha	Bloodmeal Fishmeal Inorganic
75 kg N/ha + 90 kg P_2O_5/ha	Bloodmeal Fishmeal Inorganic
150 kg N/ha	Bloodmeal Fishmeal Inorganic
150 kg N/ha + 90 kg P_2O_5/ha	Bloodmeal Fishmeal Inorganic
300 kg N/ha	Bloodmeal Fishmeal Inorganic
300 kg N/ha + 90 kg P_2O_5/ha	Bloodmeal Fishmeal Inorganic

Note: Each of the 20 treatments was replicated in four blocks.

3.3 Morphological Measurements: Greenhouse and Field Study

In the greenhouse, plant height, number of internodes and length of third internode (from soil surface) were measured every 4 days. In the field, plant height measurements were taken every two weeks. At harvest, for both settings, plant height, number of internodes (from soil surface), third internode length, diameter (at midpoint), fresh weight,

dry weight and fresh weight/dry weight ratios were determined for each sample plant. Sample internodes were stored lightly wrapped in plastic film, in individual paper bags within plastic containers at 4°C (EGC cooler, Chagrin Falls, Ohio, USA) with the exception of sectioning time at room temperature. Dry weights were measured after drying for 4 days at 65°C (Despatch Oven, Minneapolis, Minnesota).

3.4 Anatomical Measurements: Greenhouse and Field Study

Laboratory techniques followed those of Stricker (2000) and were similar to those of Mediavilla *et al.* (2001). Cross-sections were cut with razor blades and stained with TBO (toluidine blue O). TBO stains lignified tissues blue to blue-green (secondary walls), pectin stains red-purple (primary walls) and phenolic substances stain blue-green in colour. At the time of sectioning, xylem depths were measured with ocular micrometers. All cross-sections were scanned into Northern Exposure™ image analysis software 2.7 (Mississauga, Ontario) with a microscope-mounted Hitachi Color Video Camera (model VK-C370, Hitachi Ltd., Japan). Phloem measurements were made with Northern Exposure™. Phloem and xylem areas, volumes, and ratios were calculated using the equations for a cylinder. The area in which the number of primary phloem cells was counted was 0.25 mm long and the width of the primary phloem tissue (mm) wide. The unit was standardized across treatments to be the number of primary phloem cells per square millimetre. Primary phloem cell wall width was measured on the first three cells to the right of the cross-sectional image.

3.5 Statistical Analysis

SPSS™ software (SPSS, Inc., Chicago, Illinois) was used to determine means of

measurements and calculated values, analysis of variance (ANOVA) (including interaction effects) and post-hoc analyses (Tukey-HSD and Bonferroni) differences between fertilizer treatments.

4 Results

Block effects were experienced in both greenhouse and field trials of *Cannabis sativa* var. *fédrina*. Pest damage to plants was not observed, however, due to shallow application of the final fishmeal fertilizer treatment, some soil surface mould resulted. Minor fungal gnat activity was observed on the mouldy surface and ceased with its integration into the soil. In the field, minimal weed presence was either removed by hand or was naturally suppressed with increased crop canopy closure.

In the 61 to 64 days of the greenhouse study, plants grew to 19.5 to 154.4 cm heights, with 5 to 16 internodes and 0.7 to 15.4 cm long third internodes of 0.6 to 6.1 mm diameter (means, Tables 2 and 3). Greenhouse plants were watered daily and maintained at 20.1 to 23.0 °C with 44.6 to 73.9 % relative humidity. Daylength decreased from 17.0 to 13.4 hours, with phyllotaxy change apparent at 14.8 hours and flowering at 14.2 hours (Appendix A). Three greenhouse plots established only 5 sample plants, which reduced the total number of plants sampled per treatment from 24 to 23 for sea star at 150 kg N/ha and 90 kg P_2O_5/ha, fishmeal at 75 kg N/ha and 90 kg P_2O_5/ha and inorganic at 150 kg N/ha and 90 kg P_2O_5/ha.

In the 66 to 68 days of the field study, plants grew to 20.4 to 193.4 cm heights, with 4 to 16 internodes and 3.59 to 33.45 cm long third internodes of 1.4 to 9.5 mm diameter (means, Tables 7 and 8). Daily precipitation ranged from 0 to 23.4 mm with a total of 156.0 mm. Temperature ranged from 3.0 to 29.3 °C. Daylength decreased from 17.1 to 13.0 hours,

with phyllotaxy change apparent at 14.6 hours and flowering at 14.1 hours (Appendix B).

Figure 3 is a cross-sectional image showing epidermis, cortex, primary and secondary phloem and xylem of the third internode of a stem. The presence of vascular cambium retting, causing separation of secondary phloem and xylem, was apparent on some cross-sections, and offers a visual presentation of the process as described in the literature review. Pink stained cell walls of collenchyma in the cortex were well defined in some cross-sections. Although not statistically analysed, there was variation observed, even within treatment groups, for primary phloem fibre cell shape and lumen size (Figure 4), secondary phloem presence (Figure 5) and secondary xylem cell shape and wall thickness (Figure 6).

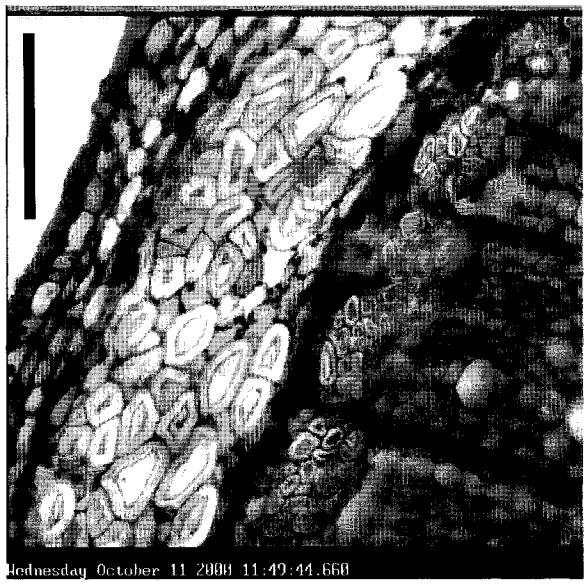

Figure 3. Cross-section of the third internode of *Cannabis sativa* var. *fédrina* stained with TBO. Epidermis is on the outside followed by cortex (pink), primary phloem (light purple), secondary phloem (blue) and xylem (blue). Colour refers to that of cell walls. Retting of vascular cambium and separation of secondary phloem and xylem is visible. Scale bar = 100 μm.

Figure 4. Example of primary phloem fibre shape and lumen size variation within one treatment (150 kg sea star N/ha + 0 kg P_2O_5/ha), on TBO stained cross-sectional images of *Cannabis sativa* var. *fédrina*. From left image to right, primary phloem fibres appear more compressed, with thinner cell walls and smaller lumens. Scale bar = 100 μm.

Figure 5. Example of secondary phloem fibre variability within one treatment (0 kg N/ha + 0 kg P_2O_5/ha), on TBO stained cross-sectional images of *Cannabis sativa* var. *fédrina*. From left image to right, secondary phloem fibre development appears to increase. Scale bar = 100 μm.

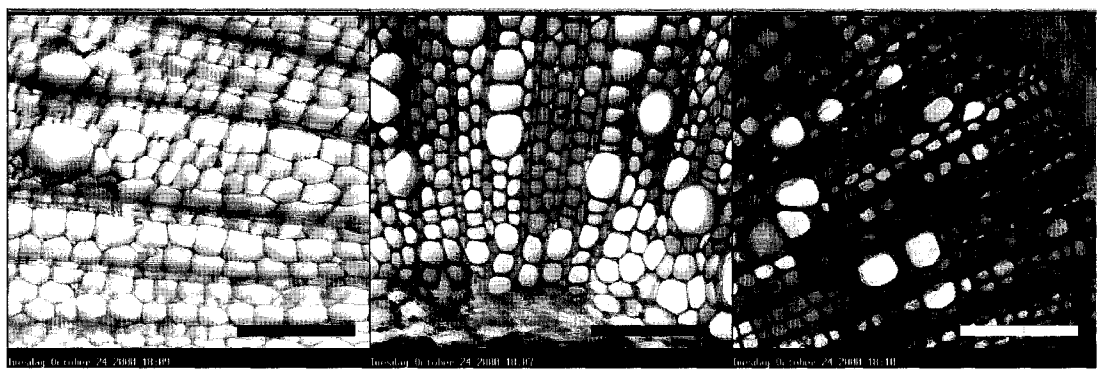

Figure 6. Example of secondary xylem cell shape and wall thickness variation on TBO stained cross-sectional images of *Cannabis sativa* var. *fédrina*. From left image to right, xylem cells appear more circular in shape with thicker cell walls. Scale bar = 100 μm.

4.1 Morphology of Greenhouse-Grown *C. sativa* var. *fédrina* (ANOVA Tables - Appendix B)

4.1.1 Stems

Stem morphology was not significantly affected by either nitrogen fertilizer type or nitrogen level (Table 2). Plants treated with 90 kg P_2O_5/ha produced significantly higher heights and number of internodes compared to those without phosphorus.

Table 2. Fertilizer effect on stem morphology of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable	Control	Sea Star	Fishmeal	Inorganic
Height (cm)	79.20 (6.24)*	87.85 (3.60)	87.41 (3.60)	84.75 (3.60)
Number of internodes	10.6 (0.3)	11.3 (0.2)	11.3 (0.2)	11.0 (0.2)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
Height (cm)	79.20 (6.24)	82.40 (3.60)	88.61 (3.60)	89.01 (3.60)
Number of internodes	10.6 (0.3)	11.0 (0.2)	11.4 (0.2)	11.1 (0.2)
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
Height (cm)	77.71 (2.79)**a**	94.14 (2.79)**b**		
Number of internodes	10.6 (0.1)**a**	11.7 (0.1)**b**		

* Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=24.

4.1.2 Internodes

The morphology of internodes was not significantly affected by either nitrogen fertilizer type or nitrogen level (Table 3). Internode diameter, fresh weight and fresh/dry weight ratio were significantly higher in treatments with the addition of 90 kg P_2O_5/ha compared to those without phosphorus.

A significant interaction occurred between the effects of nitrogen fertilizer type and phosphorus fertilizer addition on internode fresh/dry weight ratio (Figure 7). Comparing

treatments of 0 and 90 kg P_2O_5/ha, the most dramatic internode fresh/dry weight ratio increase was with inorganic nitrogen fertilizer treatment, and the least dramatic was with the fishmeal treatment.

Table 3. Fertilizer effect on third internode morphology of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable	Control	Sea Star	Fishmeal	Inorganic
Length (cm)	5.33 (0.48)*	5.12 (0.27)	5.53 (0.27)	5.26 (0.27)
Diameter (mm)	2.58 (0.21)	3.03 (0.12)	2.84 (0.12)	2.89 (0.12)
Fresh weight (g)	0.415 (0.075)	0.597 (0.043)	0.601 (0.043)	0.565 (0.043)
Dry weight (g)	0.108 (0.022)	0.132 (0.013)	0.136 (0.013)	0.112 (0.013)
Fresh / dry weight	4.73 (0.92)	5.50 (0.53)	5.17 (0.53)	5.89 (0.53)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
Length (cm)	5.33 (0.48)	5.12 (0.27)	5.57 (0.27)	5.22 (0.27)
Diameter (mm)	2.58 (0.21)	2.81 (0.12)	2.94 (0.12)	3.00 (0.12)
Fresh weight (g)	0.415 (0.075)	0.548 (0.043)	0.595 (0.043)	0.621 (0.043)
Dry weight (g)	0.108 (0.022)	0.125 (0.013)	0.134 (0.013)	0.120 (0.013)
Fresh / dry weight	4.73 (0.92)	5.36 (0.53)	5.49 (0.53)	5.71 (0.53)
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
Length (cm)	5.49 (0.21)	5.12 (0.21)		
Diameter (mm)	2.43 (0.09)**a**	3.34 (0.09)**b**		
Fresh weight (g)	0.473 (0.033)**a**	0.668 (0.033)**b**		
Dry weight (g)	0.135 (0.010)	0.114 (0.010)		
Fresh / dry weight	4.09 (0.41)**a**	6.79 (0.41)**b**		

* Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=24.

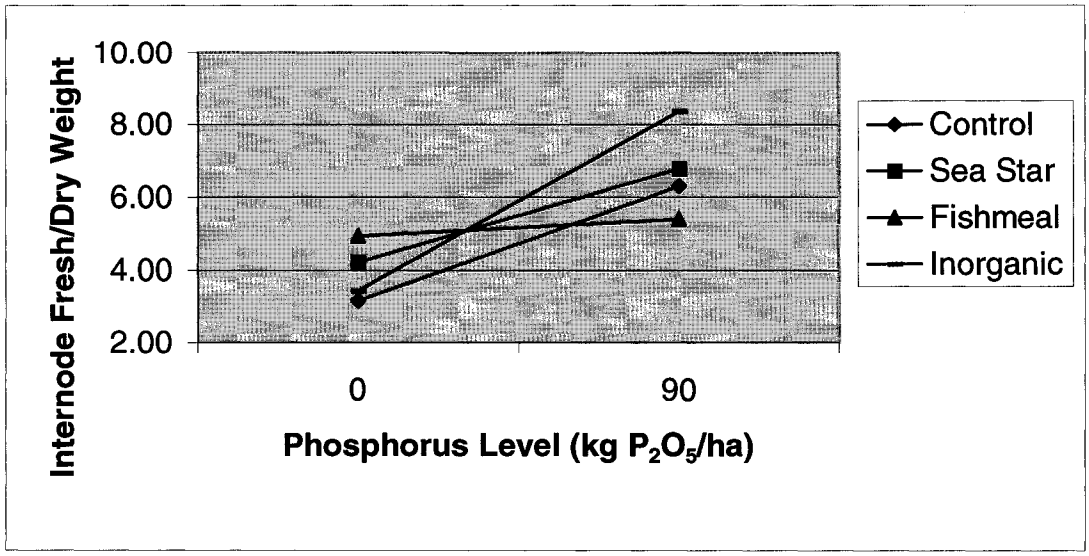

Figure 7. Interaction effects of nitrogen fertilizer type and phosphorus level on the internode fresh/dry weight of *Cannabis sativa* var. *fédrina* in the UNBC greenhouse, n=477.

4.2 Anatomy of Greenhouse-Grown *C. sativa* var. *fédrina* (ANOVA Tables - Appendix B)

4.2.1 Internodes

Internode anatomy was unaffected by either nitrogen fertilizer type or nitrogen level (Table 4). However, primary phloem fibre area, secondary phloem fibre area and volume, xylem area and volume, and total fibre area and volume were each significantly higher in treatments with phosphorus fertilizer application (Table 4, Figure 8).

Table 4. Fertilizer effect on third internode anatomy of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Control	Sea Star	Fishmeal	Inorganic
PP area (mm^2)	0.56 (0.06)†	0.65 (0.04)	0.63 (0.04)	0.63 (0.04)
PP volume (mm^3)	0.89 (0.14)	0.88 (0.08)	1.02 (0.08)	0.94 (0.08)
SP area (mm^2)	0.35 (0.09)	0.61 (0.05)	0.47 (0.05)	0.52 (0.05)
SP volume (mm^3)	0.50 (0.21)	0.87 (0.12)	0.61 (0.12)	0.79 (0.12)
X area (mm^2)	0.49 (0.12)	0.84 (0.07)	0.70 (0.07)	0.69 (0.07)
X volume (mm^3)	0.80 (0.23)	1.51 (0.14)	1.32 (0.14)	1.24 (0.14)
T area (mm^2)	1.40 (0.24)	2.09 (0.14)	1.81 (0.14)	1.84 (0.14)
T volume (mm^3)	2.18 (0.45)	3.26 (0.26)	2.95 (0.26)	2.97 (0.26)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
PP area (mm^2)	0.56 (0.06)	0.61 (0.04)	0.63 (0.04)	0.67 (0.04)
PP volume (mm^3)	0.89 (0.14)	0.92 (0.08)	0.96 (0.08)	0.96 (0.08)
SP area (mm^2)	0.35 (0.09)	0.52 (0.05)	0.51 (0.05)	0.57 (0.05)
SP volume (mm^3)	0.50 (0.21)	0.72 (0.12)	0.72 (0.12)	0.83 (0.12)
X area (mm^2)	0.49 (0.12)	0.69 (0.07)	0.72 (0.07)	0.82 (0.07)
X volume (mm^3)	0.80 (0.23)	1.22 (0.14)	1.31 (0.14)	1.53 (0.14)
T area (mm^2)	1.40 (0.24)	1.81 (0.14)	1.87 (0.14)	2.06 (0.14)
T volume (mm^3)	2.18 (0.45)	2.87 (0.26)	2.99 (0.26)	3.32 (0.26)
	0 kg P_2O_5/ha	**90 kg $_2O_5$/ha**		
PP area (mm^2)	0.51 (0.03)**a**	0.74 (0.03)**b**		
PP volume (mm^3)	0.91 (0.06)	0.97 (0.06)		
SP area (mm^2)	0.23 (0.04)**a**	0.80 (0.04)**b**		
SP volume (mm^3)	0.25 (0.09)**a**	1.22 (0.09)**b**		
X area (mm^2)	0.43 (0.05)**a**	1.00 (0.05)**b**		
X volume (mm^3)	0.75 (0.10)**a**	1.85 (0.10)**b**		
T area (mm^2)	1.17 (0.11)**a**	2.56 (0.11)**b**		
T volume (mm^3)	1.91 (0.20)**a**	4.04 (0.20)**b**		

* PP, primary phloem fibre; SP, secondary phloem fibre; X, xylem fibre; T, total fibre (PP+SP+X)

† Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=24.

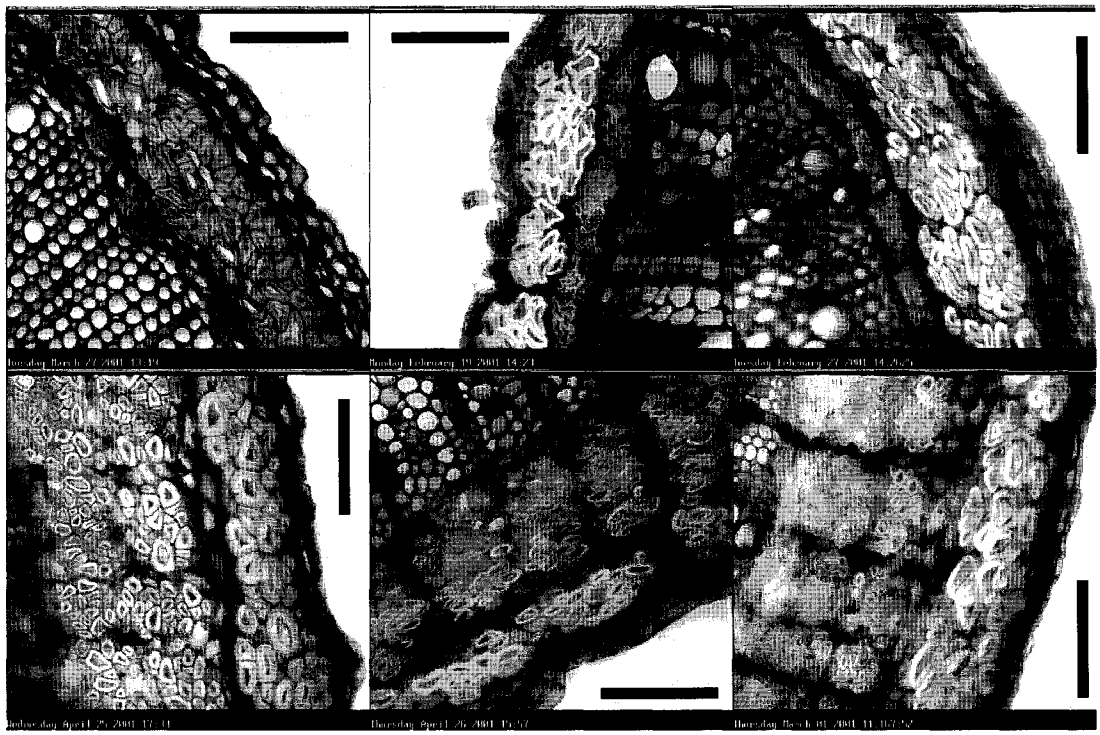

Figure 8. Representation of phosphorus level enhancement of secondary phloem fibre development on TBO stained cross-sectional images of UNBC greenhouse-grown *Cannabis sativa* var. *fédrina*. Top row: 0 kg P_2O_5/ha treatment. Bottom row: 90 kg P_2O_5/ha. Left: 0 kg N/ha. Middle: 150 kg N/ha sea star. Right: 300 kg N/ha fishmeal. Epidermis is on the outside followed by cortex (pink), primary phloem (light purple), secondary phloem (blue) and xylem (blue) tissues. Scale bar = 100 μm.

4.2.2 Primary Phloem Fibres

Primary phloem fibre cell number/mm^2 and wall width were both unaffected by either nitrogen fertilizer type or nitrogen level (Table 5). However, the number of primary phloem fibre cells/mm^2 was significantly higher in treatments without phosphorus and primary phloem fibre cell wall thickness was significantly higher with the addition of 90 kg P_2O_5/ha.

Table 5. Fertilizer effect on primary phloem fibre anatomy of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Control	Sea Star	Fishmeal	Inorganic
# PP/mm^2	3369.8 (211.1)†	2964.7 (121.9)	2908.7 (121.9)	3086.5 (121.9)
PP cell wall thickness (μm)	5.6 (0.2)	5.7 (0.1)	5.7 (0.1)	5.5 (0.1)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
# PP/mm^2	3369.8 (211.1)	3113.7 (121.9)	2902.3 (121.9)	2943.9 (121.9)
PP cell wall thickness (μm)	5.6 (0.2)	5.6 (0.1)	5.7 (0.1)	5.6 (0.1)
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
# PP/mm^2	3475.8 (94.4)**a**	2574.1 (94.4)**b**		
PP cell wall thickness (μm)	5.3 (0.1)**a**	5.9 (0.1)**b**		

* PP, primary phloem fibre; SP, secondary phloem fibre; X, xylem fibre; T, total fibre (PP+SP+X)

† Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=24.

4.2.3 Tissue Ratios

Control plants had significantly higher primary phloem fibre/total fibre area and volume ratios than any type of nitrogen fertilizer treatment (Table 6). The absence of phosphorus fertilizer application produced significantly higher primary phloem fibre/total fibre area and volume ratios, and total phloem fibre/total fibre area and volume ratios. Conversely, significantly higher secondary phloem fibre/total fibre area and volume, xylem/total fibre area and volume ratios were produced with the phosphorus treatment.

In significant interactions between the effects of nitrogen fertilizer type and phosphorus fertilizer application on primary phloem fibre/total fibre area (Figures 9) and volume (Figure 10) ratios, control or inorganic nitrogen fertilizer treatment ratios show a more dramatic decrease between the 0 or 90 kg P_2O_5/ha treatments than sea star or fishmeal treatments.

Table 6. Fertilizer effect on tissue ratios of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Control	Sea Star	Fishmeal	Inorganic
PP/T area	0.47 (0.02)**a**†	0.34 (0.01)**b**	0.39 (0.01)**b**	0.40 (0.01)**b**
PP/T volume	0.52 (0.04)**a**	0.31 (0.02)**b**	0.39 (0.02)**b**	0.41 (0.02)**b**
SP/T area	0.19 (0.02)	0.27 (0.01)	0.23 (0.01)	0.24 (0.01)
SP/T volume	0.16 (0.03)	0.23 (0.02)	0.18 (0.02)	0.20 (0.02)
X/T area	0.34 (0.02)	0.39 (0.01)	0.38 (0.01)	0.36 (0.01)
X/T volume	0.32 (0.04)	0.45 (0.02)	0.43 (0.02)	0.39 (0.02)
PP+SP/T area	0.66 (0.01)	0.61 (0.01)	0.62 (0.01)	0.64 (0.01)
PP+SP/T volume	0.68 (0.04)	0.55 (0.02)	0.57 (0.02)	0.61 (0.02)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
PP/T area	0.47 (0.02)	0.39 (0.01)	0.38 (0.01)	0.36 (0.01)
PP/T volume	0.52 (0.04)	0.39 (0.02)	0.38 (0.02)	0.35 (0.02)
SP/T area	0.19 (0.02)	0.25 (0.01)	0.24 (0.01)	0.25 (0.01)
SP/T volume	0.16 (0.03)	0.20 (0.02)	0.19 (0.02)	0.21 (0.02)
X/T area	0.34 (0.02)	0.37 (0.01)	0.38 (0.01)	0.39 (0.01)
X/T volume	0.32 (0.04)	0.40 (0.02)	0.43 (0.02)	0.44 (0.02)
PP+SP/T area	0.66 (0.01)	0.63 (0.01)	0.62 (0.01)	0.61 (0.01)
PP+SP/T volume	0.68 (0.04)	0.60 (0.02)	0.57 (0.02)	0.56 (0.02)
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
PP/T area	0.47 (0.01)**a**	0.30 (0.01)**b**		
PP/T volume	0.53 (0.02)**a**	0.24 (0.02)**b**		
SP/T area	0.17 (0.01)**a**	0.31 (0.01)**b**		
SP/T volume	0.11 (0.01)**a**	0.29 (0.01)**b**		
X/T area	0.36 (0.01)**a**	0.39 (0.01)**b**		
X/T volume	0.36 (0.02)**a**	0.47 (0.02)**b**		
PP+SP/T area	0.64 (0.01)**a**	0.61 (0.01)**b**		
PP+SP/T volume	0.64 (0.02)**a**	0.53 (0.02)**b**		

* PP, primary phloem fibre; SP, secondary phloem fibre; X, xylem fibre; T, total fibre (PP+SP+X)

† Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=24.

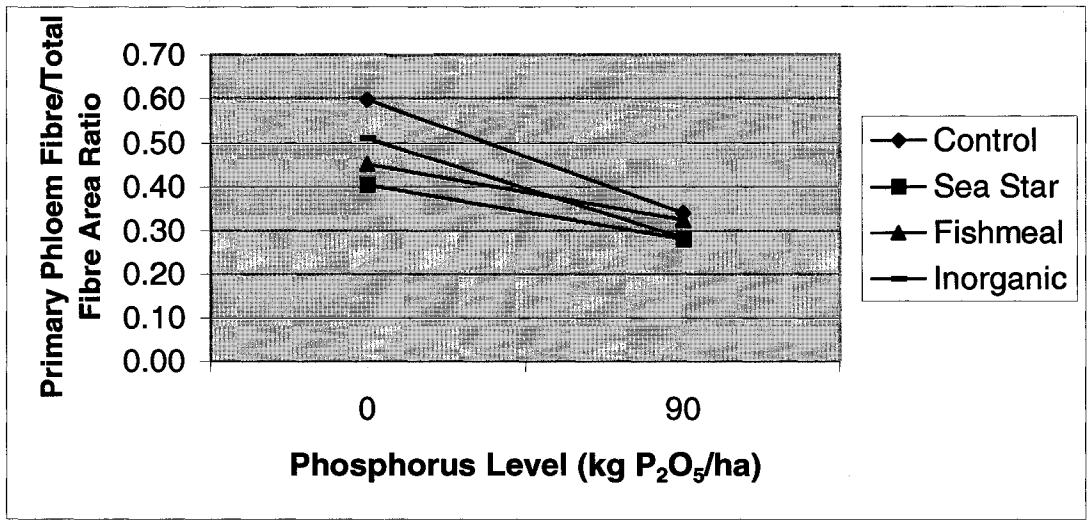

Figure 9. Interaction effects of nitrogen fertilizer type and phosphorus level on the primary phloem fibre/total fibre area ratio of *Cannabis sativa* var. *fédrina* in the UNBC greenhouse, n=477.

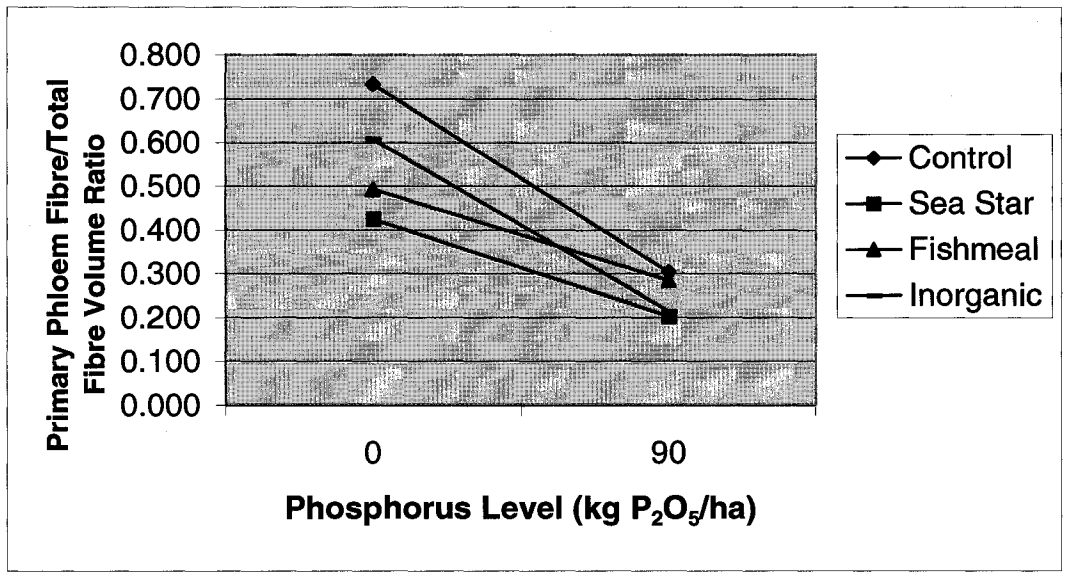

Figure 10. Interaction effects of nitrogen fertilizer type and phosphorus level on the primary phloem fibre/total fibre volume ratio of *Cannabis sativa* var. *fédrina* in the UNBC greenhouse, n=477.

4.3 Morphology of Field-Grown *C. sativa* var. *fédrina* (ANOVA Tables - Appendix C)

4.3.1 Stems

Morphological stem characteristics were significantly affected by nitrogen fertilizer level, but not by type of fertilizer applied (Table 7). Control treatment resulted in lower stem height and number of internodes than fertilized treatments. The highest stem height occurred with treatment of 150 kg N/ha. Plants without phosphorus treatment had significantly greater heights compared to plants treated with 90 kg P_2O_5/ha.

A significant interaction occurred between the effects of nitrogen fertilizer type and nitrogen level on the number of internodes (Figure 11). Means from the control treatment were consistently lower than all other treatments. Bloodmeal and fishmeal treatment means were the highest with 150 kg N/ha, while the highest inorganic treatment mean was at 75 kg N/ha.

Table 7. Fertilizer effect on stem morphology of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable	**Control**	**Bloodmeal**	**Fishmeal**	**Inorganic**
Height (cm)	97.13 (5.54)*	121.08 (3.20)	127.07 (3.20)	130.43 (3.20)
Number of internodes	9.5 (0.2)	10.3 (0.1)	10.3 (0.1)	10.4 (0.1)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
Height (cm)	97.13 (5.54)**a**	115.61 (3.20)**b**	137.29 (3.20)**c**	125.69 (3.20)**b**
Number of internodes	9.5 (0.2)**a**	10.2 (0.1)**b**	10.6 (0.1)**b**	10.2 (0.1)**b**
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
Height (cm)	128.48 (2.48)**a**	118.09 (2.48)**b**		
Number of internodes	10.4 (0.1)	10.2 (0.1)		

* Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=40.

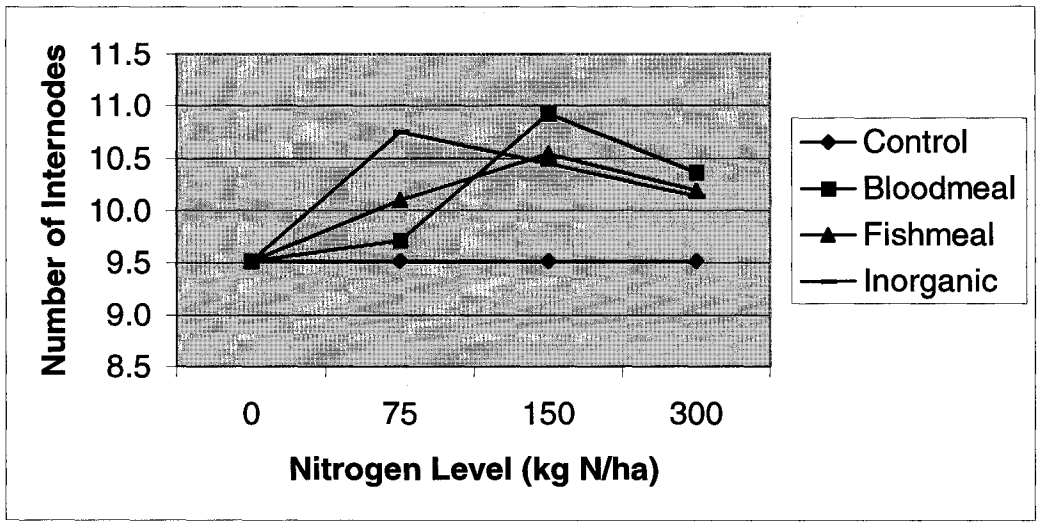

Figure 11. Interaction effects of nitrogen fertilizer type and nitrogen level on the number of internodes of *Cannabis sativa* var. *fédrina* in the Gitsegukla field, n=800.

4.3.2 Internodes

Morphological internode characteristics were significantly affected by nitrogen fertilizer level, but not by fertilizer type (Table 8). Internode diameters from the 150 or 300 kg N/ha treatments were significantly higher than those of 0 or 75 kg N/ha. Internode fresh weights were significantly higher with treatments of 150 or 300 kg N/ha compared to control. Plants without phosphorus treatment possessed significantly higher internode diameters, fresh and dry weights.

Table 8. Fertilizer effect on third internode morphology of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable	Control	Bloodmeal	Fishmeal	Inorganic
Length (cm)	10.75 (0.74)*	10.58 (0.43)	11.34 (0.43)	11.71 (0.43)
Diameter (mm)	4.12 (0.22)	4.47 (0.13)	4.85 (0.13)	4.77 (0.13)
Fresh weight (g)	1.461 (0.236)	2.091 (0.136)	2.438 (0.136)	2.424 (0.136)
Dry weight (g)	0.244 (0.040)	0.206 (0.023)	0.254 (0.023)	0.275 (0.023)
Fresh/dry weight	6.15 (1.33)	11.59 (0.77)	10.45 (0.77)	10.11 (0.77)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
Length (cm)	10.75 (0.74)	10.83 (0.43)	11.65 (0.43)	11.15 (0.43)
Diameter (mm)	4.12 (0.22)**a**	4.44 (0.13)**a**	4.93 (0.13)**b**	4.73 (0.13)**b**
Fresh weight (g)	1.461 (0.236)**a**	1.981 (0.136)**ab**	2.603 (0.136)**c**	2.369 (0.136)**bc**
Dry weight (g)	0.244 (0.040)	0.228 (0.23)	0.275 (0.023)	0.232 (0.023)
Fresh/dry weight	6.15 (1.33)	9.80 (0.77)	10.69 (0.77)	11.65 (0.77)
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
Length (cm)	11.46 (0.33)	10.87 (0.33)		
Diameter (mm)	4.83 (0.10)**a**	4.45 (0.10)**b**		
Fresh weight (g)	2.462 (0.105)**a**	2.002 (0.105)**b**		
Dry weight (g)	0.281 (0.018)**a**	0.209 (0.018)**b**		
Fresh/dry weight	9.58 (0.60)	10.94 (0.60)		

* Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=40.

4.4 Anatomy of Field-Grown *C. sativa* var. *fédrina* (ANOVA Tables - Appendix C)

4.4.1 Internodes

Nitrogen fertilizer type was found to significantly affect three anatomical characteristics, two of which did not differentiate in post-hoc tests: primary phloem fibre area and volume (Table 9). The area of primary phloem fibre was higher with fishmeal or inorganic nitrogen fertilizer treatment and lower with bloodmeal or control treatment. The volume of primary phloem fibre was highest in the control and lowest in the bloodmeal treatment. Total fibre volume was significantly higher with fishmeal or inorganic nitrogen

fertilizer treatment compared to control. Xylem area and volume were significantly higher with 150 or 300 kg N/ha treatment compared to control. Total fibre volume was significantly higher in the 150 kg N/ha treatment than the control. Primary phloem fibre area, xylem area and volume and total fibre area and volume were all significantly higher with the absence of phosphorus fertilizer compared to treatments with phosphorus.

There were significant interactions between the effects of nitrogen fertilizer type and nitrogen level for secondary phloem fibre area (Figure 12) and volume (Figure 13). Means from the control treatment were consistently lower than all other treatments. Bloodmeal and fishmeal treatment means were highest with 150 kg N/ha, while the highest inorganic treatment mean was at 75 kg N/ha.

Table 9. Fertilizer effect on third internode anatomy of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Control	Bloodmeal	Fishmeal	Inorganic
PP area (mm^2)	1.13 (0.08)†‡	1.12 (0.05)‡	1.29 (0.05)‡	1.23 (0.05)‡
PP volume (mm^3)	2.90 (0.24)‡	2.30 (0.14)‡	2.78 (0.14)‡	2.75 (0.14)‡
SP area (mm^2)	0.35 (0.09)	0.54 (0.05)	0.64 (0.05)	0.59 (0.05)
SP volume (mm^3)	0.38 (0.13)	0.59 (0.08)	0.75 (0.08)	0.70 (0.08)
X area (mm^2)	0.96 (0.15)	1.37 (0.09)	1.59 (0.09)	1.48 (0.09)
X volume (mm^3)	2.31 (0.50)	3.63 (0.29)	4.36 (0.29)	4.11 (0.29)
T area (mm^2)	2.44 (0.30)	3.04 (0.17)	3.52 (0.17)	3.29 (0.17)
T volume (mm^3)	5.58 (0.71)**a**	6.53 (0.41)**ab**	7.88 (0.41)**b**	7.56 (0.41)**b**
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
PP area (mm^2)	1.13 (0.08)	1.16 (0.05)	1.16 (0.05)	1.30 (0.05)
PP volume (mm^3)	2.90 (0.24)	2.54 (0.14)	2.83 (0.14)	2.45 (0.14)
SP area (mm^2)	0.35 (0.09)	0.54 (0.05)	0.66 (0.05)	0.58 (0.05)
SP volume (mm^3)	0.38 (0.13)	0.62 (0.08)	0.77 (0.08)	0.65 (0.08)
X area (mm^2)	0.96 (0.15)**a**	1.31 (0.09)**ab**	1.62 (0.09)**b**	1.51 (0.09)**b**
X volume (mm^3)	2.31 (0.05)**a**	3.38 (0.29)**ab**	4.61 (0.29)**b**	4.10 (0.29)**b**
T area (mm^2)	2.44 (0.30)	3.00 (0.17)	3.57 (0.17)	3.27 (0.17)
T volume (mm^3)	5.58 (0.71)**a**	6.54 (0.41)**ab**	8.22 (0.41)**b**	7.21 (0.41)**ab**
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
PP area (mm^2)	1.28 (0.04)**a**	1.13 (0.04)**b**		
PP volume (mm^3)	2.79 (0.11)	2.49 (0.11)		
SP area (mm^2)	0.61 (0.04)	053 (0.04)		
SP volume (mm^3)	0.71 (0.06)	0.59 (0.06)		
X area (mm^2)	1.56 (0.07)**a**	1.30 (0.07)**b**		
X volume (mm^3)	4.29 (0.22)**a**	3.43 (0.22)**b**		
T area (mm^2)	3.44 (0.13)**a**	2.96 (0.13)**b**		
T volume (mm^3)	7.91 (0.32)**a**	6.51 (0.32)**b**		

* PP, primary phloem fibre; SP, secondary phloem fibre; X, xylem fibre; T, total fibre (PP+SP+X)

† Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=40.

‡ Significant ANOVA results ($p \leq 0.05$) but no differentiation with post-hoc analyses.

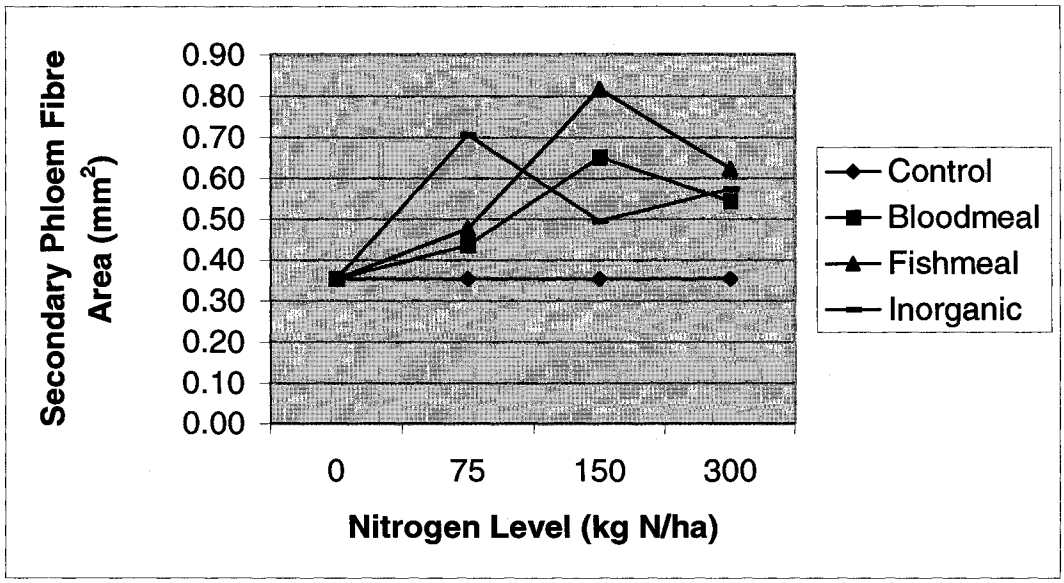

Figure 12. Interaction effects of nitrogen fertilizer type and nitrogen level on secondary phloem fibre area (mm^2) of *Cannabis sativa* var. *fédrina* in the Gitsegukla field, n=800.

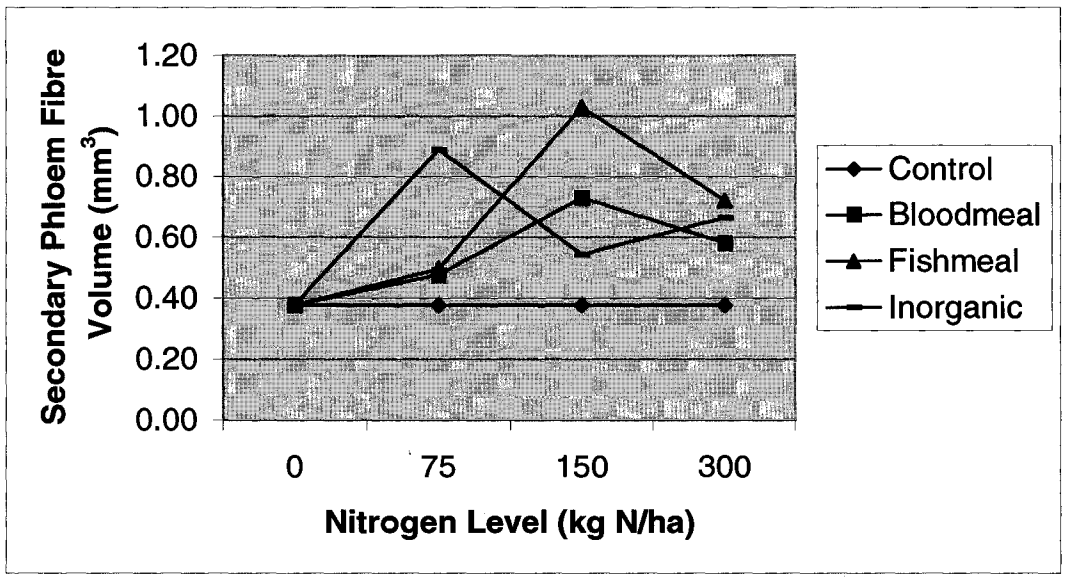

Figure 13. Interaction effects of nitrogen fertilizer type and nitrogen level on secondary phloem fibre volume (mm^3) of *Cannabis sativa* var. *fédrina* in the Gitsegukla field, n=800.

4.4.2 Primary Phloem Fibres

The number of primary phloem fibres/mm^2 and fibre wall thickness were both unaffected by either nitrogen fertilizer type or nitrogen level (Table 10). However, a significantly higher number of primary phloem fibres/mm^2 was produced in treatments with the presence of 90 kg P_2O_5/ha.

Table 10. Fertilizer effect on primary phloem fibre anatomy of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Control	Bloodmeal	Fishmeal	Inorganic
# PP/mm^2	2350.6 (103.7)†	2208.8 (59.9)	2064.5 (59.9)	2163.4 (59.9)
PP cell wall thickness (μm)	6.8 (0.3)	6.5 (0.2)	6.9 (0.2)	6.7 (0.2)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
# PP/mm^2	2350.6 (103.7)	2197.8 (59.9)	2069.6 (59.9)	2169.4 (59.9)
PP cell wall thickness (μm)	6.8 (0.3)	6.7 (0.2)	6.7 (0.2)	6.6 (0.2)
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
# PP/mm^2	2087.7 (46.4)**a**	2244.5 (46.4)**b**		
PP cell wall thickness (μm)	6.8 (0.1)	6.6 (0.1)		

* PP, primary phloem fibre; SP, secondary phloem fibre; X, xylem fibre; T, total fibre (PP+SP+X)
† Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=40.

4.4.3 Tissue Ratios

Internode tissue ratios were unaffected by either nitrogen fertilizer type or the addition of phosphorus fertilizer, but were affected by nitrogen level (Table 11). Primary phloem fibre/total fibre area and volume ratios were significantly higher for the control treatment plants compared to those with any level of nitrogen treatment. Xylem/total fibre area and volume ratios were significantly higher with 300 kg N/ha treatment compared to 75

kg N/ha, and all levels of nitrogen treatment were significantly higher than the control. Total phloem fibre/total fibre area and volume ratios were significantly higher for the control compared to all levels of nitrogen treatment, and 75 kg N/ha was higher than 300 kg N/ha treatment.

Table 11. Fertilizer effect on tissue ratios of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	**Control**	**Bloodmeal**	**Fishmeal**	**Inorganic**
PP/T area	0.52 (0.02)†	0.38 (0.01)	0.38 (0.01)	0.38 (0.01)
PP/T volume	0.60 (0.03)	0.37 (0.02)	0.37 (0.02)	0.37 (0.02)
SP/T area	0.12 (0.02)	0.17 (0.01)	0.17 (0.01)	0.18 (0.01)
SP/T volume	0.05 (0.01)	0.09 (0.01)	0.09 (0.01)	0.09 (0.01)
X/T area	0.37 (0.01)	0.44 (0.01)	0.45 (0.01)	0.45 (0.01)
X/T volume	0.35 (0.03)	0.54 (0.02)	0.54 (0.02)	0.54 (0.02)
PP+SP/T area	0.63 (0.01)	0.56 (0.01)	0.55 (0.01)	0.56 (0.01)
PP+SP/T volume	0.66 (0.03)	0.46 (0.02)	0.46 (0.02)	0.46 (0.02)
	0 kg N/ha	**75 kg N/ha**	**150 kg N/ha**	**300 kg N/ha**
PP/T area	0.52 (0.02)**a**	0.40 (0.01)**b**	0.37 (0.01)**b**	0.36 (0.01)**b**
PP/T volume	0.60 (0.03)**a**	0.42 (0.02)**b**	0.36 (0.02)**b**	0.34 (0.02)**b**
SP/T area	0.12 (0.02)	0.17 (0.01)	0.18 (0.01)	0.18 (0.01)
SP/T volume	0.05 (0.01)	0.09 (0.01)	0.09 (0.01)	0.09 (0.01)
X/T area	0.37 (0.01)**a**	0.43 (0.01)**b**	0.45 (0.01)**bc**	0.46 (0.01)**c**
X/T volume	0.35 (0.03)**a**	0.50 (0.02)**b**	0.55 (0.02)**bc**	0.57 (0.02)**c**
PP+SP/T area	0.63 (0.01)**a**	0.57 (0.01)**b**	0.55 (0.01)**bc**	0.54 (0.01)**c**
PP+SP/T volume	0.66 (0.03)**a**	0.50 (0.02)**b**	0.45 (0.02)**bc**	0.43 (0.02)**c**
	0 kg P_2O_5/ha	**90 kg P_2O_5/ha**		
PP/T area	0.39 (0.01)	0.40 (0.01)		
PP/T volume	0.38 (0.02)	0.40 (0.02)		
SP/T area	0.17 (0.01)	0.17 (0.01)		
SP/T volume	0.09 (0.01)	0.09 (0.01)		
X/T area	0.44 (0.01)	0.43 (0.01)		
X/T volume	0.53 (0.01)	0.51 (0.01)		
PP+SP/T area	0.56 (0.01)	0.57 (0.01)		
PP+SP/T volume	0.47 (0.01)	0.49 (0.01)		

* PP, primary phloem fibre; SP, secondary phloem fibre; X, xylem fibre; T, total fibre (PP+SP+X)

† Means (SE). Within rows, values followed by a different letter are significantly different ($p \leq 0.05$). Determined by ANOVA, Tukey's HSD and Bonferroni's post-hoc analyses, n=40.

5 Discussion

This thesis concentrated on literature related to *Cannabis sativa* var. *fédrina* fibre cultivation, but it is appreciated that the incorporation of fertilizer research on other fibre producing plants could only be beneficial. Comparison with results from different *C. sativa* studies must take into account that its morphological and anatomical characteristics are affected by many different factors, as discussed in the literature review. These include plant variety, sex, age, height, diameter, weight, yield, stem portion, self-thinning rate, and phyllotaxy change and flowering date. Cultivation parameters such as planting date, soil pH and type, moisture levels, daylength, nutrients such as nitrogen, phosphorus and potassium, density, harvest date and retting technique also influence *C. sativa* morphological and anatomical characteristics.

The variation in *C. sativa* sampling and measurement techniques must also be considered. This study analysed TBO stained cross-sections at the midpoint of the third internode from the soil surface to determine fibre composition of the stems. In regard to the number of internodes produced on both greenhouse and field stems, this sampling location was comparable to those of other studies which assessed fibre content at ~30 cm from the base (Correia 1999), at 20-30% of the stem height (Van der Werf *et al.* 1994b), or approximately the third of ten equal stem segments (de Meijer and Van der Werf 1994). However, if the length of internode is considered, the greenhouse stem measurement site would be below 30 cm (from the base) (Correia 1999) or 20-30% of the stem height (Van der Werf *et al.* 1994b).

Previous studies have investigated specific phloem fibre measurements through cross-sectional images and processes of TBO, carmino green of Mirande solution stained cross-

sections and gold-palladium coated samples. However, various traditional wood chemistry and manual dissection techniques have more commonly been used to determine mass fractions of stem components. The present study compared fibre-to-fibre measurements, and not fibre to whole stem cross-section measurements. Until different techniques are specifically compared with each other, their ability to produce comparable fibre proportion results will be assumed.

The greenhouse trial of this project was intended to serve as a more "controlled environment" in which to assess the effects of the fertilizer treatments. *C. sativa* research has traditionally been conducted in field settings. Of the limited greenhouse research available, no studies offered results relevant to this present study. Therefore, compared to previous field studies, stems in this greenhouse study exhibited lower heights and diameters than those of other *C. sativa* var. *fédrina* work (Scheifele 1999, Lisson and Mendham 2000, Cappelletto *et al.* 2001), but similar to those of other varieties at 60 days of growth (Kamat 2000). Internode diameters would be favourable for fibre production (Jordan *et al.* 1946, Scheifele 1999) and the number of internodes was similar to previous research (Mediavilla *et al.* 1998). In the field study, results of each noted characteristic conformed to those of previous studies. It is assumed that with a longer growing period, stems of both trials of this research would have had increased height and diameter; however, the benefit of such an increase for greater primary phloem fibre production is questionable.

Plants in the greenhouse tended to have lower heights, internode lengths, diameters, fresh and dry weights and fresh/dry weight ratios but similar number of internodes compared to field plants; however, none of these characteristics were compared statistically. It would be interesting to investigate further the differences between greenhouse and field grown plants.

In the present study, the total fibre area of a greenhouse *Fédrina* internode was

comprised of 61-66% total phloem fibre, 30-47% primary phloem fibre, 17-31% secondary phloem fibre and 34-39% xylem. In the field, the internode was composed of 54-63% total phloem fibre, 36-52% primary phloem fibre, 12-18% secondary phloem fibre and 37-46% xylem. Therefore, xylem did not generally comprise the largest portion of the stem as previously claimed (Vignon *et al.* 1996, Correia 1999). *Fédrina* studies have found stem mass fractions of 22.6-32.8% total phloem fibre, 18.9% primary phloem fibre, 3.6-45% secondary phloem fibre and 60.0-67.1 % xylem (Van der Werf *et al.* 1994a,b, Meijer 1995, Lisson and Mendham 2000, Cappelletto *et al.* 2001).

With other varieties, cross-section assessments have shown that the stem is comprised of 20% bast, 50% core and 30% pith fractions (Correia 1999) with 19.29% total phloem fibre and 77.65% xylem at 60 days of growth and 20.34% and 77.31%, respectively at 120 days of growth (Kamat 2000). At the time of highest fibre yield there was 65% primary phloem fibre area, with up to 45% secondary phloem fibre area of the cross-section at the time of flower induction (Mediavilla *et al.* 2001). Previous mass fraction assessments on other varieties have found 19.3-68% phloem fibre composed of 8.4-89% primary phloem fibre, 0-45% secondary phloem fibre and 49.6-77.7% xylem (de Meijer 1994, Cromack 1998, Scheifele 1999, Sankari 2000).

This study extrapolated measurements into volumes to offer comparison at another (third) dimension to the area results, and for future extrapolation of results into potential yields that may be attained. For example, the grand mean of all treatments results for the primary phloem fibre volume of the third internode multiplied by the number of internodes in the lower 2/3 of the stem suggests that greenhouse plants would produce 0.25 m^3 (8.93 ft^3, 0.326 yd^3) and field plants, 0.66 m^3 (23.57 ft^3, 0.863 yd^3) of primary phloem fibre on a 404,686 m^2 (100 acre) field; this would double with two crops per year.

Collenchyma was observed on stem cross-sections in this study. There are no examples of the exclusive use of collenchyma in high tensile strength fibre production (McDougall *et al.* 1993), however, the role of collenchyma in the cellulose and lignin contents of *C. sativa* bast and its pulp potential should be considered and investigated.

Cultivation conditions of *C. sativa* var. *fédrina* grown in both the greenhouse and field trials of this present study met *C. sativa* daily temperature requirements (Dempsey 1975, Bocsa and Karus 1998) and this variety's timing of phyllotaxy change in relation to photoperiod change (Lisson *et al.* 2000, Struik *et al.* 2000) and date of harvest (Van Der Werf *et al.* 1996). Greenhouse plants were watered daily, but field plants were not irrigated and as a result of the climatic conditions, total precipitation was low in the field trial (Dempsey 1975, Bocsa and Karus 1998, BCMAF 1999).

In both trials, plants in the two centre blocks were more "robust" than those of outer blocks most likely due to greater exposure to natural light. In the greenhouse, the ventilation equipment may have caused increased shade, airflow and lower temperatures on the block parallel to the outer wall. Plants were grown at a density capable of maintaining its population through to harvest (Struik *et al.* 2000), however, the mortality of three greenhouse sample plants before harvest reinforces that the potential of self-thinning should be considered when growing hemp for fibre.

In this study, one dose of nitrogen fertilizer was applied one week after ~90% germination and a second dose at one month after germination, in a powder or pellet form just below the soil surface. Phosphorus fertilizer was applied two weeks after germination. Although nitrogen fertilization schedule does not significantly affect the percentage of *C. sativa* fibre (Ritz 1972) or stem height (Scheifele 1999), fertilizer application before sowing, with subsequent doses during the period of growth (Haralanov and Babayashev 1976,

Mediavilla *et al.* 1998) could be considered. The shallow application of fertilizer resulted in fishmeal fertilizer soil surface mold; however, with re-integration into the soil, its efficacy was not expected to be negatively affected (Alaskan Fish Meal *pers. comm.* 2000). Pre-sowing fertilizer and, or, liquid fertilizer integration into the soil would be recommended for future cultivation.

5.1 Morphology of Greenhouse-Grown *C. sativa* var. *fédrina*

In the greenhouse trial, neither nitrogen fertilizer type nor level significantly affected *C. sativa* var. *fédrina* morphology. This outcome was unexpected as previous research has documented that nitrogen is the most important nutrient for *C. sativa* growth and stem yield, particularly at levels between 60-240 kg N/ha which affect stem yield, height, diameter, number of nodes and weight (Jordan *et al.* 1946, Hessler 1947, Ruzsanyi 1970, Ivonyi *et al.* 1997, Van der Werf *et al.* 1995a, Van der Werf and Van den Berg 1995, Bocsa and Karus 1998, Scheifele 1999, BCMAF 2000, Struik *et al.* 2000). If stem height is correlated with stem yield (Meijer and Keizer 1994) then the results in the present study suggest there would be no effect of nitrogen fertilizer type or level on stem yield.

The lack of difference between the effects of nitrogen fertilizer type suggests that the use of organic fertilizer produces outcomes comparable to those of inorganic nitrogen fertilizer. Benefits of organic fertilizer use are noted in the literature review of this thesis. The only evidence of previous studies comparing organic and inorganic fertilizers on *C. sativa* found barn manure to be less effective than synthetic fertilizer on stem yield (Ruzsanyi 1970). The lack of difference between the effects of nitrogen level suggests that there is the opportunity to grow *C. sativa* without nitrogen fertilizer, which can result in reduced nitrate leaching (Hendrischke *et al.* 1998) and long-term nitrate pollution (Addiscott and Darby

1991).

When 90 kg P_2O_5/ha was added to the greenhouse soil in combination with, or in the absence of a nitrogen fertilizer, plants possessed significantly greater heights, number of internodes, internode diameters, fresh weights and fresh/dry weight ratios compared to those without phosphorus. Therefore, results of this study complement previous findings that 30-100 kg P_2O_5/ha treatment increases stem yield (Ruzsanyi 1970, Ivonyi *et al.* 1997, BCMAF 2000). However, claims that on low phosphorus soils, the application of phosphorus fertilizer alone results in decreased stem height and weight (Ruzsanyi 1970), and that *C. sativa* is likely able to absorb the small quantity of phosphorus it needs from the soil (Ivonyi *et al.* 1997) were not supported. Unless the presence of phosphorus enabled the plants to maximize the use of the low nitrogen available in the soil, the lack of nitrogen fertilizer effect on stem morphology suggests that the claim that *C. sativa* requires phosphorus to aid in nitrogen-use efficiency (Bocsa and Karus 1998) was also not supported.

The significant interaction effect between nitrogen fertilizer type and phosphorus level on internode fresh/dry weight ratio complements the phosphorus level effect, as all treatments experienced an increased result with the application of phosphorus fertilizer. It can be deduced from the interaction graph that inorganic nitrogen fertilizer treatment experienced the greatest increase, and fishmeal the least. It must be noted that significant differences within interaction effects were not statistically analysed.

5.2 Anatomy of Greenhouse-Grown *C. sativa* var. *fédrina*

Anatomical characteristics of greenhouse-grown *C. sativa* var. *fédrina* were affected by nitrogen fertilizer type but not level. The ratios of primary phloem fibre/total fibre area and volume were significantly higher for control plants compared to those of any type of

nitrogen fertilizer treatment. This implies that secondary tissue development (secondary phloem and xylem) are neither enhanced nor inhibited by nitrogen application. Just as phloem fibres function in plant mechanical support, the aggregation of the less flexible secondary xylem fibres produced during secondary thickening of the stem supply vertical strength (McDougall *et al.* 1993, Bocsa and Karus 1998). Although correlation analyses between anatomical and morphological characteristics were not performed, the absence of a positive nitrogen fertilizer effect on characteristics such as stem height, weight or diameter coincides with the absence of a positive effect on secondary tissue development.

The results of the present study indicate that there are no significant differences between those of the control and/or three levels of nitrogen fertilizer, and that some primary phloem characteristics are even improved by the absence of nitrogen fertilizer, as suggested by the fertilizer type results. This is in contrast to previous findings that treatments with 56 or 112 kg N/ha increase fibre yield (Jordan *et al.* 1946), and that 80 kg N/ha produces higher bast contents than 200 kg N/ha treatment (Van der Werf *et al.* 1995a). Unless excess nitrogen application would occur above the 300 kg N/ha treatment presently analysed, the claim that it produces stems with thin phloem sections of low fibre quantity (Bocsa and Karus 1998) is also unsupported.

The proportion of phloem fibre, and a high primary and low secondary phloem fibre content in the stem, are principal quality parameters for the use of *C. sativa* in textile and paper production (Meijer 1994, Van der Werf *et al.* 1994b, Bocsa and Karus 1998, Keller *et al.* 2001, Mediavilla *et al.* 2001). Therefore, the lack of a significant nitrogen level effect and the higher ratios of primary phloem fibre/total fibre area and volume for control plants compared to those of any type of nitrogen fertilizer treatment, suggest that the absence of a nitrogen fertilizer is advantageous for primary phloem fibre production and subsequently for

textiles and paper manufacturing. In addition to, and as with greenhouse plant morphology characteristics, the absence of a difference in the effects of nitrogen fertilizer type suggest that if fertilizer use occurred, organic fertilizer would produce outcomes comparable to those of inorganic nitrogen fertilizer.

Anatomical characteristics of greenhouse-grown plants were affected by the application of phosphorus. Primary phloem fibre area, secondary phloem fibre area and volume, xylem area and volume, total fibre area and volume, primary phloem fibre cell wall thickness, secondary phloem fibre/total fibre area and volume, xylem/total fibre area and volume ratios were each significantly higher in treatments with 90 kg P_2O_5/ha application. However, the number of primary phloem fibre cells/mm^2, primary phloem fibre/total fibre area and volume ratios, and total phloem fibre/total fibre area and volume ratios were significantly higher in treatments without phosphorus addition. There is no apparent reason for the inconsistent result of primary phloem fibre area, compared to other primary phloem fibre results. Therefore, the absence of phosphorus fertilizer application is advantageous for primary phloem fibre production, and secondary phloem fibre and xylem is enhanced by phosphorus application. This improved secondary tissue development (secondary phloem and xylem) corresponds with the positive effect of phosphorus on relevant morphological characteristics such as stem height, weight and diameter.

As with the stem morphology, the lack of nitrogen fertilizer effect on stem anatomy suggests that the claim that *C. sativa* requires phosphorus to aid in nitrogen-use efficiency (Bocsa and Karus 1998) was not supported and that investigation into the effects of different levels of phosphorus fertilizer on stem morphology would be required to determine if phosphorus requirements are lower when nitrogen fertilizer is present (Ivonyi *et al.* 1997).

The significant interaction effect between nitrogen fertilizer type and phosphorus

level on primary phloem/total fibre area and volume ratios complemented both the nitrogen fertilizer type and phosphorus level effects. Control treatment results were greater than all other treatments, and all treatments experienced a decreased result with the application of phosphorus fertilizer. From the interaction graphs, it can be noted that control and inorganic nitrogen fertilizer treatments experienced greater decreases than fishmeal and sea star with the application of phosphorus fertilizer.

In general, morphological and anatomical characteristics of greenhouse plants were unaffected by nitrogen fertilizer type or level. Phosphorus application benefited plant morphology, secondary phloem fibre and xylem production, but its absence benefited primary phloem fibre content. Results from this greenhouse trial suggest that phosphorus is a more limiting factor than nitrogen on *C. sativa* growth.

5.3 Morphology of Field-Grown *C. sativa* var. *fédrina*

In the field trial, nitrogen fertilizer type did not significantly affect plant morphology, however, nitrogen level did. The lack of a nitrogen fertilizer type effect suggests that the use of organic nitrogen fertilizer produces outcomes comparable to those of inorganic fertilizer for *C. sativa* morphology.

Treatment with 150 kg N/ha resulted in significantly higher plant heights than the other nitrogen levels, and the number of internodes and internode diameters were positively affected by addition of 150 or 300 kg N/ha. Further, fresh weights were significantly higher for the 150 or 300 kg N/ha compared to the control. This confirms that nitrogen fertilization affects stem height (Jordan *et al.* 1946, Van der Werf *et al.* 1995a), diameter (Jordan *et al.* 1946, Van der Werf *et al.* 1995a, Van der Werf and Van den Berg 1995, Scheifele 1999) and number of nodes (Van der Werf and Van den Berg 1995).

Nitrogen levels assessed in this research are not equivalent to previous work, however they are somewhat similar, and can be compared. In contrast to the present results for height, previous studies have found that compared to 200 kg N/ha, treatment with 80 kg N/ha increases stem height (Van der Werf *et al.* 1995a). If stem height is indeed correlated with stem yield (Meijer and Keizer 1994), then the present results may lend support to previous work which found that compared to 0 kg N/ha, stem yield increased with 113 (Ruzsanyi 1970) or 160 kg N/ha treatments (Ivonyi *et al.* 1997), and that stem yield increased between 100 and 160 kg N/ha treatment (Struik *et al.* 2000). Stem yield decrease between 160 and 240 kg N/ha treatments (Ivonyi *et al.* 1997) may be supported, while stem yield increase between 160 and 220 kg N/ha treatments (Struik *et al.* 2000) is not.

The significant interaction effect between nitrogen fertilizer type and level on the number of internodes complemented that of the nitrogen fertilizer level effect, but offered information beyond that of the nitrogen fertilizer type, where there was no effect. From the interaction graph, it can be noted that control treatment results were lower than all other treatments, and that 150 kg bloodmeal N/ha produced the highest, and 75 kg inorganic N/ha the second highest, number of internodes.

Both in conjunction with, and in the absence of nitrogen fertilizer application, plants without phosphorus treatment had significantly greater heights, internode diameters, fresh and dry weights compared to plants treated with 90 kg P_2O_5/ha. Therefore, claims that, on low phosphorus soils, the application of phosphorus fertilizer alone results in decreased stem height and weight (Ruzsanyi 1970), that 30-100 kg P_2O_5/ha treatment increases stem yield (Ruzsanyi 1970, Ivonyi *et al.* 1997, BCMAF 2000) or that *C. sativa* requires phosphorus to aid in nitrogen-use efficiency (Bocsa and Karus 1998) were not supported. However, the suggestion that *C. sativa* plants are able to absorb the small quantity of phosphorus needed

from the soil (Ivonyi *et al.* 1997) could be warranted.

5.4 Anatomy of Field-Grown *C. sativa* var. *fédrina*

Nitrogen fertilizer type significantly affected three anatomical characteristics of field plants. The area of primary phloem fibre was higher with fishmeal or inorganic nitrogen fertilizer treatment and the volume of primary phloem fibre was highest in the control, however, the effects were not differentiated by post-hoc analysis. Effects on total fibre volume were differentiated through post-hoc tests, which noted control treatment total volume was lower than that of either fishmeal or inorganic treatment. Similar treatment effects on internode length or fibre ratios did not exist and therefore cannot explain the opposing treatment effects on primary phloem area and volume. Until otherwise determined, it will be assumed that nitrogen fertilizer type presented only a minimal, and inconsequential, effect on primary phloem production.

Nitrogen fertilizer level affected anatomical characteristics of field-grown plants. Xylem area and volume, total fibre volume and xylem/total fibre area and volume ratios were significantly higher with 150 and/or 300 kg N/ha treatment compared to the control. Primary phloem fibre/total fibre area and volume ratios and total phloem fibre/total fibre area and volume ratios were significantly higher for the control treatment plants. Therefore, primary phloem fibre development is improved in the absence of, and xylem and total fibre development are improved in the presence of, higher levels of nitrogen fertilizer. Although correlation analyses between anatomical and morphological characteristics were not performed, the increased height, diameter and number of internodes with the treatment of 150 or 300 kg N/ha coincides with the secondary tissue and total fibre development which would be required to mechanically support such increases in morphology. From these results, it can

also be assumed that increased morphological growth does not equate to increased yield of the more valuable primary phloem fibre component of the stem.

If total fibre yield is considered, the present results support previous work, which found that nitrogen treatment increases fibre yield, although present levels of 150 or 300 kg N/ha were higher than the 56 or 112 kg N/ha previously noted (Jordan *et al.* 1946). However, work which found that 80 kg N/ha produces higher bast contents than 200 kg N/ha treatment (Van der Werf *et al.* 1995a) was not supported by the present study, which found greater primary and total phloem ratios with 0 kg N/ha treatment. If excess nitrogen is considered to be that above 0 kg N/ha, then present counts of primary phloem fibre cell numbers did not support the claim that excess nitrogen application produces stems with low bast fibre quantity. However, the potential thinner phloem sections (Bocsa and Karus 1998) were supported by the greater primary and total phloem ratios with 0 kg N/ha treatment.

The significant interaction effect between nitrogen fertilizer type and level on secondary phloem area and volume offered information beyond that of the nitrogen fertilizer type or nitrogen fertilizer level, which found no effect. From the interaction graph, it can be noted that control treatment results were lower than all other treatments, and that 150 kg fishmeal N/ha produced the highest results and 75 kg inorganic N/ha the second highest.

Anatomical characteristics in the field were affected by phosphorus level. Treatments with the absence of phosphorus fertilizer produced greater primary phloem fibre areas, xylem areas and volumes and total fibre areas and volumes. The number of primary phloem fibres/mm^2 was higher in treatments with the presence of 90 kg P_2O_5/ha. These data suggest that phosphorus has a minimal effect on primary phloem fibre. The positive effect of the absence of phosphorus fertilizer on xylem and total fibre measurements corresponds with the positive effect on relevant morphological characteristics such as height and diameter.

Therefore, claims that *C. sativa* requires phosphorus to aid in nitrogen-use efficiency (Bocsa and Karus 1998) were not supported, but it may be true that *C. sativa* plants are able to absorb the small quantity of phosphorus needed from the soil (Ivonyi *et al.* 1997).

To generalize, field morphological characteristics were unaffected by nitrogen fertilizer type while any level, 150 and/or 300 kg N/ha in particular, exhibited positive results. Fishmeal or inorganic nitrogen fertilizer and 150 and/or 300 kg N/ha levels benefited some anatomical characteristics, however, primary phloem fibre results were greater in the absence of nitrogen fertilizer. The absence of phosphorus fertilizer was beneficial for field morphological and many anatomical characteristics. In contrast to the greenhouse trial results, those from this field trial suggest that phosphorus is not a more limiting factor than nitrogen on *C. sativa* growth.

5.5 Physiological Considerations

There has been limited research on the effects of plant growth regulators (PGRs) on *C. sativa* fibre production (see literature review). However, documented studies on the effect of PGRs on vascular development in other plants are available. It appears that auxin to gibberellin ratio, and cytokinin level, are important for fibre differentiation and composition within the stem (Atal 1961, Saks *et al.* 1984, Aloni 1979, 1995). Results from the present research suggest that the absence of fertilizer application is beneficial for primary phloem fibre production. It is possible that the stem density used in this study to limit lateral growth, and height variability, resulted in conditions which produced optimal PGR levels for desired primary phloem fibre composition. Investigation into potential relationships between PGR levels within the plant and nutrients such as nitrogen and phosphorus, are required to help understand the underlying physiological mechanisms of fibre production in *C. sativa.*

5.6 Conclusions

1. Greenhouse-grown *C. sativa* var. *fédrina* morphology, secondary phloem fibre and xylem were positively affected by 90 kg P_2O_5/ha treatment. Phosphorus was a more limiting factor than nitrogen on greenhouse-grown *C. sativa* var. *fédrina.*
2. Field-grown *C. sativa* var. *fédrina* morphology, secondary phloem fibre and xylem were positively affected by 150 and/or 300 kg N/ha treatment of any nitrogen fertilizer type. Nitrogen was a more limiting factor than phosphorus on field-grown *C. sativa* var. *fédrina.*
3. Greenhouse and field-grown *C. sativa* var. *fédrina* primary phloem fibre was positively affected by the absence of nitrogen or phosphorus fertilizer application. This supports the claim that *C. sativa* variety and plant density are the principal parameters to consider for *C. sativa* bast fibre cultivation (Van der Werf *et al.* 1996).
4. Unless correlation analysis proves otherwise, improvements in morphological characteristics should not be used to infer improved primary phloem fibre yields.

6 Future Research and Recommendations

This study has more data available for analysis. Within treatment variability on morphology and anatomy characteristics would be beneficial as they may affect harvesting and processing techniques. A high yield treatment with high variability may be less appealing than one of low yield and low variability. More thorough analysis of interaction effects, and correlation analysis within morphological and anatomical, and between morphological and anatomical characteristics, would also be useful. The ability to use morphological characteristics to reliably predict primary phloem fibre content would be an asset for fibre cultivation.

Additional anatomical measurements could also be performed on internode cross-sectional images. These may include analysis of collenchyma tissue, whole fibre bundle measurements, increased cells counts and cell diameters of both primary and secondary phloem, xylem cell and non-fibre tissue measurements. Variation of primary phloem fibre cell shape and lumen size, secondary xylem shape and wall thickness could also be assessed and used for research on the impact of such characteristics on fibre quality.

In this study, both greenhouse and field soil phosphorus levels were low and considered negligible, yet greenhouse phosphorus treatment results were in contrast to field results. This strongly suggests that further investigation into phosphorus effects on, requirements of, and potential soil microorganism associations with, *C. sativa* are warranted. Similar research on potassium is also recommended. Findings of the present research suggest that organic fertilizer is as effective as inorganic; therefore, research into potential organic sources to meet all *C. sativa* nutrient requirements is also recommended.

Measurements not conducted in this study, but are highly recommended for future work as they may offer additional insight into possible reasons for particular results include those of plant growth regulators, soil temperature, plant sex, root characteristics and mycorrhizal status, and self-thinning rates.

A study should be conducted with traditional wood chemistry, manual dissection mass fraction techniques and cross-section techniques to see if results from the different methodologies can be compared. Such studies may give alternate options for future *C. sativa* fibre research techniques.

The literature review and study comparison of this thesis focused on *C. sativa* fibre cultivation. As information directly relevant to this present work is very limited, and the present results appear unique, investigation into fertilizer research on other fibre producing

plants is strongly recommended.

The high number of variables that influence *C. sativa* makes it successful as a highly adaptable plant that can thrive in a multitude of conditions. The more one understands how this species can grow and develop under different regimes, the greater the potential for its serious use as a fibre-producing crop. Further, given that it exhibits a large variety of cell types in addition to different morphological and reproductive characteristics, it has great appeal for general botanical studies and as a plant example for laboratory instruction.

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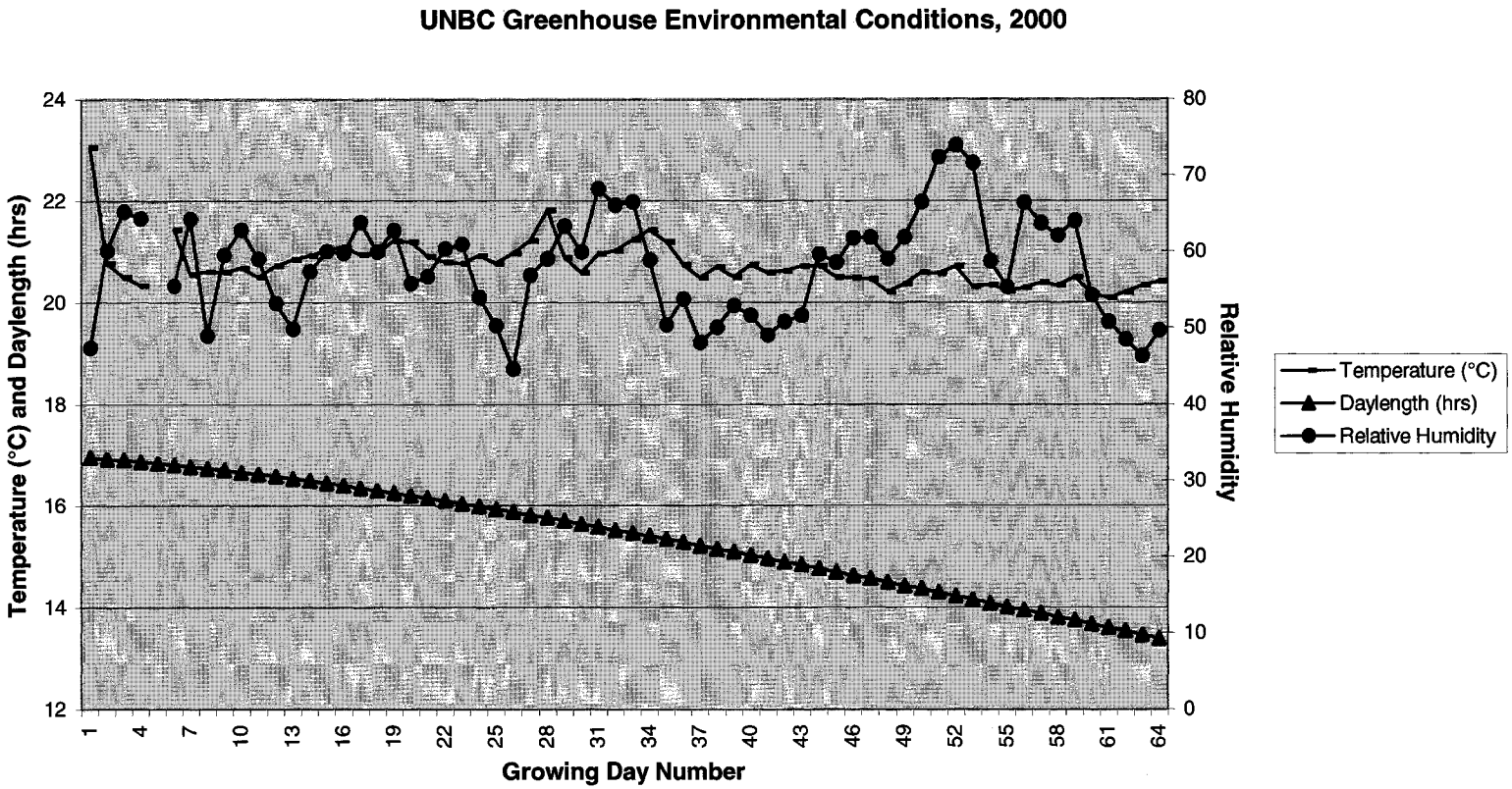
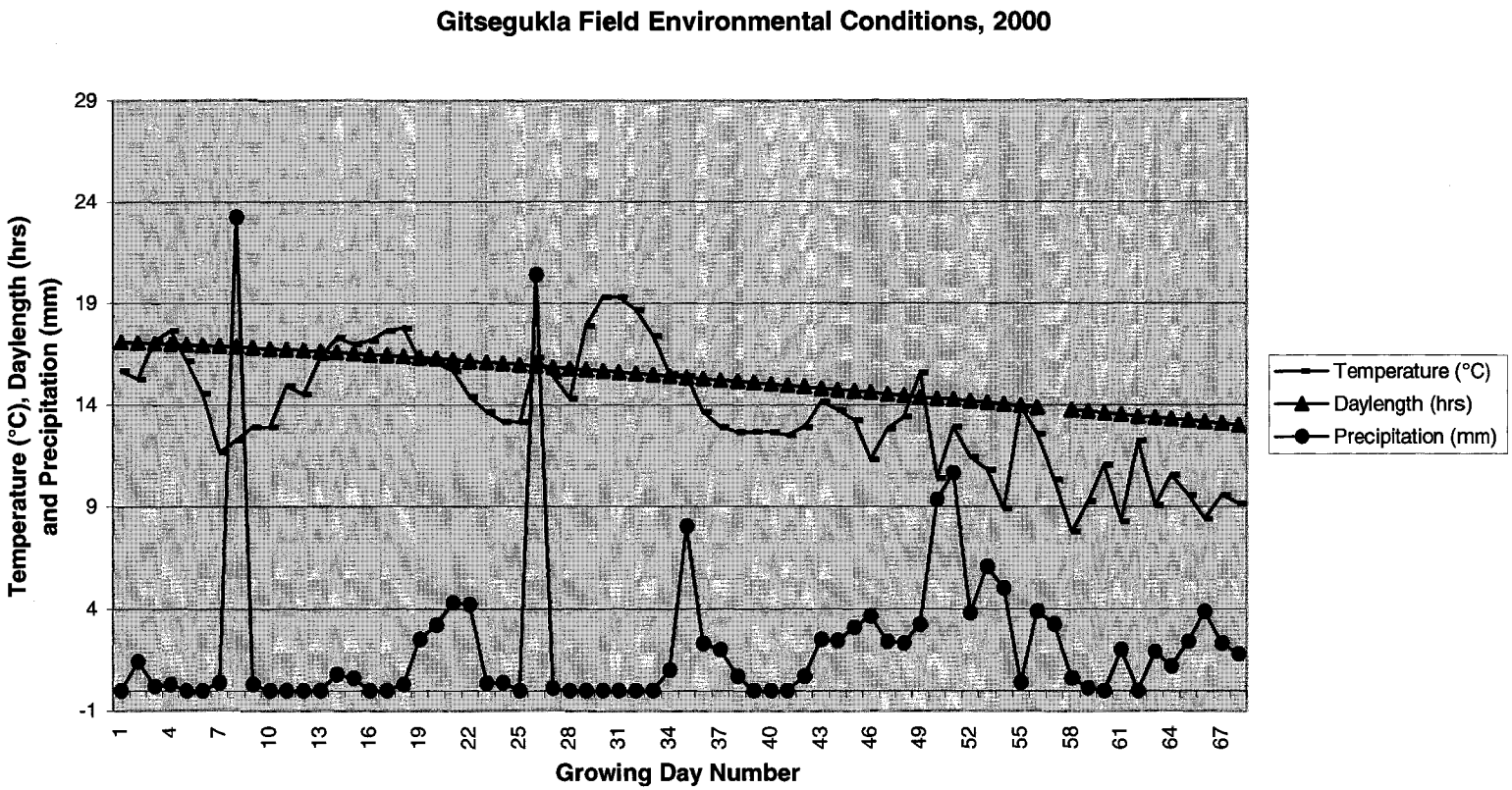


Appendix A. Temperature (°C), daylength (hrs) and relative humidity of the UNBC greenhouse during the 64 days of *Cannabis sativa* var. *fédrina* cultivation, July 4 – September 5, 2000.



Appendix B. Temperature (°C), daylength (hrs) and precipitation (mm) of the Gitsegukla field during the 68 days of *Cannabis sativa* var. *fédrina* cultivation, July 6 – September 11, 2000.

Appendix C

Randomized block ANOVA on the effects of fertilizer treatment on stem morphology of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable	Source*	df	SS	*F*	*p*
Height (cm)	B	3	17313.675	18.54	0.000
	N	2	134.580	0.22	0.806
	[N]	2	659.485	1.06	0.353
	P	1	5858.684	18.82	0.000
	N[N]	4	1367.532	1.10	0.366
	NP	2	549.166	0.88	0.419
	[N]P	2	372.844	0.60	0.553
	N[N]P	4	711.637	0.57	0.684
	Error	57	17740.355		
Number of internodes	B	3	19.371	7.50	0.000
	N	2	1.171	0.68	0.510
	[N]	2	2.544	1.48	0.237
	P	1	24.976	29.03	0.000
	N[N]	4	2.115	0.61	0.654
	NP	2	0.741	0.43	0.652
	[N]P	2	0.100	0.06	0.944
	N[N]P	4	5.523	1.60	0.186
	Error	57	49.043		

* B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer.

Randomized block ANOVA on the effects of fertilizer treatment on third internode length and diameter of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable	Source*	df	SS	*F*	*p*
Length (cm)	B	3	229.405	42.17	0.000
	N	2	2.073	0.57	0.568
	[N]	2	2.744	0.76	0.474
	P	1	2.826	1.56	0.217
	N[N]	4	9.835	1.36	0.261
	NP	2	0.788	0.22	0.805
	[N]P	2	2.178	0.60	0.552
	N[N]P	4	13.897	1.92	0.120
	Error	57	103.348		
Diameter (mm)	B	3	16.713	16.34	0.000
	N	2	0.466	0.68	0.509
	[N]	2	0.439	0.64	0.529
	P	1	15.797	46.32	0.000
	N[N]	4	2.179	1.60	0.188
	NP	2	0.231	0.34	0.715
	[N]P	2	0.046	0.07	0.935
	N[N]P	4	1.186	0.87	0.488
	Error	57	19.438		

* B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer.

Randomized block ANOVA on the effects of fertilizer treatment on third internode weights of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable	Source*	df	SS	*F*	*p*
Fresh weight (g)	B	3	3.724	27.79	0.000
	N	2	0.019	0.21	0.812
	[N]	2	0.065	0.73	0.487
	P	1	0.746	16.70	0.000
	N[N]	4	0.208	1.16	0.337
	NP	2	0.138	1.54	0.223
	[N]P	2	0.016	0.18	0.839
	N[N]P	4	0.203	1.14	0.349
	Error	57	2.546		
Dry weight (g)	B	3	0.033	2.73	0.052
	N	2	0.008	1.02	0.368
	[N]	2	0.002	0.30	0.741
	P	1	0.008	2.02	0.161
	N[N]	4	0.016	1.00	0.413
	NP	2	0.010	1.26	0.291
	[N]P	2	0.000	0.06	0.940
	N[N]P	4	0.029	1.80	0.141
	Error	57	0.229		
Fresh/dry weight	B	3	114.172	5.58	0.002
	N	2	6.237	0.46	0.636
	[N]	2	1.514	0.11	0.895
	P	1	134.620	19.72	0.000
	N[N]	4	51.162	1.87	0.128
	NP	2	59.655	4.37	0.017
	[N]P	2	0.049	0.00	0.996
	N[N]P	4	22.935	0.84	0.506
	Error	57	389.085		

* B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer.

Randomized block ANOVA on the effects of fertilizer treatment on third internode primary phloem fibre area and volume of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
PP area (mm^2)	B	3	2.190	23.01	0.000
	N	2	0.008	0.13	0.882
	[N]	2	0.040	0.62	0.539
	P	1	1.107	34.88	0.000
	N[N]	4	0.125	0.98	0.424
	NP	2	0.008	0.13	0.883
	[N]P	2	0.002	0.03	0.970
	N[N]P	4	0.114	0.89	0.473
	Error	57	1.808		
PP volume (mm^3)	B	3	23.131	49.16	0.000
	N	2	0.251	0.80	0.455
	[N]	2	0.021	0.07	0.937
	P	1	0.133	0.85	0.361
	N[N]	4	0.259	0.41	0.798
	NP	2	0.019	0.06	0.942
	[N]P	2	0.148	0.47	0.627
	N[N]P	4	1.279	2.04	0.101
	Error	57	8.939		

* PP, primary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode secondary phloem fibre area and volume of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
SP area (mm^2)	B	3	0.131	0.67	0.571
	N	2	0.230	1.77	0.180
	[N]	2	0.056	0.43	0.651
	P	1	5.894	90.80	0.000
	N[N]	4	0.344	1.32	0.272
	NP	2	0.210	1.62	0.207
	[N]P	2	0.012	0.10	0.909
	N[N]P	4	0.534	2.06	0.098
	Error	57	3.700		
SP volume (mm^3)	B	3	1.597	1.54	0.213
	N	2	0.803	1.16	0.320
	[N]	2	0.210	0.30	0.739
	P	1	16.478	47.74	0.000
	N[N]	4	1.263	0.91	0.462
	NP	2	1.155	1.67	0.197
	[N]P	2	0.116	0.17	0.845
	N[N]P	4	2.230	1.62	0.183
	Error	57	19.673		

* SP, secondary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode xylem area and volume of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
X area (mm^2)	B	3	0.673	2.12	0.108
	N	2	0.308	1.45	0.242
	[N]	2	0.232	1.10	0.341
	P	1	6.058	57.18	0.000
	N[N]	4	0.541	1.28	0.290
	NP	2	0.156	0.73	0.484
	[N]P	2	0.056	0.26	0.769
	N[N]P	4	0.332	0.78	0.541
	Error	57	6.039		
X volume (mm^3)	B	3	6.652	5.04	0.004
	N	2	0.935	1.06	0.352
	[N]	2	1.238	1.41	0.253
	P	1	21.873	49.72	0.000
	N[N]	4	2.659	1.51	0.211
	NP	2	2.016	2.29	0.110
	[N]P	2	0.563	0.64	0.531
	N[N]P	4	0.906	0.51	0.725
	Error	57	25.075		

* X, xylem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode total fibre area and volume of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
T area (mm^2)	B	3	6.439	4.50	0.007
	N	2	1.188	1.25	0.295
	[N]	2	0.813	0.85	0.432
	P	1	35.293	74.02	0.000
	N[N]	4	2.404	1.26	0.296
	NP	2	0.790	0.83	0.442
	[N]P	2	0.103	0.11	0.897
	N[N]P	4	1.923	1.01	0.411
	Error	57	27.178		
T volume (mm^3)	B	3	73.347	14.88	0.000
	N	2	1.389	0.42	0.657
	[N]	2	2.707	0.82	0.444
	P	1	82.827	50.39	0.000
	N[N]	4	5.546	0.84	0.503
	NP	2	5.412	1.65	0.202
	[N]P	2	1.147	0.35	0.707
	N[N]P	4	5.439	0.83	0.513
	Error	57	93.686		

* T, total fibre (PP+SP+X)

† B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode primary phloem fibre cell count and wall thickness of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
# PP/mm^2	B	3	10245103.728	9.58	0.000
	N	2	396832.233	0.56	0.576
	[N]	2	602100.979	0.84	0.435
	P	1	17965245.269	50.39	0.000
	N[N]	4	2141088.089	1.50	0.214
	NP	2	307707.952	0.43	0.652
	[N]P	2	533986.096	0.75	0.478
	N[N]P	4	2458545.817	1.72	0.157
	Error	57	20322961.821		
PP cell wall thickness (mm)	B	3	0.0000265	19.71	0.000
	N	2	0.0000005	0.55	0.578
	[N]	2	0.0000002	0.20	0.823
	P	1	0.0000072	16.14	0.000
	N[N]	4	0.0000012	0.66	0.620
	NP	2	0.0000010	1.08	0.345
	[N]P	2	0.0000000	0.03	0.971
	N[N]P	4	0.0000019	1.04	0.392
	Error	57	0.0000255		

* PP, primary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode primary phloem fibre/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
PP/T area	B	3	0.018	1.44	0.241
	N	2	0.040	4.87	0.011
	[N]	2	0.006	0.75	0.475
	P	1	0.575	139.03	0.000
	N[N]	4	0.005	0.33	0.858
	NP	2	0.040	4.86	0.011
	[N]P	2	0.005	0.61	0.548
	N[N]P	4	0.027	1.65	0.175
	Error	57	0.236		
PP/T volume	B	3	0.086	2.15	0.104
	N	2	0.125	4.70	0.013
	[N]	2	0.020	0.75	0.478
	P	1	1.669	125.67	0.000
	N[N]	4	0.006	0.11	0.980
	NP	2	0.129	4.86	0.011
	[N]P	2	0.014	0.53	0.593
	N[N]P	4	0.086	1.61	0.183
	Error	57	0.757		

* PP, primary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode secondary phloem fibre/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
SP/T area	B	3	0.010	0.98	0.407
	N	2	0.019	2.80	0.069
	[N]	2	0.001	0.13	0.879
	P	1	0.371	110.15	0.000
	N[N]	4	0.014	1.02	0.406
	NP	2	0.020	3.00	0.058
	[N]P	2	0.006	0.91	0.409
	N[N]P	4	0.026	1.90	0.122
	Error	57	0.192		
SP/T volume	B	3	0.024	0.97	0.413
	N	2	0.033	1.95	0.152
	[N]	2	0.003	0.18	0.835
	P	1	0.657	78.24	0.000
	N[N]	4	0.019	0.57	0.687
	NP	2	0.029	1.74	0.185
	[N]P	2	0.008	0.50	0.609
	N[N]P	4	0.060	1.78	0.145
	Error	57	0.478		

* SP, secondary phloem fibre;

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode xylem/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
X/T area	B	3	0.014	1.85	0.148
	N	2	0.007	1.43	0.247
	[N]	2	0.004	0.72	0.489
	P	1	0.022	8.93	0.004
	N[N]	4	0.008	0.80	0.530
	NP	2	0.005	1.08	0.347
	[N]P	2	0.000	0.03	0.975
	N[N]P	4	0.005	0.48	0.750
	Error	57	0.142		
X/T volume	B	3	0.050	1.55	0.212
	N	2	0.043	2.03	0.141
	[N]	2	0.019	0.91	0.408
	P	1	0.232	21.77	0.000
	N[N]	4	0.033	0.77	0.552
	NP	2	0.042	1.95	0.151
	[N]P	2	0.001	0.04	0.961
	N[N]P	4	0.016	0.38	0.821
	Error	57	0.608		

* X, xylem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode total phloem fibre/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the UNBC greenhouse.

Dependent Variable*	Source†	df	SS	*F*	*p*
PP+SP/T area	B	3	0.014	1.85	0.148
	N	2	0.007	1.43	0.247
	[N]	2	0.004	0.72	0.489
	P	1	0.022	8.93	0.004
	N[N]	4	0.008	0.80	0.530
	NP	2	0.005	1.08	0.347
	[N]P	2	0.000	0.03	0.975
	N[N]P	4	0.005	0.48	0.750
	Error	57	0.142		
PP+SP/T volume	B	3	0.050	1.55	0.212
	N	2	0.043	2.03	0.141
	[N]	2	0.019	0.91	0.408
	P	1	0.232	21.77	0.000
	N[N]	4	0.033	0.77	0.552
	NP	2	0.042	1.95	0.151
	[N]P	2	0.001	0.04	0.961
	N[N]P	4	0.016	0.38	0.821
	Error	57	0.608		

* T, total fibre (PP+SP+X)

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Appendix D

Randomized block ANOVA on the effects of fertilizer treatment on stem morphology of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable	Source*	df	SS	*F*	*p*
Height (cm)	B	3	34026.403	46.12	0.000
	N	2	1077.711	2.19	0.121
	[N]	2	5649.188	11.49	0.000
	P	1	2167.502	8.81	0.004
	N[N]	4	1557.684	1.58	0.191
	NP	2	1005.270	2.04	0.139
	[N]P	2	347.015	0.71	0.498
	N[N]P	4	1147.674	1.17	0.335
	Error	57	14106.640		
Number of internodes	B	3	26.861	32.39	0.000
	N	2	0.362	0.65	0.523
	[N]	2	2.968	5.37	0.007
	P	1	0.416	1.51	0.225
	N[N]	4	5.281	4.78	0.002
	NP	2	0.200	0.36	0.698
	[N]P	2	0.641	1.16	0.321
	N[N]P	4	0.276	0.25	0.909
	Error	57	15.755		

* B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer.

Randomized block ANOVA on the effects of fertilizer treatment on third internode length and diameter of *Cannabis sativa* var. *fédrina a* grown in the Gitsegukla field.

Dependent Variable	Source*	df	SS	*F*	*p*
Length (cm)	B	3	138.562	10.43	0.000
	N	2	16.064	1.81	0.172
	[N]	2	8.236	0.93	0.400
	P	1	5.650	1.28	0.263
	N[N]	4	14.979	0.85	0.502
	NP	2	11.139	1.26	0.292
	[N]P	2	10.152	1.15	0.325
	N[N]P	4	11.488	0.65	0.630
	Error	57	252.308		
Diameter (mm)	B	3	24.409	20.71	0.000
	N	2	1.957	2.49	0.092
	[N]	2	2.839	3.61	0.033
	P	1	2.364	6.02	0.017
	N[N]	4	2.533	1.61	0.184
	NP	2	0.867	1.10	0.338
	[N]P	2	0.053	0.07	0.935
	N[N]P	4	1.299	0.83	0.513
	Error	57	22.388		

* B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer.

Randomized block ANOVA on the effects of fertilizer treatment on third internode weights of *Cannabis sativa* var. *fédrina a* grown in the Gitsegukla field.

Dependent Variable	Source*	df	SS	*F*	*p*
Fresh weight (g)	B	3	22.510	16.87	0.000
	N	2	1.851	2.08	0.134
	[N]	2	4.747	5.34	0.008
	P	1	3.520	7.91	0.007
	N[N]	4	1.481	0.83	0.510
	NP	2	1.129	1.27	0.289
	[N]P	2	0.127	0.14	0.868
	N[N]P	4	1.119	0.63	0.644
	Error	57	25.353		
Dry weight (g)	B	3	0.404	10.44	0.000
	N	2	0.059	2.31	0.109
	[N]	2	0.032	1.25	0.293
	P	1	0.107	8.33	0.005
	N[N]	4	0.011	0.22	0.925
	NP	2	0.018	0.71	0.494
	[N]P	2	0.015	0.58	0.565
	N[N]P	4	0.056	1.09	0.370
	Error	57	0.735		
Fresh/dry weight	B	3	82.319	1.94	0.134
	N	2	28.798	1.02	0.369
	[N]	2	41.147	1.45	0.243
	P	1	34.195	2.41	0.126
	N[N]	4	36.620	0.65	0.632
	NP	2	61.441	2.17	0.124
	[N]P	2	33.188	1.17	0.318
	N[N]P	4	19.822	0.35	0.843
	Error	57	808.151		

* B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer.

Randomized block ANOVA on the effects of fertilizer treatment on third internode primary phloem fibre area and volume of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
PP area (mm^2)	B	3	4.059	28.02	0.000
	N	2	0.352	3.64	0.033
	[N]	2	0.282	2.92	0.062
	P	1	0.330	6.84	0.011
	N[N]	4	0.353	1.83	0.136
	NP	2	0.060	0.62	0.543
	[N]P	2	0.080	0.82	0.444
	N[N]P	4	0.099	0.51	0.725
	Error	57	2.753		
PP volume (mm^3)	B	3	31.288	22.44	0.000
	N	2	3.493	3.76	0.029
	[N]	2	1.925	2.07	0.135
	P	1	1.615	3.47	0.068
	N[N]	4	0.461	0.25	0.910
	NP	2	1.441	1.55	0.221
	[N]P	2	2.345	2.52	0.089
	N[N]P	4	2.235	1.20	0.320
	Error	57	26.494		

* PP, primary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode secondary phloem fibre area and volume of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
SP area (mm^2)	B	3	0.993	5.62	0.002
	N	2	0.109	0.93	0.402
	[N]	2	0.163	1.38	0.259
	P	1	0.138	2.35	0.131
	N[N]	4	0.674	2.86	0.031
	NP	2	0.133	1.13	0.329
	[N]P	2	0.073	0.62	0.542
	N[N]P	4	0.067	0.28	0.888
	Error	57	3.357		
SP volume (mm^3)	B	3	1.880	4.58	0.006
	N	2	0.292	1.07	0.351
	[N]	2	0.286	1.05	0.358
	P	1	0.270	1.97	0.166
	N[N]	4	1.622	2.96	0.027
	NP	2	0.143	0.52	0.595
	[N]P	2	0.112	0.41	0.666
	N[N]P	4	0.294	0.54	0.709
	Error	57	7.805		

* SP, secondary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode xylem area and volume of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
X area (mm^2)	B	3	5.976	10.88	0.000
	N	2	0.571	1.56	0.219
	[N]	2	1.222	3.34	0.043
	P	1	1.055	5.76	0.020
	N[N]	4	1.571	2.15	0.087
	NP	2	0.488	1.33	0.272
	[N]P	2	0.080	0.22	0.805
	N[N]P	4	0.329	0.45	0.772
	Error	57	10.432		
X volume (mm^3)	B	3	74.398	12.63	0.000
	N	2	6.474	1.65	0.201
	[N]	2	18.460	4.70	0.013
	P	1	12.445	6.34	0.015
	N[N]	4	12.343	1.57	0.194
	NP	2	5.121	1.30	0.279
	[N]P	2	0.106	0.03	0.973
	N[N]P	4	7.140	0.91	0.465
	Error	57	111.958		

* X, xylem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode total fibre area and volume of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
T area (mm^2)	B	3	29.5421	13.96	0.000
	N	2	2.79908	1.98	0.147
	[N]	2	3.90051	2.76	0.071
	P	1	3.89601	5.52	0.022
	N[N]	4	6.97644	2.47	0.055
	NP	2	1.44663	1.03	0.365
	[N]P	2	0.52834	0.37	0.689
	N[N]P	4	1.2448	0.44	0.778
	Error	57	40.2082		
T volume (mm^3)	B	3	240.307	19.98	0.000
	N	2	24.019	3.00	0.058
	[N]	2	34.006	4.24	0.019
	P	1	28.283	7.05	0.010
	N[N]	4	26.173	1.63	0.179
	NP	2	8.954	1.12	0.334
	[N]P	2	2.272	0.28	0.754
	N[N]P	4	20.800	1.30	0.282
	Error	57	228.517		

* T, total fibre (PP+SP+X)

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode primary phloem fibre cell count and wall thickness of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
# PP/mm^2	B	3	5718589.417	22.16	0.000
	N	2	261274.089	1.52	0.228
	[N]	2	217761.028	1.27	0.290
	P	1	451337.577	5.25	0.026
	N[N]	4	788397.421	2.29	0.071
	NP	2	97680.498	0.57	0.570
	[N]P	2	69591.534	0.40	0.669
	N[N]P	4	338101.938	0.98	0.424
	Error	57	4902944.923		
PP cell wall thickness (mm)	B	3	0.0000236	11.95	0.000
	N	2	0.0000024	1.81	0.172
	[N]	2	0.0000002	0.18	0.837
	P	1	0.0000007	1.07	0.305
	N[N]	4	0.0000036	1.37	0.255
	NP	2	0.0000016	1.20	0.308
	[N]P	2	0.0000009	0.70	0.499
	N[N]P	4	0.0000014	0.55	0.702
	Error	57	0.0000375		

* PP, primary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode primary phloem fibre/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
PP/T area	B	3	0.012	1.11	0.352
	N	2	0.000	0.06	0.940
	[N]	2	0.024	3.36	0.042
	P	1	0.003	0.95	0.335
	N[N]	4	0.014	1.02	0.403
	NP	2	0.008	1.10	0.339
	[N]P	2	0.005	0.68	0.513
	N[N]P	4	0.004	0.26	0.902
	Error	57	0.199		
PP/T volume	B	3	0.023	0.85	0.473
	N	2	0.000	0.01	0.993
	[N]	2	0.082	4.52	0.015
	P	1	0.012	1.27	0.264
	N[N]	4	0.037	1.03	0.399
	NP	2	0.019	1.08	0.348
	[N]P	2	0.024	1.31	0.279
	N[N]P	4	0.006	0.18	0.949
	Error	57	0.515		

* PP, primary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode secondary phloem fibre/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
SP/T area	B	3	0.003	0.56	0.642
	N	2	0.000	0.08	0.921
	[N]	2	0.001	0.42	0.656
	P	1	0.000	0.10	0.750
	N[N]	4	0.012	1.66	0.172
	NP	2	0.002	0.54	0.584
	[N]P	2	0.003	0.73	0.484
	N[N]P	4	0.003	0.42	0.790
	Error	57	0.100		
SP/T volume	B	3	0.001	0.27	0.848
	N	2	0.000	0.15	0.864
	[N]	2	0.000	0.09	0.916
	P	1	0.000	0.07	0.796
	N[N]	4	0.010	1.82	0.138
	NP	2	0.001	0.26	0.768
	[N]P	2	0.002	0.80	0.455
	N[N]P	4	0.005	1.00	0.417
	Error	57	0.076		

* SP, secondary phloem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode xylem/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable*	Source†	df	SS	*F*	*p*
X/T area	B	3	0.004	1.43	0.243
	N	2	0.001	0.37	0.689
	[N]	2	0.014	7.01	0.002
	P	1	0.002	1.90	0.174
	N[N]	4	0.004	0.99	0.423
	NP	2	0.002	1.16	0.319
	[N]P	2	0.003	1.56	0.219
	N[N]P	4	0.000	0.09	0.984
	Error	57	0.058		
X/T volume	B	3	0.025	1.53	0.218
	N	2	0.000	0.04	0.965
	[N]	2	0.073	6.78	0.002
	P	1	0.010	1.77	0.188
	N[N]	4	0.025	1.15	0.343
	NP	2	0.015	1.35	0.267
	[N]P	2	0.021	1.94	0.153
	N[N]P	4	0.001	0.03	0.998
	Error	57	0.307		

* X, xylem fibre

† B, block; N, nitrogen fertilizer type; [N], nitrogen level;
P, phosphorus fertilizer

Randomized block ANOVA on the effects of fertilizer treatment on third internode total phloem fibre/total fibre area and volume ratio of *Cannabis sativa* var. *fédrina* grown in the Gitsegukla field.

Dependent Variable	Source†	df	SS	*F*	*p*
PP+SP/T area	B	3	0.004	1.43	0.243
	N	2	0.001	0.37	0.689
	[N]	2	0.014	7.01	0.002
	P	1	0.002	1.90	0.174
	N[N]	4	0.004	0.99	0.423
	NP	2	0.002	1.16	0.319
	[N]P	2	0.003	1.56	0.219
	N[N]P	4	0.000	0.09	0.984
	Error	57	0.058		
PP+SP/T volume	B	3	0.025	1.53	0.218
	N	2	0.000	0.04	0.965
	[N]	2	0.073	6.78	0.002
	P	1	0.010	1.77	0.188
	N[N]	4	0.025	1.15	0.343
	NP	2	0.015	1.35	0.267
	[N]P	2	0.021	1.94	0.153
	N[N]P	4	0.001	0.03	0.998
	Error	57	0.307		

* T, total fibre (PP+SP+X)

† B, block; N, nitrogen fertilizer type; [N], nitrogen level; P, phosphorus fertilizer