Hemp for textiles: plant size matters

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Abstract

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Fibre hemp (*Cannabis sativa* L.) may be an alternative to cotton and synthetic fibres as a raw material for textile yarn production in the European Union. The agronomic options to manipulate plant development and crop growth with the aim to optimise hemp long fibre production were investigated. Field trials with factors sowing density, sowing date, harvest time and variety were conducted. Stems were traditionally processed by retting, drying, breaking, and scutching. Following standard protocols, almost 1500 hemp stem samples were analysed. Varieties differ widely in their fibre content, but this thesis shows that when variety and plant size are known, the amounts of fibres, wood, and retting losses are known. The dry weight of the stems at harvest, not the factors underlying this weight, are determinant. In retted stems the dry matter is split–up into fibres and wood in a fixed way. The options to manipulate this ratio by crop management, given variety, are very small and for practical reasons they can be neglected. In fibre hemp two bast fibre types occur. Primary or long fibres are valuable for yarn spinning. Secondary fibres are too short and their presence hampers the production of fine yarns. This thesis shows that the secondary fibre front height increases with plant weight. Although a causal relationship between secondary fibre formation and flowering does not exist, the secondary fibre front is found higher in flowering plants when compared to non–flowering plants of the same height. This is likely to be caused by the higher weight or momentum of flowering plants as compared with non–flowering plants of the same height. Consequently, a harvest before flowering is preferable. This was shown in a greenhouse experiment, in which the short–day response of hemp was used to create size ranges of flowering and non–flowering plants. To produce high–quality raw materials for textile production, short crops should be grown. The options to produce plants with the desired size are manifold. Since sowing density, sowing date, and harvest time do not have an additional effect on the primary fibre content besides the indirect effect through stem weight, any combination of these factors could be chosen to optimize plant size.

Key words: *Cannabis sativa* L., day length sensitivity, fibre hemp, genotype, harvest time, plant density, plant weight, primary fibres, secondary fibres, sowing date, textiles.

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Chapter 1

General introduction

1 Hemp for textile applications

The world market demand for bast fibres for high–value textile applications is increasing. Part of this demand could be provided by fibre hemp (*Cannabis sativa* L.). At present however, there is no large–scale production of high–quality hemp yarns and fabrics in the European Union. First because primary producers cannot supply the homogeneous, high–quality long–fibre raw materials the yarn spinners require, and second because fibre processing is still suboptimal (Nebel, 1995; Keller, 1997; Ranalli, 1999; Cappelletto, 2001; Amaducci, 2003, 2006; Liberalato, 2003; Ranalli and Venturi, 2004; Esposito and Rondi, 2006; Traina and Rondi, 2006).

Little seems to be known about the causal relationships between the primary production process, the visible or easily measurable characteristics of harvested hemp stems, and the amount and characteristics of the fibres that can be extracted (Ranalli and Venturi, 2004).

The absence of a reliable high–quality hemp fibre supply holds back textile industries to invest in new processing technologies, and European farmers have little incentive to grow fibre hemp, and to improve the raw materials, unless there is a market willing to pay a higher price for high quality. A break–through in this status quo is needed to establish a competitive, innovative, and sustainable hemp fibre textile industry in the European Union (Van der Schaaf, 1966; Anonymous, 1994b; Bócsa and Karus, 1998; Van Dam, 1999; Amaducci, 2003, 2005, 2006).

The renewed interest in high–quality hemp fibres calls for an agronomic study based on in–depth knowledge of the botany and physiology of the plant. Yarn spinners have high demands with respect to fibre characteristics such as fineness, refinability, strength, fibre length distribution, cohesiveness, homogeneity, and cleanliness (low shiv content); hence, it should be known how the amount of fibres with the desired qualities can be maximised within a single plant and within a crop (Sultana, 1992;

Anonymous, 1994b; Nebel, 1995; Van Dam, 1999; Wulijarni–Soetjipto *et al.*, 1999; Liberalato, 2003; Allam, 2004; Hann, 2005; Sponner *et al.*, 2005).

The main objective of this thesis is to outline the agronomic options for manipulating plant development and crop growth of fibre hemp, in order to optimise high–quality long textile fibre production. In this first chapter, the crop, the plant, and its fibres are introduced, the state of knowledge prior to this research is described, and an outline of each of the chapters of this thesis is presented.

2 History of hemp

2.1 An old and important crop

Hemp is one of the first plants cultivated and one of the oldest non–food crops. It is a multi–purpose crop, grown for its bast and wood fibres, its seeds, its oil, and its cannabinoids. The historical evidence for medicinal and narcotic use of *Cannabis sativa* L. dates back at least 5000 years, and the use of the seed oil for at least 3000 years. As a textile fibre, however, its history is probably longer; the oldest remains of hemp cloth are estimated to be about 6000 years old (Schultes, 1970; Clarke, 1999; Wulijarni–Soetjipto *et al.*, 1999).

Hemp bast fibres are among the strongest and most durable of all vegetable fibres, and particularly in Asia, Central Europe, and North America, fibre hemp has widely been used as a textile fibre for hundreds of years. Cultivation in Europe became widespread from 500 AD onwards, and reached its maximum between the sixteenth and eighteenth century. Hemp, flax (*Linum usitatissimum* L.), stinging nettle (*Urtica dioica* L.), and wool were the most important raw materials for the European textile industries by that time (Dewey, 1913; Schultes, 1970; Pounds, 1979; Dempsey, 1975; Wulijarni–Soetjipto *et al.*, 1999; Herer, 2000).

In the Netherlands, fibre hemp production peaked in the seventeenth and eighteenth century, because the basic equipment of sailing ships was largely dependent upon the cultivation and processing of hemp. Canvas sails, rigging, ropes, fishing nets, but also uniforms were made of the weather–proof fibres. Moreover, hemp farmers processed their own twine for binding and yarns for weaving household textiles and clothing (Hoogendoorn, 1993; Bócsa and Karus, 1998; Herer, 2000).

2.2 Decline

The decline of the sailing industry, the large–scale development of tropical fibre production, e.g., cotton (*Gossypium* ssp.), jute (*Corchorus* ssp.), and sisal (*Agave sisalana* Perrine), the competition with other profitable crops or livestock, the absence of labour–saving machinery, and the difficulties in securing sufficient skilled labour caused the decline of the crop in North America and Western Europe. Markets disappeared and hemp was replaced by cheaper alternatives, which in general were less durable (Dewey, 1913; Garland, 1946; Dempsey, 1975; Brink *et al*., 2003).

The prospects of hemp as a textile fibre went down quickly with two labour–saving inventions that made cotton textiles a lot cheaper. In 1768, a series of aligned spinning wheels, the 'spinning Jenny', was developed by Hargreaves and improved by Arkwright. Cotton and wool were more easily spun by such machinery than hemp. In 1793, Whitney invented the cotton gin, a machine for removing the seeds from harvested cotton bolls. Handicraft was replaced by cotton industries. Hemp fibre production, however, remained labour–intensive, the yarns became too expensive, and hemp lost its position as a widely used textile fibre crop (Dewey, 1913; Garland, 1946; Bócsa and Karus, 1998; Herer, 2000; Brink *et al.*, 2003).

The availability of cheap cotton and the large scale production of cheap synthetic textile fibres in the twentieth century made a return of hemp as a textile fibre crop improbable. Moreover, cultivation of hemp was prohibited in many countries due to the association of the crop with the production of illegal narcotics (Hoffmann, 1957; Bócsa and Karus, 1998; Wulijarni–Soetjipto *et al.*, 1999; Herer, 2000; Ranalli and Venturi, 2004; Amaducci, 2005).

When supplies of tropical fibres were interrupted by World War II, however, a fibre hemp industry was quickly re–established in among others the United States of America ('Hemp for Victory') and Germany to produce raw materials for, e.g., tents, parachutes, and uniforms. Also in the Netherlands the interest in de crop revived for a short time. However, when tropical fibre imports re–established, the ban on hemp was re–imposed (Anonymous, 1942; Anonymous, 1943; De Jonge, 1944; Bócsa and Karus, 1998; Herer, 2000).

Cannabis sativa L. as a fibre crop fell into oblivion in North America and Western Europe, with the exception of France, where the production of fibre hemp for a niche market, specialty papers, survived after the textile industries withdrew from hemp in the 1960s. Also in Eastern Europe only a small hemp industry survived (Anonymous, 1994a; Bócsa and Karus, 1998).

As a consequence of the diminished importance, important knowledge and varieties went lost, research withdrew from hemp, and the development of new machinery for cultivation, harvesting, and processing almost came to a standstill (Bócsa and Karus, 1998; Cappelletto, 2001; Liberalato, 2003). Nowadays hemp fibres are mainly produced for low and medium–value applications, with China, North Korea and India as the largest producers (Wulijarni–Soetjipto *et al.*, 1999; Liberalato, 2003; Van Dam, 2014).

3 The comeback of hemp

3.1 Sustainability

Reintroduction of fibre hemp as a rotation crop has been advocated and researched occasionally in the past century in among others the Netherlands (De Jonge, 1944; Van der Schaaf, 1963; Friederich, 1964; Du Bois, 1982), but the efforts remained without success until the last decade of the twentieth century when hemp made a remarkable worldwide comeback.

This global rediscovery of hemp was among others catalysed by the publication in 1985 of Jack Herer's 'The emperor wears no clothes' (Herer, 2000). In this provocative book, Herer advocates the use of *Cannabis sativa* L. for social as well as economic purposes, outlines the history of the crop and its uses, and crusades the 'conspiracy' against hemp. For the rest, in the last decades of the twentieth century the demand of fashion designers and consumers for linen, a natural fibre from flax and in many aspects comparable with hemp fibre, also increased (Hann, 2005).

In the Netherlands (e.g., Van Berlo, 1993; De Meijer, 1993; Van der Werf, 1994), in other European countries (e.g., Höppner and Menge–Hartmann, 1994; Cromack, 1998; Sankari and Mela, 1998; Struik *et al.*, 2000; Cappelletto, 2001), but also elsewhere in the world (e.g., Lisson, 1998; Ehrensing, 1998), research programmes were initiated to investigate the feasibility of resumed domestic fibre hemp production.

Most EU member states released the ban on industrial hemp between 1993 and 1996. Even in the United States of America, a number of States allow the cultivation of fibre hemp nowadays. In 2013, the first legal industrial hemp crop in 56 years was harvested in de U.S.A. (Carus *et al.*, 2013; Raabe, 2013; Anonymous, 2014).

The world–wide cultivation area of industrial hemp reached about 85,000 ha in 2011, with approximately 60,000 ha for fibres (mainly grown in China and Europe), and 25,000 ha for seeds (mainly grown in Canada, China, and Europe). It is currently (2014) cultivated on 17,000 ha in the European Union, which is the largest area since 10 years. France is the main producer in the EU (Carus *et al.*, 2013; Anonymous, 2014; Van Dam, 2014).

Most of the experiments described in this thesis, were conducted within the framework of the EU–funded HEMP–SYS project, which aimed to promote the development of a competitive, innovative, and sustainable hemp fibre textile industry in the European Union. Universities, research centres, agricultural societies, private businesses, and industries collaborated in designing and developing an integrated processing chain for hemp textile fibres (Amaducci, 2003, 2006). Commercial enterprises also rediscovered the crop and its wide range of marketable bio–based end products. Most of the recent developments are related to the public concern for the environment and the endeavour towards sustainability:

- The Common Agricultural Policy of the European Union underwent a major reorientation. To combat agricultural surpluses, low prices, and budgetary expenditure, and to support the world trade relations and the environment, the subsidies for food production were gradually reduced, and diversification and developments in non–food crops were supported. Fibre hemp fits well in this European policy (Rexen, 1993; Thomas, 1993; Liberalato, 2003; Amaducci, 2003, 2006; Ranalli and Venturi, 2004).

- The narrow crop rotation in the intensive agriculture in, e.g., the Netherlands increased the incidence of plant diseases, lowered the yields, and enhanced the use of biocides. To stop these trends, 'new' crops were identified to widen conventional crop rotations. These new crops should reduce the incidence of pathogens, require less biocides, and besides be profitable in the non–food market. Fibre hemp could be such a crop (Van der Schaaf, 1966; Du Bois, 1982; Van der Werf, 1994; Crowley, 2001; Von Francken–Welz and Léon, 2003).

- Fibre hemp was also advocated to relieve the pressure of the paper industry on remaining natural forests, as an alternative textile fibre to a high–input crop such as cotton, and to replace glass fibres, rock wool, synthetic fibres or asbestos in a wide range of applications. The technical natural fibre market is growing, and fibre hemp could take its share (Dewey and Merrill, 1916; Du Bois, 1982; Van Berlo, 1993; Van der Werf, 1994; Bócsa and Karus, 1998; Lisson and Mendham, 2000; Scheer–Triebel and Léon, 2000; Leupin, 2001; Ebskamp, 2002; Kamat *et al.*, 2002; Liberalato, 2003; Von Francken–Welz and Léon, 2003; Blackburn *et al.*, 2004; Ranalli and Venturi, 2004; Anonymous, 2010, 2014; Van Dam, 2014).

- Finally, the ecology–conscious consumer's demand for healthy, environmental–friendly, and preferably visibly 'natural' products supported the rediscovery of hemp. Trends in fashion and taste, however, might change. Possibly this is not a growing market, but only a cyclical niche market in which the fickleness of the public taste plays an important role (Bócsa and Karus, 1998; Van Dam, 1999; Anonymous, 2000; Ranalli and Venturi, 2004; Burczyk *et al.*, 2005; Sponner *et al.*, 2005; De Boo, 2006; Esposito and Rondi, 2006).

The background of the recent interest in fibre hemp shows that environmentally–friendly production chains are preferable, if not a prerequisite, to be successful. Certified organic production should be aimed at, to obtain a higher market price. Within the framework of the HEMP–SYS project (Amaducci, 2003, 2006) therefore the major environmental impacts associated with the production of hemp yarns were quantified. A Life Cycle Analysis (LCA) by Van der Werf and Turunen (2008) showed that the ecological footprint of hemp yarn production can be reduced by reducing the relatively high energy use in the processing and yarn production stages, and by reducing eutrophication during crop growth. Further, the use of biocides, chemical defoliants, and retting agents should be avoided (Keller, 1997; Ranalli and Venturi, 2004; Amaducci *et al.*, 2008a).

3.2 Added value

History has shown that there should be solid economic prospects as well, which means that on the longer term, new crops should be able to survive in the market without subsidies (Thomas, 1993). For this reason, new hemp projects are often focused on regional development and cooperative local production chains in order to secure a larger share of the added value for the primary producers (Beerepoot, 2003; Blackburn *et al.*, 2004; De Boo, 2006; Janszen *et al.*, 2007). A market price comparable with linen seems necessary to escape from the niche market (Van Dam, 1999; Esposito and Rondi, 2006).

After the reintroduction of hemp as a field crop in Europe in the 1990s, research aimed particularly at a high dry matter production for paper and composites. Hemp was sown as soon as the risk of frost damage was acceptably low, and late flowering varieties with a long vegetative growing stage were selected to make optimal use of the length of the growing season (Dempsey, 1975; Van der Werf *et al.*, 1994a; Meijer *et al.*, 1995; Sankari and Mela, 1998; Ranalli, 1999; Lisson and Mendham, 2000; Struik *et al.*, 2000).

Bulk products with low added value still account for the vast majority of Europe’s hemp fibre market, and without any significant technical progress or new fields of application, little economic growth of the sector is to be expected (Liberalato, 2003; Karus and Vogt, 2004; Carus *et al.*, 2013).

To be competitive in the world market, farmers in the European Union have to provide quality instead of quantity: tailor–made raw materials with high added value. The costs of labour and land are too high for bulk production. With technically superior raw materials, luxury markets have to be explored where the price of the end products depends only for a small part upon the costs of the raw materials (Van Dam, 1999; Liberalato, 2003; Ranalli and Venturi, 2004; Janszen *et al.*, 2007).

3.3 End products

Fibre hemp can be grown for a multitude of end products and semi–manufactures in non–food, food, feed, and pharmaceuticals. Seeds, oil, wood, and bast fibres can be cashed. Waste streams of production processes can be converted into, e.g., bio fuels (Hoffmann, 1957; Bócsa and Karus, 1998; Van Dam, 1999; Herer, 2000; Wulijarni–Soetjipto *et al.*, 1999).

The highly nutritious hemp seeds can be used for bird, fish, and cattle feed as well as for human consumption (bakery products, vegetable oil). The food and feed market is growing fast nowadays, especially in Canada and the U.S.A. Chopped whole plants are fit as provender for cattle. The seed oil, like linseed oil, can also be used as lighting oil or as a basis for, e.g., paint, lubricants, wood preservatives, and body care products (e.g., Pate, 1999; Karus and Vogt, 2004; Debergh, 2009; Anonymous, 2014).

The woody parts of the stem, the ‘shives’ or ‘shiv’ (Dutch: ‘scheven’) that remain after fibre extraction, are mainly (≈ 95% in 2004) applied as livestock bedding in stables (animal litter), but they are also increasingly converted into, e.g., medium density fibre board (MDF) or other building (e.g., Hempcrete, Isochanvre) and woodworking applications (e.g., Dewey, 1913; Dewey and Merrill, 1916; Friederich, 1964; Du Bois, 1982; Anonymous, 2000; Crowley, 2001; Beerepoot, 2003).

Bast fibre applications are numerous. Combined with the woody fibres they are used in pulp for paper and carton, but the fibres can also be used for the production of specialty papers, ropes, twines, canvas, geotextiles, filters, and building and insulation materials. A growing market, aimed at fuel savings, is the use of natural fibre composites for lightweight interior parts in the automotive industry. Weight reduction as compared to glass fibre enforced composites is around 30% (Dewey and Merrill, 1916; Haepp, 1996; Keller, 1997; Karus, 2005; Anonymous, 2000, 2007, 2010, 2014).

Economically the most interesting prospects for hemp bast fibres, however, seem to be in the apparel (clothing) sector (Van Dam, 1999; Cappelletto *et al.*, 2001; Amaducci, 2003; Liberalato 2003; Ranalli and Venturi, 2004; Esposito and Rondi, 2006; Janszen *et al.*, 2007).

3.4 Textiles

The bulk of our demands for textile fibres is currently met by synthetic fibres and cotton (*Gossypium* sp.). Together they account for more than 90% of the world's textile fibre market, with about equal shares. Both however, are associated with environmental problems. Synthetic fibre production depletes fossil energy resources, and for the production of cotton alarming amounts of biocides are used. Cotton is cultivated on approximately 2.4% of the world's arable land. Nevertheless about 25% of the global use of insecticides is applied in cotton. Moreover, around 50% of the total amount of pesticides used in developing countries, and even in the United States of America, is applied in this crop. Besides, cotton needs large amounts of fertiliser and water, and causes depletion of the natural water resources, as well as salinization. Unless produced organically (≈ 0.04% of the global production in 2006, ≈ 0.7% in 2011), cotton is not an environmentally friendly crop (Anonymous, 1999; Kalliala and Nousiainen, 1999; Kerkhoven and Mutsears, 2003; Kooistra *et al.*, 2006; Truscott *et al.*, 2013; Van Dam, 2014).

Fibre hemp could be an excellent substitute for cotton and different processing techniques can be used to convert hemp fibres into textiles. 'Cottonized' hemp fibres, refined by chemical–physical methods (e.g., STEX or steam–explosion, ultrasound) can be processed on cotton spinning machines, and hemp–cotton blended fabrics can be used for the production of, e.g., jeans. Already in the 1920s, 1930s, and 1940s Germany considered this 'cottonized hemp' (German: 'Flockenbast') as a serious alternative for all imported cotton and in recent years Chinese textile industries started to replace cotton by hemp fibres (Dempsey, 1975; Nebel, 1995; Keller, 1997; Bócsa and Karus, 1998; Van Dam, 1999; Keller *et al.*, 2001; Leupin, 2001; Ebskamp, 2002; Beerepoot, 2003; Blackburn *et al.*, 2004; De Boo, 2006; Anonymous, 2007, 2010).

The highest added value in fibre hemp production, however, can be obtained by producing high–quality long fibres for the finest yarns for fashion textiles, a luxury niche market (Van Dam, 1999; Cappelletto *et al.*, 2001; Amaducci, 2003; Ranalli and Venturi, 2004). The flax fibre production chain for high–quality linen in Western Europe has shown that with high–quality standards it is possible to compete with imported raw materials from outside the European Union (Van Dam, 1999; Liberalato, 2003). To introduce hemp into the fashion textile sector, however, serious improvements should be made with respect to fibre quality, especially fineness (Nebel, 1995; Ranalli and Venturi, 2004).

The tensile strength and durability of hemp fibres, the resistance to wear and tear, are superior to cotton fibres, and the hemicellulose content contributes to the highly valued textile features breathability and thermal insulation. Hemp long fibres are in many aspects comparable with the yarns spun from the bast fibres of flax. The fabrics woven from hemp yarns wrinkle and breathe like linen, and have the same 'natural' irregular structure (Robinson, 1996; Ebskamp, 2002; Anonymous, 1994b; Preti, 2006). New applications of fibre hemp for textiles, however, are surely not restricted to the apparel section. Because the absorbency is superior to cotton, hemp fibre is an excellent material for, e.g., bedcovers, table–linens, bath towels, padded seats, baby clothing, and diapers (Robinson, 1996; Preti, 2006).

Although high–quality yarn production economically seems to have the most interesting prospects, even here valorisation of waste streams (cascade principle) might be necessary to establish a competitive hemp fibre textile industry in Europe (Van Dam, 1999; Liberalato, 2003; Karus, 2005).

4 Plant and crop

4.1 Biology

Hemp belongs to the Cannabaceae family, which includes only one genus, *Cannabis*, and one species, *Cannabis sativa* L. It originates from the temperate parts of Asia; probably the centre of origin is China. Both botanical classification and the exact geographical origin, however, are subject to on–going debates. These disputes are among others complicated by the early and widespread cultivation of hemp (Dewey, 1913; Hoffmann, 1957; Schultes, 1970; Dempsey, 1975; Vavilov, 1992; Raman, 1998; Wulijarni–Soetjipto *et al.*, 1999).

Fibre hemp is a vigorous annual crop, propagated by seed. The seed is an achene: a dry, hard–shelled, one seeded fruit, formed from a single carpel. The thousand seed weight of the oval, grey to brown mottled nuts is around 16–22 g. There is no vernalisation response. Germination is epigeal, and seeds usually germinate and emerge within a week (Dewey, 1913; Hoffmann, 1957; Heslop–Harrison and Heslop–Harrison, 1969; Dempsey, 1975; Höppner and Menge–Hartmann, 1994; Wulijarni–Soetjipto *et al.*, 1999; Höppner *et al.*, 2004).

After a period of relatively slow growth in the first weeks after germination, a fast growth period starts, in which the requirements for water and nutrients are high; more than 50% of the final plant height usually is produced in about a month (Höppner and Menge–Hartmann, 1994; Bócsa and Karus, 1998; Sankari and Mela, 1998). During this period the plants are very susceptible to lodging (knock–down)(author's personal experience…).

Stem length at maturity is very variable, and is strongly affected by environmental factors. A height up to six meters can be reached in a four months growing season, and under favourable growing conditions length increases of up to 10 cm in one day were recorded (Hoffmann, 1957; Friederich, 1964; Höppner and Menge–Hartmann, 1994; Bócsa and Karus, 1998; Clarke, 1999; Höppner *et al.*, 2004).

In hemp grown for textiles the seeding rates are high. Consequently, branching is suppressed, and leaves are only present on the hollow main stem. The two cotyledons are sessile, but all true leaves have a long petiole. The characteristic hemp leaves are palmately compound and composed of 3–11 lanceolate serrate leaflets with a length of 5–15 cm and a width of 1–2 cm. The number of leaflets and their size increase progressively from node to node until the start of flowering, when this trend is reversed (Hoffmann, 1957; Heslop–Harrison and Heslop–Harrison, 1969; Wulijarni–Soetjipto *et al.*, 1999). The fibres and their development during the growing season will be discussed in Section 6 of this chapter.

4.2 Dioecious and monoecious varieties

In its origin the species is dioecious, meaning that male and female flowers develop on separate plants (Picture 1A). The two sexes are morphologically indistinguishable before the development of inflorescences, but in the generative phase sexual dimorphism is extremely pronounced. Male hemp has a branched inflorescence in the top of the plant, with few or no leaves. The many easily recognisable flowers have five white to yellowish–green petals of about 5 mm long, and five stamens which at maturity discharge abundant yellow pollen for wind–pollination. The much smaller female flowers are tightly clustered in unbranched, leafy, resinous, sticky inflorescences. Although the female plants at flowering are easily distinguishable from the male plants, the individual female flowers are inconspicuous because green bracts surround the ovary. At flowering two protruding pistils, only a few millimetres long, are visible (Kundu, 1942; Hoffmann, 1957; Heslop–Harrison and Heslop–Harrison, 1969; Raman, 1998; Clarke, 1999; Wulijarni–Soetjipto *et al.*, 1999).

A variable fraction of monoecious plants, with male and female flowers on the same plant, is usually present in natural populations and crops (Hoffmann, 1957; Dempsey, 1975; Clarke, 1999). Such plants resemble the more robust female habitus (Horkay and Bócsa, 1996), which makes sense because these plants should be strong enough to bear the relatively heavy tops of the plants with the seeds. Stems of female plants on average are a little shorter and thicker (Schumann *et al.*, 1999), and the tap root system of the female plant is also more developed (Kundu, 1942; Bócsa and Karus, 1998), presumably for the same reason.

Picture 1. *Cannabis sativa* L.
A) In its origin the species is dioecious. In the generative phase sexual dimorphism is extremely pronounced. Inflorescences of a male plant (left) and a female plant (right).
B) In a densely sown crop branching is suppressed and approximately 80% of the above ground dry matter is located in the erect main stem.

Heterogeneity in a dioecious hemp crop is partially an inevitable consequence of sexual dimorphism. At harvest two populations with different characteristics (e.g., biometry, flowering time, fibre characteristics) are present. In a dioecious crop, male and female plants are generally present in similar numbers, but depending on growing conditions and cultivar, deviations might occur. Male plants die soon after anthesis, whereas female plants live three to five weeks longer, until seed ripeness. Therefore, the ratio might shift in favour of the females at the end of the growing season (Kundu, 1942; Anonymous, 1943; Borthwick and Scully, 1954; Heslop–Harrison and Heslop–Harrison, 1969; Anonymous, 1994a; Van der Werf and Van der Berg, 1995; Schumann *et al.*, 1999; Wulijarni–Soetjipto *et al.*, 1999).

Monoecious varieties have been bred for reasons of homogeneity and mechanisation (Dempsey, 1975; Mediavilla *et al.*, 1999). Without continued selection, however, such artificial varieties return to the dioecious state in two or three generations (Anonymous, 1994). Monoecious hemp is considered appropriate, especially when crops are grown for both seed and fibre. Harvest in such a double use crop is relatively late, because the seeds have to ripen. In dioecious hemp varieties by that time the male plants would be deteriorated, which causes yield losses and hampers harvesting (Anonymous, 1994a; Mediavilla *et al.*, 2001a).

Dioecious varieties historically are considered most fit for textile purposes. However, because the fibre quality of male plants is considered superior to the fibre quality of female plants, the timing of the harvest of dioecious varieties is complicated with respect to fibre quality, quantity, homogeneity, and suitability for harvesting (Hoffmann, 1957; Horkay and Bócsa, 1996; Bócsa and Karus, 1998; Anonymous, 1994a).

Male plants are also reported to have a higher fibre percentage and higher quality. In the past, when harvests were carried out manually, male plants were often harvested earlier than the female plants, which had to wait until seed ripeness. The fibres from male plants were used for finer household purposes, whereas fibres from female plants were used for sacking or canvas (Rowlandson, 1849; Hoffmann, 1957; Jakobey, 1965; Höppner and Menge–Hartmann, 1994; Horkay and Bócsa, 1996).

In the experiments described in this thesis monoecious as well as dioecious varieties were used. Agronomic characteristics and genetic background of fibre hemp varieties were summarised by, e.g., De Meijer (1995) and Bócsa and Karus (1998).

4.3 Short–day response

Hemp is a short–day plant. Tournois (1912) used the species in the first unequivocal demonstration of photoperiodic induction of flowering. Photoperiod sensitivity is very different for varieties and most varieties have a flowering response that is typical for a quantitative short–day plant: short days accelerate flowering. However, there are also varieties with an absolute short–day requirement and a true critical day length. Lisson *et al.* (2000) showed for two varieties that in photoperiods less than 14 hours flowering occurs in a minimum constant thermal time, while at longer photoperiods flowering is progressively delayed. With increasing temperature the time between first primordia to flowering becomes shorter (Borthwick and Scully, 1954; Heslop–Harrison and Heslop–Harrison, 1969; Lisson *et al.*, 2000).

The critical day length generally increases with the latitude of adaptation. When a certain variety is cultivated at lower latitude it will flower earlier; when it is cultivated at higher latitude it will flower later. To avoid a rapid genetic shift towards a different phenological pattern, sowing–seed production has to take place at the appropriate latitude (Heslop–Harrison and Heslop–Harrison, 1969; Bócsa and Karus, 1998; De Meijer and Keizer, 1994).

For quality reasons, fibre hemp is harvested around the time of flowering (Bócsa and Karus, 1998). To maximise stem dry matter and fibre yield, however, it is important not to choose a variety that flowers too early at the chosen site, because around flowering the allocation of dry matter to the stem decreases (De Meijer and Keizer, 1994; Van der Werf *et al.*, 1994a; Meijer *et al.*, 1995; Mediavilla, 1999 *et al.*; Ranalli, 1999; Struik *et al.*, 2000; Cooper, 2003).

In Chapter 5, we make use of the short–day response of hemp to demonstrate that secondary fibre formation depends on plant weight, not on flowering.

4.4 Psychoactive compounds

The major disadvantage of fibre hemp is its association with the production of illegal narcotics, like hashish and marijuana (weed). Before seed set it is very difficult to discriminate between drug and fibre types of *Cannabis sativa* L. Consequently, hemp was put under a ban in many countries in the twentieth century, regardless of the intended use or the concentration of psychoactive compounds (De Meijer *et al.*, 1992; Bócsa and Karus, 1998; Herer, 2000; Cappelletto *et al.*, 2001).

Hemp synthesizes about sixty chemicals belonging to the cannabinoids family, and members of this phytochemical group have never been found in other species. Some cannabinoids are psychoactive, and the most important of these intoxicants is Δ9–tetrathydrocannabinol (THC), which is found in all parts of the plant, with highest concentrations in the tops of female plants, especially in the bracts, its glandular hairs, and their resin around pollination. THC does not contribute to the characteristic smell of hemp; it is odourless. The distinctive smell of hemp plants is among others caused by terpenes, which appear in both drug and non–drug types (Turner *et al*., 1980; Pate, 1994; Wulijarni–Soetjipto *et al.*, 1999; Niesink *et al.*, 2002).

The THC content of a hemp strain mainly depends on its genetic background, but is modified by environmental conditions like latitude, elevation, nutrient levels, temperature, and moisture (De Meijer *et al.*, 1992; Pate, 1994; Bócsa and Karus, 1998; Niesink *et al.*, 2002). Hemp without any THC seems utopian: although the role cannabinoids play in plant development is not well understood, they seem to be indispensable for the growth of the plant (Bócsa and Karus, 1998). Possibly, cannabinoids offer protection against insects or micro–organisms, or play a role in suppressing the growth of surrounding vegetation or in the defence against ultraviolet radiation, drought, or high temperatures. Evidence for such claims, however, is lacking (Schultes, 1970; De Meijer *et al.*, 1992; Pate, 1994; Mediavilla *et al.*, 1999).

Fibre hemp varieties cannot be used as a drug. Low THC content is one of the main breeding objectives, because most countries that allow the cultivation of fibre hemp restricted the THC content to about 0.20–0.35% (De Meijer *et al.*, 1992; Höppner and Menge–Hartmann, 1994; Bócsa and Karus, 1998; Lisson and Mendham, 2000; Cappelletto *et al.*, 2001; Crowley, 2001; Burczyk *et al.*, 2005), which is far below the THC content of drug types (Niesink *et al.*, 2002). Nevertheless, the association of fibre hemp with its narcotic relatives has hampered the reintroduction of the crop, when its economic prospects increased (Anonymous, 1994a; Bócsa and Karus, 1998; Herer, 2000; Cappelletto *et al.*, 2001).

4.5 Pests and diseases

Fibre hemp is relatively pest–tolerant (McPartland, 1999) and can be cultivated without the use of biocides (Höppner and Menge–Hartmann, 1994; Gutberlet and Karus, 1995; Cappelletto *et al.*, 2001; Crowley, 2001; Keller *et al.*, 2001; Mediavilla *et al.*, 2001a). Although many pests and diseases have been described, some of which are specific for hemp (McPartland, 1996a, 1996b, 1999; Bócsa and Karus, 1998; Gottwald, 2002), serious economic losses are rarely reported (Anonymous, 1943;

Gutberlet and Karus, 1995; Ranalli, 1999).

In the field experiments described in this thesis, no biocides were applied, and pests and diseases of significance did not occur. Incidentally, seedlings died from wilting diseases caused by different oomycetes or fungi (Friederich, 1964; Dempsey, 1975; Van der Werf *et al.*, 1994a; Meijer *et al.*, 1995; McPartland, 1999). The early canopy closure in high density stands for textile applications increases the humidity around the stems, hence the risk of fungal attack, especially under unfavourable weather conditions (Friederich, 1964; Meijer *et al.*, 1995; Crowley, 2001).

Rabbits, hares and roe deer can be serious threats for an establishing crop as well, and to protect seeds and seedlings, care must also be taken to scare away birds (author's personal experience).

4.6 Weed control

Fibre hemp, unlike flax, suppresses weeds efficiently, especially when it is grown at high plant densities. The fast growing crop outcompetes and smothers most weeds before they set seed. Consequently, weed seed banks are reduced, which is of benefit for the subsequent crops in the rotation. Hemp therefore is recommended to precede crops susceptible to weed problems and for organic crop rotations. Herbicides are not needed (Rowlandson, 1849; Dewey, 1913; Hoffmann, 1957; Meijer *et al.*, 1995; Höppner and Menge–Hartmann, 1994, 1995; Bócsa and Karus, 1998; Crowley, 2001).

A future increase of the fibre hemp acreage might increase the incidence of pests and diseases. Also hemp broomrape (*Orobanche ramosa* L.), a parasitic weed, might cause problems, as it does in, e.g., France, Russia, and Italy already. This so–called 'hemp killer' spread from Asia to Europe in the seventeenth century with the increasing cultivation of its host. The roots of the broomrape species penetrate hemp roots, extract nutrients, and eventually kill the plants. Clean seed, resistant varieties, and crop rotation are recommended to avoid these problems (Dewey, 1913; Friederich, 1964; Dempsey, 1975; Bócsa and Karus, 1998; Wulijarni–Soetjipto *et al.*, 1999; Gonsior *et al.*, 2004).

4.7 Crop management

Fibre hemp is an easy–to–grow, low maintenance crop; it requires no field operations between sowing and harvest (Van der Schaaf, 1966; Anonymous, 1994b; Struik *et al.*, 2000; Anonymous, 2003). The crop can be grown organically, without major problems (Menge–Hartmann and Höppner, 1996; Ranalli and Venturi, 2004; Danckaert *et al.*, 2006). Hemp is known for its 'plasticity' or high capacity to adapt to various cultivation and environmental factors (Friederich, 1964; Schultes, 1970; Bócsa and Karus, 1998; Struik *et al.*, 2000; Amaducci *et al.*, 2002a).

Fibre hemp may yield up to 20 Mg stem dry matter per hectare, depending on agronomy and environment. Usually stem dry matter yields between 7–15 Mg ha^{-1} are reported in field experiments (e.g., Bredemann *et al.*, 1961; Van der Werf, 1994; Mediavilla *et al.*, 1999; Struik *et al.*, 2000; Scheer–Triebel and Léon, 2000; Svennerstedt and Landström, 2000; Crowley, 2001; Amaducci *et al.*, 2002a; Von Francken–Welz and Léon, 2003; Höppner *et al.*, 2004; Burczyk *et al.*, 2005, 2009; Amaducci, 2006b). Fibre yields are usually between 2 and 4 Mg ha^{-1} (Dempsey, 1975; Svennerstedt and Landström, 2000; Mediavilla *et al.*, 2001; Von Francken–Welz and Léon, 2003; Höppner *et al.*, 2004; Deleuran and Flengmark, 2005). A survey in 2010, covering 99% of the production area, showed that hemp farmers in the European Union on average harvested 7.3 Mg stems ha^{-1} (Carus *et al.*, 2013).

Van der Werf (1994) extensively described crop physiology, dry matter production, and dry matter distribution, and a basic crop growth and development model to predict total and stem dry matter yield of fibre hemp was developed by Lisson *et al.* (2000b). For textile destinations, however, the focus should not be on quantity, but rather on fibre quality and homogeneity (Van der Werf and Van der Berg, 1995; Ranalli, 1999; Liberalato, 2003).

Although under favourable conditions a height up to 6 m can be reached, such tall plants are unfit for textile yarn production, with respect to quality (Jakobey, 1965) as well as suitability for harvesting and processing (Friederich, 1964; Van der Werf and Van der Berg, 1995; De Maeyer and Huisman, 1995; Schulz, 1998). Usually, canopy height at harvest is between 1.5 and 3 m (e.g., Höppner and Menge–Hartmann, 1994; Cromack, 1998; Mediavilla *et al.*, 1999; Struik *et al.*, 2000; Vetter *et al.*, 2002; Amaducci *et al.*, 2002a), but little seems to be known about the causal relationships between visible or easily measurable characteristics of the stems (e.g., plant height and stem diameter) and the characteristics (quantity and quality) of the fibres that can be extracted.

A well–prepared seedbed, ploughed, and subsequently harrowed, is necessary to get the top–soil into fine tilth. This supports a rapid and uniform emergence, which is important for the establishment of a uniform crop as well as for weed suppression. Fibre hemp is very sensitive to poor soil structure; water logging and soil compaction hamper emergence, and cause irregular crops. The crop needs less water than common rotation crops such as wheat, sugar beet or maize (Rowlandson, 1849; Dewey, 1913; Friederich, 1964; Sankari and Mela, 1998; Struik *et al.*, 2000; Anonymous, 2003; Venturi and Ranalli, 2004).

The recommended seeding rates for stem production of fibre hemp vary widely (50–750 seeds m^{-2}) depending on the production goal, the expected plant density, the expected yield, and regional traditions (Dempsey, 1975; Van der Werf *et al.*, 1995a; Ranalli, 1999; Amaducci *et al.*, 2002ab; Burczyk *et al.*, 2009). For quality reasons, hemp for textile destinations traditionally is sown in densities that are higher than the lowest plant density that gives maximum stem dry matter yield at the site (Rowlandson, 1849; Jakobey, 1965; Van der Werf *et al.*, 1995a; Struik *et al.*, 2000; Amaducci *et al.*, 2002a;). With increasing plant density, individual plant size is reduced (Kira *et al.*, 1953; De Wit, 1960; Amaducci *et al.* 2002a), which is reported to improve the bast fibre content and the fineness of the fibres (Jakobey, 1965; Van der Werf *et al.*, 1995a).

The effect of sowing density on stem yield, however, is limited, because of inter–plant competition (Kira *et al.*, 1953) and 'self–thinning' or density–induced mortality (Van der Werf *et al.*, 1995a). An increase in biomass yield in time is accompanied by a reduction of the number of plants. The death rate caused by self–thinning in hemp is high as compared with other dicotyledons (Van der Werf *et al.*, 1995a). With increasing amounts of applied nitrogen, self–thinning increases (Van der

Werf, 1994; Höppner and Menge–Hartmann, 1994; Menge–Hartmann and Höppner, 1995; Amaducci *et al.*, 2002a). Such dying plants cause irregularities in the crop which might hamper a clean and parallel harvest of the stems.

In a densely sown crop branching is suppressed (Dewey, 1913; Clarke, 1999; Amaducci *et al.*, 2002a), and approximately 80% of the above ground dry matter (Van der Werf, 1994; Meijer *et al.*, 1995; Struik *et al.*, 2000) is located in the erect main stem (Picture 1B), which contains the valuable long fibres (Section 6.1). Smooth unbranched stems also facilitate parallel, aligned harvesting and processing, which is necessary for long fibre extraction in hemp (Van Dam, 1999; Van Dam and Van den Oever, 2006; Venturi *et al.*, 2007), like it is in flax (Hann, 2005). In a maturing crop sown at a high density, the leaves from the lower stem part gradually become yellow and drop off (Dewey, 1913).

In fibre hemp crops often an understorey of suppressed plants, called 'underhemp' (German: 'Unterhanf'), exists (Hoffmann, 1957). These very small plants under certain circumstances survive relatively well in the low–light environment under the canopy. They do not contain any valuable textile fibres, and will completely be lost during processing (Van der Schaaf, 1963; Van der Werf and Van den Berg, 1995; Bócsa and Karus, 1998).

As for flax, a surplus of nitrogen increases the risk of lodging. This should be avoided to keep an easy–to–harvest high–quality crop. In the experiments described in this thesis, nitrogen fertilisation was limited to avoid this problem, and also to reduce size variability and self–thinning (Friederich, 1964; Dempsey, 1975; Van der Werf and Van der Berg, 1995; Bócsa and Karus, 1998; Cappelletto *et al.*, 2001; Amaducci, 2005).

Hemp does not exhaust the soil and can be grown many years in succession with low amounts of fertiliser and little reduction in yield (Dewey, 1913). However, the advantages of fibre hemp in a crop rotation, e.g., improved soil structure (Bócsa and Karus, 1998) and weed eradication (Section 4.6), in this case are not utilized and the risk of diseases, pests, and weeds, e.g., hemp broomrape increases.

The fibre content depends among others on variety, but there are many contrasting results presented in the literature about crop management factors that would or would not affect the quality of hemp fibres and the productivity of fibre hemp. It is one of the objectives of the present thesis to understand the underlying principles.

5 Fibre processing

5.1 Fibre releasing

Before hemp fibres can be used for yarn spinning, the fibre bundles have to be released from the surrounding tissues by biological, mechanical or chemical processes. Processing of fibre hemp into high–quality yarns in principle is similar to linen production from flax (Sponner *et al.*, 2005), for which the industrial production chain is accurately described by, e.g., Hann (2005) and Salmon–Minotte and Franck (2005).

Traditionally, hemp fibre extraction for textile purposes consists of retting, breaking and scutching (Ranalli, 1999). Mechanical fibre releasing without retting or 'green decortication' seems unfit for fine textile purposes (Dewey and Merrill, 1916; Keller, 1997; Keller *et al.*, 2001; Hobson *et al.*, 2001; Sponner *et al.*, 2005). Altering the sequence of retting (or 'bio–degumming') and decortication, however, might be a serious alternative to the traditional method (Leupin, 2001; Tofani, 2006; Anonymous, 2007).

We used a traditional fibre–extraction method (Picture 2) for the experiments described in this thesis, because of the small size of the samples as compared to industrial processing batches. However, with respect to the procedural steps and the final products, the method is comparable to industrial processing.

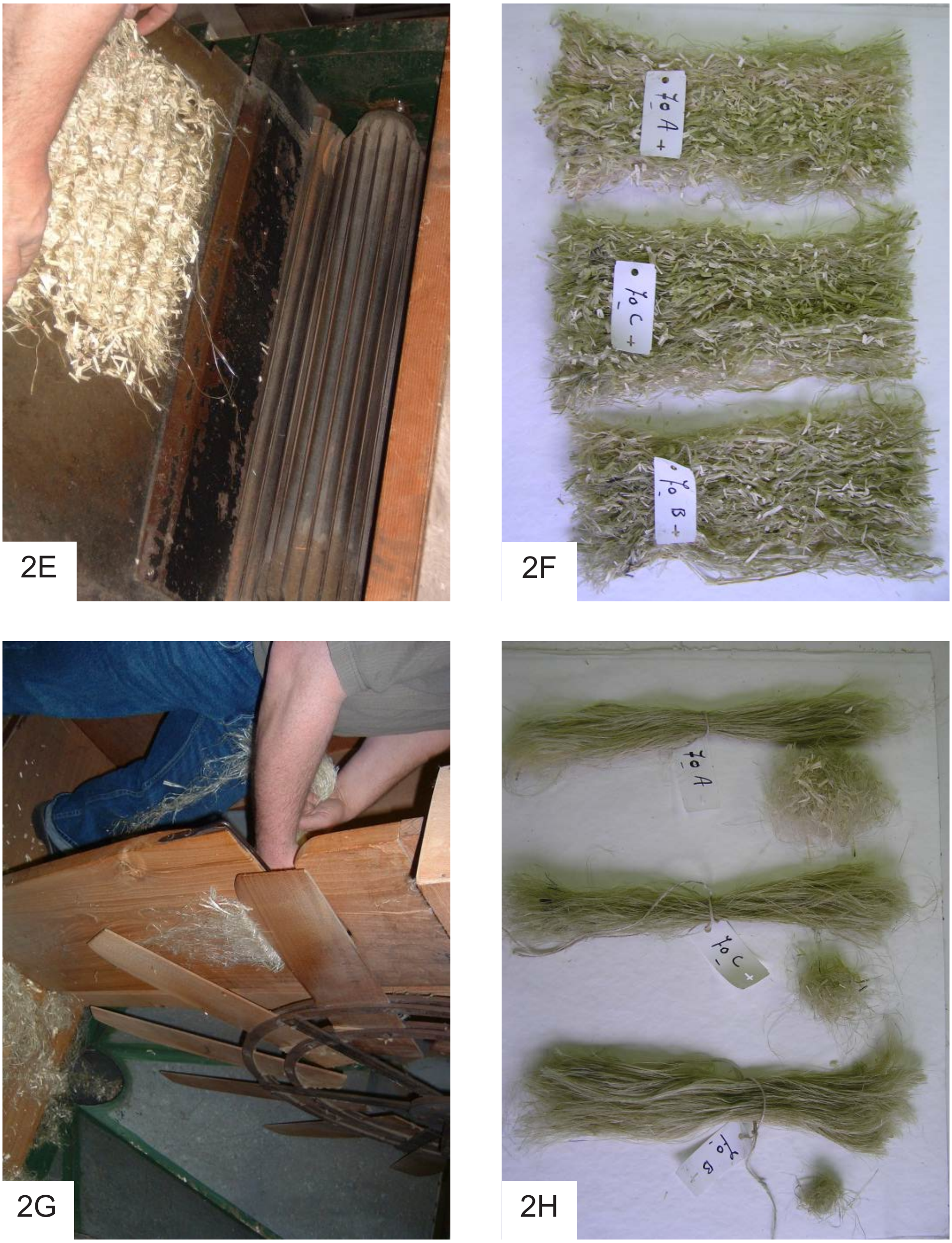
5.2 Retting

The main retting techniques are dew retting and (warm–)water retting. When hemp is to be dew retted, the stems after mowing with a cutter bar are spread in the field in uniform layers ('swaths'), and turned manually or mechanically once or several times during the 10–40 days retting period to provide a uniform end product. It is a relatively cheap method in which the fibres are released by fungi that are excessively present under moist field circumstances. However, the quality of dew–retted fibres is difficult to control, because of the extreme weather dependency of the process. Especially temperature and degree of humidity are important factors in combination with swath height. Wet circumstances might hinder a timely gathering of the retted stems, which eventually leads to over–retting and loss of fibre quality and quantity. Dew retting gives rise to heterogeneous fibres, hence is unfit for the production of high–quality yarns. Stand or dry–line retting, in which the stems are only harvested in spring after the moist and frosty autumn and winter (Finland, Sweden), also produces fibres with too low quality for yarn production (Dempsey, 1975; Anonymous, 1994; Ranalli, 1999; Van Dam, 1999; Hobson *et al.*, 2001; Müssig and Martens, 2003; Allam, 2004; Pasila, 2004; Hann, 2005; Salmon–Minotte and Franck, 2005; Sponner *et al.*, 2005).

For the production of textile yarns, water retting is recommended. This takes place in ponds, ditches, lakes, tanks or basins in which the stems are submerged. Fibre releasing is performed by the spontaneous enzymatic action of anaerobic bacteria. The method pollutes the water and is accompanied by a characteristic, foul odour (butyric acid, hydrogen sulphide, methane) for which reasons retting in open water was abandoned in many countries (Ranalli, 1999; Van der Werf and Turunen, 2008).

In our experiments, after harvesting (Picture 2A), groups of 50 stems are cut into 50 cm stem parts and bundled (Picture 2B) after which the fibres are extracted indoors by a controlled warm–water retting procedure (Pictures 2C and 2D) to avoid the extreme weather dependency that comes along with dew retting. It is the most suitable method for such fibre extraction experiments (Hoffmann, 1957). The method is relatively expensive, but it benefits the final quality of the fibres and the yarns that can be spun (Dempsey, 1975; Van Dam, 1999; Tofani, 2006). It also enabled us to determine retting losses under controlled conditions. After drying the retted stems on a drying floor (e.g., Van der Werf *et al.*, 1994b), the fibres and wood of the stems were separated from each other in two mechanical steps, breaking and scutching.

Picture 2 A) Hemp stems were cut at soil level. B) Per plot 50 cm parts of 50 stems were bundled. The stems were weighed after conditioning (2 days, 19 °C, 73% humidity). C) The bundles were warm water retted in PVC tubes with closed bottoms for 4 days at 34 °C. D) After retting the stems merely consist of fibres and wood. The stems were washed with tepid water and dried on a drying floor for 4 days at 27 °C. The stems were weighed after conditioning.



E and F) To separate fibres and wood the stems were fed into a flax breaker consisting of a double series of ribbed breaking rollers. The wood was broken into shives, while the fibres passed under the rollers easily. G and H) Scutching was performed on a Flemish mill with rotary blades that beat the broken stems in such a way that shives and tow were separated from the long fibres. Long fibres and tow were weighed after conditioning.

5.3 Breaking and scutching

After breaking the stems on a flax breaker (Picture 2E and 2F), scutching is performed with a Flemish mill (Van den Oever *et al.*, 2003) with rotary blades that beat the broken stems in such a way that remaining shives and scutching tow are separated from the long fibres (Pictures 2G and 2H). Tow, the fibres that are lost during breaking and scutching, in our experiments was collected and manually separated from remaining shives. The amounts of scutching tow and scutched long fibres are weighed to determine the relative amounts of fibre and wood prior to breaking. The final product in the experiments described in this thesis is scutched long fibre. To produce hemp yarns the next processing steps would be hackling and spinning.

5.4 Hackling and spinning

Hackling is a combing process with wire pins of increasing fineness and closeness, which aligns and refines the long fibres, with the aim to produce a continuous fine strand of fibres or 'sliver' for spinning. Losses are high; hackling yields are reported to be around 40% only (Sponner *et al*., 2005; Tofani, 2006).

The slivers undergo a series of doubling and drafting operations with the aim to attenuate the fibres and to reduce the yarn count, which means that the fineness is enhanced. To produce the finest yarns the spinning technique and the fineness of the fibre material are important. Yarns can be spun either on a dry or a wet basis. The first procedure is cheaper, the latter, however, produces yarns with higher quality. When hemp fibres pass through hot water before spinning, drawing out is greater and a finer hence more valuable yarn can be spun (Allam, 2004; Hann, 2005; Salmon–Minotte and Franck, 2005; Sponner *et al.*, 2005; Van Dam and Van den Oever, 2006).

The final fineness of the fibre material depends on the dimensions of individual fibres or ultimates, their grouping into fibre bundles or fibre collectives and the refinability of such bundles, which is the division into two or more bundles consisting of less ultimates. In flax the fibre strand fineness is affected more by the grouping than by the thickness of the ultimates in the stem (Shepherd, 1956; Müssig and Martens, 2003; Chernova and Gorshkova, 2007).

Fibre fineness can be improved with chemical retting agents as well, however, this does not fit in sustainable textile production chains (Leupin, 2001; Venturi *et al.*, 2007; Amaducci *et al.*, 2008a; Van der Werf and Turunen, 2008).

6 Fibres and fibre development

6.1 Fibres

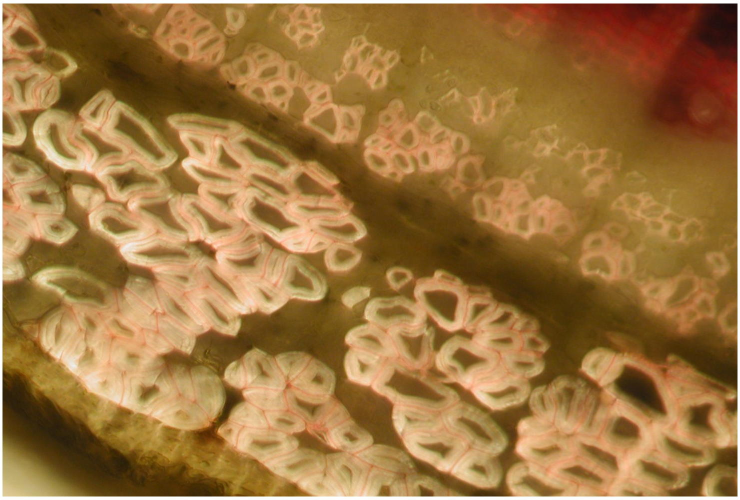
Fibres are slender strands of natural or man–made material, usually having a length of at least 100 times their diameter, and characterised by flexibility, cohesiveness, and strength. These characteristics make a wide range of plant fibres fit to be spun into yarns, and then woven or knitted into fabrics. In the European Union, fibre hemp and flax, both annuals, and stinging nettle, a perennial crop, are fit to be cultivated for this purpose (Lipton, 1995; Scheer–Triebel and Léon, 2000; Brink *et al.*, 2003; Vogl and Hartl, 2003).

Botanically, the valuable textile long fibres that can be extracted from these crops, are phloem (bast) fibres. They belong to the sclerenchyma, the tissue giving mechanical support to plants. Fibre formation in plants is a highly orchestrated, but yet poorly understood process, which involves stages of cell initiation, elongation, cell wall layer formation, and maturation (Kundu, 1942; McDougall *et al.*, 1993; Van Dam and Gorshkova, 2003; Brink *et al.*, 2003; Amaducci *et al.*, 2005).

6.2 Primary fibres are valuable, secondary fibres are unwanted

In fibre hemp two types of bast fibres occur, primary and secondary fibres. The classification is made according to their origin. The cell walls of both types are enforced with layers of cellulose and both types are organised in bundles. In a cross–section of a hemp stem the outer fibre bundle layer consists of primary fibres, the inner layer, if present, of secondary fibres (Picture 3) (Kundu and Preston, 1940; Kundu, 1942; Van Dam and Gorshkova, 2003, Sponner *et al.*, 2005).

For spinning high–quality textile hemp yarns only the primary or ‘long’ fibres (average length 20 mm) are valuable. Secondary or ‘short’ fibres (average length 2



Picture 3. A cross–section (100 × magnified) of a hemp stem with primary and secondary fibres, stained with phloroglucinol (0.1 g in 20 ml 15% HCl), which colours lignin red.

mm) are unwanted (Hoffmann, 1957; Bredemann *et al.*, 1961; Ranalli, 1999; Mediavilla, 2001; Brink *et al.*, 2003; Schäfer and Honermeier, 2003). These fibres are too short for spinning and contain too much lignin (Kundu and Preston, 1940; Hoffmann, 1957; Schäfer and Honermeier, 2003) which is detrimental for the production of fine, flexible, and homogeneous yarns (Bócsa and Karus, 1998; Cappelletto, 2001).

Because it is technically difficult to separate the secondary fibres from the primary fibres during commercial fibre processing (Kundu and Preston, 1940; Van Dam and Gorshkova; 2003), it should be known how the development of secondary fibres above stubble height can be avoided in the raw materials aimed at textile yarn production.

6.3 Fibre development

Primary fibre bundles are already present in very young hemp stems. They run longitudinally along the stem from bottom to top and can reach almost the full length of the plants (Van Dam and Gorshkova, 2003; Hernandez *et al.*, 2006). The primary fibres have to be strong enough before the bundles can be extracted, and their maturity or ‘ripeness’ progresses from bottom to top (Kundu, 1942; Mediavilla *et al.*, 2001; Schäfer and Honermeier, 2003; Van Dam and Gorshkova, 2003; Amaducci *et al.,* 2005). Hemp for textiles is usually harvested around the time of flowering, when the primary fibres are ‘ripe’ and stem dry matter yield and fibre yield are highest (Bócsa and Karus, 1998; Mediavilla *et al.*, 2001; Schäfer and Honermeier, 2003; Amaducci *et al.*, 2005; Burczyk *et al.*, 2009).

Secondary fibres might form when a stem part has reached its maximum length (Kundu and Preston, 1940; Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005; Chernova and Gorshkova, 2007). They are absent in young hemp plants or only present in a thin layer at the stem base (Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005, 2008a; Hernandez *et al.*, 2006). Around flowering, however, secondary fibres are found higher up along the stem, with a layer thickness that decreases from bottom to top (Van der Werf, 1994b; Mediavilla *et al.*, 2001a; Schäfer and Honermeier, 2003; Amaducci *et al.*, 2005). Mediavilla *et al.* (2001a) stated that with the induction of the generative phase secondary fibre formation increases quickly. Schäfer and Honermeier (2003) also related an increased secondary fibre formation and a thicker secondary fibre layer to a phenological stage of the plant, the period between flowering and seed.

6.4 Timing of the harvest

The observed coincidence of enhanced secondary fibre formation with the transition from the vegetative to the generative growing stage of the plants does not necessarily point at a causal relationship between these phenomena.

Botanically the bast fibres in hemp belong to the sclerenchyma tissue which gives mechanical support to the plants (Kundu, 1942; McDougall *et al.*, 1993; Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005) and the need for such support increases when plants grow taller and tops become heavier, due to the development of inflorescences and filling of the seeds. Therefore it seems likely that the increasing size of the plant is involved in secondary fibre formation.

Harvest time of fibre hemp should be optimised on the basis of insight into the progress of the secondary fibre front from bottom to top, the ripening of the primary fibres and the thickness of the layers. The rates of the processes over time are not well quantified, nor are the factors determining the onset of changes herein. To avoid the presence of the unwanted secondary fibres above stubble height and to optimise the production of valuable primary fibres, we need to know whether hemp should be harvested before flowering or before the plants become too tall.

7 Objective and structure of the thesis

7.1 Problem definition

The main objective of this thesis is to outline the agronomic options for manipulating plant development and crop growth of fibre hemp, in order to produce high–quality long textile fibres. We need to know how the amount of primary fibres with the desired quality can be maximised within a plant or crop, while at the same time contamination of these valuable fibres with the unwanted secondary fibres must be avoided.

At the start of this thesis research many contrasting results were found in the literature about factors that would or would not affect the primary fibre content. Important factors seemed to be sowing density, sowing date, harvest time, variety, and site, but although many experiments had been conducted, literature was still far from conclusive.

Increased sowing densities have been reported to increase primary fibre percentages (Jakobey, 1965; Dempsey, 1975; Van der Werf *et al.*, 1995a; Cromack, 1998; Von Francken–Welz and Léon, 2003), but also to have no effect on primary fibre percentages at all (Van der Schaaf, 1963; Van der Schaaf, 1966; Höppner and Menge–Hartmann, 1994; Vetter *et al.*, 2002; Von Francken–Welz and Léon, 2003; Burczyk *et al.*, 2009). Delayed harvesting has been reported to increase (Van der Schaaf, 1963) and decrease (Schäfer and Honermeier, 2003) primary fibre percentages, or to have no effect at all (Burczyk *et al.*, 2009). Moreover, primary fibre percentages for a given variety vary widely within (e.g., Vetter *et al.*, 2002) and between (e.g., cf. Cromack, 1998; Mediavilla *et al.*, 1999; Vetter *et al.*, 2002) experiments.

Little seems to be known about the causal relationships between the primary production process, the visible or easily measurable characteristics of harvested hemp stems, and the amount and characteristics of the fibres that can be extracted (Ranalli and Venturi, 2004). Extraction of the fibres is inevitable if one wants to know the fibre content, as no correlations are known between the fibre content and the visible characteristics of the stems (Hoffmann, 1957).

The options for improvement of fibre yields and fibre quality hence are unknown as underlying processes are poorly understood. Therefore, a reliable raw material qualification system, on which pricing could be based, has not been developed yet (Van Dam, 1999; Amaducci, 2003; Traina and Rondi, 2006; Van Dam and Van den Oever, 2006), and agronomic choices largely are made based on practical experience, local traditions, hearsay, and unproven rules of thumb.

7.2 Approach

In Chapters 2, 3, and 4 experiments are described in which we investigated which factors or interactions between factors determine the amount of primary fibres in hemp stems, using a new approach.

Fibres give mechanical support to the plants hence the amount of fibres present is likely to be related to the size of the plant and the forces the plant is subjected to. It is questionable therefore, whether the effects of sowing density, sowing date, harvest time or any other factor affecting the size of the plants, are essentially different. It could also be an indirect effect: these factors all affect stem weight and thereby fibre content. To determine whether the factors affect plant characteristics independently, or through similar mechanisms based on their effects on biometry, their interactions are studied.

Because fibre quantity (Bredemann, 1940; Hoffmann, 1957; Van der Werf *et al.*, 1994b; Amaducci *et al.*, 2008a) and quality (Cappelletto *et al.*, 2001; Amaducci *et al.*, 2008a) show patterns along the stem which possibly interact with the other factors that are investigated, differences between stem parts are also taken into account. It is necessary anyway to cut the stems, as complete stems cannot be processed with our equipment: processed stem parts need to be of equal length.

In contrast to many other fibre hemp experiments, retting losses were determined separately. Subsequently, we relate the fibre content to the dry weight of the stems after retting. This method avoids differences in retting losses to appear as differences in fibre content, and focuses on the ratio in which fibre and wood are produced. Possibly this ratio is a variety characteristic, whereas retting losses presumably are different for different stem parts and harvest times. Basically, the method we have chosen, provides a split up of the hemp stems into fibres, wood, and retting losses, whereas in other publications the wood and the retting losses are considered as a whole.

Quality is of highest importance when hemp fibres are to be used for textile purposes. Yarn spinners have high demands with respect to fibre characteristics as fineness, refinability, strength, fibre length distribution, homogeneity, and cleanliness (low shiv content) (Sultana, 1992; Nebel, 1995; Van Dam, 1999; Wulijarni–Soetjipto *et al.*, 1999; Allam, 2004; Anonymous, 1994b; Hann, 2005; Sponner *et al.*, 2005).

To introduce hemp long fibres into the fashion textile sector, fibres should be produced allowing the spinning of yarns between Nm 20 and Nm 40 (Nebel, 1995; Ranalli and Venturi, 2004). Nm is the metric yarn number: the yarn length in meters per 1 gram of mass ($m \cdot g^{-1}$) . The finer the yarns that can be spun, the higher the value of the raw material is (Allam, 2004; Ranalli and Venturi, 2004; Van Dam and Van den Oever, 2006).

Along the production chain from harvested hemp stems to such high–quality textile yarns, however, most of the fibre material is lost, especially during hackling and scutching (Sponner *et al.*, 2005; Tofani, 2006). Although the waste products of the different processing steps can be used for lower value applications, with respect to high–quality yarn spinning they should be considered worthless. Therefore, measuring fibre characteristics as fineness in scutched hemp, the end product of the experiments described in this thesis, is not adequate to describe the fitness of the fibres for high–

quality yarns.

The fibre characteristics are only important with respect to the fibres that have passed all processing steps before spinning. The first step in which fibres are lost is the scutching procedure, hence only the long fibres surviving this step are valuable. Their share in the total fibre fraction can be considered a quality parameter of the raw material (Hoffmann, 1957; Allam, 2004). Therefore this long fibre/total fibre ratio is determined.

Because secondary fibres are detrimental for high–quality yarn spinning, it should be known how to produce fibre hemp without these short fibres. In literature indications are found that secondary fibre formation depends on flowering (Mediavilla *et al.*, 2001a; Schäfer and Honermeier, 2003), but it seems more likely that the increasing plant size triggers secondary fibre formation. Therefore the presence or absence of secondary fibres along the hemp stem in relation to the flowering status and plant size will be taken into account as the second quality parameter in this research.

7.3 Outline of the thesis

Chapter 2 – Sowing density and harvest time affect fibre content in hemp (*Cannabis sativa* L.) through their effects on stem weight

Sowing density and harvest time are considered very important factors affecting the quality of hemp fibres and the productivity of fibre hemp, but although many experiments have been conducted, the optimal combination is still unknown. To determine whether sowing density and harvest time affect plant characteristics independently or through similar mechanisms based on their effects on biometry, their interactions were studied.

In Chapter 2, it is demonstrated that the amount of fibre in a hemp stem part is almost completely determined by the weight and the position of that stem part. When the plant grows, the increase in dry matter is split up into fibres and wood in a fixed way. Sowing density and harvest time effects on fibre content are indirect: through stem weight.

Chapter 3 – Postponed sowing does not alter the fibre/wood ratio or fibre extractability of fibre hemp (*Cannabis sativa* L.)

For reasons of fibre quality (low secondary fibre content) as well as suitability for processing (processing on existing flax scutching and hackling lines), it could be interesting to grow shorter hemp crops. Because hemp is a short–day plant, postponing the sowing date might be a suitable strategy to obtain smaller plants around flowering, when primary fibres are ‘ripe’ enough to be harvested.

In Chapter 3, it is shown that postponing the sowing date does not alter the ratio in which a fibre hemp variety produces fibre and wood and that it technically should be possible to grow two successive textile hemp crops to compensate for the lower stem yields of shorter crops.

Chapter 4 – Site does not affect the fibre content ranking order among fibre hemp (*Cannabis sativa* L.) varieties

In growing fibre hemp for textile applications selecting the right fibre hemp variety is very important, as it affects fibre content, fibre quality as well as stem dry matter yield. Although some varieties consistently show relatively high fibre percentages, whereas other varieties are known for their consistently low fibre content, the absolute values for a given variety vary widely within and between experiments.

In Chapter 4, it is shown that previously reported genotype × environment interactions with respect to fibre percentages presumably are largely due to the method of analysis. When fibre percentages are based on the dry weight of stems after retting and retting losses are calculated separately, the effect of the environment on the fibre content of varieties, if any, is very small and for practical reasons can be neglected.

Chapter 5 – Plant size determines secondary fibre development in hemp (*Cannabis sativa* L.)

In literature indications are found that with the induction of the generative phase the height up to which secondary fibres are present in hemp stems increases quickly. However, the formation of these unwanted fibres could also be related to plant size or weight. A greenhouse experiment was conducted, in which flowering and plant size effects on secondary fibre growth were disentangled. The short–day response of the plant was used to produce the required range of plant sizes.

In Chapter 5, it is shown that flowering as such does not induce secondary fibre formation in hemp; it is again the size of the plant that matters.

Chapter 6 – Limited options to manipulate fibre content in hemp (General discussion)

In the final chapter, the obtained knowledge is condensed and considered in a broader perspective.

This thesis shows that given variety and plant size, the amounts of primary fibres, wood, and retting losses and the height up to which secondary fibres can be found are determined to a high degree. Options to manipulate are very limited. The main tasks of the primary producer hence are choosing the right variety and performing crop management aimed at controlling plant size in order to avoid the presence of secondary fibres above stubble height.

Chapter 2

Sowing density and harvest time affect fibre content in hemp (*Cannabis sativa* L.) through their effects on stem weight

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Abstract

Sowing density and harvest time are considered important crop management factors influencing fibre quantity and quality in hemp (*Cannabis sativa* L.). We investigated whether the effects of these factors are essentially different or that both factors affect stem weight and thereby total and long fibre content. The effects of all combinations of three sowing densities and three harvest times were studied for six different stem parts. Almost 500 samples consisting of stem parts from 50 plants and with a length of 50 cm were tested. Fibres were extracted by a controlled warm–water retting procedure, followed by breaking and scutching. The initial sample weight was fractionated into retting losses, wood, tow, and long fibres. In both Italy and the Netherlands, crops were successfully established, with different stem densities (99–283 m^{-2}), plant heights (146–211 cm), and stem diameters (4.5–8.4 mm) at harvest. Stem dry matter yields (6.8–11.7 Mg ha^{-1}) increased with a delay in harvest time but were not affected by sowing density. Retting loss percentages were lower in lower stem parts and decreased with later harvest. Within a certain stem part, however, the absolute retting losses were constant with harvest time. Multiple linear regression analyses showed that the amount of fibre in a hemp stem part is almost completely determined by the weight and the position of that stem part. When the plant grows, the increase in dry matter is split up into fibres and wood in a fixed way. This total fibre/wood ratio was highest in the middle part of the stem, and lower towards both bottom and top. Sowing density and harvest time effects were indirect, through stem weight. The long fibre weight per stem part increased with the total fibre weight per stem part and hence with stem part weight. Stem weight increased with harvest time and as harvest time did not affect plant density, the highest long fibre yields were obtained at the last harvest time. The long fibre/total fibre ratio was lowest in the bottom 5 cm of the stems, but similar for all other stem parts that were studied. Sowing density and harvest time effects again were indirect. Fibre percentages in retted hemp decreased with increasing stem weights, towards a level that is presumably a variety–characteristic. The dry matter increase between harvests, however, is much more important with respect to total and long fibre yield.

Key words: *Cannabis sativa* L., decorticating, fibre hemp, fibre percentage, fibre quality, production chain, retting, scutching, textiles, tow.

1 Introduction

There are many contrasting results presented in the literature about factors that would or would not influence the quality of hemp fibres and the productivity of fibre hemp (*Cannabis sativa* L.).

Sowing density and harvest time are considered very important factors (Jakobey, 1965; Dempsey, 1975; Bócsa and Karus, 1998; Struik *et al.*, 2000; Amaducci *et al.*, 2008a), but although many experiments have been conducted, information in the literature is still far from conclusive with respect to the optimal sowing density and harvest time, or the optimal combination thereof.

With a new approach, we investigated whether the effects of sowing density and harvest time are essentially different, or that both factors affect stem weight and thereby fibre content. To our knowledge, the interaction between factors has never been studied in such detail before in hemp, or in other bast fibre crops. A renewed interest in high–quality hemp fibres, however, makes a thorough, botanically sound underpinning of production strategies relevant. We need to know how the amount of fibres with the desired homogeneity and quality can be maximised within a single plant and within a crop. In this paper, we present a conceptual model and field data to underpin the proposed model.

1.1 A competitive fibre production chain

To be competitive in the world fibre market, farmers in the European Union have to provide tailor–made raw materials with high added value for specialised niche markets. The flax (*Linum usitatissimum* L.) fibre production chain for high–quality linen in Western Europe has shown that only with high–quality standards it is possible to compete with imported raw materials from outside the European Union (Van Dam, 1999).

The highest added value in fibre hemp production can be obtained by producing high–quality ‘long’ fibres for the finest yarns for fashion textiles, a luxury niche market (Van Dam, 1999; Cappelletto *et al.*, 2001; Amaducci, 2003; Ranalli and Venturi, 2004). Although the world market demand for natural bast fibres for high–

value textile applications is growing, and the market conditions to relaunch hemp fibre in the textile production chain are favourable (Amaducci, 2003; Esposito and Rondi, 2006), there is at present no large–scale production of high–quality hemp yarns and fabrics in Western Europe. First because primary producers cannot supply the homogeneous high–quality raw material the processors require, and second because fibre processing is suboptimal (Amaducci, 2003; Esposito and Rondi, 2006; Traina and Rondi, 2006).

1.2 Quality from crop to fibre

The spinning industries have high demands with respect to the fibre characteristics fineness, refinability, strength, and homogeneity, as these determine the quality of the yarns that can be spun (Sultana, 1992; Van Dam, 1999; Hann, 2005; Sponner *et al.*, 2005). However, little is known about the causal relationships between the primary production process, the visible or easily measurable characteristics of harvested hemp stems, and the characteristics of the fibres that can be extracted. Therefore, a reliable raw material qualification system, on which pricing could be based, has not been developed yet (Van Dam, 1999; Amaducci, 2003; Traina and Rondi, 2006).

To stimulate the development of a competitive hemp fibre textile production chain, and to provide relevant decision support to primary producers, it is necessary to identify those plant and crop characteristics that determine hemp fibre quality. Quality should be acknowledged as a critical factor through the entire production chain.

1.3 Sowing density and harvest time

Increased sowing densities have been reported to increase fibre percentages (Jakobey, 1965; Cromack, 1998), but also to have no effect on fibre percentage at all (Van der Schaaf, 1963; Höppner and Menge–Hartmann, 1994; Vetter *et al.*, 2002). Delayed harvestings have been reported to increase (Van der Schaaf, 1963) and decrease (Schäfer and Honermeier, 2003) primary fibre percentages or to have no effect at all (Burczyk *et al.*, 2009).

The same ambiguity is present for the percentage of bark or bast, the tissue in which the fibres are embedded. To increase the fibre percentage, the bast percentage should be increased (Hoffmann, 1957), but Lisson and Mendham (2000) showed that the bast percentage was not affected by sowing density in a range of 50 – 300 plants m^{-2}, and based on a regression analysis of the same data they even suggested a small linear decline with increasing sowing density. Van der Werf *et al.* (1994a,b), however, concluded that the bast percentage depends on sowing density and harvest time and their interaction, and that the bast percentage increases with increasing sowing density, earlier harvest, and decreasing stem weight. Interactions in their experiments, however, are difficult to interpret, because of limitations to the experimental design, the small sample size, and because the fibre/bark ratio is unknown.

It is questionable whether the effects of sowing density and harvest time are essentially different or that both factors affect stem weight and thereby fibre content. Sowing density affects interplant competition and thus plant biometry (plant height, stem diameter, and plant weight) and population characteristics (size distribution and self–thinning). However, plant and crop characteristics also change during the growing season because of growth and development. Biometry, population characteristics (plant density and size distribution), and physiological stage (generative or vegetative, fibre ‘ripeness’) therefore will be different at different harvest times. To determine whether sowing density and harvest time affect plant characteristics independently or through similar mechanisms based on their effects on biometry, their interactions should be studied.

1.4 Stem part

Fibre quantity (Bredemann, 1940; Hoffmann, 1957; Van der Werf *et al.*, 1994b; Amaducci *et al.*, 2008a) and quality (Cappelletto *et al.*, 2001; Amaducci *et al.*, 2008a) show patterns along the stem, and these possibly interact with the effects of both sowing density and harvest time. Hence, to determine what the ideal crop looks like, when it should be harvested, and which part of the crop should be used to produce high–quality yarns, any patterns along the stem should be taken into account.

1.5 Sowing density × harvest time × stem part

In this paper, we therefore analyse the effects of all combinations of three sowing densities and three harvest times on the fibre quantity and quality of six different stem parts. We chose sowing densities in a range that is considered appropriate for textile destinations (Amaducci *et al.*, 2002a). We chose harvest times around flowering, because these are generally recommended (e.g., Dempsey, 1975; Bócsa and Karus, 1998), not only because of the development or 'ripeness' of the primary fibres and the expected yield, but also because of quality aspects beyond the scope of this paper, for instance lignin and secondary fibre content. We chose stem parts with a length of 50 cm, which is the minimum length our scutching device requires, to describe the patterns along the stem in as much detail as possible.

1.6 Sites

The experiments were conducted at two sites with contrasting environmental characteristics (Table 1), because in the past differences between sites were reported to affect plant biometry, stem yield, and fibre content (Schulz, 1998; Lisson and Mendham, 2000; Struik *et al.*, 2000; Vetter *et al.*, 2002).

Table 1. Site–specific information for Cadriano (I) and Achterberg (NL).

	Cadriano (I)	Achterberg (NL)
Latitude, longitude	43°33' N, 11°21' E	51° 58' N, 5°35' E
Soil	Silt–clay–loam	Sand
Preceding crop	Wheat	Spring barley
N applied (kg ha^{-1})	60	40
Organic matter (%)	1.7	4.1
Soil pH in H_2O	7.2	5.6
Sowing date	6 April	29 April
Harvested area per plot	5 m^2	2 m^2
Border	2.5 m	1 m

1.7 Fibre processing

Processing of fibre hemp into high–quality yarns in principle is similar to linen production from flax (Sponner *et al.*, 2005), and production for a niche market implies the use of the linen production chain and systems for manufacturing hemp yarns (Esposito and Rondi, 2006; Traina and Rondi, 2006). Hann (2005) and Salmon–Minotte and Franck (2005) accurately described this linen production chain.

After harvesting, the fibres have to be liberated from the surrounding tissues by retting the stems, a process in which moulds (dew retting) or bacteria (water retting) degrade pectic substances. In addition, other substances, for example proteins, sugars, starch, fats, waxes, tannins, and minerals are removed (Hann, 2005). Cellulose is not decomposed easily, and hence, the woody part of the stems and the cellulose–filled fibre bundles survive retting. These two components can be separated from each other in two mechanical steps, breaking and scutching (Hann, 2005; Salmon–Minotte and Franck, 2005; Sponner *et al.*, 2005).

Because of the small sample size as compared to industrial processing, we use a 'traditional' way to process hemp or flax stems into hands of long fibre: after breaking the stems on a flax breaker, scutching is performed on a Flemish mill (Van den Oever *et al.*, 2003). However, with respect to the procedure and its final products our method is comparable to modern scutching turbines.

1.8 Fibre percentage

Bast fibre yield or fibre quantity in general can be assessed based on the stem dry matter yield and the fibre percentage (Sankari, 2000). Several authors (Bredemann, 1940; Höppner and Menge–Hartmann, 1994; Van der Werf *et al.*, 1994b; Sankari, 2000; Amaducci *et al.*, 2008a) calculated fibre percentages based on the 'Reinfaser' method of Bredemann (1922). However, they did not determine the weight of the woody part of the stems for which a method is described in the same paper. Presumably because the importance was not recognized and the method is rather laborious. Consequently, retting losses are unknown, and the weight of the fibres can only be related to the total weight of the air–dried stems prior to fibre extraction.

The calculated fibre percentage, to our opinion, is an inadequate variable to compare crop management factors or combinations thereof and to understand underlying botanical processes, because no distinction is made between the wood and the materials that are lost during retting; they are considered as a whole. We want to study these amounts independently, because we expect that the amounts of fibre and wood are interrelated, whereas retting losses presumably are different for different stem parts and harvest times (see below). Therefore, in our analysis, the total weight of harvested stems is subdivided into amounts of retting losses, wood, and fibre.

1.9 Fractionation into retting losses, wood, and fibre

During processing from harvested stems to long fibres, three subdivisions are made between main product and waste or by–product. Fibres are extracted by a controlled warm–water retting procedure to avoid the extreme weather dependency that comes along with field retting (Dempsey, 1975; Van Dam, 1999; Hann, 2005; Salmon–Minotte and Franck, 2005; Sponner *et al.*, 2005), and because it enables us to determine retting losses per treatment under controlled conditions.

After retting, the stems merely consist of fibres and wood (Hann, 2005). These are separated from each other during breaking and subsequent scutching. While scutching, another subdivision is made as well; fibres either remain in the valuable long fibre bundle or fall away as scutching tow. ‘Tow’, the fibre material beaten out of the bundles, can be used for other applications, but not for long fibre spinning. The long fibre/total fibre ratio after scutching therefore characterises the quality of the raw material (Hoffmann, 1957). When retting losses and the total fibre amounts are known, the fractionation of total dry matter into fibres and wood can also be calculated.

1.10 A botanical model

For different combinations of sowing density, harvest time, and stem part, we expect to find differences in the relative and absolute amounts of retting losses, long fibre, tow, and total fibre. Figure 1 schematically shows our main expectations. Especially between different stem parts, we expect differences in retting loss percentages,

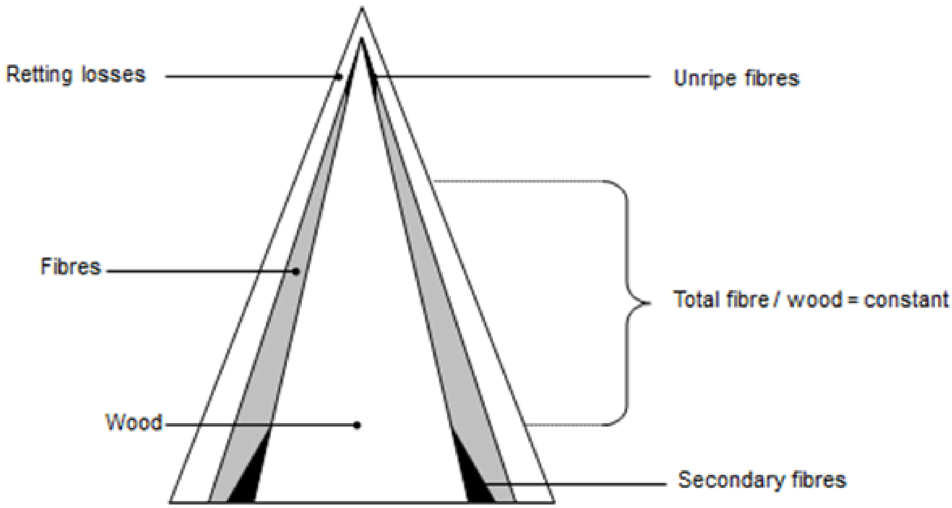


Figure 1. A botanical model of a hemp stem.

because the top part is relatively herbaceous, whereas the bottom part is relatively woody. Cappelletto *et al.* (2001) showed the differences in chemical composition.

The hemp fibres used for spinning yarns are the primary bast or phloem fibres. The single fibre cells are organised in bundles, which run longitudinally along the stem from bottom to top and can reach almost the full length of the plants (Van Dam and Gorshkova, 2003). As plants age, these primary fibres are gradually filled with cellulose, and their strength depends on the cellulose filling degree or 'ripeness' which progresses with time from bottom to top (Mediavilla *et al.*, 2001; Schäfer and Honermeier, 2003; Amaducci *et al.*, 2005, 2008a). 'Ripeness' in this sense is not so much a physiological stage, but a threshold value above which the fibres are strong enough to allow proper extraction.

We do not expect to find a 'ripeness front' below which fibre filling has ceased, because Amaducci *et al.* (2005, 2008a) showed that even in the lowest internode fibre filling continues between full flowering and end of flowering. Possibly, the weight increase of the primary fibres in a certain stem part keeps pace with the weight increase of the wood, in which case the fibre/wood ratio would be constant with harvest time. The ratio in which total dry matter is split up into fibres and wood in a fixed way might be a variety characteristic.

We expect different total fibre/wood ratios along the stem. Bredemann (1940) showed that the fibre percentage differed along the stem with highest percentages in the middle of the stems and lower percentages towards both bottom and top. A more detailed analysis by Van der Werf *et al.* (1994b) confirms these results but also suggests that the fibre percentage is practically stable at a maximum level that is found in the range between 10–20% and 50–60% of the total plant height at seed ripeness.

In the experiments of Bredemann (1940) and Van der Werf *et al.* (1994b), retting losses were not taken into account; therefore, it is questionable whether the pattern described is also present in the total fibre/wood ratio along the stem. Although sampling on length percentiles has an advantage when studying the patterns along the stem into detail, the disadvantages for our purposes are insuperable, since retting losses and the weight of the wood are not determined separately and no difference can be made between long fibres and tow. Besides the samples cannot be processed any further, because fibres are not aligned anymore and their chemical composition has been changed. The method we use does not have these disadvantages. However, scutching requires stem parts of equal length (see Materials and methods).

The long fibre/total fibre ratio is expected to be lower in the bottom part of the stems, because this part contains more tow (Cappelletto *et al.*, 2001), which is presumably related to secondary fibre forming in the basal internodes (Amaducci *et al.*, 2005, 2008a; Hernandez *et al.*, 2006).

1.11 Hypotheses

We will test the following hypotheses:

1. Retting loss percentages decrease as stem parts mature because maturation is associated with increasing amounts of fibres and wood, whereas the amount of material that is lost during retting is constant. Retting loss percentages therefore are lower in lower stem parts and decrease with a delay in harvest.
2. Sowing density and harvest time affect fibre content through their effect on stem weight only.

3. The total fibre/wood ratio per stem part does not depend on sowing density and harvest time.
4. The total fibre/wood ratio is highest in the middle part of the stems and lower towards bottom and top.
5. The long fibre/total fibre ratio decreases from the middle part of the stems towards the bottom.

2 Materials and methods

2.1 Experimental design

Field experiments were carried out at Azienda Università Bologna in Cadriano (Italy) and Achterberg (the Netherlands) in 2005. The experimental set–up at both sites was a completely randomized four–replicate split–plot design, with three plant densities as main plots, and three harvest times as sub–plots. Site–specific information is given in Table 1. Applied nitrogen fertiliser doses on both sites were based on successful hemp experiments on the same sites (Struik *et al.*, 2000; Amaducci *et al.*, 2002b).

Seeds of *Cannabis sativa* L. cv. Futura 75, a late–maturing monoecious variety, originated from the same batch purchased from La Fédération Nationale des Producteurs de Chanvre, Les Mans, France. Seeds were sown with a precision drill at a depth of approximately 3–4 cm, at target plant populations of 120, 240, and 360 plants m^{-2} (coded D120, D240, and D360). The crop was not thinned when target densities were exceeded. Distance between rows was 12.5 cm. No biocides were used. The crop was not irrigated.

2.2 Harvests

At harvest, stems were cut at soil level. Dead plants and shed leaves were not collected. In Italy, harvests took place when 50% of the plants had reached a predetermined stage of growth: beginning (H1), full (H2), and end (H3) of flowering (Mediavilla *et al.*, 2001b). In the Netherlands, H2 was planned at the time when 50% of the plants ≥ 100 cm were flowering, meaning that at least one flower, either male or female, was open. This moment was predicted based upon flowering dates in earlier experiments. H1 took place two weeks before this date, and H3 two weeks after this date.

2.3 Post–harvest measurements

Per harvested plot, 100 (the Netherlands) or 50 (Italy) plants were randomly taken and measured for stem diameter at 10 cm above soil level, and for plant height. In the Netherlands, also flowering status per plant was recorded. Per harvested plot, plants were divided into two groups: plants with a height ≥ 100 cm and smaller plants. Fresh weights of both groups were determined, and the number of plants per group was counted. The smaller plants or 'underhemp' were discarded (Hoffmann, 1957; Lisson and Mendham, 2000; [Chapter 1]).

Plants with a height ≥ 100 cm were further processed. The dry weights of both stems and remainder, i.e. leaves and inflorescences, were determined on 20 of these plants following drying for 24 hours at 105 °C in an oven. The other plants were dried on a drying floor for 4 days at 27 °C, in order to prevent them from decaying during storage. After storage, stems were prepared for extracting the fibres.

2.4 Preparation of the stems

Scutching requires stem parts of equal length (see Fibre extraction), and the minimum length required for our Flemish mill is about 50 cm. To examine the fibre development along the stem, per harvested plot, 200 stems were randomly taken and were defoliated. Four comparable groups of 50 stems were composed with about the same size distribution and total weight (Figure 2A).

Two of these groups were used to study the bottom part of the stem. Stems were laid parallel with the bottom parts at equal height. From the first group, the stems were cut into a B0–50 cm part and the subsequent B50–100 part, where B0 is soil level. From the second group, the B25–75 cm part was cut. The other two groups were used to study the top parts of the stem. Stems were laid parallel with the tops at equal height. From one group, the 50 stems were cut into a T0–50 cm part and a T50–100

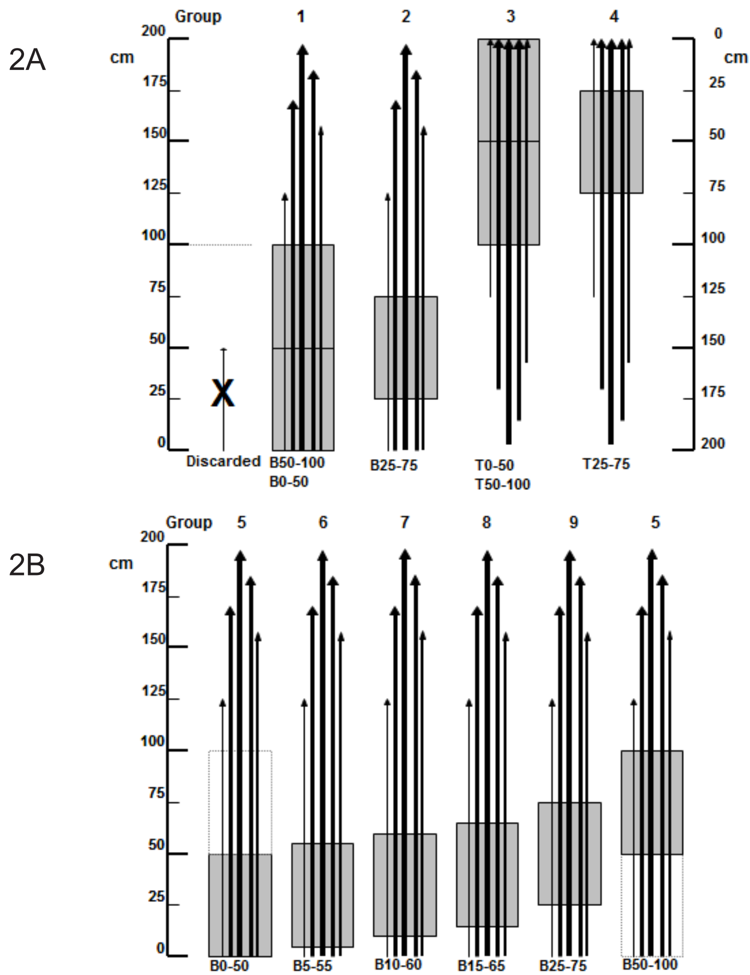


Figure 2.
A) Stem partitioning in the main experiment. Plants smaller than 100 cm were discarded (×). Per harvested plot, 200 stems were used. Four comparable groups of 50 stems (only 5 per group are drawn) were composed. Groups 1 and 2 were used to study the bottom part of the stem. Stems were laid parallel with the bottom parts at equal height. From group 1, the stems were cut into a B0–50 cm part and a B50–100 part. From group 2 the B25–75 cm part was cut. Groups 3 and 4 were used to study the top parts of the stems. Stems were laid parallel with the tops (▲) at equal height. From group 3, the stems were cut into a T0–50 cm part and a T50–100 cm part. From group 4, the T25–75 cm part was cut.
B) Stem partitioning in the detailed bottom part experiment. As in the main experiment the plants smaller than 100 cm were discarded (not shown). Six comparable groups of 50 stems were composed, with about the same size distribution and total weight. Stems were laid parallel with the bottom parts at equal height. From the different groups, stems were cut into B0–50 (group 5), B5–55 (group 6), B10–60 (group 7), B15–65 (group 8), B25–B75 (group 9), and B50–B100 stem parts (group 5). Samples were tied up with tie–ribs and remainders were discarded.

cm part, where T0 is the utmost top of the plant. From the other group, the T25–75 cm part was cut. Samples were tied up with tie–ribs and remainders were discarded.

2.5 Fibre extraction

Fibre extraction consisted of four subsequent steps: (A) retting, (B) breaking, (C) scutching, and (D) cleaning. Before retting, before breaking, and after cleaning, weighing took place to determine respectively the initial weight, the retting losses, and the amounts of long fibre and tow. The weight of the wood was estimated by subtracting retting losses and total fibre weight from the stem part weight before retting. To compare the different batches properly, weighing was always preceded by conditioning the materials at 19 °C and 73% humidity for at least 48 hours (Van den Oever *et al.*, 2003), and machinery was never adjusted during the experiment.

A. Retting

Warm–water retting took place in cylindrical PVC tubes with open top and closed bottom, a height of 120 cm and a diameter of 16 cm. The six bundles originating from the same harvest plot were put together in the same cylinder. Prior to retting, the cylinders were filled with tepid tap water. This water, used to wash away solubles and irregularities such as dust and sand, was drained after 2 hours. The cylinders were then placed in a retting basin. Basin and cylinders were both filled with tap water of 34 °C up to a height of approximately 110 cm. Stems were completely submerged, but water exchange between cylinders was avoided. Retting was performed at 34 °C in 96 hours by the spontaneous enzymatic action of anaerobic bacteria. After retting, the bundles were carefully washed with tepid water. Excess water dripped off by placing the bundles vertically on a grating above a drain. Next, the bundles were dried on a drying floor for 4 days at 27 °C.

B. Breaking

To separate fibres and wood, the tie–ribs were removed and the stems were arranged in an even layer, and then fed into a flax breaker consisting of a double series of ribbed breaking rollers. These heavyweight rollers put pressure on the stems by means of a spring system. As a result, stems were flattened and the brittle wood was broken into shives, most of which fell through the machine, while the flexible fibres passed under the rollers easily.

C. Scutching

Scutching (decorticating) was performed on a Flemish mill with rotary blades that beat the broken stems in such a way that shives and tow were separated from the long fibres in the sample. Both sides of the samples, the upper and lower part of the stem part, were manually fed through the rotary blades eight times; after four times the bundle was turned inside out. Because the end of the sample has to be held in the hand while scutching the other side of the sample, all stem parts in a sample have to be of uniform length. If shorter stem parts were accepted, all the fibre material in these shorter parts, both long fibre and tow, would end up in the tow section. Our aim, however, was to distinguish between long fibre and tow.

D. Cleaning

After scutching, the long fibres and tow were cleaned by hand to remove any remaining shives. After fibre extraction, conditioning, and weighing, the amounts of long fibre and tow were determined and the weight of the wood was estimated. T0–50 tow was not cleaned, because tow could not easily be separated from the wood. Consequently, total fibre could not be calculated for this stem part.

2.6 Detailed bottom part experiment

To study the bottom part in more detail, an extra experiment was carried out in the Netherlands on the same field as the main experiment. The experimental set–up was a completely randomized design with four replicates and three plant densities (D120, D240, and D360), and one harvest on August 16, which was one day after H2 in the main experiment. Fibre extraction was the same as in the main experiment, cutting of the stems in 50 cm parts, however, was different (Figure 2B). Six comparable groups of 50 stems were composed with about the same size distribution and total weight. Stems were laid parallel with the bottom parts at equal height. From the different groups, stems were cut into B0–50, B5–55, B10–60, B15–65, B25–B75, and B50–B100 stem parts. Samples were tied up with tie–ribs and remainders were discarded.

2.7 Statistical analysis

Statistical analyses of the data ($P < 0.05$) were conducted using GENSTAT® release 9.2. Following tests for normality:

- Multiple linear regression analyses were performed for total fibre/wood ratio and for long fibre/total fibre ratio. Stepwise addition or subtraction of terms was carried out to define the most suitable model to use in general linear modelling i.e. the model with the minimum residual mean squares. B–part and T–parts were analysed separately. There were no statistical or biological reasons to test non–linear models.
- Analyses of variance (ANOVAs) were calculated for all other variables. Means, standard errors of differences of means (SEDs), and degrees of freedom were reported.

3 Results

In both Italy and the Netherlands, crops were successfully established with even stand densities. No signs of water or nutrient stresses were visible. Weeds were suppressed well by the crops, and pests and diseases of significance did not occur.

3.1 Plant density

At full emergence, plant densities were close to the target densities in the Netherlands (Table 2). Although some plants died because of crowding, differences between treatments persisted throughout the entire experiment at both sites. Harvest time did not affect plant density; density–related plant death at both sites took place before the first harvest. The plant density at harvest was higher in the Netherlands than in Italy;

the number of plants ≥ 100 cm m^{-2}, however, was not significantly different.

3.2 Timing of the harvest

In the Netherlands, the flowering percentage at H2 in plants ≥ 100 cm on average was 43%, which was close to the targeted 50%. H1 took place at the beginning of flowering (22%), and H3 took place at the end of flowering (81%). Flowering percentages were similar for sowing densities, with one exception. At H1, the flowering percentage in D120 (32%) was higher than in D360 (12%).

In Italy, the hemp was harvested at predetermined stages of growth (see Materials and Methods). Therefore, in Italy there were three weeks between H2 and H3 instead of two in the Netherlands. Because of this, and because of the larger daily temperature sum in Italy, the differences between harvests in terms of temperature sums were much larger in Italy than in the Netherlands (Table 3).

3.3 Plant biometry

Plant height varied with sowing density and harvest time (Table 4). The lower the sowing density and the later the harvest, the taller the plants were. There was no interaction with site, while for the processed plants ≥ 100 cm stems from Italy were smaller.

The percentage of plants ≥ 100 cm decreased with increasing sowing density and was higher in Italy than in the Netherlands. These processed plants account for 96.7–99.7% of the harvested volume, assuming the stems have a conical shape (Amaducci *et al.*, 2002).

Stem diameter showed an interaction between sowing density and site. The lower the sowing density, the thicker the stems were. In D240 and D360 both sites showed equal stem diameters, in D120, however, stems were thicker in the Netherlands. In Italy, a delay in harvest time resulted in thicker stems, whereas in the Netherlands harvest time did not affect stem diameter.

Table 2. Plant density at full emergence in the Netherlands, plant density at harvest, and the number of plants ≥ 100 cm m^{-2} at harvest in the Netherlands and Italy.

	Plant density (m^{-2})		
	Full emergence (NL)	Harvest	Plants ≥ 100 cm
D120	129	109	103
D240	261	199	183
D360	371	261	226
SED	7.5	6.3	7.0
d.f.	6	12	12
Italy	-	181	n.s.
The Netherlands	-	198	
SED	-	7.1	
d.f.	-	6	

SED, standard errors of differences of mean; n.s., not significant; d.f., degrees of freedom.

Table 3. Harvest dates, Temperature sums (growing degree days (GDD);°C days) at harvest, and stem dry matter yield (Mg ha^{-1}), across sowing densities for 3 harvests and 2 sites. Temperature sums were calculated using a base temperature of 2 °C (Van der Werf, 1997).

	Harvest date		Temperature sum (GDD; °C days)		Stem DM yield (Mg ha^{-1})	
	I	NL	I	NL	I	NL
H1	10–6	1–8	948	1149	6.8	9.2
H2	24–6	15–8	1182	1303	7.6	10.6
H3	14–7	29–8	1648	1476	10.4	11.7
SED					0.66	
d.f.					9	

SED, standard errors of differences of mean; d.f., degrees of freedom.

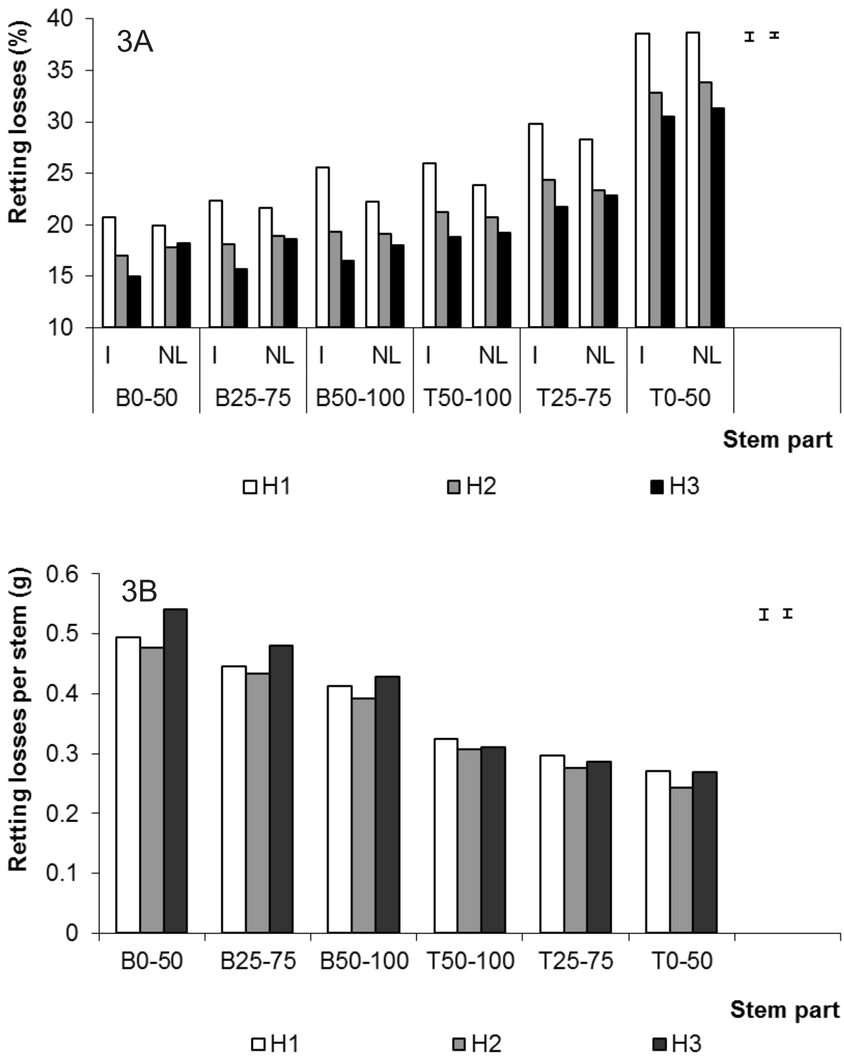
Table 4. Plant height and stem diameter for harvested and processed plants (≥ 100 cm), percentage of plants ≥ 100 cm and volume percentage of plants ≥ 100 cm.

	Harvested plants			Processed plants (≥ 100 cm)					
	Plant height (cm)	Stem diameter (mm)		Plant height (cm)	Stem diameter (mm)		%	Volume%	
		I	NL		I	NL		I	NL
D120	193	6.9	8.1	199	7.0	8.4	94.9	99.7	99.0
D240	176	5.9	6.1	183	6.0	6.4	93.8	99.7	98.2
D360	159	5.3	5.1	170	5.5	5.4	88.1	98.8	96.7
SED	4.5	0.23		4.06	0.22		0.01	0.00	
d.f.	12	17		12	17		12	14	
H1	165	5.4	6.4	171	5.5	6.7			
H2	177	6.0	6.2	186	6.1	6.6			
H3	187	6.8	6.6	194	6.9	6.9			
SED	3.2	0.23		2.58	0.22				
d.f.	36	24		36	30				
I				177			96.1		
NL				190			88.3		
SED				3.97			0.01		
d.f.				6			6		

SED, standard errors of differences of mean; d.f., degrees of freedom.

3.4 Retting losses

As expected (Hypothesis 1), retting loss percentages decreased as plants matured. Retting loss percentages were lower in lower stem parts and decreased with a delay of harvest. The effect of harvest time was more pronounced in Italy than in the Netherlands (Figure 3A).



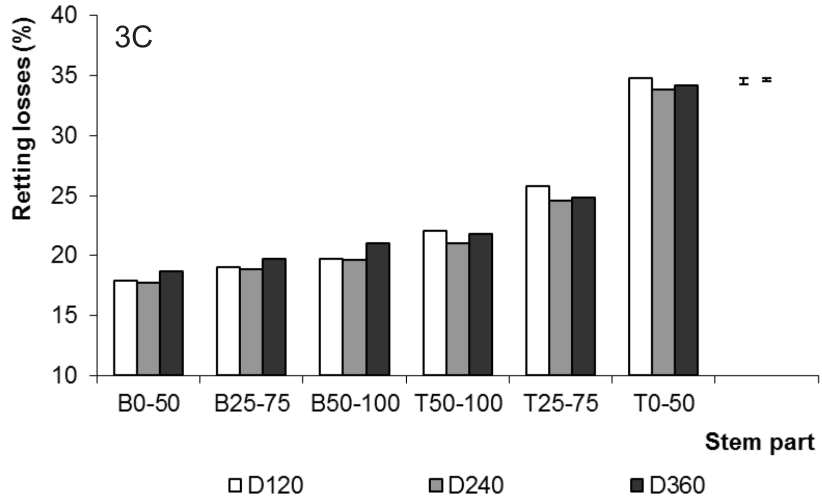


Figure 3.
A) Relative retting losses (%) per stem part across densities for 2 sites × 3 harvests. Bars indicate the overall SED and SED within site respectively (n = 4).
B) Absolute retting losses per stem (g) per stem part across densities and sites for 3 harvests. Bars indicate the overall SED and SED within harvest respectively (n = 8).
C) Relative retting losses (%) per stem part across sites and harvests for 3 sowing densities. Bars indicate the overall SED and SED within sowing density respectively (n = 8).
SED, standard errors of differences of mean.

The differences in retting loss percentages between harvests were mainly because of the weight increase of the stem parts. The amounts of materials that were lost during retting in a certain stem part were in the same order of magnitude (cf. Figure 3A and 3B). For these absolute retting losses, no interactions between site and harvest time were present. The effect of sowing density on retting loss percentages was small, and without a clear pattern (Figure 3C).

3.5 Total fibre/wood ratio

Because we expected that sowing density and harvest time only affect fibre content through their effects on stem weight (Hypothesis 2) and that the fractionation into fibre and wood per stem part does not depend on these factors (Hypothesis 3), the total fibre weight per stem part was plotted against the wood weight per stem part. Strong correlations became apparent, for both bottom and top (Figure 4).

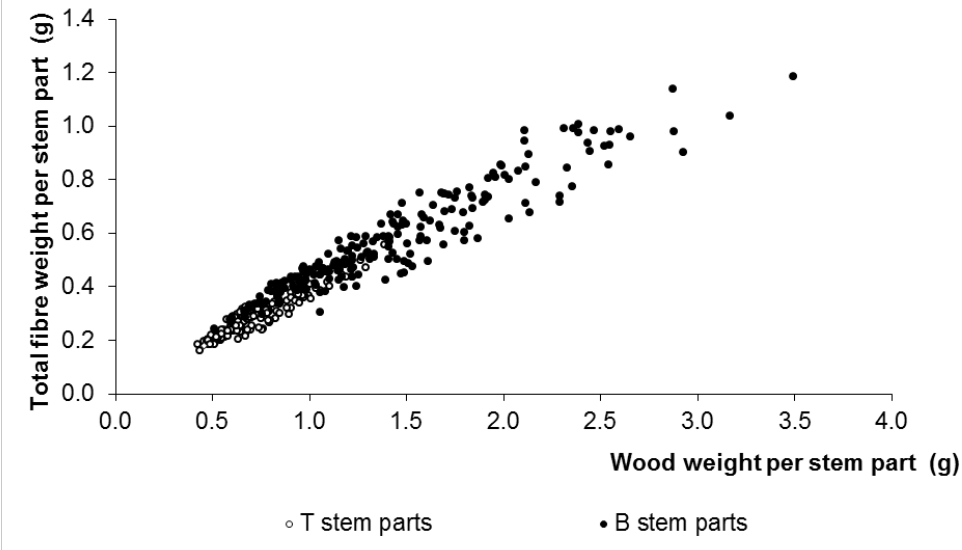


Figure 4. Total fibre weight per stem part against wood weight per stem part for all B stem parts, T25–75 and T50–100. $R^2 = 0.898$ (B) and 0.879 (T).

To determine whether sowing density and harvest time had an extra effect besides their effects through stem weight, multiple linear regression analyses were performed for both bottom and top, with factors sowing density, harvest time, stem part, and site. Stem part was taken into account because we expected differences between different stem parts (Hypothesis 4). Site was taken into account because differences in fibre content between sites were reported by several authors (see Introduction).

In Figures 5 and 7, we present both the total fibre/wood ratio and the fibre percentage based on the stem part weight after retting. These fibre percentages were derived from the linear regression analyses as follows:

Fibre percentage = total fibre/(total fibre + wood) × 100%
= ((a × wood weight per stem) + b)/((1+ a) × wood weight per stem + b) × 100%

The analyses (Table 5) showed that the factors wood weight per stem (1), stem part (2) and site (3), and their interactions together accounted for 95.6% (B) and 95.2% (T) of the variance in total fibre weight per stem. Sowing density and harvest time (4), and interactions with these factors together increased the percentage of variance accounted for with only 0.5% (B) and 0.9% (T) to 96.1% (B) and 96.0% (T). All these factors, however, contributed significantly to the model as tested by the variance ratios ($P < 0.05$). The following patterns occurred:

1. More wood, more fibre. The wood weight per stem part accounted for 89.8% (B) and 87.9% (T) of the variance in the total fibre weight per stem part (Figure 5A and Figure 5B), which means that an increased total fibre weight per stem part was mainly the result of an increased weight of that stem part (i.e. total fibre + wood).

2. Highest total fibre/wood ratios in the middle. The total fibre/wood ratio was highest in the middle part of the stems, and lower towards both bottom (Figure 5A) and top (Figure 5B). This general pattern was not fundamentally changed by effects of site, sowing density, and harvest time, because no interactions were present between stem part and these factors. As intercepts *b* were positive, the fibre percentage decreased with increasing wood weight per stem part.

3. Higher total fibre/wood ratios in Italy. For comparable treatments, the intercept *b* (B–part) or the slope a (T–part) is slightly higher for the Italian hemp. The total fibre percentage is also a little higher in Italy (Figure 6). General patters, however, were the same for both sites.

4. *Sowing density and harvest time effects were indirect.* Besides the effects via stem weight, the individual effects of sowing density (Figure 7A) and harvest time (Figure 7B) were marginal. When wood weight per stem is dropped from the regression models, however, sowing density and harvest time become the main factors explaining the total amount of fibres in a hemp stem part. Note that in Figures 7A and 7B the fibre percentages differed considerably with increasing stem part weight, while the ratio in which fibres and wood were produced did not.

Table 5. Multiple linear regression models for the main experiment.

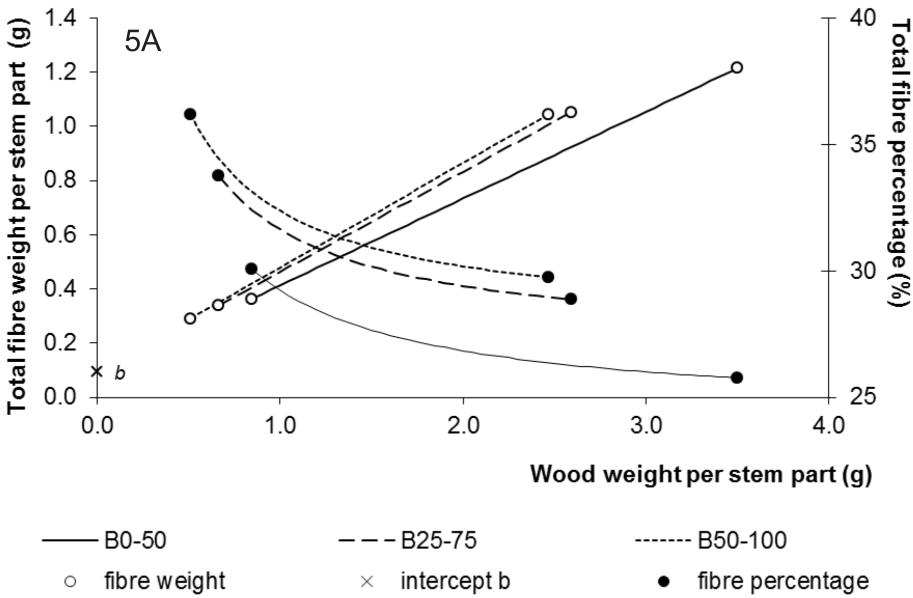
	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
5A) Total fibre/Wood (Bottom)	1. Wood weight per stem part	1	7.1688	7.1688	4953.92	<0.001	89.8
	2. Wood weight per stem part × Stem part	2	0.4040	0.2020	139.59	<0.001	94.8
	3. Site	1	0.0617	0.0617	42.66	<0.001	95.6
	4. Wood weight per stem part × Sowing density	2	0.0345	0.0173	11.92	<0.001	96.0
	5. Wood weight per stem part × Harvest	2	0.0106	0.0053	3.65	0.028	96.1
	Residual	207	0.2996	0.0015			
	Total	215	7.9791	0.0371			
5B) Total fibre/Wood (Top)	1. Wood weight per stem part	1	0.6929	0.6929	3136.94	<0.001	87.9
	2. Stem part	1	0.0385	0.0385	174.34	<0.001	92.8
	3. Wood weight per stem part × Site	1	0.0190	0.0190	86.11	<0.001	95.2
	4. Wood weight per stem part × Harvest	2	0.0072	0.0036	16.24	<0.001	96.0
	Residual	136	0.0300	0.0002			
	Total	141	0.7877	0.0056			

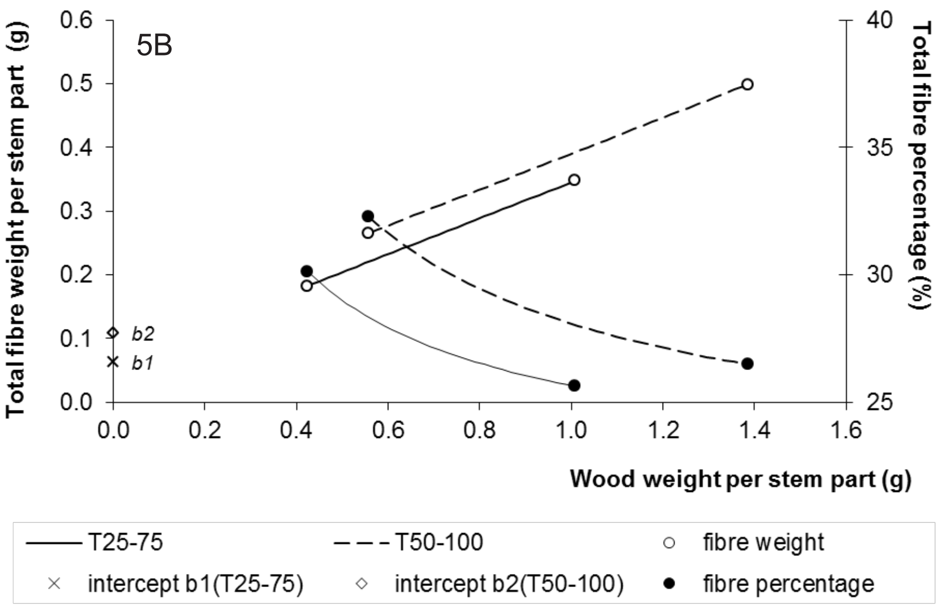
d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

Table 5 –continued–.

	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
5C) Long fibre/Total fibre (Bottom)	1. Total fibre weight per stem part	1	4.2561	4.2561	6084.99	<0.001	92.2
	2. Total fibre weight per stem part × Stem part	2	0.1675	0.0837	119.72	<0.001	95.8
	3. Total fibre weight per stem part × Site	1	0.0246	0.0246	35.11	<0.001	96.4
	4. Total fibre weight per stem part × Harvest	2	0.0058	0.0029	4.16	0.017	96.5
	5. Total fibre weight per stem part × Site × Harvest	2	0.0110	0.0055	7.85	<0.001	96.7
	6. Harvest	2	0.0048	0.0024	3.44	0.034	96.7
	Residual	205	0.1434	0.0007			
	Total	215	4.6131	0.0215			
5D) Long fibre/Total fibre (Top)	1. Total fibre weight per stem part	1	0.5882	0.5882	4283.86	<0.001	96.4
	2. Sowing density	2	0.0012	0.0006	4.37	0.015	96.5
	3. Total fibre weight per stem part × Sowing density	2	0.0021	0.0011	7.63	<0.001	96.8

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.


5A
Total fibre weight per stem part (g)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
b
0.0
1.0
2.0
3.0
4.0
Wood weight per stem part (g)
Total fibre percentage (%)
40
35
30
25
B0-50
B25-75
B50-100
fibre weight
intercept b
fibre percentage


5B
Total fibre weight per stem part (g)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
b2
b1
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Wood weight per stem part (g)
Total fibre percentage (%)
40
35
30
25
T25-75
T50-100
fibre weight
intercept b1(T25-75)
intercept b2(T50-100)
fibre percentage

◄ Figure 5.
A) Total fibre weight per stem part (open symbols, left Y-axis) and total fibre percentage (closed symbols, right Y-axis) against the wood weight per stem part for I–D120–H3 and stem parts B0–50 (a = 0.32), B25–75 (a = 0.37) and B0–50 (a = 0.39), all with intercept *b* = 0.09. I–D120–H3 is chosen as an example. The differences between stem parts are the same for the other treatments, but with a slightly different intercept (NL: –0.03) and/or slope (D240: –0.02, D360: –0.03, H1: +0.00, H2: +0.01). The wood weight per stem part range shown is the full range for the stem parts, irrespective of treatment.
B) Total fibre weight per stem part (open symbols, left Y-axis)) and total fibre percentage (closed symbols, right Y-axis) against the wood weight per stem part for I–H3, all sowing densities and stem parts T25–75 (b = 0.06) and T50–100 (b = 0.11), both with slope *a* = 0.31. The intercept difference between stem parts is significant (P < 0.05). I–H3 is chosen as an example. The differences between stem parts are the same for the other treatments, but with a slightly different slope (NL: –0.03, H1: –0.03, H2: –0.02). The wood weight per stem part range shown is the full range for the stem parts, irrespective of treatment.

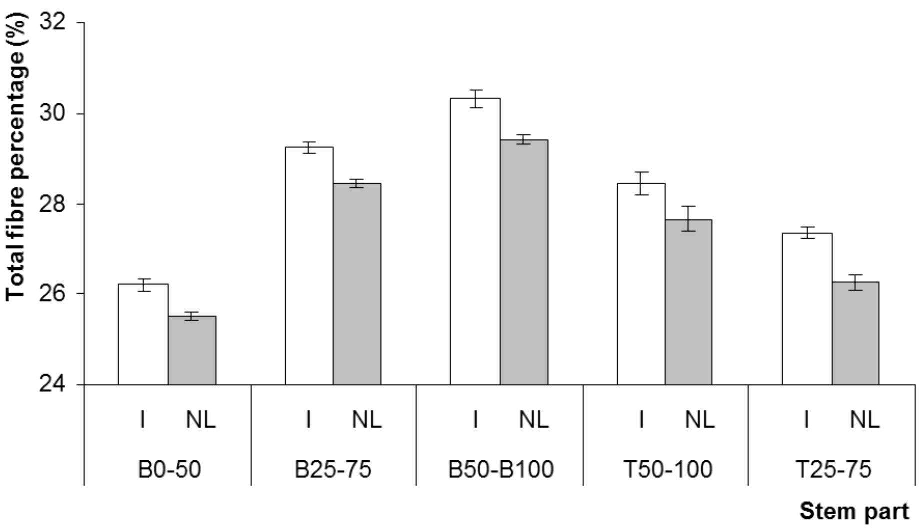
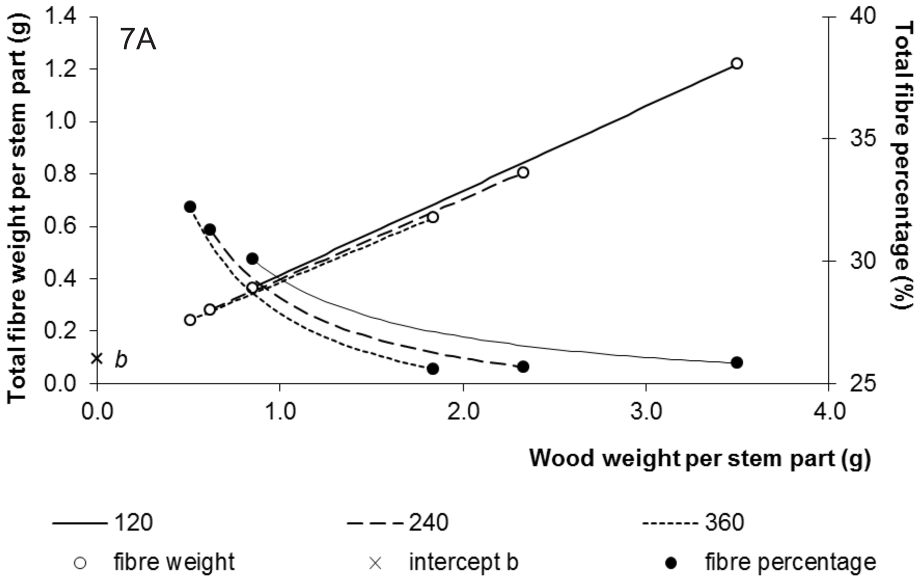
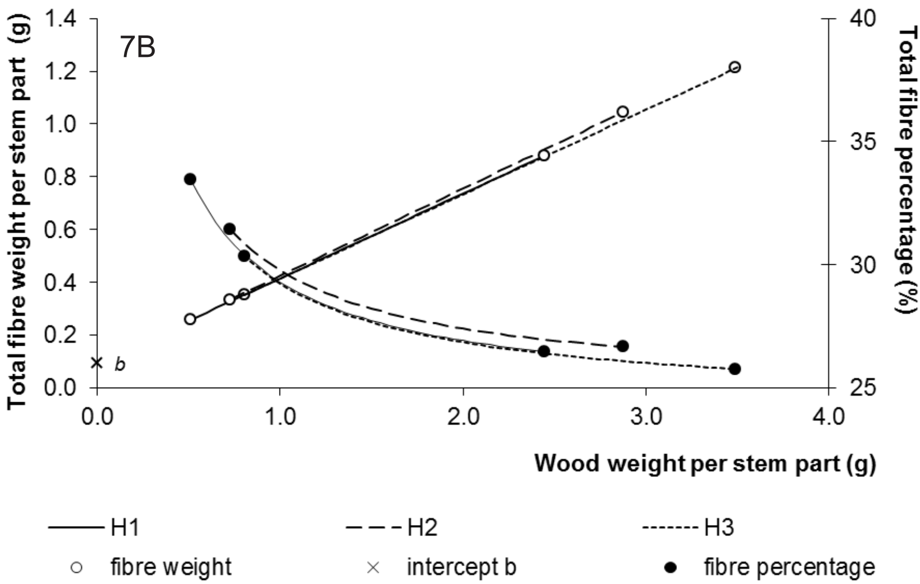


Figure 6. Total fibre as a percentage of total stem part weight after retting derived from the linear regression models for D120–H3 for 5 stem parts and 2 sites. Bars indicate standard error of mean.


7A
Total fibre weight per stem part (g)
Total fibre percentage (%)
Wood weight per stem part (g)
b
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
25
30
35
40
0.0
1.0
2.0
3.0
4.0
120
240
360
fibre weight
intercept b
fibre percentage

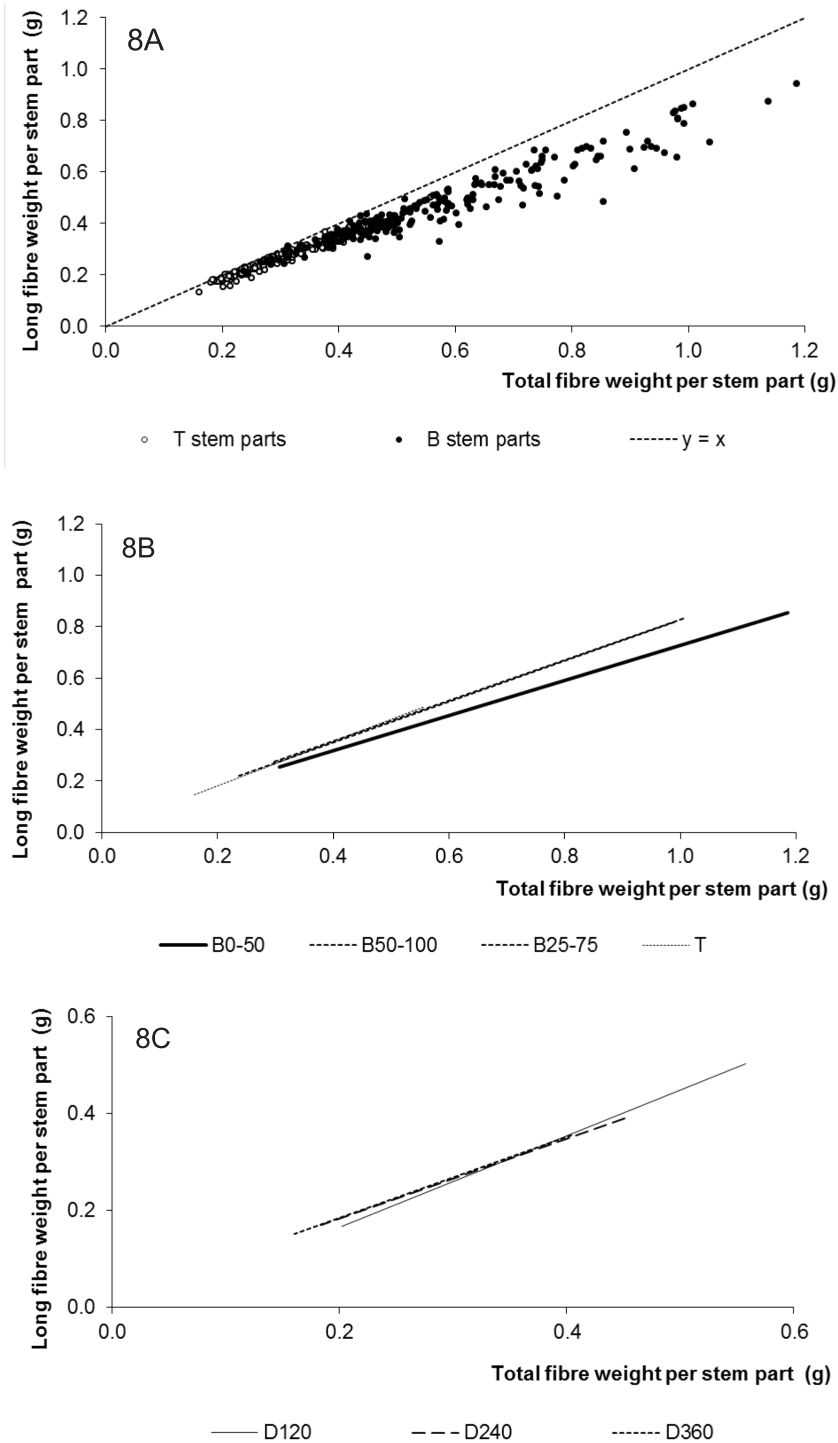

7B
Total fibre weight per stem part (g)
Total fibre percentage (%)
Wood weight per stem part (g)
b
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
25
30
35
40
0.0
1.0
2.0
3.0
4.0
H1
H2
H3
fibre weight
intercept b
fibre percentage

◄ Figure 7.
A) Total fibre weight per stem part (open symbols, left Y-axis) and total fibre percentage (closed symbols, right Y-axis) against the wood weight per stem part for I–H3–B0–50 and densities D120 (a = 0.32), D240 (a = 0.30) and D360 (a = 0.29), all with intercept *b* = 0.09. I–H3–B0–50 is chosen as an example. The differences between densities are the same for other treatments, but with a slightly different intercept (NL: – 0.03) and/or slope (B25–75: + 0.05, B50–100: + 0.07, H1: + 0.00, H2: + 0.01). The wood weight per stem part range shown is the full range for the densities, irrespective of treatment.
B) Total fibre weight per stem part (open symbols, left Y-axis) and total fibre percentage (closed symbols, right Y-axis) against the wood weight per stem part for I–D120–B0–50 and harvest H1 (a = 0.32), H2 (a = 0.33) and H3 (a = 0.32), all with intercept *b* = 0.09. I–D120–B0–50 is chosen as an example. The differences between densities are the same for other treatments, but with a slightly different intercept (NL: – 0.03) and/or slope (B25–75: +0.05, B50–100: +0.07, D240: –0.02, D360: –0.03). The wood weight per stem part range shown is the full range for the harvests, irrespective of treatment.

3.6 Long fibre/total fibre ratio

The long fibre/total fibre ratio after scutching is one of the main quality features of the raw material because it shows which share of the harvested fibre becomes available for the next processing step in long fibre production: hackling (Hoffmann, 1957). When the long fibre weight per stem part was plotted against the total fibre weight per stem part, strong correlations became apparent (Figure 8A). To determine which other factors affected the long fibre weight, again multiple linear regression analyses were performed with factors sowing density, harvest time, stem part and site (Table 5).

The analyses showed that the factors total fibre weight per stem part, stem part, and site, and their interactions together accounted for 96.4% of the variance in long fibre weight per stem for both B– and T–part. Sowing density and harvest time and interactions with these factors, together increased the percentage of variance accounted for with only 0.4% (B) and 0.5% (T) to 96.7% (B) and 96.8% (T). All these factors, however, contributed significantly to the model as tested by the variance ratios ($P < 0.05$). The following patterns occurred:


8A
Long fibre weight per stem part (g)
Total fibre weight per stem part (g)
T stem parts
B stem parts
y = x
8B
Long fibre weight per stem part (g)
Total fibre weight per stem part (g)
B0-50
B50-100
B25-75
T
8C
Long fibre weight per stem part (g)
Total fibre weight per stem part (g)
D120
D240
D360

◄ Figure 8.
A) Long fibre weight per stem part against total fibre weight per stem part for all B stem parts, T25–75 and T50–100. R^2 = 0.922 (B) and 0.964 (T).
B) Long fibre weight per stem part (g) against the total fibre weight per stem part (g). Linear regression lines for stem parts B0–50, B25–75, B0–50, and both T–parts. For the T–parts one line is drawn, because stem part is not in the linear regression model (Table 5D).
C) Long fibre weight per stem part (g) against the total fibre weight per stem part (g). Linear regression lines for the T–part of the stem sowing densities D120, D240, and D360.

1. More fibre, more long fibre. The total fibre weight per stem part accounted for 92.2% (B) and 96.4% (T) of the variance in the long fibre weight per stem part (Figure 8A), which means that an increased long fibre weight per stem part was mainly the result of an increased total fibre weight (i.e. long fibre + tow) of that stem part.

2. Lowest long fibre/total fibre ratio in B0–50. The long fibre/total fibre ratio was lowest in the lowest stem part examined, but similar for all other stem parts. This became visible when only total fibre weight and stem part were taken into account in the relevant ranges, and the resulting linear regression lines were plotted in Figure 8B. For all treatments, except T–D120 (b = –0.02) a small positive intercept b was found, which means that the long fibre share decreased a little with increasing total fibre weight.

3. *Sowing density and harvest time effects were indirect.* Besides the interaction through stem weight, which determined total fibre weight, the individual effects of sowing density and harvest time were marginal in explaining which share of the harvested fibres are long fibres.

Sowing density had no effect in the B–part and in the T–part the effects of sowing density on slope and intercept (Table 5) practically cancelled each other out in the relevant ranges (Figure 8C).

Harvest did not affect the long fibre/total fibre ratio in the T–part (Table 5); hence, long fibre weight in the T–part in practice depends on one factor: total fibre weight. Harvest time was involved in three regression factors (Table 5C) in the B–part, which together increased the percentage of variance accounted for by only 0.3%. The combined effect of these factors was that in Italy harvest time caused practically no differences in the long fibre/total fibre ratio. In the Netherlands H1 and H2 were on a lower level, but H3 was on a slightly higher level, comparable to the Italian level.

At H3 intercepts b in the B (0.016) and T (–0.023) parts were close to zero. Consequently, along the H3 regression lines the long fibre shares in the total fibre fraction were similar.

3.7 Detailed bottom part experiment

The bottom part of the stem was studied into more detail at H2 on the Dutch site. The multiple linear regression models for the total fibre/wood ratio and the long fibre/total fibre ratio (Table 6) were very similar to the models in the main experiment (Table 5). Because the detailed bottom part study was carried out at one site and for one harvest time, these factors were not in the model. The remaining factors were in the same order and caused effects in the same order of magnitude as in the main experiment.

Wood weight per stem part and stem part together accounted for 98.0% of the variance in total fibre weight per stem part. Sowing density added 0.1%. As in the main experiment, the total fibre/wood ratio was slightly higher in D120 as compared to both other densities.

The total fibre/wood ratio decreased from B50–100 towards B0–50. Moving downwards along the stem, the decrease accelerated. Figure 9A shows the slopes a for the different stem parts. Total fibre weight per stem part was equal for B0–50, B5–55, B10–60, and B15–65 for all densities (0.84, 0.53, and 0.42 g for D120, D240, and D360, respectively).

Total fibre weight per stem part and stem part together accounted for 93.9% of the variance in long fibre weight per stem part. Sowing density, as in the main experiment, did not affect retting loss percentages or the long fibre/total fibre ratio.

The bottom 5 cm of the stems is different from all other stem parts. B0–50 showed a lower long fibre/total fibre ratio (Figure 9B) and a lower retting loss percentage (16.4%) than all other stem parts (17.4–17.6%).

Table 6. Multiple linear regression models for the detailed bottom part experiment.

	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
6A) Total fibre/Wood (Bottom)	1. Wood weight per stem part	1	1.9181	1.9181	3455.81	<0.001	91.2
	2. Wood weight per stem part × Stem part	5	0.1423	0.0285	51.26	<0.001	98.0
	3. Sowing density	2	0.0047	0.0024	4.27	<0.001	98.1
	Residual	62	0.0344	0.0006			
	Total	70	2.0995	0.0300			
6B) Long fibre/Total fibre (Bottom)	1. Total fibre weight per stem part	1	0.9016	0.9016	1016.34	<0.001	88.8
	2. Total fibre weight per stem part × Stem part	5	0.0549	0.0110	12.37	<0.001	93.9
	Residual	64	0.0568	0.0009			
	Total	70	1.0133	0.0145			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

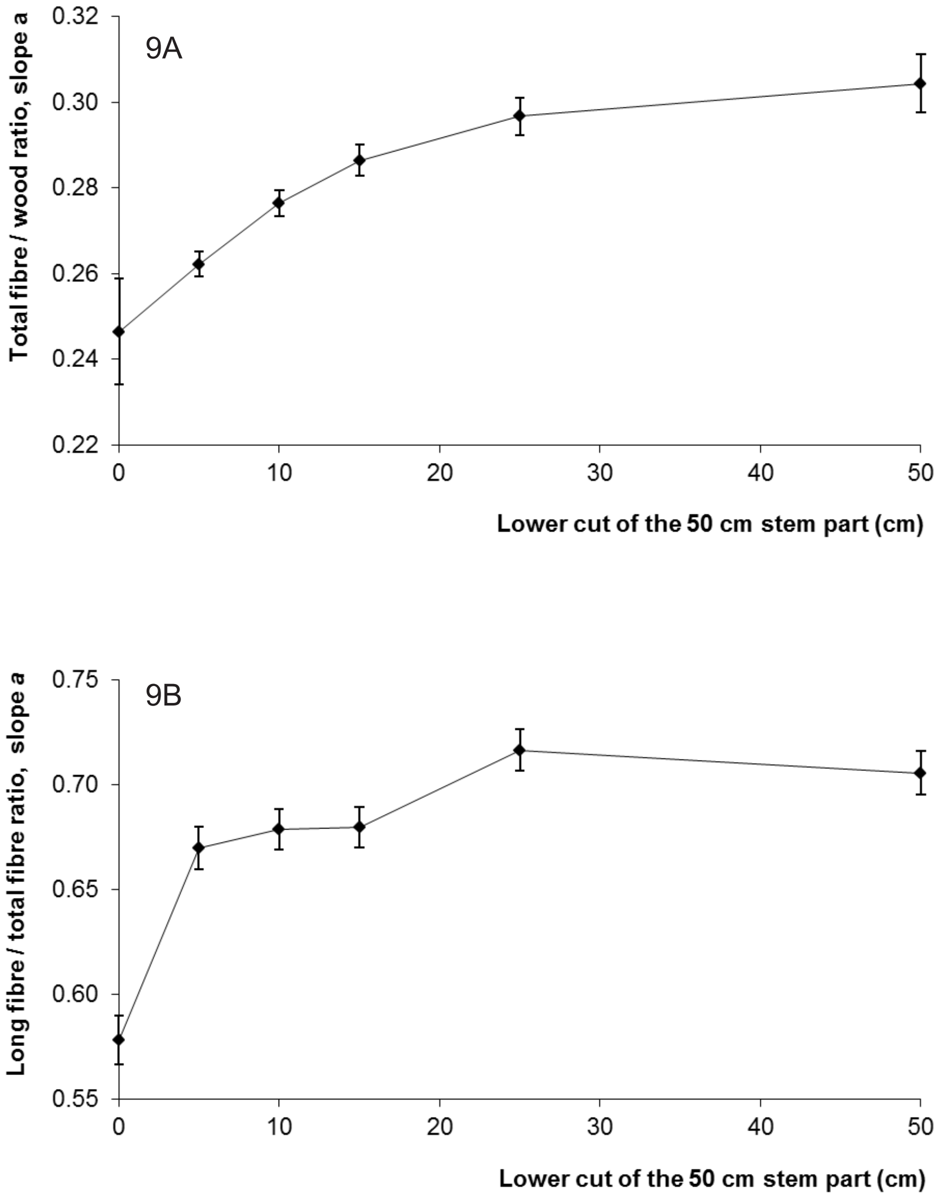


Figure 9. Detailed bottom part experiment.
A) Slopes *a* derived from the total fibre/wood ratio against the lower cut height of the stem parts. Error bars indicate SE.
B) Detailed bottom part experiment. Slopes *a* derived from the long fibre/total fibre ratio against the lower cut height of the stem parts. Error bars indicate SE.

4 Discussion

4.1 Variability in raw materials

The field experiments were successful in establishing hemp crops with different plant densities at harvest. Although plant mortality occurred, and was most pronounced in the highest sowing density, stand density differences between sowing density treatments persisted throughout the entire experiment. Remarkably, plant densities were not different for different harvest dates, as on both sites all mortality occurred before the first harvest. This is in contrast with Meijer *et al.* (1995), who concluded that stand densities at harvest were practically irrespective of sowing density.

A broad range of raw materials was created, differing not only in treatment but also in related visible or easily measurable characteristics. To illustrate this, the average plant weight at harvest varied with a factor 4.3 between treatments, being the ratio between NL–120–H3 (11.1 g) and I–360–H1 (2.6 g). These treatments also showed the largest differences in stem diameter (8.4 vs. 4.5 mm), and plant height (211 vs. 146 cm). The plant density at harvest varied with a factor 2.9, being the ratio between NL–360–1 (283 plants m^{-2}) and I–120–H2 (99 plants m^{-2}). Because of these differences, we considered the raw material batches to cover the variability that could be expected to show any treatment effects. Our observed lack of major sowing density and harvest time effects could therefore not be because of a lack of variability in plant characteristics at harvest.

4.2 Representative batches

The number of small plants (< 100 cm) or 'underhemp', was considerable in some of the treatments; their weight, however, was negligible. Although up to 16.8% (NL–D360) small plants were discarded, the processed plants accounted for 96.7–99.7% of the harvested volume (Table 4) and in the Netherlands for 99.0% (D120), 98.0% (D240) and 96.4% (D360) of the harvested fresh weight. The processed plants ≥ 100 cm therefore can be considered representative for the harvested plot. After discarding the smaller plants, the majority of the plants that were processed had a plant height close to average canopy height (cf. Figure 2A and Table 4). Nevertheless, variability between raw material batches was maintained (Table 4).

4.3 Plant biometry

Stems were thinner and shorter with increasing plant density. Van der Schaaf (1963), Jakobey (1965), Höppner and Menge–Hartmann (1994), Van der Werf *et al.* (1995), Schulz (1998), and Amaducci *et al.* (2002b; 2008a) reported the same. Stem elongation in hemp largely depends on competition for light, nutrients and so forth (de Meijer and Keizer, 1994; Amaducci *et al.*, 2002b), which is increased at higher densities. Furthermore, stem diameter is positively correlated with plant height (Hoffmann, 1957). In our experiments, this relationship is also present within all harvested plots.

A delay in harvest in general increases plant height (Hoffmann, 1957; De Meijer and Keizer, 1994), as well as stem diameter (Amaducci *et al.*, 2002b). This was confirmed in our experiments. In the Netherlands, harvest time did not affect stem diameter, which was also found by Van der Schaaf (1963), but in Italy stem diameter increased with a delay of harvest.

Remarkably, site did not affect plant height when all harvested plants were taken into account, whereas the plants ≥ 100 cm were taller in the Netherlands than in Italy. The reason was a much higher percentage of plants < 100 cm in the Netherlands (11.7%; cf. Table 4) as compared with Italy (3.9%), which implies a larger variability in plant height at harvest in the Netherlands. A higher mortality rate of small plants in Italy could be the reason for this difference, which corresponds with the overall lower plant numbers (Table 2).

4.4 Stem yield

Plant density did not affect stem dry matter yield, which confirmed the results obtained by Meijer *et al.* (1995), Struik *et al.* (2000), Amaducci *et al.* (2001, 2002ab, 2008a), and Vetter *et al.* (2002) in comparable density ranges. Amaducci *et al.* (2002a) reported that plant density did not affect total dry matter yield at different harvest times. The increase in stem dry matter yield (Table 3) with a delay in harvest, was caused by an increase in individual plant weight that was inversely proportional to plant density.

The stability of the plant density at different levels, while stem yield increases with increasing individual plant weight, did not necessarily violate the concept of 'self–thinning', as described by Van der Werf *et al.* (1995). The stem yield level, at which such self–thinning takes place, presumably was not reached yet. Van der Werf *et al.* (1995) neither observed density effects at stem yields as low as the ones presented in Table 3, nor did they observe density effects in such early harvests. Apparently, below a certain stem yield level, different stable plant densities can produce similar stem yields by distributing the yield increase in time over the available stems.

4.5 Retting losses

Retting loss percentages decreased as stem parts matured, because maturation was associated with increasing amounts of fibre and wood, whereas the amount of material that was lost during retting in a certain stem part was constant. Retting loss percentages therefore were lower in lower stem parts and decreased with later harvest, hence Hypothesis 1 can be maintained. In Italy, this effect is larger because stem dry weight increased relatively more with delayed harvest than in the Netherlands, because of the larger temperature sum (Table 3) and radiation sum between harvests.

Reported increasing fibre percentages with delayed harvest time (Van der Schaaf, 1963), therefore might be because of relatively lower retting losses. Because earlier harvested crops lose relatively more weight during processing than later harvested crops, maturity should be taken into account with respect to price fixing of unretted hemp stems.

Sowing density affected retting loss percentages slightly, but a clear pattern was not recognized and the differences seemed unimportant.

4.6 Long fibre yield

To produce highest quality hemp yarns, only the long fibres are valuable. The maximum amount of these long fibres at both sites and for all sowing densities is obtained at the last harvest time:

- Because a delay in harvest time did not affect plant density (Table 2), the highest long fibre yield was obtained when long fibre weight per stem was highest.
- The long fibre weight per stem part increased with the total fibre weight of that stem part (Figure 8B, Table 5).
- The total fibre weight per stem part increased with the wood weight of that stem part (Figure 5A, Figure 5B and Table 5), hence with stem part weight (i.e. total fibre + wood).
- Stem part weight was highest at H3, because stem dry matter yield was highest at H3 (Table 3), and the plant density was unaffected by harvest time (Table 2).

These weight factors in the regression models were by far more important for long fibre production than all other sowing density and harvest time effects. Plant weight decreased with increasing sowing density, and increased with a delay of harvest (see Stem yield), but the effects of sowing density and harvest time were not essentially different; both factors affected stem part weight and thereby fibre content, hence Hypothesis 2 is maintained.

4.7 Fibre percentage

Another weight effect, of less importance, was caused by the positive intercepts *b* in the total fibre/wood ratio. With increasing sowing density, individual plant weight decreased. This has a positive effect on the total fibre percentage. Because plant density did not affect stem dry matter yield (Table 3) the highest fibre yields should be expected in the highest sowing density, D360. The positive effect of intercept *b* on the fibre percentage, however, levels off towards a fibre percentage equivalent to $a/(1+a) \times 100\%$ with increasing stem part weight. At H1 therefore, the differences caused by sowing density were larger than at H3. In the B–part, where most of the weight is located, no effect was found at H3 and in the T–part, total fibre percentage was increased with only 1 – 2 per cent point, showing that this weight effect is of minor importance with respect to fibre production. It confirms, however, the findings of Hoffmann (1957), who concluded from a literature study that fibre percentages always decrease with increasing stem weight. For the differences between sites, we could not find a morphological or botanical explanation.

For long fibre production, also the intercepts of the regression lines for the long fibre/total fibre ratio could be important. At H3 however, the intercepts were close to zero (-0.02–0.02), hence the long fibre shares in the total fibre fraction were similar along the H3 regression lines.

The total fibre percentage based on the weight of the stem parts after retting decreased with a delay of harvest, because the positive effect of intercept *b* on the fibre percentages decreased inevitably with increasing stem weight. The fibre percentage as such, therefore, is not an adequate indicator for long fibre yield. There is no reason to harvest the crop when the fibre percentage is highest (H1); the crop should be harvested when the maximum amount of long fibre is produced per unit area (H3), unless other quality aspects, beyond the scope of this paper, give rise to other decisions.

Fibre percentages based on the stem dry weight before retting are even more difficult to interpret because two opposite effects play a role in maturing plants. Besides the negative effect of increasing stem weight, the decreasing retting loss percentage (Figure 3A) causes a positive effect on the fibre percentage, and these processes cannot be disentangled. For future research, we therefore recommend the stepwise characterisation of the different stem components as introduced in this paper.

4.8 Total fibre/wood ratio

The positive intercept *b* means that a) there is no linear correlation between fibre and wood production in very small plants or b) a hypothetical very small plant starts producing fibres before it produces wood. When it grows, beyond a certain minimum weight, the increase in dry matter is split up into fibres and wood in a fixed way.

Sowing density and harvest time effects on the total fibre/wood ratio were absent or very small and in practice irrelevant. We therefore surmise that the total fibre/wood ratio per stem part is similar for different sowing densities and harvests and maintain Hypothesis 3.

It is questionable whether other factors that affect stem weight, for example nitrogen fertilisation, can change this total fibre/wood ratio. Vetter *et al.* (2002) concluded from an extensive trial (4 years, 4 sites, 4 varieties, 4 nitrogen levels) that nitrogen level did not affect the total fibre percentage, although large differences were found in plant height and stem diameter. Höppner and Menge–Hartmann (1994) neither found differences in fibre percentage. We assume that comparing fibre percentages in these experiments was not a problem. In both experiments one harvest was carried out, hence differences in retting loss percentages because of differences in maturity were unimportant. Besides, the plants were relatively tall as compared to the ones in our experiments. Therefore, we assume the effect of intercept b on fibre percentages to be low.

Chemical stem analysis by Cromack (1998) indicated that genetic differences have a greater impact on the bast fibre content than plant density. We assume the total fibre/wood ratio to be a variety characteristic, with a bow–shaped pattern along the stem. To calculate the total fibre production per stem part, this ratio has to be multiplied by the stem part weight, which depends on the interaction of sowing density, harvest time, fertilisation, and so forth. We plan to test different varieties in future studies.

4.9 Patterns along the stem

The total fibre/wood ratio increased towards the middle part of the stem, and decreased towards both bottom and top, hence we maintain Hypothesis 4. The results of the detailed bottom part study (Figure 9A) suggested a levelling–off towards the middle part of the stem. The bow–shaped patterns that occurred for the fibre percentages (Figure 6 and Figure 10) confirmed the laboratory results of Bredemann (1940) and Van der Werf *et al.* (1994b) for the 'Reinfaser' percentage. The decrease towards the top was possibly caused by losses of unripe fibres during processing (Figure 1).

The long fibre/total fibre ratio decreased from the middle of the stems towards the bottom hence Hypothesis 5 is maintained. The decrease however, is very abrupt in the bottom 5 cm (Figure 9B), roughly the length of the epicotyls. The different composition of the bottom 5 cm was also observed during scutching. When the lower end of a B0–50 stem part was scutched, a net–like fibre structure was scutched off at once. The remaining bundles of long fibres therefore were shorter.

Because of the 90% overlap between subsequent stem parts B0–50, B5–55, B10–60, and B15–65, the real difference between B0–50 and the other stem parts is much larger than the differences in retting loss percentages and slopes *a* suggest: the bottom 5 cm contained practically no long fibres. This is most likely related to secondary fibre forming in the epicotyls and first internodes (Amaducci *et al.*, 2005; Hernandez *et al.*, 2006; Amaducci *et al.*, 2008a; Figure 1). In practice, the bottom 5 cm will be in the stubble and cause no difficulties in fibre processing.

When hemp was a traditional crop in Italy, only the middle parts of the stems were used for textiles. The base of the hemp stem contained too much tow and the top was used for paper production (Cappelletto *et al.*, 2001). We also found more tow in the bottom part and a lower long fibre/total fibre ratio. Further we did not analyse the T0–50 stem part, because fibres and wood could not be separated easily in the top 25 cm of the stems. The middle part is most fit for textile applications.

4.10 Filling degree

The number of primary fibre cells in a cross–section does not change when fibre elongation has ceased and cell wall thickening has started (Gorshkova *et al.*, 2003; Amaducci *et al.*, 2005). Between H1 and H3, the average weight increase of retted stems was 40%, 46%, and 54% for B0–50, B25–75, and B50–100 respectively. In this bottom 1m of the stems, stem elongation has ceased before H1 (data not shown). We therefore assume that the average weight increase of the primary fibres, or their filling degree, kept pace with the weight increase of the wood.

4.11 Next step in processing: hackling

The final product in our experiments is scutched long fibre. To produce high–quality hemp yarns, these long fibres should be hackled in the next processing step. Hackling is a combing process with wire pins of increasing fineness and closeness, which aligns and refines the long fibre bundles, with the aim to produce a continuous fine strand of fibres or 'sliver' for spinning. The scutched long fibre is split up into hackled long fibre, which can be used for spinning high–quality yarns, and hackling tow, which can only be used for low quality applications. The ratio hackled long fibre/scutched long fibre depends on the degree of hackling to which the fibres are subjected (Hann, 2005) and the quality of the starting material. Scutching as well as hackling yields are indicative for the quality of the crops being processed (Van Dam and Van den Oever, 2006). The quality of the hackled fibre is decisive for quality of the yarns that can be spun.

A standardised objective method to determine hackled long fibre/scutched long fibre ratios for small samples has not been developed yet. Fineness and strength of the fibres, however, should be determined after hackling, because fibre characteristics will change during hackling, the procedure is selective and hackling long fibre yields are reported to be around 40% only (Sponner *et al.*, 2005).

5 Conclusions

The separate determination of retting losses, wood, tow, and long fibre at different heights along the stem enabled us to verify a botanical model (Figure 1) and demonstrated that:

1. Sowing density and harvest time affect fibre content in hemp through their effects on stem weight only. In a stem, beyond a certain minimum weight, the increase in dry matter is split up into fibres and wood in a fixed way, which varies with height along the stem.
2. This fibre/wood ratio is highest in the middle part of the stem and lower towards both bottom and top.

3. The long fibre share in the total fibre fraction does not depend on sowing density and harvest time.
4. The long fibre/total fibre ratio is lowest in the bottom 5 cm of the stems, but similar for all other parts that were measured.
5. Retting loss percentages decrease as stem parts mature, because maturation is associated with increasing amounts of fibres and wood, whereas for each stem part the amount of material that is lost during retting is constant.

Chapter 3

Postponed sowing does not alter the fibre/wood ratio or fibre extractability of fibre hemp (*Cannabis sativa* L.)

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Abstract

Because hemp is a short–day plant, postponing the sowing date might be a suitable strategy to obtain shorter and smaller plants around flowering, when primary fibres are 'ripe' enough to be harvested. Smaller plants can be processed on existing flax scutching and hackling lines, and might have fibre characteristics that are desirable for producing high–quality long fibres for yarn spinning. It was investigated whether sowing beyond the normal sowing period in the Netherlands affects the ratio in which fibres and wood are produced, and what proportion of these fibres are long fibres, suitable for long fibre spinning. About 400 stem samples were fractioned into retting losses, wood, tow, and long fibres, and the ratios between fractions were analysed with multiple linear regression analyses. A normal sowing date at the end of April was compared with a postponed sowing date at the end of May. The total fibre/wood ratio was not affected. Over 95% of the variance in total fibre per stem part was accounted for by the wood weight per stem part (55.5%), the variety (+ 33.3%), and the stem part (+ 6.5%). The amount of long fibre per stem part mainly depended on the amount of the total fibre per stem part (95.4% variance was accounted for) and the stem part (+ 2.0%). For economic reasons, it could be interesting to grow two successive high–quality hemp crops in one growing season. Therefore, in an additional experiment with one variety, the effect of sowing fibre hemp up to 12 weeks later than normal on the quantity and quality of the fibres was studied. Postponing the sowing date up to 12 weeks had no important effects on retting losses, the total fibre/wood ratio, and the long fibre/total fibre ratio. It is therefore technically possible to grow two successive hemp crops. Whether this fits well in farming systems remains to be studied.

Key words: baby hemp, *Cannabis sativa* L., fibre hemp, fibre percentage, fibre quality, harvest time, long fibre, retting, scutching, sowing date, textiles, tow.

1 Introduction

The highest added value in fibre hemp (*Cannabis sativa* L.) production can be obtained by producing high–quality 'long' fibres for the finest yarns for fashion textiles, a luxury niche market (Van Dam, 1999; Cappelletto *et al.*, 2001; Amaducci, 2003; Liberalato, 2003). The renewed interest in these high–quality hemp fibres (Amaducci, 2003; Esposito and Rondi, 2006), calls for an agronomic study based on knowledge of the botany and physiology of the plant. It should be known how the amount of fibres with the desired quality could be maximised within a single plant and within a crop.

1.1 Processing on flax lines

The limited market for high–quality hemp yarns does not justify the development of specialised hemp scutching and hackling lines, hence existing flax (*Linum usitatissimum* L.) processing lines should be used. To process hemp on such processing lines, the stems have to be cut into two or more parts, or the cultivation technique has to be adjusted to grow hemp with the size of flax (Liberalato, 2003; Ranalli and Venturi, 2004; Amaducci, 2005; Esposito and Rondi, 2006; Venturi *et al.*, 2007; Amaducci *et al.*, 2008a).

1.2 Baby hemp

An attempt to produce smaller hemp plants by stopping their growth with glyphosate at the desired plant height of 1.2 m failed. The straw yields were low, the cultivation costs high and the 'baby hemp' fibres were heterogeneous and had a low quality, probably because the plants were immature (Liberalato, 2003; Amaducci, 2005). Further, the use of a herbicide increased the environmental impact and did not fit in the environmentally friendly image of hemp (Venturi *et al.*, 2007; Van der Werf and Turunen, 2008).

1.3 Maturity in shorter plants

Because hemp is a short–day plant (Tournois, 1912; Borthwick and Scully, 1954; Heslop–Harrison and Heslop–Harrison, 1969), there is a more natural way to reduce the plant height. The flowering date, given variety and latitude, can be predicted rather precisely (De Meijer and Keizer, 1994; Amaducci *et al.*, 2008a). Hence, the length of the vegetative growth phase can be reduced by postponing the sowing date. As soon as the transition to the generative phase is achieved, it is likely that stem elongation slows down or stops (De Meijer and Keizer, 1994), the primary fibre formation has ceased and that these fibres are ripe (Mediavilla *et al.*, 2001a; Amaducci *et al.*, 2005).

1.4 Quality

These smaller plants might have more desirable fibre characteristics than larger plants. In hemp two types of bast fibres occur:

Primary fibres run longitudinally along the stem from bottom to top and can reach almost the full length of the plants (Van Dam and Gorshkova, 2003). These fibres are desired for the production of high–quality yarns.

Secondary fibres are unwanted, because they are too short for spinning and reduce the fibre quality (Bócsa and Karus, 1998; Schäfer and Honermeier, 2003; Amaducci *et al.*, 2005). Secondary fibres derive from tangential division of cambium cells when a stem part has reached its maximum length. In young plants, they are absent or only present in a thin layer at the stem base (Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005, 2008a; Hernandez *et al.*, 2006).

Amaducci *et al.* (2005) showed that the thickness of the secondary fibre layer and the height up to which secondary fibres were present, increased with decreasing plant density, and with the time passed since the end of internode elongation. Possibly the weight of the stem, which in general increases with decreasing plant density and increases with thermal time, is the key factor.

1.5 More stem, more wood, more (long) fibre

Westerhuis *et al.* (2009a [Chapter 2]) showed that the total amount of bast fibre in a hemp stem part is almost completely determined by the weight and the position of that stem part. Multiple linear regression analyses showed that in a growing plant, beyond a certain minimum weight, additional dry matter was allocated to phloem (bast) fibres and xylem (wood) in fixed fractions. This total fibre/wood ratio was highest in the middle part of the stem and lower towards both bottom and top. Sowing density and harvest time affected the fibre content in a hemp stem only indirectly, through their effects on individual stem weight. It is expected, that sowing date has a similar effect.

To investigate this for the Dutch growing season, a normal sowing at the end of April (Van der Schaaf, 1963; Friederich, 1964; Meijer *et al.*, 1995; Van der Werf, 1994; Van der Werf *et al.*, 1995a; Struik *et al.*, 2000) was compared with a postponed sowing at the end of May. Different harvests were carried out to create a larger range of stem weights. Different varieties were tested to ensure that observed phenomena were independent of variety.

Literature on postponing sowing dates in hemp is scarce. Lisson and Mendham (2000) carried out different experiments and observed no effect or a small decline in the bark percentage when postponing the sowing date. According to Hoffmann (1957), the amounts of fibre and bark are correlated. Friederich (1964) stated that sowing hemp in the Netherlands after 1 May results in shorter stems and delayed ripeness, but did not present experimental data to support these statements. The shorter stems, however, is what is aimed for.

1.6 An additional late hemp crop

Although primarily aiming at quality, for economic reasons the importance of yield should not be neglected. The more the sowing date is delayed, the more the potential stem yield drops. Kamat *et al.* (2002) and Liberalato (2003) suggested growing an additional fibre hemp crop in the same field after harvesting the first, to make optimal use of the length of the growing season. Hemp is, within limits, self–tolerant. It can be cultivated three years in succession without significant yield losses (Bócsa and Karus, 1998). However, the second crop then should be sown in summer.

To determine whether a successful crop can be grown that late in the Dutch growing season, an additional experiment was carried out to compare crops sown in April, May, June, and July.

1.7 Fibre extraction

Because of the small sample size as compared with industrial processing, traditional methods to extract the fibres are used. However, with respect to the procedure and its final products, the methods are comparable (Hann, 2005; Salmon–Minotte and Franck, 2005; Sponner *et al.*, 2005). The fibres are liberated from the surrounding tissues by a controlled warm–water retting procedure hence retting losses can be determined under controlled conditions. During retting, bacteria degrade pectic substances, and in addition proteins, sugars, starch, fats, waxes, tannins, and minerals are removed (Hann, 2005). Westerhuis *et al.* (2009a [Chapter 2]) showed that retting loss percentages gradually decreased with increasing stem weight, and this seemed irrespective the cause of the higher stem weight. It is therefore expected that the stem weight before retting is the only important factor accounting for the variation in stem weight after retting. This will be analysed with multiple linear regression.

Because cellulose is not decomposed as easily, merely wood and fibres survive retting. These will be separated by breaking the stems on a flax breaker and scutching on a Flemish mill (Van den Oever *et al.*, 2003). While scutching, fibres either remain in the valuable long fibre bundle, or fall away as scutching tow. Since only long fibres are valuable for high–quality yarn spinning, the long fibre/total fibre ratio is an important quality characteristic (Hoffmann, 1957; Allam, 2004), and will be determined.

1.8 Hypotheses

The following hypotheses are addressed:

1. Postponing the sowing date affects the biometry of hemp plants. Plants become shorter and thinner at harvest, and have a lower individual stem weight.

2. Retting loss percentages gradually decrease with increasing stem weight, irrespective the cause of the higher stem weight. There is a linear relationship between the stem weight before and after retting.
3. Sowing date and harvest time affect total fibre content through their effects on stem weight only.
4. The total fibre/wood ratio per stem part does not depend on sowing date or harvest time.
5. Sowing date and harvest time affect long fibre content through their effects on total fibre weight only.
6. With respect to the hypotheses above no effects of or interactions with the factor variety are expected.

2 Materials and methods

2.1 Experimental design

Field experiments were carried out in Achterberg, the Netherlands, latitude 51° 58’ N, longitude 5°35’ E, in 2005 and 2007, on adjacent fields with comparable characteristics (sandy soil, organic matter 4.1%, pH (H_2O) 5.6).

The experimental set–up in both years was a randomized four–replicate split–plot design, with sowing date–variety combinations as main plots, and harvest times as subplots. Harvest plots were 2 m^2, surrounded by 1 m border rows to avoid edge effects.

Seeds were sown with a precision drill at a depth of approximately 3–4 cm at target plant populations of 240 plants m^{-2}, a density in the range appropriate for textile destinations (Amaducci *et al.*, 2002a). Distance between rows was 12.5 cm.

Experiments were carried out with three varieties. Seeds of variety Fedora were purchased from La Fédération Nationale des Producteurs de Chanvre (FNCP), Les Mans, France. Seeds of varieties Beniko and Bialobrzeskie were purchased from the Institute of Natural Fibres (INF), Poznań, Poland. Fedora and Bialobrzeskie were tested in both years, Beniko only in 2007.

A normal sowing date at the end of April (S1) and a postponed sowing date at the end of May (S2, + 4 weeks) were compared in a 2–year experiment (Experiment 1). In 2007, with variety Fedora an additional experiment was carried out with sowings in April (S1), May (S2, + 4 weeks), June (S3, + 8 weeks), and July (S4, + 12 weeks).

Nitrogen fertiliser at a rate of 50 kg ha^{-1} was applied manually per plot, directly after sowing. The amount was based on successful hemp experiments at the same site (Struik *et al.*, 2000; Westerhuis *et al.*, 2009a [Chapter 2]).

At full emergence, plant density in Experiment 1 was assessed in two squares of 1 m^2 per plot. No biocides were used. Because of the dry conditions in April 2007, the field was irrigated one and two days after the first sowing date that year, on both occasions with approximately 15 mm of water, to ensure uniform germination and emergence.

2.2 Harvests

At harvest, stems were cut at soil level. Dead plants and shed leaves were not collected.

In Experiment 1 (Table 1), three harvests were carried out with 2–week intervals. The intermediate harvest (H2) was planned at the time when 50% of the plants ≥ 100 cm were expected to flower, meaning that at least one flower, either male or female, was open.

In Experiment 2 (Table 2), plants were harvested after 73 field days for all sowings and after 87 field days for S1, S2, and S3. Harvest times were not related to flowering status, because flowering was expected to occur before the plants were tall enough to process.

2.3 Postharvest measurements

Per harvested plot, 100 plants were measured for stem diameter at 10 cm above soil level and for plant height. Flowering status was recorded per plant. Per harvested plot, plants were divided into two groups: plants with a height ≥ 100 cm and shorter plants. Fresh weights of both groups were determined and the number of plants per group was counted. As in Westerhuis *et al.* (2009a [Chapter 2]) the shorter plants, 'underhemp', were discarded.

Plants with a height ≥ 100 cm were further processed. The dry weights of both stems and remainder, i.e. leaves and inflorescences, were determined on 20 plants, following drying for 24 hours at 105 °C in an oven. The other plants were dried on a drying floor for 4 days at 27 °C in order to prevent them from decaying during storage.

Table 1. Experiment 1. Sowing dates, harvest times, and temperature sums (Growing Degree Days; °C days) in a replicated fibre hemp experiment on postponed sowing in 2005 and 2007. T–sums were calculated using a base temperature of 2 °C (Van der Werf, 1997).

	2005				2007			
	Sowing	Harvest	Date	T–sum (GDD)	Sowing	Harvest	Date	T–sum (GDD)
S1	29–4	H1	25–7	1045	27–4	H1	9–7	1063
		H2	8–8	1219		H2	23–7	1293
		H3	22–8	1399		H3	6–8	1509
S2	27–5	H1	25–7	783	25–5	H1	23–7	926
		H2	8–8	957		H2	6–8	1142
		H3	22–8	1136		H3	20–8	1357

Table 2. Experiment 2. Sowing dates, harvest times, temperature sums (Growing Degree Days; °C days), and flowering percentages in a fibre hemp experiment on postponed sowing with variety Fedora in 2007. T–sums were calculated using a base temperature of 2 °C (Van der Werf, 1997).

Sowing			Harvest	Date	Field days	T–sum (GDD)	Flowering (%)
S1	27–4	Normal	H1	9–7	73	1063	6
			H2	23–7	87	1293	42
S2	25–5	+ 4 weeks	H1	6–8	73	1142	78
			H2	20–8	87	1357	94
S3	22–6	+ 8 weeks	H1	2–9	73	1108	90
			H2	17–9	87	1278	96
S4	20–7	+ 12 weeks	H1	1–10	73	998	99

2.4 Preparation of the stems

Scutching requires stem parts of equal length. Stem parts with a length of 50 cm, which is the minimum length required for the Flemish mill, were processed to study the patterns along the stem in as much detail as possible. Per harvested plot, 100 stems were defoliated. Two comparable groups of 50 stems were assembled. Stems were laid out parallel with the bases level. From the first group, the stems were cut into a B0–50 cm part and a B50–100 cm part, where B0 is soil level. From the second group the B25–75 cm part was cut. Samples were tied up with tie–ribs and remainders were discarded.

2.5 Fibre extraction

Fibre extraction was identical to fibre extraction in Westerhuis *et al.* (2009a [Chapter 2]). Before retting, before breaking, and after cleaning, weighing took place to determine respectively the initial dry weight, the retting losses, and the amounts of long fibre and tow. The weight of the wood was estimated by subtracting retting losses, and total fibre weight (i.e. long fibre + tow) from the stem dry weight before retting. To compare the different batches properly, weighing was always preceded by conditioning the materials at 19 °C and 73% humidity for at least 48 hours (Van den Oever *et al.*, 2003), and the machinery was not adjusted during the experiment.

Retting

Warm–water retting took place in 120 cm high polyvinylchloride (PVC) tubes with a 16 cm diameter and closed bottom. Prior to retting, the cylinders were filled with tepid tap water. This water, used to wash away contaminants, was drained after 2 hours. The cylinders were placed in a retting basin and filled with tap water of 34 °C. Stems were completely submerged, but water exchange between cylinders was avoided. Retting was performed at 34 °C in 96 hours, after which the bundles were carefully washed with tepid water. Excess water was drained away by placing the bundles vertically on a grating above a drain. Next, the bundles were dried on a drying floor for 4 days at 27° C.

Breaking

To separate fibres and wood, the tie–ribs were removed and the stems were arranged in an even layer, and then fed into a flax breaker consisting of a double series of ribbed breaking rollers. These heavy–weight rollers put pressure on the stems by means of a spring system. As a result, stems were flattened and the brittle wood was broken into shives, most of which fell through the machine, while the flexible fibres passed under the rollers easily.

Scutching

Scutching was performed on a Flemish mill with rotary blades that beat the broken stems in such a way that shives and tow were separated from the long fibres in the sample. Both sides of the samples, the upper and lower part of the stem part, were manually fed through the rotary blades eight times; after four times the bundle was turned inside out. Because the end of the sample had to be held in the hand while scutching the other side of the sample, all stem parts in a sample had to be of uniform length. If shorter stem parts were accepted, all the fibre material in these shorter parts, both long fibre and tow, would end up in the tow section. The aim, however, was to distinguish between long fibre and tow.

Cleaning

After scutching, the long fibres and tow were cleaned by hand to remove any remaining shives. After fibre extraction, conditioning, and weighing, the amounts of long fibre and tow were determined, and the weight of the wood was estimated.

2.6 Statistical analysis

Statistical analyses of the data were conducted using GENSTAT® release 10.2. Treatment differences are reported as significant when $P < 0.05$. Following tests for normality:

- Multiple linear regression analyses were performed to analyse the ratios between the stem part weight before and after retting, between the total fibre weight per stem part and the wood weight per stem part, and between the long fibre weight per stem part and total fibre weight per stem part. Stepwise addition or subtraction of terms was carried out to define the most suitable model to use in general linear modelling, i.e. the model with the minimum residual mean squares. There were no statistical or biological reasons to test non–linear models.

- Analyses of variance were calculated for all other variables. Means, standard errors of differences of means (SEDs), F probabilities and degrees of freedom are reported. Because variety Beniko was sown in 2007 only, analyses of variance were calculated for both years separately to avoid non–orthogonality. Unless otherwise stated, the results described refer to the processed plants.

3 Results

Crops were successfully established. Although density was below target in some treatments, plant stands were even at harvests. No signs of water or nutrient stresses were visible, weeds were suppressed well by the crops, and pests and diseases of significance did not occur.

Experiment 1. Postponing the sowing by 4 weeks

Harvests were carried out as planned at approximately beginning (4–36% flowering), middle (39–66%), and end (84–93%) of flowering (Table 3).

Plant density (Table 4)

In 2005, for both sowing dates plant density at full emergence in Bialobrzeskie was close to the targeted 240 plants per m^2, while it was lower in Fedora. At harvests, the differences between varieties were still present, and in the postponed sowing date treatment (S2) more plants had survived than in the normal sowing date treatment (S1). Plant density was equal for the first (H1) and intermediate (H2) harvest, but lower at the final harvest (H3). At H2, more plants ≥ 100 cm per unit area could be selected.

In 2007, plant densities at full emergence were far below target for Beniko and Bialobrzeskie. For Fedora, however, plant density was above target. Emergence was higher in S2 than in S1. Harvest time did not affect plant density, but a variety × sowing date interaction was present. Fedora showed the highest densities, and no differences between the sowing date treatments. In Beniko and Bialobrzeskie, at harvest plant densities were higher in S2 than in S1. Bialobrzeskie showed higher plant densities than Beniko in S2, but not in S1.

3.1 Plant biometry (Table 3)

Plant height

In 2005, plant height showed an interaction between sowing date and harvest time. Plant height increased with a delay of harvest. The increase between harvests, however, was much larger in the postponed sowing date treatment (S2) than in the normal sowing date treatment (S1). At the first (H1) and intermediate harvest (H2), plants were taller in the normal sowing date treatment (S1), but at the final harvest time (H3), plant heights were similar for both sowing date treatments.

In 2007, plant height also showed an interaction between sowing date and harvest time. In the normal sowing date treatment (S1), plants were smaller at H1 than at both other harvests, which did not differ. In the postponed sowing date treatment (S2), plant height increased with a delay in harvest time. At H1 and H3, plant height was not different between the sowing date treatments. At H2, however, plants were taller in the normal sowing date treatment (S1). Plant height also showed an interaction between sowing date and variety. In the normal sowing date treatment (S1), varieties were different in plant height, in the postponed sowing date treatment (S2) they were not. Plant heights for Beniko and Fedora were not different between both sowing date treatments. Bialobrzeskie was taller in the normal sowing date treatment (S1).

Stem diameter

In 2005, stem diameters were similar at the first (H1) and intermediate (H2) harvest; at the final harvest (H3), however, stems were slightly thicker. Sowing date and variety did not affect stem diameter.

In 2007, stem diameter was similar for Beniko and Bialobrzeskie, but smaller for Fedora. Stem diameter also showed an interaction between sowing date and harvest time. In the normal sowing date treatment (S1), stems were slightly thicker at H2 than at H1. All other treatments showed similar stem diameters.

Plant weight

In 2005, plant weight showed a harvest time × sowing date × variety interaction. However, for each sowing date–variety combination, plant weight increased between the first (H1) and the last harvest (H3). This increase was faster in the postponed sowing date treatment (S2) than in the normal sowing date treatment (S1). In none of the comparable H × S treatments, the varieties were different.

In 2007, plant weight showed a harvest time × sowing date interaction. For both sowing dates, plant weight increased with harvest, but at H2 and H3, plants from the postponed sowing date treatment (S2) had a lower plant weight than plants from the normal sowing date treatment (S1).

Plant weight also depended on a sowing date × variety interaction. Sowing date did not affect the plant weight for Beniko and Fedora, but Bialobrzeskie had a lower plant weight in S2 than in S1. For both sowing dates Beniko and Bialobrzeskie had similar plant weights; Fedora had much lower individual plant weight than the other varieties.

3.2 Stem dry matter yield (Table 3)

In 2005, stem dry matter yield showed an interaction between sowing date and harvest time. It increased with a delay of harvest time, at the postponed sowing date, however, the increase was larger. The last harvest time in the postponed sowing date treatment (S2–H3) showed stem dry matter yields similar to all three harvests of the normal sowing date (S1).

In 2007, stem dry matter yield increased with a delay of harvest, and was lower for Beniko than for Bialobrzeskie and Fedora. No sowing date effect was observed.

Table 3. Experiment 1. Flowering, plant height, stem diameter, plant weight, stem dry matter yield, and the fresh weight proportion of the processed plants in a replicated fibre hemp experiment on postponed sowing in 2005 (2 sowing dates × 3 harvest times × 2 varieties) and 2007 (2 sowing dates × 3 harvest times × 3 varieties). Analyses of variance were calculated per year. Only relevant main effects and relevant interactions are presented. F probabilities of all main effects and all interactions are presented. F pr. = F probability, d.f. = degrees of freedom, SED = standard error of differences of means, S = sowing, H = harvest, V = variety, n.s. = not significant at P < 0.05. D.f. and SED when comparing means with the same level of sowing are placed between brackets.

2005		Flowering (%)		Plant height (cm)		Stem diameter (mm)	Plant weight (g)				Stem DM yield (Mg ha^{-1})		Processed plants fresh weight	
							Bialobrzeskie		Fedora					
		S1	S2	S1	S2		S1	S2	S1	S2	S1	S2	S1	S2
Harvest 1		36	4	163	139	5.8	5.3	3.4	5.5	3.8	8.5	5.4	97.4	92.1
Harvest 2		66	46	176	164	5.9	5.3	4.5	5.7	4.5	8.9	7.6	98.2	96.9
Harvest 3		90	84	185	179	6.2	6.5	5.0	6.3	6.5	9.7	8.8	98.9	97.3
df		28 (24)		15 (24)		24	13 (24)				18 (24)		18 (24)	
SED		4.0 (3.6)		5.5 (3.2)		0.11	0.69 (0.36)				0.52 (0.37)		0.46 (0.32)	
F pr.	H	<0.001		<0.001		<0.001	<0.001				<0.001		<0.001	
	S	<0.001		0.015		n.s.	0.027				0.003		<0.001	
	V	n.s.		n.s.		n.s.	n.s				n.s.		n.s.	
	H x S	<0.001		<0.001		n.s.	0.009				<0.001		<0.001	
	H x V	n.s.		n.s.		n.s.	n.s				n.s.		n.s.	
	V x S	n.s.		n.s.		n.s.	n.s				n.s.		n.s.	
	H x V x	n.s.		n.s.		n.s.	0.029				n.s.		n.s.	

Table 3 –continued–.

2007		Flowering (%)		Plant height (cm)		Stem diameter (mm)		Plant weight (g)		Stem DM yield (Mg ha^{-1})	Processed plants fresh weight
		S1	S2	S1	S2	S1	S2	S1	S2		
Harvest 1		9	9	151	151	5.4	5.5	4.0	3.8	5.5	96.9
Harvest 2		39	65	174	160	5.7	5.4	5.8	4.6	7.6	97.9
Harvest 3		92	93	172	166	5.5	5.6	6.7	5.6	8.9	97.6
df		39 (32)		40 (34)		34 (34)		37 (34)		34	34
SED		5.0 (4.3)		3.5 (3.1)		0.15 (0.12)		0.32 (0.27)		0.25	0.16
Beniko		44		163	159	5.9		5.9	5.4	6.7	97.9
Bialobrzeskie		56		180	160	5.8		6.8	4.9	7.5	97.6
Fedora		54		154	157	4.8		3.9	3.7	7.8	96.8
df		14		14		14		14		14	14
SED		4.3		4.3		0.14		0.41		0.28	0.25
Sowing 1											97.8
Sowing 2											97.1
df											14
SED											0.2
F pr.	H	<0.001		<0.001		n.s.		<0.001		<0.001	<0.001
	S	0.019		0.017		n.s.		0.002		n.s.	0.005
	V	0.033		0.001		<0.001		<0.001		0.006	0.002
	H x S	<0.001		0.018		0.037		0.031		n.s.	n.s.
	H x V	n.s.		n.s.		n.s.		n.s.		n.s.	n.s.
	V x S	n.s.		0.005		n.s.		n.s.		n.s.	n.s.
	H x S x V	n.s.		n.s.		n.s.		n.s.		n.s.	n.s.

Table 4. Experiment 1. Plant densities at full emergence, plant densities at harvest and the number of plants ≥ 100 cm m-2 at harvest, in a replicated fibre hemp experiment on postponed sowing in 2005 (2 sowing dates × 3 harvest times × 2 varieties) and 2007 (2 sowing dates × 3 harvest times × 3 varieties). Analyses of variance were calculated per year. Only relevant main effects and relevant interactions are presented. F probabilities of all main effects and all interactions are presented. F pr. = F probability, d.f. = degrees of freedom, SED = standard error of differences of means, S = sowing, H = harvest, V = variety, n.s. = not significant at $P < 0.05$.

Plant density (m^{-2})	2005			2007				
	Emergence	Harvest	≥100 cm	Emergence	Harvest		≥100 cm	
					S1	S2	S1	S2
Beniko	–	–	–	149	130	151	111	127
Bialobrzeskie	234	205		175	138	182	117	149
Fedora	207	185		270	242	249	205	206
d.f.	12	9		18	14		14	
SED	9.4	7.9		8.9	6.7		5	
Sowing 1		181		172				
Sowing 2		209		224				
d.f.		9		18				
SED		7.9		7.3				

Table 4 –continued–.

Plant density (m^{-2})		2005			2007				
		Emergence	Harvest	≥100 cm	Emergence	Harvest		≥100 cm	
						S1	S2	S1	S2
Harvest 1		–	202	156	–				
Harvest 2		–	201	166	–				
Harvest 3		–	182	156	–				
d.f.		–	24	24	–				
SED		–	5.1	4.2	–				
F pr.	H	–	< 0.001	0.025	–		n.s.		
	S	n.s	0.005	n.s.	< 0.001		< 0.001		< 0.001
	V	0.014	0.032	n.s.	< 0.001		< 0.001		< 0.001
	H x S	–	n.s.	n.s.	–		n.s.		n.s.
	H x V	–	n.s.	n.s.	–		n.s.		n.s.
	V x S	–	n.s.	n.s.	n.s.		< 0.005		0.01
	H x S x V	–	n.s.	n.s.	–		n.s.		n.s.

Analyses of variance were calculated per year. Only relevant main effects and relevant interactions are presented. F probabilities of all main effects and all interactions are presented. F pr. = F probability, d.f. = degrees of freedom, SED = standard error of differences of means, S = sowing, H = harvest, V = variety, n.s. = not significant at $P < 0.05$.

3.3 Retting losses (Table 5A)

There was a linear relationship between the stem part weights before and after retting, and retting loss percentages were higher in stem parts with lower weight (Figure 1).

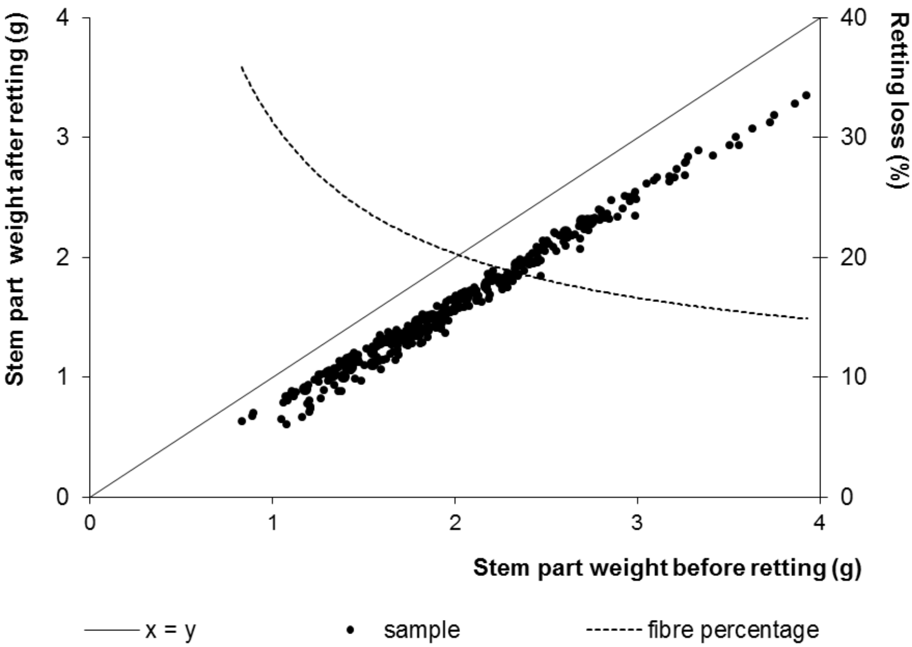


Figure 1. The stem part weight after retting plotted against the stem part weight before retting for 350 samples (•, left Y-axis) in a replicated fibre hemp experiment on postponed sowing in 2005 (2 sowing dates × 3 harvest times × 2 varieties × 3 stem parts) and 2007 (2 sowing dates × 3 harvest times × 3 varieties × 3 stem parts). The linear regression line for all samples is $y = 0.91x - 0.22$ ($R^2 = 0.984$), the line is not drawn. The dotted line (right Y-axis) indicates the retting loss percentage derived from the linear regression line.

Multiple linear regression showed that stem part weight before retting accounted for 98.4% of the variance in stem part weight after retting. A small difference between years was present (+1%). Six other terms together increased the percentage of variance accounted for by 0.4% to 99.8%. The two sowing date terms in the model (+ 0.2%) resulted in regression lines with slightly different slopes and intercepts for the four sowing date × year combinations. The lines cross in the valid ranges and the differences between the lines are too small to visualise. The effects of variety and harvest were even smaller. Stem part terms were not in the model.

Table 5. Multiple linear regression models.

	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
5A) Retted/Unretted stem part weight	1. Stem part weight before retting	1	91.7733	91.7733	150479.74	<0.001	98.4
	2. Year	1	0.8425	0.8425	1381.37	<0.001	99.3
	3. Sowing	1	0.1794	0.1794	294.24	<0.001	99.5
	4. Harvest	2	0.1270	0.0635	104.10	<0.001	99.6
	5. Stem part weight before retting × Year	1	0.0525	0.0525	86.02	<0.001	99.7
	6. Stem part weight before retting × Variety	2	0.0757	0.0378	62.06	<0.001	99.7
	7. Sowing × Year	1	0.0132	0.0132	21.72	<0.001	99.8
	8. Variety	2	0.0140	0.0699	11.46	<0.001	99.8
	Residual	339	0.2067	0.0006			
	Total	350	93.2843	0.2665			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

Table 5 –continued–.

	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
5B) Total Fibre/Wood	1. Wood weight per stem part	1	7.3383	7.3383	6649.95	<0.001	55.5
	2. Wood weight per stem part × Variety	2	4.3809	2.1904	1984.98	<0.001	88.8
	3. Wood weight per stem part × Stem part	2	0.8532	0.4266	386.60	<0.001	95.3
	4. Wood weight per stem part × Year	1	0.0279	0.0279	25.26	<0.001	95.5
	5. Wood weight per stem part × Stem part × Year	2	0.0544	0.0272	24.65	<0.001	95.9
	6. Harvest	2	0.0201	0.0100	9.09	<0.001	96.0
	7. Wood weight per stem part × Harvest	2	0.0408	0.0204	18.47	<0.001	96.3
	8. Wood weight per stem part × Variety × Harvest	4	0.0263	0.0066	5.97	<0.001	96.5
	9. Year	1	0.0199	0.0199	18.04	<0.001	96.6
	10. Year × Harvest	2	0.0335	0.0167	15.12	<0.001	96.9
	11. Wood weight per stem part × Year × Variety	1	0.0131	0.0131	11.85	<0.001	97.0
	12. Wood weight per stem part × Stem part × Variety	4	0.0161	0.0040	3.65	0.006	97.1
	Residual	326	0.3597	0.0011			
	Total	350	13.1841	0.0377			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

Table 5 –continued–.

	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
5C. Long fibre/Total fibre	1. Total fibre weight per stem part	1	9.7132	9.7132	20932.64	<0.001	95.4
	2. Stem part	2	0.2091	0.1045	225.29	<0.001	97.4
	3. Year	1	0.0332	0.0332	71.64	<0.001	97.7
	4. Stem part × Year	2	0.0294	0.0147	31.71	<0.001	98.0
	5. Total fibre weight per stem part × Sowing date	1	0.0146	0.0146	31.52	<0.001	98.2
	6. Total fibre weight per stem part × Harvest	2	0.0081	0.0040	8.73	<0.001	98.2
	7. Total fibre weight per stem part × Variety	2	0.0119	0.0060	12.86	<0.001	98.4
	8. Total fibre weight per stem part × Harvest × Variety	4	0.0070	0.0018	3.79	0.005	98.4
	Residual	335	0.1554	0.0005			
	Total	350	10.1821	0.0291			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

3.4 Total fibre/wood ratio (Table 5B)

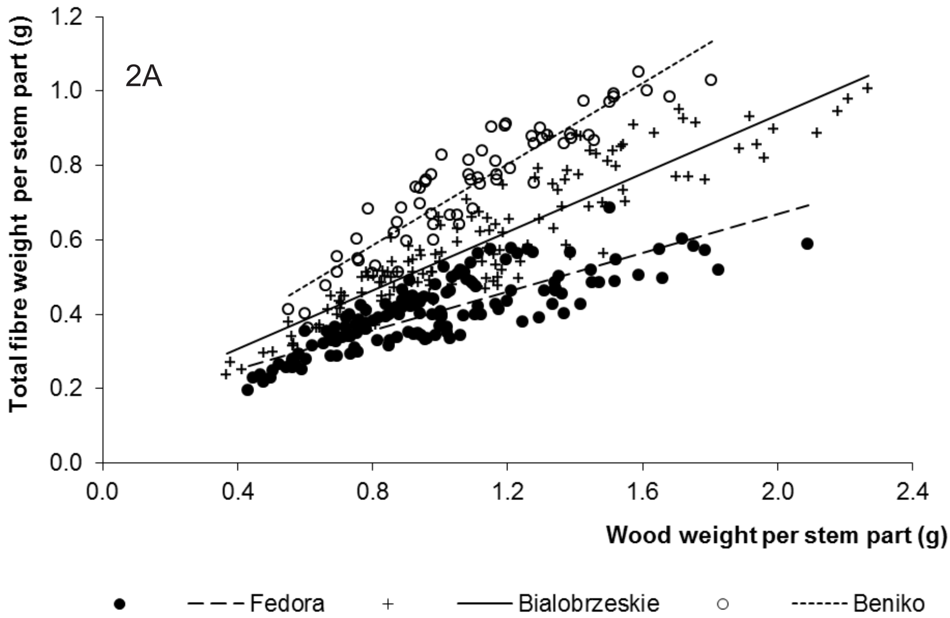
The weight of the wood per stem part accounted for 55.5% of the variance in total fibre weight per stem part. Variety (+33.3%) was the second most important factor. The slopes of the regression lines were very different for Beniko (0.55), Bialobrzeskie (0.39), and Fedora (0.26). Along the stem, the ratio in which fibres and wood were produced was also different (+6.5%) with lowest total fibre/wood ratios in the lowest stem part examined. Nine further terms showed significant contributions to the final regression model, increasing the percentage of variance accounted for by 1.8% to 97.1%. Their effects on slopes and intercepts, however, were marginal.

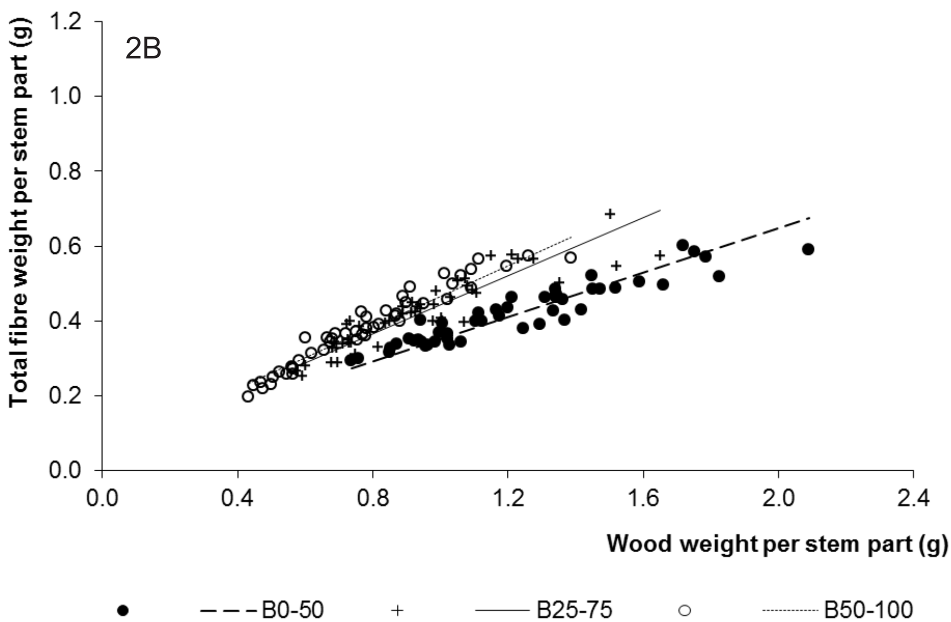
In three figures (Figures 2A, 2B, and 2C), the improvement of the model is shown. Postponing the sowing date did not have a significant effect on the total fibre/wood ratio, as there were no sowing date terms in the final regression model.

3.5 Long fibre/total fibre ratio (Table 5C)

An increased long fibre weight per stem part was mainly the result of an increased total fibre weight of that stem part: 95.4% of the variance was accounted for. A difference between stem parts was observed (+2.0%). The long fibre/total fibre ratio in all treatments was lowest in B0–B50, but similar for both other stem parts (Figure 3A). In stem part B0–50, the variability in the long fibre/total fibre ratio was much larger than in both other stem parts. This was caused by a difference between years, especially in this stem part (terms 3 and 4). Six further terms showed significant contributions to the final regression model, increasing the percentage of variance accounted for by 1.0% to 98.4%. Their effects on slopes and intercepts were marginal.

A small sowing date effect increased the percentage of variance accounted for by 0.2%. In the postponed sowing date treatment (S2), the long fibre/total fibre ratio was slightly lower than in the normal sowing date treatment (S1). Figure 3B shows, as an example, the difference between S1 (a = 0.80) and S2 (a = 0.77) for variety Fedora, stem part B0–50 at H2 in 2005. Because in the final linear regression model, the factor sowing date did not interact with other factors, this 0.03 slope difference between S1 and S2 was valid for all stem parts, all varieties, all harvests and both years.


2A
Total fibre weight per stem part (g)
Wood weight per stem part (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
Fedora
Bialobrzeskie
Beniko


2B
Total fibre weight per stem part (g)
Wood weight per stem part (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
B0-50
B25-75
B50-100

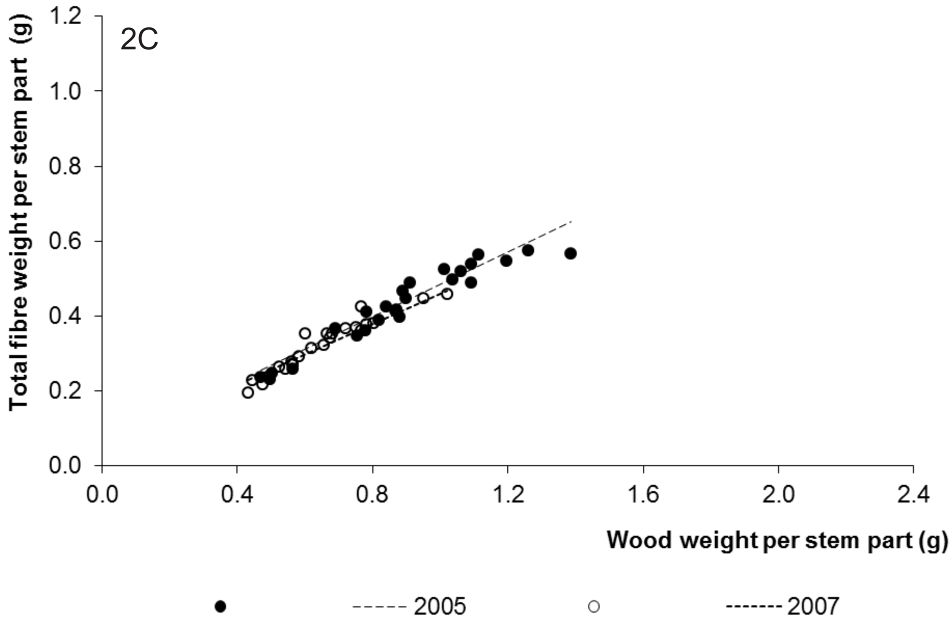


Figure 2. Hemp samples from 2 experiments on postponed sowing in 2005 (2 sowing dates × 3 harvest times × 2 varieties × 3 stem parts) and 2007 (2 sowing dates × 3 harvest times × 2 varieties × 3 stem parts) were fractioned into retting losses, wood, tow, and long fibre. A multiple linear regression analysis was performed with variable wood weight per stem part and factors sowing date, harvest time, variety, stem part, and year, which showed that 55.5% of the variance in the total fibre weight (i.e. long fibre + tow) was accounted for by the wood weight per stem part. Twelve terms (Table 5B) contributed significantly to the model as tested by the variance ratios ($P < 0.05$). In this series of figures, the improvement of the model (added terms; accumulated percentage of variance accounted for) is shown.
A) The slopes of the regression lines are different for varieties (2; 88.8%).
B) The slopes are different for stem parts (3; 95.3%). Variety Fedora is chosen as an example.
C) The slopes are different for both years (4 + 5; 95.9%). Variety Fedora, stem part B50–100 is chosen as an example.

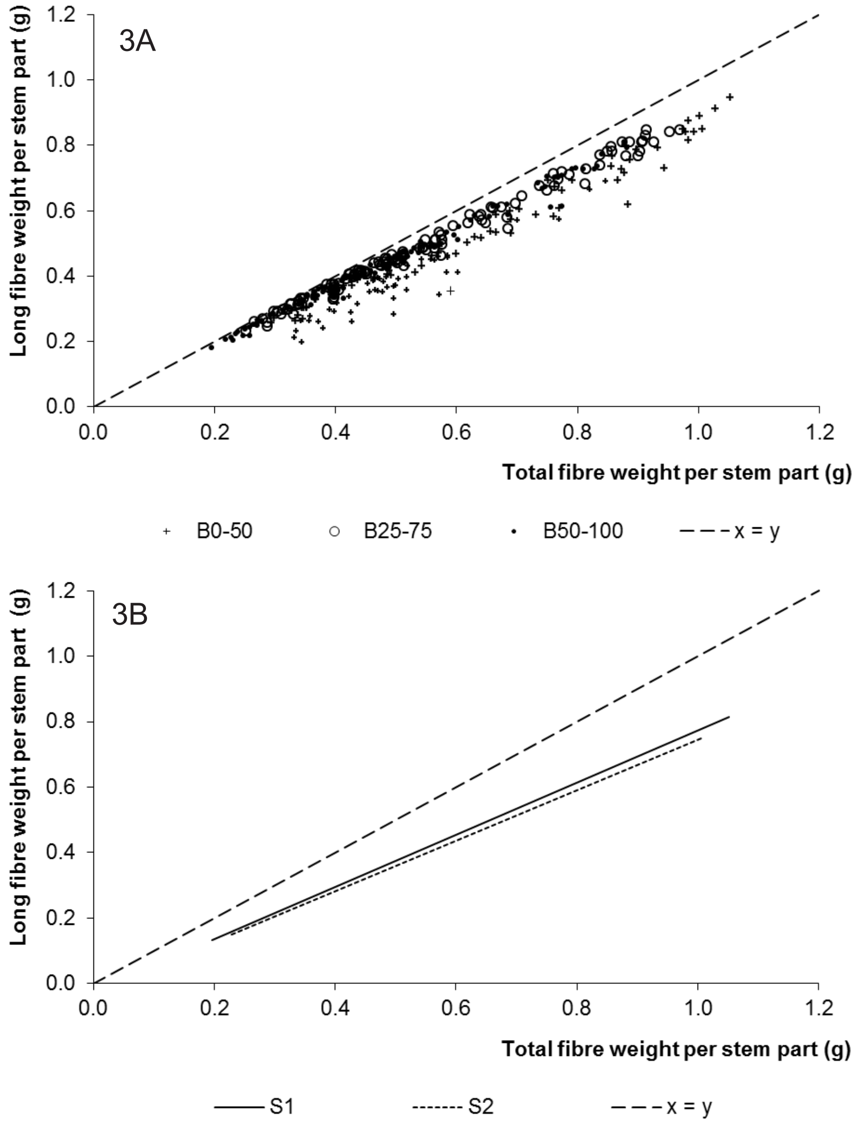


Figure 3. Long fibre weight per 50 cm stem part plotted against total fibre weight per stem part in 2 experiments on postponed sowing in 2005 (2 sowing dates × 3 harvest times × 2 varieties × 3 stem parts) and 2007 (2 sowing dates × 3 harvest times × 2 varieties × 3 stem parts).

A) In stem part B0–50, the variability in the long fibre/total fibre ratio was much larger than in both other stem parts.

B) In the postponed sowing date treatment (S2), the long fibre/total fibre ratio was slightly lower than in the normal sowing date treatment (S1). The drawn lines are valid for variety Fedora 2005–H2–B0–50. The 0.03 slope differences between the lines, however, are equal for all stem parts, all varieties, all harvests, and both years. The total fibre weight per stem ranges shown are the full ranges for S1 and S2, irrespective further treatment specifications.

Experiment 2. An additional late hemp crop

For both harvests, the temperature sums for the sowing date treatments were in the same order of magnitude (Table 2).

3.6 Plant density, biometry and dry matter yield (Table 6)

Plant densities were lower for S3 and S4 than for S1 and S2, while no thinning occurred between harvests. The number of discarded plants (< 100 cm) was similar for treatments, and their proportion in the harvested fresh weight was small (2.3–5.7%). Plant height decreased with later sowing, with the exception of the first harvest in S1, which did not fit in this pattern. Stem diameters were small, in a narrow range (4.3–4.8 mm) and similar for treatments. Selecting stems for processing created a small difference: for S3, the processed stems were slightly thicker than for S1 and S2. Stem diameter, plant height, individual stem weight, and dry matter yield, however, were not different for the processed stems of S1, S2, and S3 with a harvest after 87 days. In S3 and S4, the stem fraction was low as compared with S1 and S2.

3.7 Retting losses, total and long fibre (Table 7)

Multiple regression analyses were performed with the aim to determine the effect of postponing the sowing date on the retting losses, the total fibre/wood ratio, and the long fibre/total fibre ratio. In none of these analyses was sowing date an important factor.

Retting losses (Table 7A)

The regression lines for the different sowing dates crossed in the valid range as is shown for S1 and S4 (73 field days, stem part B25–75) in Figure 4A. The differences between the sowing dates were too small to present all four lines. These differences were equal for all stem parts and both harvests.

Total fibre/wood ratio (Table 7B)

The total fibre/wood ratios were slightly lower in S1 and S3 than in S2 and S4. The regression lines for S1 and S4, and the resulting fibre percentages based on the dry weight after retting are presented in Figure 4B. The differences between the sowing dates were equal for all stem parts and both harvests.

Long fibre/total fibre ratio (Table 7C)

The regression lines for the different sowing dates crossed in the valid range as is shown in Figure 4C for stem part B25–75. The lines are valid for both harvests, because harvest time was not a factor in the final regression model. The total fibre × stem part × sowing date term in the regression model was mainly caused by the very low long fibre/total fibre ratio in stem part B0–50 for the same S3 plot on both harvest times. In this plot, plant density was low and individual plant weight high. Consequently, the ranges in Figure 4C are wider for S3.

Table 6. Experiment 2. Biometry and yield characteristics in a fibre hemp experiment on postponed sowing with variety Fedora in 2007. A normal sowing date at the end of April (S1) was compared with postponed sowings in May (S2, + 4 weeks), June (S3, + 8 weeks) and July (S4, + 12 weeks). Harvests were carried out after 73 and 87 field days. Because S4–H87 is missing, two analyses of variance were performed: comparison three sowings (S1, S2, S3) and two harvests (H73, H87) and comparison four sowings (S1, S2, S3, S4) and one harvest (H73). F pr., F probability; d.f., degrees of freedom; SED, standard error of differences of means; S, sowing.

		Harvest	Sowing date				Comparison				Comparison		
							3 sowings and 2 harvests				4 sowings and 1 harvest		
			S1	S2	S3	S4		F pr.	SED	d.f.	F pr.	SED	d.f.
Density (m^{-2})	All	H73	235	255	190	186	S	<0.001	10.5	2	0.004	15.7	3
		H87	242	252	195	–	H	n.s.					
							S×H	n.s.					
Density (m^{-2})	≥ 100	H73	196	215	147	141	S	<0.001	8.8	2	<0.001	29.4	3
		H87	208	205	149	–	H	n.s.					
							S×H	n.s.					
Plant height (cm)	All	H73	131	146	134	118	S	0.036	3.5	2	0.001	4.6	3
		H87	152	144	136	–	H	0.025	2.9	1			
							S×H	0.013	5.0	2			
Plant height (cm)	≥ 100	H73	142	160	155	133	S	n.s.			0.003	5.5	3
		H87	163	160	158	–	H	0.041	3.5	1			
							S×H	n.s.					

Table 6 –continued–.

		Harvest	Sowing date				Comparison 3 sowings and 2 harvests				Comparison 4 sowings and 1 harvest		
			S1	S2	S3	S4		F pr.	SED	d.f.	F pr.	SED	d.f.
Stem diameter	all	H73	4.3	4.5	4.8	4.5	S	n.s.			n.s.		
(mm)		H87	4.6	4.5	4.5	–	H	n.s.					
							S×H	n.s.					
Stem diameter	≥ 100	H73	4.6	4.8	5.5	5.2	S	0.021	0.20	2	0.047	0.027	3
(mm)		H87	4.9	4.9	5.2	–	H	n.s.					
							S×H	n.s.					
Stem weight	≥ 100	H73	2.7	3.5	4.6	3.6	S	0.011	0.34	2	0.01	0.41	3
(g)		H87	4.3	4.4	4.8	–	H	0.005	0.28	1			
							S×H	n.s.					
Dry matter yield	≥ 100	H73	6.6	9.2	8.8	7.2	S	0.005	0.41	2	0.001	0.50	3
(Mg ha^{-1})		H87	10.7	11.3	10.4	–	H	<0.001	0.33	1			
							S×H	0.015	0.58	2			
Stem fraction	≥ 100	H73	0.79	0.81	0.71	0.67	S	<0.001	0.008	2	<0.001	0.01	3
		H87	0.80	0.77	0.65	–	H	<0.001	0.006	1			
							S×H	0.002	0.011	2			
Stem dry matter yield	≥ 100	H73	5.2	7.6	6.6	5.1	S	<0.001	0.34	2	<0.001	0.43	3
(Mg ha^{-1})		H87	8.8	9.1	7.0	–	H	<0.001	0.28	1			
							S×H	<0.001	0.48	2			

Table 7. Multiple linear regression models.

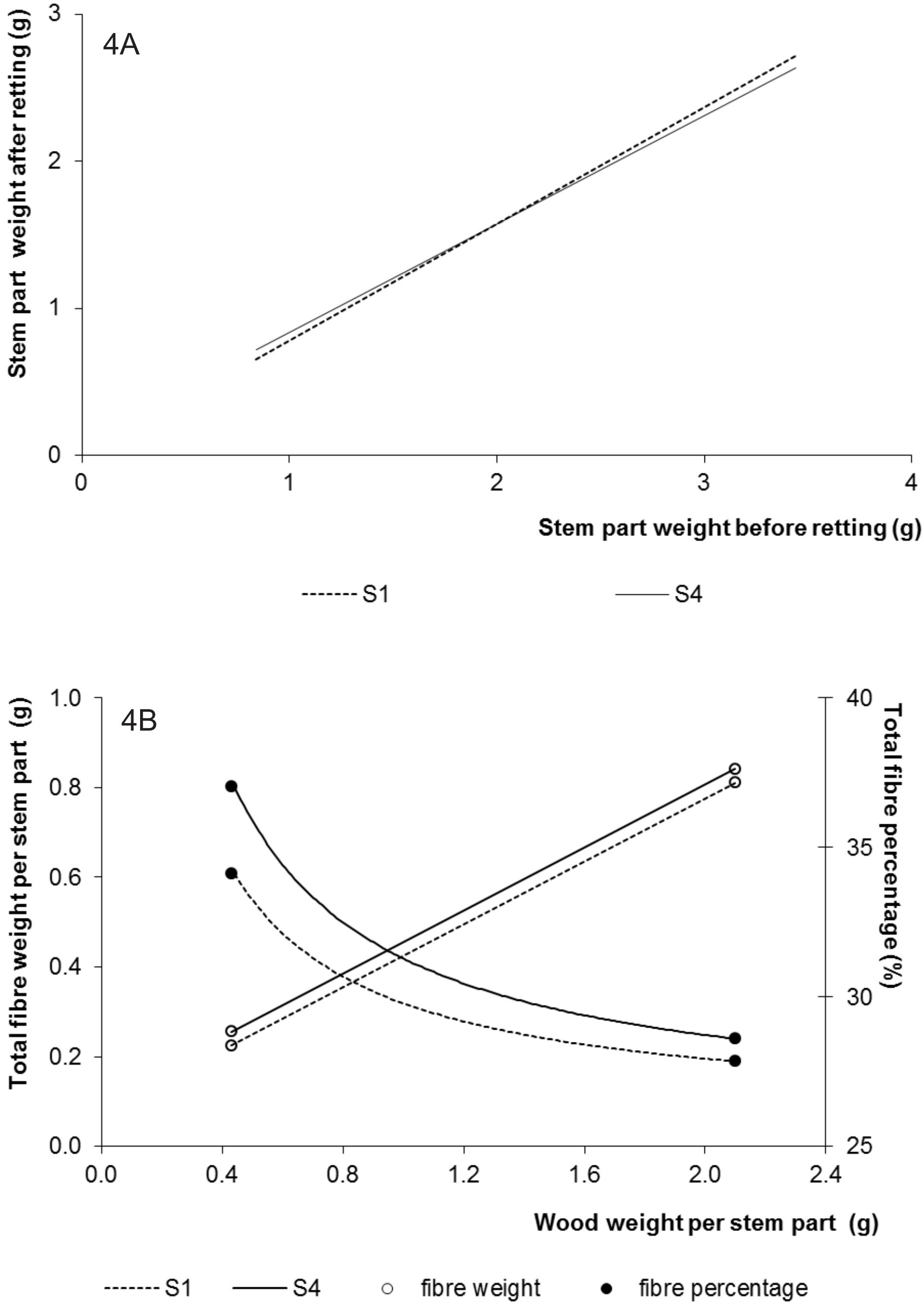
	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
7A) Retted/Unretted stem part weight	1. Stem part weight before retting	1	11.9615	11.9615	40751.07	<0.001	99.4
	2. Stem part weight before retting × Stem part	2	0.0216	0.0108	36.86	<0.001	99.6
	3. Stem part weight before retting × Harvest	1	0.0085	0.0085	29.02	<0.001	99.7
	4. Sowing date	3	0.0106	0.0035	12.02	<0.001	99.8
	5. Stem part weight before retting × Sowing date	3	0.0039	0.0013	4.47	0.006	99.8
	Residual	73	0.0214	0.0003			
	Total	83	12.0276	0.1449			
7B) Total fibre/Wood	1. Wood weight per stem part	1	0.5039	0.5039	1256.69	<0.001	74.1
	2. Wood weight per stem part × Stem part	2	0.1214	0.0607	151.34	<0.001	92.1
	3. Sowing date	3	0.0098	0.0033	8.13	<0.001	93.3
	4. Wood weight per stem part × Harvest	1	0.0081	0.0081	20.26	<0.001	94.5
	5. Wood weight per stem part × Stem part × Harvest	2	0.0042	0.0021	5.18	0.008	95.1
	Residual	74	0.0297	0.0004			
	Total	83	0.6770	0.0082			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

Table 7 –continued– .

	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
7C) Long fibre/Total fibre	1. Total fibre weight per stem part	1	0.2856	0.2856	1210.79	<0.001	70.2
	2. Total fibre weight per stem part × Stem part	2	0.0597	0.0299	126.64	<0.001	84.7
	3. Stem part	2	0.0275	0.0137	58.27	<0.001	91.6
	4. Total fibre weight per stem part × Sowing date	3	0.0091	0.0030	12.86	<0.001	93.7
	5. Total fibre weight per stem part × Stem part × Sowing date	6	0.0047	0.0008	3.32	0.006	94.6
	6. Sowing date	3	0.0027	0.0009	3.83	0.014	95.2
	Residual	66	0.0156	0.0002			
	Total	83	0.4049	0.0049			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.


4A
Stem part weight after retting (g)
Stem part weight before retting (g)
0
1
2
3
4
S1
S4
4B
Total fibre weight per stem part (g)
Total fibre percentage (%)
Wood weight per stem part (g)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.6
2.0
2.4
25
30
35
40
fibre weight
fibre percentage

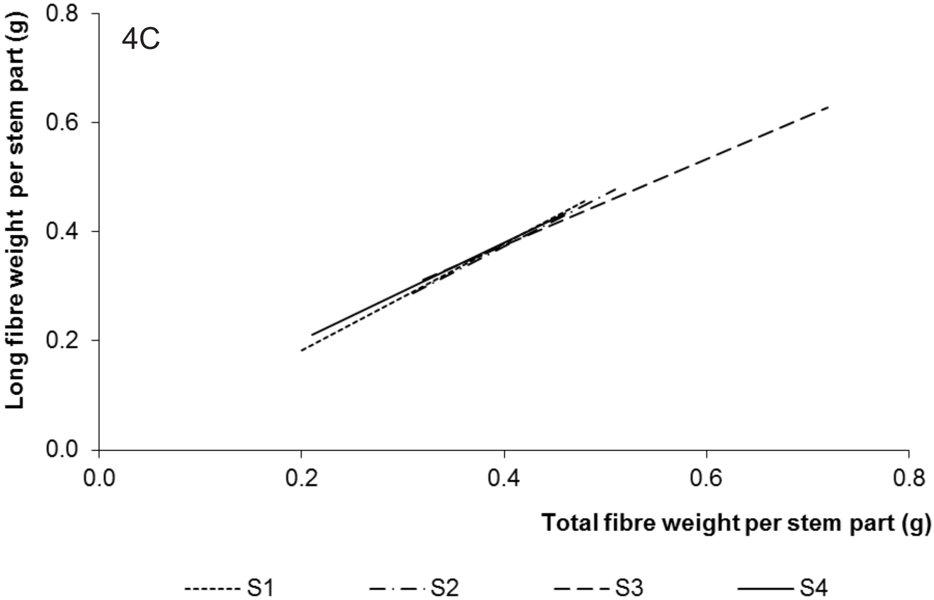


Figure 4. An experiment on postponed sowing in 2007 (4 sowing dates × 3 harvest times × 3 stem parts) with variety Fedora.
A) The stem part weight after retting plotted against the stem part weight before retting for sowing dates S1 (27–4–2007) and S4 (20–7–2007). The regression lines for S2 (25–5–2007) and S3 (22–6–2007) are not drawn because the differences with S4 and S1 respectively were too small to visualise. The drawn lines are valid for stem part B25–75 and a harvest after 73 field days. The differences between the lines, however, are equal for the other stem parts (B0–50 and B50–100), and for harvests after 87 field days. The shown range in stem part weight before retting is the full range for stem parts, harvests, and sowing dates.
B) Total fibre weight per stem part (open symbols, left Y-axis) and total fibre percentage based on the dry weight after retting (closed symbols, right Y-axis) against the wood weight per stem for S1 and S4. The regression lines for S2 and S3 are not drawn, because the differences with S4 and S1 respectively were too small to visualise. The drawn lines are valid for stem part B25–75 and a harvest after 73 field days. The differences between the lines, however, are equal for the other stem parts (B0–50 and B50–100), and for harvests after 87 field days.
C) Long fibre weight per stem part against total fibre weight per stem part for sowing dates S1, S2, S3, and S4. The drawn lines are valid for stem part B25–75 and both harvests.

4 Discussion

4.1 Raw materials

The batches of raw materials were considered to be fit for studying the effects of postponing the sowing date in hemp and the processed plants to be representative for the harvested plots. Although the number of discarded plants (< 100 cm) was considerable, their contribution to fresh weight was negligible (Table 3 and 6), which corresponds with the conclusions of Lisson and Mendham (2000) and Westerhuis *et al.* (2009a [Chapter 2]). Plant densities were always within the same range with no significant differences in the studied phenomena as was found previously (Westerhuis *et al.* (2009a [Chapter 2]).

The predictions of flowering time were sufficiently accurate to plan harvests around flowering in Experiment 1 (Table 3) and, as Westerhuis *et al.* (2009a [Chapter 2]) has shown the effects of harvest time to be unimportant, comparisons between sowing date treatments based on field days (Experiment 2) were considered justified.

4.2 Biometry and stem yield

It was expected that plant height, stem diameter and stem weight would decrease with postponing the sowing date. The current experiments, however, do not support hypothesis 1.

In Experiment 1, stem diameter was not affected by sowing date, and the differences in plant height and plant weight were, especially at the final harvest, absent or small. In Experiment 2, unintentional differences in plant densities between sowing date treatments caused differences in biometry, which probably have overruled the effects of postponing the sowing date. Plant densities were lower for S3 and S4 than for S1 and S2. With decreasing plant density, hemp stems in general are thicker and longer (see next paragraph) and these effects are opposite to the effects expected of postponed sowing.

To obtain smaller plants at harvest, besides postponing the sowing date, increasing the sowing density is a suitable strategy (e.g., Venturi *et al.*, 2007). Hemp stems are thinner and shorter with increasing plant density (Van der Schaaf, 1963; Jakobey, 1965; Höppner and Menge–Hartmann, 1994; Van der Werf *et al.,* 1995a; Schulz, 1998; Amaducci *et al.,* 2002b, 2008a; Westerhuis *et al.*, 2009a [Chapter 2]). An advantage of this strategy over postponing the sowing date is that stem dry matter yield is not affected (Meijer *et al.*, 1995; Struik *et al.*, 2000; Amaducci *et al.*, 2001, 2002a,b, 2008a; Vetter *et al.*, 2002; Westerhuis *et al.*, 2009a [Chapter 2]). Consequently, lower stem yields caused by postponing the sowing date cannot be compensated for by increasing the sowing density (Lisson and Mendham, 2000).

In Experiment 2, the stem fraction in S3 and S4 was low as compared with S1 and S2 (Table 6). These late sown crops flowered early (Table 2), which presumably caused an increased allocation of dry matter to the inflorescences as described by Van der Werf *et al.* (1994). To avoid or delay this effect of postponed sowing a later flowering variety could have been chosen. However, this will not only affect stem fraction hence stem yield, but also plant biometry and fibre ripeness. Fine–tuning will be necessary to find the ideal combination of sowing date, harvest time, and variety at any latitude. To study the effect of sowing date per se on the ratios of interest a single variety was used.

4.3 Retting losses

There was a strong linear relationship between the stem part weight before and after retting, and retting loss percentages gradually decreased with increasing stem part weight (Figure 1), practically irrespective the cause of the higher stem part weight hence hypothesis 2 is maintained. Postponing the sowing date had no important effects on retting losses, besides the indirect effect via stem weight. Sowing date terms in the regression models (Tables 5a and 7a) contributed very little to the explained variance and there was no consistent trend with sowing date.

Analyses of variance (ANOVAs) on the retting data of Experiment 1 (analysis not shown) showed large differences in retting loss percentages between treatments. They were lower for lower stem parts, later harvests and earlier sowing, and were higher for Fedora in 2007 than for the other varieties. The multiple linear regression analysis revealed that stem part weight was the key factor explaining these differences. The differences in retting loss percentages between stem parts as calculated by ANOVAs, for example, were only caused by weight differences: there are no stem part terms in the regression model (Table 5A). An additional reason for the differences in retting loss percentages between treatments in the ANOVAs is that with increasing stem part weight the retting loss percentages decrease, because the regression lines have an intercept (Figure 1). For the difference between years, no explanation was found.

The different composition of stem parts, with increasing relative amounts of materials that are lost during retting towards the top of the plant confirms the findings of a chemical analysis by Cappelletto *et al.* (2001). For price fixing of unretted hemp stems, the higher relative retting losses in stems with a lower weight should be taken into account.

4.4 Total fibre/wood ratio

The weight of an individual plant, hence the weight of a certain stem part, depended on sowing date × harvest time interactions (Table 3). Sowing date and harvest time affected total fibre content through their effects on this stem weight only.

Besides this indirect effect, there are no relevant sowing date effects. In Experiment 1 (Table 5B), there were no sowing date terms in the final regression model. In Experiment 2 sowing date contributed slightly but significantly to the explained variance, but no consistent trend with sowing date was observed (Table 7, Figure 4B). Harvest time terms in the final regression models for Experiment 1 and 2 were statistically significant yet in practice irrelevant (Table 5B, 7B).

It can be concluded that the total fibre/wood ratio per stem part is similar for different sowing dates and harvests hence hypotheses 3 and 4 are maintained.

The total fibre/wood ratios were very different for the different varieties in Experiment 1. This was expected, based on the large differences in fibre percentages reported by other authors when comparing these varieties (Bócsa and Karus, 1998; Cromack, 1998; Mediavilla *et al.*, 1999; Sankari, 2000; Vetter *et al.*, 2002). The aim, however, was not to reveal these differences, but to determine whether the effects of sowing date, harvest time and stem part were similar for different varieties. They were, because important interactions between variety and these other factors were not present in the final regression model (Table 5B).

The main terms in the regression analysis for the total fibre/wood ratio in Experiment 1 were in the same order and caused effects in the same order of magnitude as in Westerhuis *et al.* (2009a [Chapter 2]). The weight of the wood per stem and the variety together accounted for 88.8% of the variance in total fibre weight per stem. In Westerhuis *et al.* (2009a [Chapter 2]), 89.8% of this variance was accounted for by the term wood weight per stem, which makes sense, since in that experiment only one variety was tested.

4.5 Long fibre/total fibre

Sowing date and harvest time affected the long fibre content mainly through their effects on total fibre weight and the amount of long fibre increased with the total amount of fibre (Table 5C).

The extra effects of sowing date, besides the effect via total fibre weight were unimportant (Figures 3B, 4C), and no trend with postponed sowing was present: in practice, the four regression lines in Figure 4C can be considered one line. For this reason hypothesis 5 is maintained.

The long fibre/total fibre ratio was lowest in B0–50. In Westerhuis *et al.* (2009a [Chapter 2]), we concluded that this was caused by the different composition of the bottom 5 cm of stems. In practice, this part will be in the stubble. The variety effect on the long fibre total fibre ratio was negligible and consistent: varieties that produced more long fibre also produced more tow.

In the processing chain, after scutching the fibres are hackled, a combing process to align and refine the fibres. A standardised objective method to determine hackled long fibre/scutched long fibre ratios for small samples has not been developed yet. This however, could be very important, because hackling long fibre yields are reported to be around 40% (Sponner *et al.*, 2005; Tofani, 2006) only. Optimising genotype, crop management, and environment might improve this.

5 Conclusions

The results confirmed the main conclusions of Westerhuis *et al.* (2009a [Chapter 2]) and showed that the same principles were applicable on a wider scale:

- Besides the relative amounts of wood, tow, and long fibre in a hemp stem, also the retting losses could accurately be described with multiple linear regression analyses.
- As sowing density and harvest time, sowing date affected the fibre content in hemp only indirectly, via the effect on individual stem weight.
- The three tested varieties showed large differences in total fibre/wood ratio, but were remarkably similar with respect to retting losses, the patterns along the stem and the long fibre/total fibre ratio, which confirmed hypothesis 6. Further testing of a wider genetic range, however, could be relevant to establish the further scope for breeding.

Technically, it is possible to grow two successive hemp crops; however, it cannot be decided whether two successive crops with a short growing season should be preferred over one crop with a long growing season. Whether this fits well in farming systems remains to be studied. Because the total fibre/wood ratio and the long fibre/total fibre ratio do not depend on the sowing date, other conclusive factors should be present. The total stem yield, hackling yield, the desired plant height for processing, quality aspects beside the scope of this paper (e.g., fibre strength, fineness, refinability, secondary fibre content), and the willingness of the industries to pay a higher price for tailor made hemp stems will be decisive.

Chapter 4

Site does not affect the fibre content ranking order among fibre hemp (*Cannabis sativa* L.) varieties

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Abstract

In growing fibre hemp for textile applications, selecting the variety is very important, as it affects fibre content, fibre quality as well as stem dry matter yield. It was investigated whether the ranking of varieties with respect to their total and long fibre content was affected by the environment. Experiments in Finland (2004) and the Netherlands (2007) were compared. Samples of the bottom, middle, and top of stems were fractioned into wood, long fibre, and tow. Retting losses were determined separately, and fibre percentages were calculated based on the dry weight of stems after retting. This method avoids differences in retting losses to appear as differences in fibre percentage and focuses on the ratio in which varieties produce fibre and wood. When the five selected varieties were ranked from low to high total or long fibre percentage, the order was the same for both sites. Highest fibre percentages were found in the middle stem part. The scutched long fibre/total fibre ratio in this stem part was around 90%, irrespective of variety or site. It is concluded that the effect of the environment on the fibre content of varieties, if any, is small and for practical reasons can be neglected.

Key words: *Cannabis sativa* L., fibre hemp, fibre content, genotype, retting, textiles.

1 Introduction

The highest added value in fibre hemp (*Cannabis sativa* L.) production can be obtained by producing high–quality long fibres for the finest yarns for fashion textiles, a small but growing market (Van Dam, 1999; Cappelletto *et al.*, 2001; Amaducci, 2003; Liberalato, 2003; Esposito and Rondi, 2006). To provide relevant decision support to primary producers, it should be known how the amount of fibres of the desired quality can be maximised within a single plant and within a crop.

Farmers producing hemp for textile applications should aim at the optimal combination of stem dry matter yield × fibre content × fibre quality to maximise their profits. The choice of the variety is very important, as it affects all three factors (Bócsa and Karus, 1998). Many varieties have been described (e.g., De Meijer, 1995; Bócsa and Karus, 1998; Mediavilla *et al.*, 1999) and fibre hemp is grown in many different environments over a wide range of latitudes. Selecting the most suitable variety for any environment, however, seems complex, because of the possible genotype × environment interactions.

The interaction between genotype (variety) and latitude is important with respect to stem dry matter yield. Some varieties are suitable at certain latitude while others are not. With respect to the effect of the environment on the total and long fibre content of varieties, the picture is less clear. The main objective of the experiments described in this paper is to investigate whether the ranking of varieties with respect to their total and long fibre content is affected by environment. Two contrasting sites at different latitudes, one in Finland, the other in the Netherlands, are compared.

1.1 Stem dry matter yield

To maximise stem dry matter production, hemp should be sown as soon as the risk of frost damage is acceptably low, and varieties with a long vegetative growing period should be selected to make optimal use of the length of the growing season (Dempsey, 1975; Van der Werf *et al.*, 1994a; Meijer *et al.*, 1995; Ranalli, 1999; Lisson and Mendham, 2000). The length of the vegetative growing period, however, depends on the interaction between genotype and environment. Hemp is a short–day plant

(Tournois, 1912; Borthwick and Scully, 1954; Heslop–Harrison and Heslop–Harrison, 1969), varieties show a wide range of critical day lengths (Amaducci *et al.*, 2008b), and day length obviously differs for different latitudes. To optimise stem dry matter production it is important not to choose a variety that flowers too early at the chosen site, because around flowering, the allocation of dry matter to the stem decreases (De Meijer and Keizer, 1994; Van der Werf *et al.*, 1994a; Ranalli, 1999; Westerhuis *et al.*, 2009b [Chapter 3]).

The effect of genotype on stem dry matter yield will not be discussed in this paper. Stem dry matter yields for different varieties were published for many different sites. A comparison, e.g., between sites in Italy, the Netherlands, and the United Kingdom was published by Struik *et al.* (2000).

1.2 Fibre content

Although some varieties consistently show relatively high fibre percentages, e.g., Beniko and Bialobrzeskie (Mediavilla *et al.*, 1999; Sankari, 2000b; Bennett *et al.*, 2006), other varieties are known for their consistently low fibre content, e.g., Tiborszállási (Amaducci, 2006b; Tofani, 2006). However, the absolute values for a given variety vary widely within and between experiments. Vetter *et al.* (2002), for instance, found in an extensive variety trial in Germany (12 varieties, 4 years, 5 sites) wide ranges in fibre percentages, e.g., for Fedora (12.7−22.6%) and Futura (15.4−22.6%). Presumably, these wide ranges are largely due to differences in dew retting losses and the weight of the processed stem parts (Westerhuis *et al.*, 2009 a,b [Chapter 2 and 3]).

1.3 Retting losses

Hemp retting, which is comparable to flax (*Linum usitatissimum* L.) retting, is the process in which the fibres are liberated from the surrounding tissues. Moulds (dew retting) or bacteria (water retting) degrade pectins, and in addition, other substances, including proteins, sugars, starch, fats, waxes, tannins, and minerals, are removed from the biomass. Cellulose is not readily decomposed, hence merely the woody part of the stems and the cellulose–filled fibre bundles survive retting (Hann, 2005).

Fibre percentages are usually calculated by dividing the dry weight of the extracted fibres by the dry weight of the stems before retting (e.g., Sankari, 2000b; Vetter *et al.,* 2002). Consequently, differences in retting losses cause differences in fibre percentages. The thus calculated fibre percentage might be suitable to determine the fibre yield, but it is an inadequate variable to understand underlying botanical processes. Westerhuis *et al*. (2009a,b [Chapters 2 and 3] therefore proposed to distinguish between the retting loss percentage (1a) and the fibre percentage after retting (1b):

1a. Retting loss percentage = *100 × (1 − dry weight retted stems/dry weight stems)*

1b. Fibre percentage = *100 × dry weight fibres/dry weight retted stems*

This fibre percentage (1b), shows in fact the ratio in which fibres and wood are produced and therefore is an important botanical characteristic for fibre hemp. It is different for varieties, but for a given variety independent of sowing density, sowing date, and harvest time (Westerhuis *et al.*, 2009a,b [Chapter 2 and 3]). In contrast with this, Westerhuis *et al.* (2009b [Chapter 3]) reported large variability in retting loss percentages and showed that retting loss percentages gradually decreased with increasing stem weight, irrespective of the cause of the higher stem weight, e.g., lower plant density, later harvest, or different stem part.

To compare the samples from both sites properly, a controlled warm–water retting procedure was used to avoid the extreme weather dependency that comes along with field retting (Dempsey, 1975; Van Dam, 1999; Hann, 2005; Salmon–Minotte and Franck, 2005; Sponner *et al.*, 2005). Over–retting or under–retting and other possible sources of unintended and undesirable differences in fibre content are excluded in this way and retting losses can be determined under controlled conditions.

In earlier investigations retting losses were not reported or not in as much detail as needed to calculate the ratio in which fibres and wood were produced. Therefore a proper comparison between the results we obtained with the results found in literature for the same varieties unfortunately is not possible. (e.g., Cromack, 1998; Mediavilla *et al.,* 1999; Sankari, 2000b; Vetter *et al.*, 2002; Amaducci, 2006b; Bennett *et al.*, 2006).

1.4 Stem part

The total fibre content shows a bow–shaped pattern along the stem, with highest fibre percentages in the middle and lower fibre percentages towards both bottom and top (Bredemann, 1940; Van der Werf *et al.*, 1994b; Westerhuis *et al.*, 2009a,b [Chapters 2 and 3]). For this reason, different stem parts should be taken explicitly into account.

1.5 Fibre quality

One particularly important aspect of fibre quality must also be taken into account. To introduce hemp into the fashion textile sector, fibres should be produced allowing the spinning of yarns between Nm 20 and Nm 40. Nm is the metric yarn number: the yarn length in meters per 1 gram of mass ($m \cdot g^{-1}$). The finer the yarn that can be spun, the higher the value of the raw material is (Ranalli and Venturi, 2004; Van Dam and Van den Oever, 2006). Yarn spinners have high demands with respect to the underlying fibre characteristics fineness, refinability, strength, and homogeneity (Sultana, 1992; Van Dam, 1999; Allam, 2004; Hann, 2005; Sponner *et al.*, 2005).

However, these characteristics are only important with respect to the fibres that have passed all processing steps before spinning. The first threshold in processing is scutching and only the fibres surviving this step (‘scutched long fibres’) are valuable. Their share in the total fibre fraction can be considered a quality parameter of the raw material (Hoffmann, 1957; Allam, 2004). ‘Scutching tow’, the fibre material beaten out of the bundles, can be used for other applications, but not for long fibre spinning.

The total fibre (i.e. ‘scutched long fibres’ + ‘scutching tow’) percentage, the scutched long fibre percentage, and the ratio between them will be determined to investigate whether genotype × environment interactions for these variables are present.

2 Materials and methods

2.1 Experimental design

Field experiments were carried out at contrasting sites in Jokioinen (60° 49' N, 23° 28' E), Finland and in Achterberg (51° 58' N, 5° 35' E), the Netherlands. In Jokioinen, twelve fibre hemp varieties were sown on 17 May 2004: Beniko*, Bialobrzeskie*, Chamaeleon, Dioica, Epsylon, Fedora*, Felina, Ferimon, Fibranova, Futura*, Lovrin, and Tiborszállási*. Yield data of this experiment were reported by Pahkala *et al.* (2008). On 27 April 2007, five of these varieties (those marked with an asterisk), covering the wide range in fibre content that was found in the Finnish experiment, were sown in Achterberg.

The experimental set–up at both sites was a randomized four–replicate split–plot design with varieties as main plots and harvest dates as sub–plots. The field was ploughed the previous autumn. Prior to sowing the field was harrowed. Seeds were sown with a precision drill at a depth of approximately 3–4 cm at target plant populations of 240 plants m^{-2}, a density within the range appropriate for textile hemp (Amaducci *et al.*, 2002a). Distance between rows was 12.5 cm. Harvest plots were 3 m^2 surrounded by at least 1 m border rows to avoid edge effects. No biocides were used. At harvests, dead plants and shed leaves were not collected.

In Finland, the experiment was carried out on a silty clay soil with 4.7% organic matter and pH (H_2O) 6.3. Nitrogen fertiliser was applied at a rate of 120 kg N ha^{-1}. This amount was based on an experiment in 2003 at the same site (Pahkala *et al.,* 2008). No irrigation was applied. A single harvest was carried out at the beginning of flowering (Mediavilla *et al.,* 1998), as the harvest planned at the end of flowering was compromised by a severe frost on 11 October 2004. At harvest, stems were cut close to soil level (stubble < 5 cm), using a Honda garden tiller with a saw tool (F410/560 S). One replication each of cultivars Beniko and Fedora was discarded, because of damage caused by stormy weather. Per harvested plot, 50 plants were randomly taken and measured for plant height and stem diameter at 10 cm above cut height.

In the Netherlands, the experiment was carried out on a sandy soil with 4.1% organic matter and pH (H_2O) 5.6. Nitrogen fertiliser was applied manually per plot at a rate of 50 kg N ha^{-1}, directly after sowing. This amount was based on a successful hemp experiment at the same site in 2005 (Westerhuis *et al.*, 2009a [Chapter 2]). Because of the dry conditions in April 2007, the field was irrigated one and two days after sowing, on both occasions with approximately 15 mm of water, to ensure uniform germination and emergence. Three harvests were planned with two weeks between subsequent harvests. The middle harvest was planned at the time when 50% of the plants ≥ 100 cm were flowering, meaning that at least one flower, either male or female, was open. This moment was predicted based upon flowering data from earlier experiments. At harvests, stems were manually cut at soil level with pruning shears (no stubble). Per harvested plot, 100 plants were randomly taken, flowering status was recorded and plant height and stem diameter at 10 cm above cut height were measured.

At both sites the dry weights of both stems and remainder, i.e. leaves and inflorescences, were determined on 20 plants following drying for 24 hours at 105 °C in a stove. The other plants were dried on a drying floor for 4 days at 27 °C in order to prevent them from decaying during storage and shipment.

2.2 Stem selection and sample preparation

Stem selection and sample preparation were different for the sites.

From the Finnish trial, for some of the plots a limited number of plants were available. The minimum length for processing on the Flemish mill was 50 cm and all stem parts in a sample should have equal length. To study bottom, middle, and top of the same plants, only plants with a height ≥ 150 cm could be used. The smaller plants were discarded. A variable number of stems (minimal 15) were processed, depending on the availability of undamaged stems, their height, and the diameter of the PVC tubes that were used for retting. The stems were defoliated and from each individual stem the bottom 50 cm, the exact middle 50 cm, and the top 50 cm stem part were cut (Figure 1A). Samples were tied up with tie–ribs and remainders were discarded.

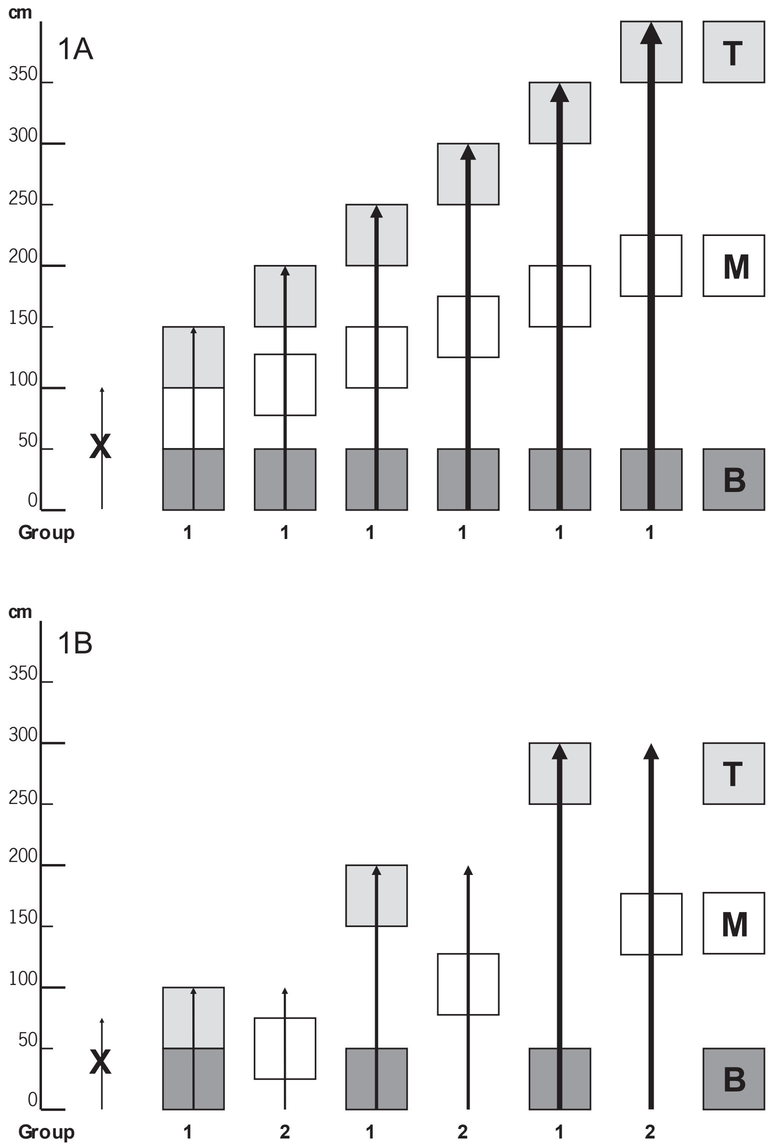


Figure 1. Stem partitioning before processing.
A) Finland: plants shorter than 150 cm were discarded (X). A variable number of plants (minimal 15) were processed, depending on availability and size. Stem parts originated from the bottom (B), middle (M), and top (T) 50 cm of the same stems.
B) The Netherlands: plants shorter than 100 cm were discarded (X). Two comparable groups (1, 2) of 50 stems were assembled. Stem parts originated from the bottom (B), middle (M), and top (T) 50 cm of the stems. B and T stem parts were cut from the same stems (group 1); M stem parts were cut from group 2.

From the Dutch trial, a large share of the plants was shorter than 150 cm hence too short to cut into three 50 cm parts. Therefore, it was decided to cut bottom and top part from the same plants, but the middle section from a parallel group of plants.

Consequently, as in Westerhuis *et al.*(2009a,b [Chapters 2 and 3]), plants < 100 cm were discarded and plants ≥ 100 cm were processed. Per harvested plot, 100 stems were randomly taken and were defoliated. Two comparable groups of 50 stems were assembled. From the first group the bottom 50 cm and top 50 cm stem part were cut, from the second group the exact middle 50 cm stem part was used (Figure 1B). Samples were tied up with tie–ribs and remainders were discarded.

2.3 Fibre extraction

Industrial processing of fibre hemp into high–quality yarns in principle is similar to linen production from flax. Sponner *et al.* (2005), Hann (2005), and Salmon–Minotte and Franck (2005) described this linen production chain accurately and in detail. Because of the small sample size as compared to industrial processing, a traditional fibre extraction method was used. With respect to the procedural steps and the final products, the methods, however, are identical.

The method consisted of four steps: retting, breaking, scutching, and cleaning. Before retting, before breaking, and after cleaning, weighing took place to determine the initial dry weight, the retting losses, and the amounts of scutched long fibre and scutching tow. The weight of the wood was estimated by subtracting retting losses and total fibre weight (i.e. scutched long fibre + scutching tow) from the stem dry weight before retting. To compare the different batches properly, weighing was always preceded by conditioning the materials at 19 °C and 73% humidity for at least 48 h (Van den Oever *et al.*, 2003) and the machinery was not adjusted during the experiment.

Retting

Warm–water retting took place in 120 cm high PVC tubes with a 16 cm diameter and closed bottom. Prior to retting the cylinders were filled with tepid tap water. This water, used to wash away contaminants, was drained after 2 hours. The cylinders were placed in a retting basin and filled with tap water of 34 °C. Stems were completely submerged, but water exchange between cylinders was avoided. Retting was performed at 34 °C in 96 hours after which the bundles were carefully washed with tepid water. Excess water was drained away by placing the bundles vertically on a grating above a drain. Next, the bundles were dried on a drying floor for 4 days at 27 °C.

Breaking

To separate fibres and wood the tie–ribs were removed and the stems were arranged in an even layer, and then fed into a flax breaker consisting of a double series of ribbed breaking rollers. These heavyweight rollers put pressure on the stems by means of a spring system. As a result, stems were flattened, and the brittle wood was broken into shives, most of which fell through the machine, while the flexible fibres passed under the rollers easily.

Scutching

Scutching was performed on a Flemish mill with rotary blades that beat the broken stems in such a way that remaining shives and tow were separated from the long fibres. Both sides of the samples, the upper and lower part of the stem parts, were manually fed through the rotary blades eight times; after four times the bundle was turned inside out. Because the end of the sample had to be held in the hand while scutching the other side of the sample, all stem parts in a sample had to be of uniform length. If shorter stem parts were accepted, all the fibre material in these shorter parts, both scutched long fibre and scutching tow, would end up in the tow section. The aim, however, was to distinguish between these fractions.

Cleaning

After scutching, the long fibres and tow were cleaned by hand to remove any remaining shives and tow. After fibre extraction, conditioning, and weighing, the amounts of scutched long fibre and scutching tow were determined, and the weight of the wood was estimated. Top 50 cm was not cleaned, because in this part tow could not easily be separated from the wood. Consequently, total fibre weight and hence total fibre percentage could not be calculated for this stem part.

2.4 Statistical analysis

Statistical analyses of the data ($P < 0.05$) were conducted using GENSTAT® release 11.1. Following tests for normality:

- Multiple linear regression analyses were performed to analyse the ratios between the stem part weight before and after retting and between the total fibre and wood fractions for the hemp produced in the Netherlands. Stepwise addition or subtraction of terms was carried out to define the most suitable model to use in general linear modelling, i.e. the model with the minimum residual mean squares. There were no statistical or biological reasons to test non–linear models.
- Analyses of variance (ANOVAs) were calculated for all other variables. Means, standard errors of differences of means (SEDs), and degrees of freedom are reported.

3 Results

First it is explained which method was used to compare the two sites. Next, the results of the Finnish trial with twelve varieties are reported to show the large differences in long and total fibre content between the varieties and the similarity between them with respect to the patterns along the stem. This is followed by a comparison between the sites for the five varieties that were grown at both sites. Finally, retting losses are presented. Characteristics of the harvested and processed hemp are presented in Table 1.

Table 1. Varieties, harvest dates, temperature (T–) sum at harvest, plant height, stem diameter, volume share of processed plants, density at full emergence, density at harvest, and flowering percentage in two experiments on fibre hemp in Jokioinen (Finland) in 2004 and Achterberg (the Netherlands) in 2007. The volume share of the processed plants is calculated according to Amaducci *et al.* (2002a). Flowering data were only recorded in the Netherlands. Temperature sums were calculated using a base temperature of 2 °C (Van der Werf, 1997).

Variety	Harvest	Date	T–sum	Plant height (cm)	Stem Diameter (mm)	Volume share (%)	Density at Emergence (m^{-2})	Density at harvest (m^{-2})	Flowering (%)
Jokioinen, Finland									
Fedora	H1	31–8	1241	209	8.1	92	182	107	-
Ferimon	H1	2–9	1264	214	7.9	91	157	105	-
Bialobrzeskie	H1	3–9	1280	222	8.2	95	217	108	-
Felina	H1	7–9	1327	219	8.0	92	179	133	-
Epsylon	H1	9–9	1338	226	8.8	94	164	107	-
Beniko	H1	10–9	1349	213	8.3	94	206	100	-
Futura	H1	14–9	1396	236	9.5	95	160	84	-
Tiborszállási	H1	15–9	1407	250	9.7	96	198	91	-
Chamaeleon	H1	16–9	1413	239	10.4	97	135	83	-
Lovrin	H1	20–9	1450	243	9.9	96	144	112	-
Dioica	H1	23–9	1473	260	11.5	98	133	71	-
Fibranova	H1	29–9	1521	247	10.6	98	88	62	-

Table 1 –continued–.

Variety	Harvest	Date	T–sum	Plant height (cm)	Stem Diameter (mm)	Volume share (%)	Density at Emergence (m^{-2})	Density at harvest (m^{-2})	Flowering (%)
Achterberg, the Netherlands									
Beniko	H1	9–7	1061	154	6.2	98	130	130	2
	H2	23–7	1291	173	6.3	99		121	27
	H3	6–8	1507	176	6.2	98		126	84
Bialobrzeskie	H1	9–7	1061	162	5.6	98	136	148	24
	H2	23–7	1291	190	6.2	98		129	51
	H3	6–8	1507	188	5.9	99		124	94
Fedora	H1	9–7	1061	142	4.6	96	250	235	6
	H2	23–7	1291	163	4.9	98		242	42
	H3	6–8	1507	157	4.8	97		250	93
Futura	H1	30–7	1394	162	5.0	98	244	228	4
	H2	13–8	1614	176	5.2	98		250	56
	H3	27–8	1832	180	5.4	98		240	97
Tiborszállási	H1	30–7	1394	213	7.8	99	91	89	16
	H2	13–8	1614	232	8.1	99		87	82
	H3	27–8	1832	232	7.7	99		77	97

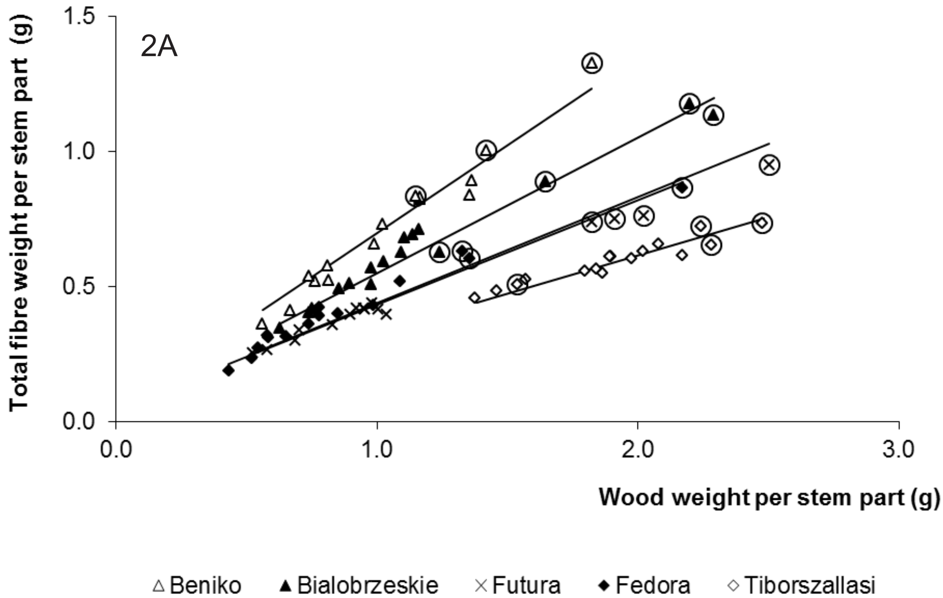
3.1 How to compare the sites: a problem and a solution

In Westerhuis *et al.* (2009a,b [Chapters 2 and 3]) the ratios between the total fibre weight per stem part and the weight of the wood per stem part were analysed with multiple linear regression analyses. Based on these papers different total fibre/wood ratios were expected for different varieties and stem parts. The aim was to investigate and quantify the differences between sites using the same statistical method. In Figures 2A (middle stem part) and 2B (bottom stem part) therefore, the total fibre weight per stem part was plotted against the wood weight per stem part for the five varieties. The figures clearly show the large differences between varieties and the absence of an important variety × stem part interaction.

A linear regression analysis (Table 2A) of the Dutch trial revealed that the weight of the wood (53.9%), the variety (+ 42.7%) and the stem part (+1.6%) together accounted for 98.3% of the variance in total fibre weight.

However, the aim was to investigate whether or not the ratio in which fibres and wood were produced was affected by site. Although Figures 2A and 2B indicate that the differences between sites are very small, as compared to the differences between varieties, they also show that multiple linear regression analysis is not suitable to analyse the differences between sites. The stems in the Finnish experiment, on average were taller and thicker than the stems in the Dutch experiment (Table 1), hence their weight was higher. Consequently, the majority of the data points from the Finnish experiment (encircled) are found on the right side of Figures 2A and 2B, which means that the distribution is unbalanced with respect to site. Moreover, the small number of data points from the Finnish experiment, only one harvest was carried out, did not warrant the calculation of regression lines per site.

This problem was tackled as follows. Westerhuis *et al.* (2009a [Chapter 2]) showed that with increasing stem weight the total fibre percentage decreased. The reason is that the linear regression line *total fibre weight* = a × *wood weight*+ b, had a positive intercept (b), which means that with increasing stem weight the positive effect of intercept b on the fibre percentage levels off towards a fibre percentage equivalent to $a/(1+a) \times 100\%$ (Figure 3).



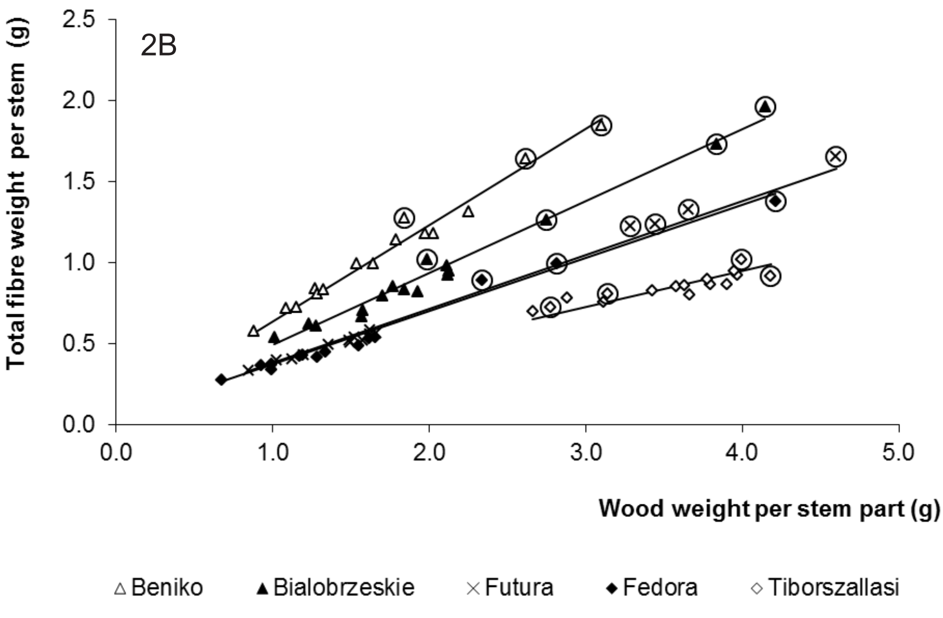


Figure 2. The total weight of the fibre per stem part plotted against the weight of the wood per stem part. Data obtained from fibre hemp experiments in Achterberg (NL) in 2007 (5 varieties × 3 harvests × 4 replicates), and in Jokioinen (F) in 2004 (5 varieties × 4 replicates). The data obtained in the Finnish experiment are encircled.
A) The middle 50 cm of stems.
B) The bottom 50 cm of stems.

Table 2. Multiple linear regression models for a fibre hemp experiment in the Netherlands in 2007 (5 varieties × 3 harvest times × 3 stem parts). All non–significant terms are included in the residual term. a. regression of total fibre weight per stem on wood weight per stem b. regression of the stem part weight after retting on the stem part weight before retting.

	Fitted terms ($P < 0.05$)	d.f.	s.s.	m.s.	v.r.	F pr.	%
2A) Total fibre/Wood	1. Wood weight per stem part	1	8.7937	8.7937	17072.71	<.001	62.7
	2. Wood weight per stem part × Variety	4	4.5530	1.1383	2209.89	<.001	95.3
	3. Wood weight per stem part × Stem part	2	0.4380	0.2190	425.14	<.001	98.5
	4. Wood weight per stem part × Variety × Stem part	8	0.0736	0.0092	17.86	<.001	99.0
	5. Variety	2	0.0295	0.0148	28.65	<.001	99.3
	6. Stem part	4	0.0069	0.0017	3.33	0.012	99.3
	7. Variety × Stem part	8	0.0088	0.0011	2.14	0.035	99.3
	Residual	150	0.0773	0.0005			
	Total	179	13.9807	0.0781			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

Table 2 –continued–.

	Fitted terms (P < 0.05)	d.f.	s.s.	m.s.	v.r.	F pr.	%
2B) Retted/Unretted stem part weight	1. Stem part weight before retting	1	205.9289	205.9290	657862.44	<.001	99.8
	2. Variety	4	0.2597	0.0649	207.44	<.001	99.9
	3. Stem part weight before retting × Harvest	2	0.0387	0.0193	61.75	<.001	99.9
	4. Stem part weight before retting × Stem part	2	0.0758	0.0379	121.00	<.001	99.9
	5. Stem part weight before retting × Variety	4	0.0272	0.0068	21.72	<.001	100.0
	6. Stem part weight before retting × Stem part × Harvest	4	0.0256	0.0064	20.47	<.001	100.0
	Residual	162	0.0507	0.0003			
	Total	179	206.4067	1.1531			

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability; %, percentage of variance accounted for.

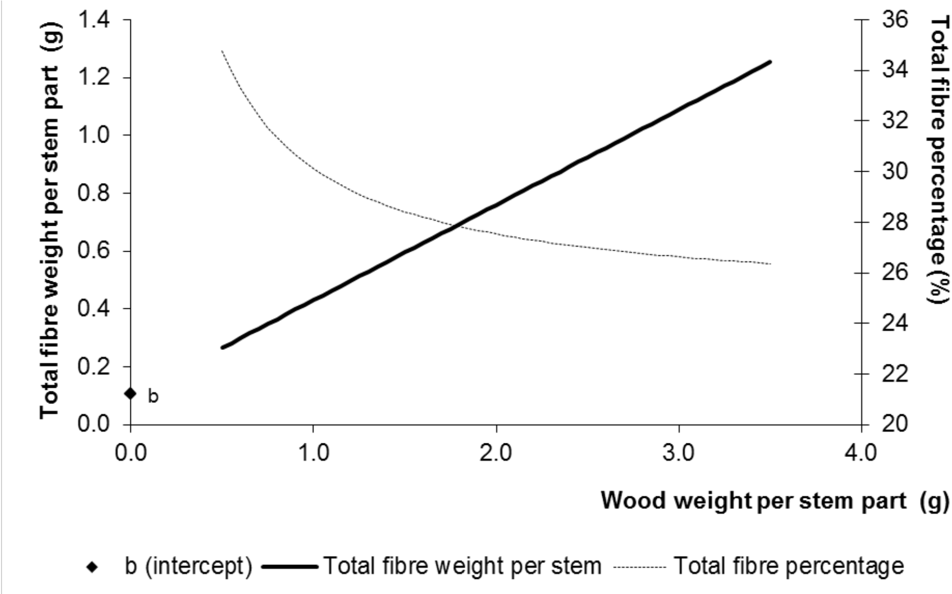


Figure 3. Total fibre weight per stem part (straight line, left Y-axis) and total fibre percentage (dotted line, right Y-axis) against the wood weight per stem part (g) for a linear regression line y = *a*x + *b*, with *a* = 0.33 and *b* = 0.1. With increasing stem part weight the fibre percentage decreases, although the ratio in which fibres and wood are produced does not change.

In the Dutch experiment, the stem weight was highest at the last harvest time (H3). Unless a harvest time effect would be present, an extra effect of harvest time besides its effect on stem weight, it would be reasonable to compare the H3 samples by means of an analysis of variance (ANOVA) with the Finnish samples to investigate whether there is a site effect. Because three harvests were carried out in the Netherlands, the number of samples per variety was sufficiently high to warrant a multiple linear regression analysis to check whether the ratio in which fibres and wood were produced depended on harvest time or not. Table 2A shows that there were no harvest time terms in the final regression model. This means that for all five varieties and all three stem parts the ratio in which fibres and wood were produced was not significantly different for different harvest times, confirming the results from Westerhuis *et al.* (2009a,b [Chapters 2 and 3]). Therefore, it was decided to compare the samples obtained at H3 in the Netherlands with the samples of the Finnish experiment by means of analyses of variance.

3.2 Comparisons between the twelve varieties in Finland

There were large differences in total fibre and scutched long fibre percentage between the twelve varieties (Table 3). Differences were also present in the ratio between them. For these three characteristics, a variety × stem part interaction was found.

The twelve varieties showed large differences in total fibre percentage. The ranges in bottom (20−39%) and middle (24−42%) stem parts were in the same order of magnitude. In both stem parts, Beniko showed the highest total fibre percentage and Tiborszállási the lowest. For Chameleon, Dioica, Fibranova, and Futura the total fibre percentage was not significantly different in the bottom and middle stem part, whereas the other eight varieties showed highest total fibre percentages in the middle stem part.

The twelve varieties also showed large differences in scutched long fibre percentage for bottom (13−32%), middle (22−37%) and top (16−24%) stem parts. In all three stem parts, Beniko showed the highest long fibre percentage. Tiborszállási showed the lowest long fibre percentage, but the differences with Lovrin (middle and top stem part), and Futura and Epsylon (top stem part) were not significant. For all varieties, except Dioica, the highest scutched long fibre percentage was found in the middle stem part. In Dioica, no significant difference was found between the bottom and middle stem part.

In the bottom stem part, the scutched long fibre/total fibre ratio was different for varieties (64−81%). It was highest in, among others, the four varieties with the highest total fibre percentages (Beniko, Bialobrzeskie, Dioica, and Chamaeleon) while it was lowest in, among others, Tiborszállási, the variety with the lowest total fibre percentage. In the middle stem part, the scutched long fibre/total fibre ratio was not different for varieties (range 84−91%). It was higher in the middle stem part than in the bottom stem part. For Chamaeleon, Dioica, and Beniko, however, this difference was not significant.

3.3 Comparisons between the two sites

For the five varieties that were selected for sowing at both sites the samples obtained at the third harvest time in the Netherlands were compared by means of analyses of

Table 3. Total fibre percentage, scutched long fibre percentage and scutched long fibre/total fibre ratio for 12 fibre hemp varieties, in a variety trial in 2004 in Jokioinen, Finland. Varieties are ranked in order of total fibre percentage of the bottom stem part. Varieties marked with an asterisk were also used in the experiment in Achterberg, the Netherlands.

Variety	Total fibre (%) Stem part			Scutched long fibre (%) Stem part				Ratio (%) Stem part		
	B	M	Average	B	M	T	Average	B	M	Average
Tiborszállási*	19.9	23.6	21.7	13.1	21.5	15.6	16.7	66	91	78
Lovrin	23.6	26.7	25.1	17.1	23.9	17.4	19.5	73	89	81
Fedora*	26.0	30.3	28.2	18.0	26.9	18.3	21.0	68	88	78
Futura*	26.7	28.0	27.3	19.8	24.1	16.6	20.1	74	86	80
Epsylon	27.1	29.9	28.5	17.4	25.2	17.7	20.1	64	84	74
Felina	27.5	31.3	29.4	17.8	26.4	19.7	21.3	64	84	74
Ferimon	28.3	31.6	30.0	19.4	27.1	18.5	21.7	68	85	77
Fibranova	30.0	30.2	30.1	22.6	25.8	18.7	22.4	75	86	80
Chamaeleon	31.4	32.0	31.7	25.1	27.9	18.9	24.0	80	87	83
Dioica	31.7	31.3	31.5	25.4	27.1	19.8	24.1	80	87	83
Bialobrzeskie*	32.1	34.2	33.2	25.8	30.1	21.4	25.8	80	88	84
Beniko*	38.8	41.7	40.3	31.7	37.0	24.1	30.9	81	88	84
Average	28.6	30.9	29.8	21.1	26.9	18.9	22.9	73	87	80
s.e.d.		0.78				1.33			3.5	
d.f.		65				99			65	
F pr. Variety × Stem part		< 0.001				< 0.001			< 0.001	

B, bottom stem part; M, middle stem part; T, Top stem part; s.e.d., standard error of differences; d.f., degrees of freedom; F pr., F probability.

variances with the samples obtained at the single harvest time in Finland (Table 4).

Total fibre

There was no main site effect, but the site × variety × stem part interaction was significant ($P < 0.05$). The effects of site and stem part on the total fibre percentage, however, were small as compared to the effect of variety. When the five varieties per stem part were ranked from low to high total fibre percentage, the ranking was the same for both sites. Lowest total fibre percentages were found in Tiborszállási and highest in Beniko. The differences between Futura and Fedora were small and not significant in the bottom stem part.

The total fibre percentages were higher in the middle stem parts than in the bottom stem parts for all varieties at both sites. For Futura in Finland the difference, however, was not significant. In Finland, the difference between the bottom and middle stem part was small as compared to the Netherlands.

In the bottom stem part, Tiborszállási, Fedora, Futura, and Bialobrzeskie showed similar total fibre percentages at both sites, whereas Beniko showed slightly higher total fibre percentages at the Finnish site. In the middle stem part, Tiborszállási and Beniko showed similar total fibre percentages at both sites, whereas Fedora, Futura, and Bialobrzeskie showed higher total fibre percentages at the Dutch site.

Scutched long fibre

As for the total fibre percentage, the effects of site and stem part on the scutched long fibre percentage were small in comparison with the effect of variety.

For all stem parts, the scutched long fibre percentage was higher at the Dutch site than at the Finnish site and the difference between the sites increased from the bottom stem part to the top stem part. At both sites and for all varieties, the scutched long fibre percentage was highest in the middle stem part.

For all varieties, the scutched long fibre percentages were higher at the Dutch site than at the Finnish site. For Tiborszállási, however, the difference was not significant. When the five varieties are ranked from low to high scutched long fibre

Table 4. Total fibre percentage, scutched long fibre percentage and scutched long fibre/total fibre ratio for 5 fibre hemp varieties in two variety trials in 2004 in Jokioinen, Finland and in 2007 in Achterberg, the Netherlands.

Variety	A. Total fibre (%) Stem part			B. Scutched long fibre (%) Stem part				C. Ratio (%) Stem part		
	B	M	Avg	B	M	T	Avg	B	M	Avg
Jokioinen, Finland										
Tiborszállási	19.9	23.6	21.7	13.1	21.5	15.6	16.7	66	91	78
Fedora	26.0	30.3	28.2	18.1	27.0	18.4	21.0	69	88	79
Futura	26.7	28.0	27.3	19.8	24.1	16.6	20.1	74	86	80
Bialobrzeskie	32.1	34.2	33.2	25.8	30.1	21.4	25.8	80	88	84
Beniko	38.8	41.7	40.3	31.8	37.1	24.2	30.9	81	88	85
Average	28.7	31.5	30.1	21.7	28.0	19.2	23.0	74	88	81
Achterberg, the Netherlands										
Tiborszállási	18.8	23.5	21.2	14.7	21.1	17.0	17.6	78	90	84
Fedora	24.5	32.6	28.6	20.2	29.7	25.8	25.2	82	91	86
Futura	25.9	31.1	28.5	23.1	29.0	26.7	26.3	89	93	91
Bialobrzeskie	30.7	37.3	34.0	26.3	33.2	28.0	29.2	86	89	87
Beniko	37.2	40.8	39.0	31.9	37.6	28.6	32.7	86	92	89
Average	27.4	33.1	30.3	23.2	30.1	25.2	26.2	84	91	88
All										
Tiborszállási	19.4	23.5	21.5	13.9	21.3	16.3	17.2	72	90	81
Fedora	25.3	31.5	28.4	19.1	28.3	22.1	23.2	75	90	83
Futura	26.3	29.5	27.9	21.4	26.5	21.6	23.2	82	90	86
Bialobrzeskie	31.4	35.7	33.6	26.1	31.7	24.7	27.5	83	89	86
Beniko	38.0	41.2	39.6	31.9	37.4	26.4	31.9	85	90	87
Average	28.1	32.3	30.2	22.5	29.0	22.2	24.6	79	90	84

(further statistics next page)

Table 4 – continued -.

Variety	A. Total fibre (%) Stem part			B. Scutched long fibre (%) Stem part				C. Ratio (%) Stem part		
	B	M	Avg	B	M	T	Avg	B	M	Avg
s.e.d.		0.73								
d.f.		49								
F pr. Site × Variety × Stem part		0.012			n.s.				n.s.	
s.e.d.					0.78					
d.f.					19					
F pr. Site × Variety		0.004			< 0.001				n.s.	
s.e.d.					0.69				1.8	
d.f.					12				11	
F pr. Site × Stem part		< 0.001			< 0.001				< 0.001	
s.e.d.					0.72				2.1	
d.f.					78				50	
F pr. Variety × Stem part		< 0.001			< 0.001				< 0.001	

B, bottom stem part; M, Middle stem part; T, Top stem part; Avg, average, s.e.d., standard error of differences; d.f., degrees of freedom; F pr., F probability. Varieties are ranked in order of total fibre percentage of the bottom stem part.

percentage, at both sites the order was the same as for the total fibre percentage: lowest long fibre percentages were found in Tiborszállási and highest in Beniko. At both sites, the differences between Futura and Fedora were not significant. In the middle stem part, no differences between sites or varieties were present with respect to the scutched long fibre/total fibre ratio. In all cases, it was around 90%. In the bottom stem parts, the scutched long fibre/total fibre ratio was lower at the Finnish site (74%) than at the Dutch site (84%), and Tiborszállási (72%) and Fedora (73%) showed a significantly lower ratio than Futura (82%), Beniko (83%), and Bialobrzeskie (85%).

3.4 Retting losses

Retting losses were analysed with multiple linear regression (Table 2B) as in Westerhuis *et al.* (2009b [Chapter 3]). The analysis was only performed for the Dutch experiment for reasons mentioned above.

There was a linear relationship between the stem part weights before and after retting. Figure 4 shows that with increasing stem part weight the absolute retting losses increased. The retting loss percentage, however, decreased with increasing stem part weight and levelled off towards about 15%.

The analysis (Table 2B) showed that stem part weight before retting accounted for 99.8% of the variance in stem part weight after retting. A small but significant difference between varieties was present (+ 0.1% explained variance). Variability was largest in the top part of the stems.

4 Discussion

4.1 Raw materials

The Finnish hemp on average was taller and thicker than the hemp produced in the Netherlands (Table 1). This was probably mainly due to differences in plant density at emergence and to severe thinning (on average 40%) of the crop between full emergence and harvest in Finland, whereas in the Netherlands almost no thinning (on average 4%) occurred. Hemp stems are thinner and shorter with increasing plant

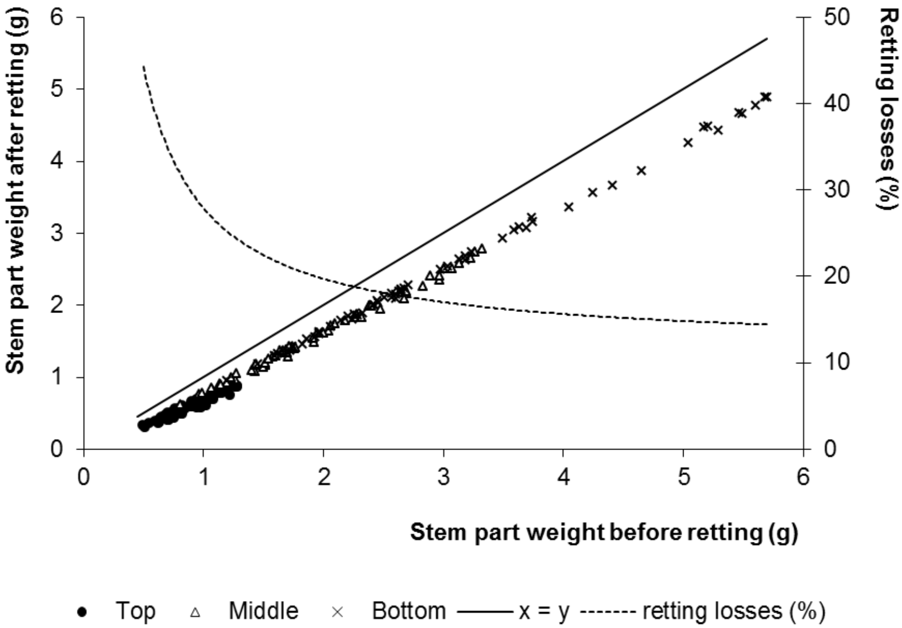


Figure 4. The stem part weight after retting plotted against the stem part weight before retting for top, middle, and bottom 50 cm stem parts in a fibre hemp experiment (3 harvest times × 5 varieties × 3 stem parts × 4 replicates) in the Netherlands in 2007 (symbols, left Y-axis). The distances between the symbols and the line x = y indicate the absolute retting losses. With increasing stem part weight the retting loss percentage (---, right Y-axis) decreased.

density (Van der Schaaf, 1963; Jakobey, 1965; Höppner and Menge–Hartmann, 1994; Van der Werf *et al.,* 1995a; Amaducci *et al.,* 2002b, 2008a; Westerhuis *et al.*, 2009a [Chapter 2]).

The severe thinning in Finland was possibly caused by the higher amount of nitrogen that was applied, because thinning in hemp increases with increasing N–fertilisation (Höppner and Menge–Hartmann, 1994; Van der Werf and Van den Berg, 1995; Amaducci *et al.*, 2002a; Vetter *et al.*, 2002).

The processed plants were considered representative for the harvested plots. Although the number of discarded plants was considerable, their contribution to the harvested volume (Amaducci *et al.*, 2002a) was small. From the Finnish plots on average 5% (range 2–9%) of the harvested volume could not be used for processing, from the Dutch plots on average 2% (range 1–4%).

4.2 Total fibre percentage

The experiment in Finland showed that varieties were very different with respect to their total fibre content (Table 3). Based on that experiment five varieties were selected, covering the wide range in fibre content found, for a comparative study on a contrasting site in the Netherlands.

The main objective was to investigate whether the ranking of varieties with respect to their total fibre content was affected by site. A main site effect, however, was absent, showing that neither of the sites on average showed significantly higher fibre percentages. Although total fibre percentage depended on a site × stem part × variety interaction (Table 4), the effect of variety was very dominant and not compromised by the interaction (cf. Figure 2). Consequently, the ranking of the varieties with respect to their total fibre percentage based on the dry weight of the stems after retting was not affected by site.

Disentangling the three–way interaction site × stem part × variety, differences were encountered, which were possibly due to differences in harvest technique and plant size between the sites, rather than to differences in the ratio in which any variety produced fibres and wood.

4.3 Harvest technique

Based on the findings of Bredemann (1940), Van der Werf *et al.* (1994b) and Westerhuis *et al.* (2009a,b [Chapters 2 and 3]) higher total fibre percentages were expected in the middle stem part than in the bottom stem part. For all varieties grown at the Dutch site this difference was present (4−8 per cent point).

In Finland however, the differences were smaller (1−4 per cent point) and not significant for Futura. Also in Chamaeleon, Dioica, and Fibranova, varieties that were only grown in Finland, no significant difference between bottom and middle stem part was found (Table 3).

Probably this difference between sites was caused by the different harvest techniques. Whereas in the Netherlands the stems were cut manually at soil level with pruning shears (no stubble), in Finland the hemp was harvested using a garden tiller with a saw tool, which inevitably created a stubble of a few centimetres (< 5 cm).

Westerhuis *et al.* (2009a [Chapter 2]) showed that the fibre content of the bottom 5 cm of hemp stems is very low and that the fibre content increases towards the middle of stems. Consequently, cutting the stems a few centimetres higher increases the fibre percentage. This might explain why the differences between bottom and middle part were smaller in Finland than in the Netherlands, and why the fibre percentage in the bottom part on average was a little higher, but only significantly higher in Beniko, in the Finnish hemp.

4.4 Plant size

For reasons explained above the five varieties that were grown at both sites were compared with ANOVAs instead of multiple linear regression analyses. It was decided to compare the results of the last harvest time (H3) in the Netherlands with the hemp grown in Finland, because at H3 the stem weight was highest. However, the average stem weight at H3 was still lower than the average stem weight of the Finnish hemp. Westerhuis *et al.* (2009a [Chapter 2]) showed that with increasing stem weight, the fibre percentage decreased, although the ratio in which fibres and wood were produced did not change (Figure 3). This effect might have caused the slightly lower total fibre percentages in the middle stem part in Fedora, Futura, and Bialobrzeskie in Finland as compared to the Netherlands.

4.5 Scutched long fibre

The second aim of our experiments was to determine whether genotype × environment effects were present for the scutched long fibre percentage. When the five varieties were ranked from low to high scutched long fibre percentage, at both sites the ranking order was the same as for the total fibre percentage.

For the scutched long fibre/total fibre ratio, differences between sites and between varieties were only present in the bottom part of stems. In this bottom part, the ratio was lower than in the middle stem part, though differences were not significant for Chamaeleon, Dioica, and Beniko in the Finnish experiment. The lower ratio in the bottom part was expected, because in Westerhuis *et al.* (2009a,b [Chapters 2 and 3]), the bottom 50 cm of stems always showed lower scutched long fibre/total fibre ratios than all other stem parts examined, irrespective of sowing density, variety, sowing date or harvest time.

In Westerhuis *et al.* (2009a [Chapter 2]), this was ascribed to the different composition of the bottom 5 cm of the stems. However, this was based on an experiment with only one variety at one site. The experiments described in this paper showed that the scutched long fibre/total fibre ratio in the bottom part was different for varieties and sites. Possibly this was due to differences in plant height (Table 1) and differences between varieties with respect to the length of the middle section (see below), or the interaction between these factors. The data set obtained in this experiment, however, was not suitable to investigate the background of the differences between varieties and sites in more detail.

The scutched long fibre/total fibre ratio in the middle stem part was around 90%, regardless site or variety. To produce homogeneous, high–quality textile fibres preferentially only this middle part of the stems should be used to extract the fibres (Cappelletto, 2001; Van Dam and Van den Oever, 2006) hence this is the most valuable part. It is therefore very important to know the length of this middle section as related to plant height. The relative weight share of this high–quality stem part should be maximised in order to maximise profits. Upper and lower limits should be known to determine cut heights, and to adjust machinery. In this respect not only the relative long–fibre yield should be taken into account, but also all other important quality aspects that might show patterns along the stem.

In addition, the presence or absence of the unwanted secondary fibres, especially in the bottom part of stems (Bócsa and Karus, 1998; Schäfer and Honermeier, 2003; Amaducci *et al.*, 2005) should be taken into account. Obviously, a homogenous crop, in all aspects, is preferable for such optimisations. Future research should focus on this, as primary producers need to know the optimal combination of plant height and stem diameter, and the optimal heights to cut the stems at harvest in order to provide the homogeneous high–quality raw material the processors require.

Stubbles that are too short decrease the quality of the raw materials, stubbles that are too long lower the profits (Bredemann, 1940).

4.6 Retting losses

Whereas large differences were found in the ratio in which varieties produced fibre and wood, variety was an unimportant factor with respect to retting loss percentages. Stem part weight before retting accounted for 99.8% of the variance in stem part weight after retting (Table 2B), which confirms the conclusions of Westerhuis *et al.* (2009b [Chapter 3]). The differences between varieties, although statistically significant, are in practice unimportant.

Largest variability was found in the top part of the stems (Figure 4), but the absolute fibre weight in this part is low, as is the fibre quality. These fibres are not suitable for spinning high–quality yarns (Cappelletto, 2001; Van Dam and Van den Oever, 2006). Variability in this stem part might also partly be due to the relative low weight of this stem part and structural differences caused by differences in flowering stage (e.g., internode length, branching, and the presence of resins).

4.7 Crop management strategy

It can be concluded that the effect of the environment on the fibre content of varieties, if any, is small and for practical reasons can be neglected. Differences in fibre content found in the literature, given variety, were probably mainly due to weight differences in the processed stem parts and differences caused by under- or overretting in the field, but not to differences in the ratio in which any variety produces fibres and wood. With respect to price fixing of unretted hemp stems, it is therefore important to know the variety and to determine the retting losses under controlled conditions, for which a protocol should be developed that sellers and buyers agree on.

The experiments described in this paper and those in Westerhuis *et al.* (2009a,b [Chapters 2 and 3]) have shown that the effects of site, sowing density, sowing date, and harvest time on the ratio in which any variety produces fibre and wood are absent or minimal. Improving the total fibre content, given variety, is not a promising strategy. To improve the fibre content of a hemp crop at any site, a variety with higher fibre content should be chosen. Crop management, given site, should be focused on optimising stem dry matter yield and possible other fibre quality traits not studied here. Moreover, the large varietal differences and lack of interactions with the environment show good prospects for breeding. As genotype × environment interactions are unimportant with respect to fibre content, testing this characteristic on only one site, should be conclusive.

Chapter 5

Plant weight determines secondary fibre development in fibre hemp (*Cannabis sativa* L.)

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Abstract

In fibre hemp (*Cannabis sativa* L.) grown for the production of high–quality textile yarns the presence of secondary fibres is unwanted. These fibres are too short for spinning and their presence hampers the production of fine and homogeneous yarns from the primary or long fibres. Primary fibres are present along the stem from bottom to top and hemp is traditionally harvested around the time of flowering, when the cell walls of these fibres are sufficiently thickened with cellulose to be extracted. In literature indications are found that the height up to which secondary fibres are present, moves upwards along the stem during the growing season, and that this process accelerates around flowering. To optimise the length of the stem part with primary fibres, but without secondary fibres, the background of secondary fibre development should be elucidated. It can be hypothesised that either flowering or the increasing plant size accelerates the formation of secondary fibres. To investigate this, an indoor experiment was conducted in greenhouses with mobile covers in which the day–length sensitivity of hemp was used to create size ranges of flowering and non–flowering plants for a single cultivar, Futura 75. Secondary fibre formation was recorded using microscopic techniques. The height up to which secondary fibres were present, depended on plant weight. The higher secondary fibre front in flowering plants was most likely caused by the higher weight of these plants as compared with non–flowering plants of the same height. Results from a field experiment confirmed the correlation between plant size and the height of the secondary fibre front. Therefore, to optimise the length of the stem part with primary fibres, but without secondary fibres above stubble height, for Futura 75 a relatively short crop of around 1.3–1.4 m should be harvested before flowering. This ideal crop height is likely to differ between varieties.

Key words: *Cannabis sativa* L., day length sensitivity, fibre hemp, fibre quality, flowering, plant height, plant weight, primary fibres, secondary fibres, textiles.

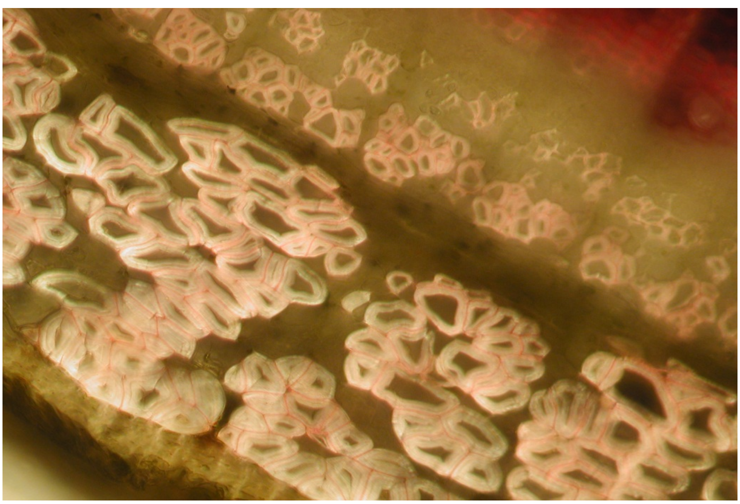
1 Introduction

1.1 Primary fibres are valuable, secondary fibres are unwanted

In fibre hemp (*Cannabis sativa* L.) two types of bast fibres occur, primary and secondary fibres. The classification is made according to their origin. The cell walls of both types are enforced with layers of cellulose, and both types are organised in bundles. In a cross–section of a hemp stem, the outer fibre bundle layer consists of primary fibres, the inner layer, if present, of secondary fibres (Picture 1) (Kundu and Preston, 1940; Kundu, 1942).

For spinning high–quality textile hemp yarns only the primary or 'long' fibres are valuable. Secondary or 'short' fibres are unwanted because these fibres are too short for spinning and their presence hampers the production of fine and homogeneous yarns from the primary or long fibres (Hoffmann, 1957; Bredemann *et al.*, 1961; Ranalli, 1999; Mediavilla, 2001; Schäfer and Honermeier, 2003).

Kundu (1942) stated that the presence of a few layers of parenchyma cells between the primary and secondary fibre bundles enables the isolation of the primary



Xylem

Secondary fibre layer

Primary fibre layer

Picture 1. A cross–section (100 × magnified) of a hemp stem with primary and secondary fibres stained with phloroglucinol (0.1 g in 20 ml 15% HCl), which colours lignin red.

fibre bundles by a retting process, but although secondary fibres can easily be distinguished under a microscope, and methods are available to isolate these short fibres in the laboratory (Bredemann *et al*., 1961; Van der Werf *et al*., 1994b), it is technically difficult to separate them from the primary fibres during commercial fibre processing (Kundu and Preston, 1940; Van Dam and Gorshkova, 2003). For this reason it should be known how the development of secondary fibres above stubble height can be avoided in the raw materials aimed at textile yarn production.

1.2 Primary fibre development

Primary bast fibre cells develop in the primary phloem that is differentiated from the procambium (Kundu, 1942; Aloni, 1987). Cells are initiated before or during stem elongation and fibre cells mainly grow with the surrounding tissue; they elongate when stems elongate (Kundu, 1942; Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005; Chernova and Gorshkova, 2007). While the neighbouring cells continue to divide, the multinuclear primary fibre cells can reach a length of 5−55 mm (average 20 mm) and a width of 10–60 μm before cell division occurs (Kundu, 1942; Brink *et al.*, 2003; Van Dam and Gorshkova, 2003).

The individual or elementary primary fibre cells are held together in bundles by binding substances that mainly consist of hemicelluloses, lignin, and pectins (McDougall *et al.*, 1993; Vincent, 2000; Keller, 2001; Brink *et al.*, 2003; Van Dam and Gorshkova, 2003; Sponner *et al.*, 2005). The long fibres of hemp, desired for textile processing, are collectives of such primary bast fibre bundles (Müssig and Martens, 2003; Chernova and Gorshkova, 2007). The high length–to–diameter ratio of the cells, between 250–1000 (Brink *et al.*, 2003), and the cell and bundle architecture make these fibres fit to be spun into yarns, and then woven or knitted into fabrics.

To introduce hemp long fibres into the fashion textile sector, fibres should be produced allowing the spinning of yarns between Nm 20 and Nm 40 (Nebel, 1995; Ranalli and Venturi, 2004), in which Nm is the Number metric, the yarn length in meters per 1 gram of mass ($m \cdot g^{-1}$). The finer the yarns that can be spun, the higher the value of the raw material is (Allam, 2004; Ranalli and Venturi, 2004).

Primary fibre bundles are already present in very young hemp stems. They run longitudinally along the stem from bottom to top and can reach almost the full length of the plants (Van Dam and Gorshkova, 2003; Hernandez *et al.*, 2006). These fibres have to be strong enough before the bundles can be extracted. This so–called 'ripeness' or maturity of the primary fibres is closely connected with the cellulose filling degree of the cells, which progresses from bottom to top, and from the outer to the inner part of the stem. At maturity the lumen of the tapering fibre cells is very small and protoplasm is absent (Kundu, 1942; Mediavilla *et al.*, 2001; Schäfer and Honermeier, 2003; Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005).

Hemp is usually harvested around the time of flowering, when the primary fibres are filled and stem dry matter yield and fibre yield are highest (Bócsa and Karus, 1998; Mediavilla *et al.*, 2001; Schäfer and Honermeier, 2003; Amaducci *et al.*, 2005; Burczyk *et al.*, 2009).

1.3 Secondary fibre development

Secondary fibres might derive from tangential division of vascular cambium cells when a stem part has reached its maximum length (Kundu and Preston, 1940; Kundu, 1942; Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005; Chernova and Gorshkova, 2007). These uninucleate fibre cells are no longer than 2 mm (Kundu, 1942; Hoffmann, 1957), which is too short for spinning. Besides, secondary fibres contain too much lignin (Kundu and Preston, 1940; Schäfer and Honermeier, 2003), which is detrimental for the production of fine, flexible, and homogeneous yarns (Bócsa and Karus, 1998; Cappelletto, 2001).

Secondary fibres are absent in young hemp plants or only present in a thin layer at the stem base (Van Dam and Gorshkova, 2003; Amaducci *et al.*, 2005, 2008a; Hernandez *et al.*, 2006). However, around the usual harvest time, hence around flowering, secondary fibres are found higher up along the stems, with a thicker layer towards the bottom part of the stem (Van der Werf, 1994b; Mediavilla *et al.*, 2001a; Schäfer and Honermeier, 2003, 2006; Amaducci *et al.*, 2005, 2008a).

Mediavilla *et al.* (2001a) stated that with the induction of the generative phase secondary fibre formation increases quickly, at first in female plants. Schäfer and Honermeier (2003, 2006) also related the observed increased secondary fibre formation and thicker secondary fibre layers to a phenological stage of the plant: the beginning of flowering. However, the observed coincidence of enhanced secondary fibre formation with the transition from the vegetative to the generative growing stage of the plants does not necessarily point at a causal relationship between the two.

1.4 Coincidence or cause?

Although it cannot be excluded yet that flowering accelerates the process of secondary fibre formation, it seems more likely that the size of the plant determines the amount of secondary fibres. Botanically the bast fibres in hemp belong to the sclerenchyma tissue which gives mechanical support to the plants (Kundu, 1942; McDougall *et al.*, 1993; Van Dam and Gorshkova, 2003) and the need for such support increases when plants grow taller and when tops become heavier, due to the development of inflorescences and the filling of the seeds. It could be considered an example of 'mechanoperception', the perception of mechanical stimuli that keep plants in balance with their physical environment (Telewski, 2006).

The findings of Bredemann *et al.* (1961), Van der Werf *et al.* (1994b), Höppner *et al.* (2004), and Amaducci *et al.* (2005, 2008a) that increased amounts of secondary fibres were present in plants with higher stem dry weight and in lower internodes could support our view. However, secondary fibre formation was not related to phenological stage in these experiments, hence flowering as an accelerator of secondary fibre formation cannot be excluded. Flowering as the exclusive trigger to secondary fibre formation must be excluded as secondary fibres are also observed in non–flowering hemp (Amaducci *et al.*, 2005; Hernandez *et al*., 2006).

It can be hypothesised that either the change from the vegetative to the generative phase accelerates the formation of secondary fibres higher up along the stems or that the increasing height and weight of the plant, which obviously are strongly correlated, cause the formation of secondary fibres. To avoid the presence of the unwanted secondary fibres above stubble height and to optimise the production of uncontaminated valuable primary fibres, we need to know whether hemp should be harvested before flowering or before the plants become too tall. Therefore, the background of the development of secondary fibre formation during the growing cycle should be elucidated.

1.5 A size range with flowering and non–flowering plants

To discriminate between flowering and plant size as the cause of enhanced secondary fibre formation, a broad size range of flowering and non–flowering plants is required. Such a test set cannot easily be achieved in a field experiment, as flowering plants grown under natural climate and day–length conditions at higher latitudes in general are relatively tall at the end of the growing season. However, in a greenhouse with day–length control it is rather simple to disconnect phenological stage and plant size.

The reason is that hemp is a short–day plant (Tournois, 1912; Borthwick and Scully, 1954; Heslop–Harrison and Heslop–Harrison, 1969) and can be kept vegetative and growing for a prolonged period of time under long day conditions, while a transition to short–days triggers the plant to flower within days (Heslop–Harrison and Heslop–Harrison, 1969). Around this transition, the longitudinal growth of the plant slows down or stops (Hoffmann, 1957; De Meijer and Keizer, 1994; Meijer *et al.*, 1995; Schäfer and Honermeier, 2003), hence by transferring plants from a long–day compartment to a short–day compartment at different moments in time, the desired test set with flowering and non–flowering plants of different sizes can be achieved.

Harvesting plants from different treatments and microscopic analyses from stem cross– sections regarding the presence or absence of secondary fibres then provides the opportunity to find out whether or not flowering and secondary fibre formation are related. The results of an earlier conducted field experiment were analysed for comparison.

The aim of the day–length treatments thus is simply and solely to obtain a size–range of flowering and non–flowering plants. Microscopic measurements on plants of this test set were not related to the day–length treatments the plants underwent, but only to the result of the treatments: the size characteristics of the plants and their phenological stage.

2 Materials and Methods

Sites and seeds

Both the field and the greenhouse experiment were carried out in Wageningen (51° 58' N, 5° 4' E), with a monoecious variety, Futura 75, to reduce plant–to–plant variation (Van der Werf and Van der Berg, 1995). Seeds were purchased from La Fédération Nationale des Producteurs de Chanvre (FNCP), Les Mans, France.

Preparation of cross–sections

Cross–sections of stems were cut by hand with razor blades and then placed on glass slides. The cuts were stained with phloroglucinol (0.1 g in 20 ml 15% HCl), which colours lignin red, hence facilitating the distinction between different tissues. After 12 minutes the excess of the solution was removed with filter paper and replaced with a droplet of 50% glycerol to prevent the sections from drying (Kundu and Preston, 1940; Ko *et al.*, 2004; Hernandez *et al.*, 2006). Light microscopes (Zeiss) with lenses of ×40 and ×100 magnification were used to study the cross–sections.

2.1 Greenhouse experiment

The greenhouse experiment was carried out between 19 May (day 1) and 25 July 2005 (day 68) in two similar adjacent greenhouses with horizontal airflow, in which the period of natural day–light was controlled by means of a mobile cover.

Substrate and sowing

148 plastic containers of 29 cm × 29 cm × 28 cm (l × w × h) were filled with potting soil (1.5 mg $N \cdot l^{-1}$) mixed with 2 $g \cdot l^{-1}$ Osmocote® (13% N, 13% P_2O_5, 13% K_2O, 2% Mg, and 1% Fe). Per container ± 30 seeds were evenly distributed over the surface and covered with 3–4 cm of the substrate. At full emergence (day 12), the seedlings were manually thinned to 17 per container. The resulting density of approximately 200 plants m^{-2} suppresses branching and lies within the density range appropriate for textile destinations (Dempsey, 1975; Amaducci *et al.*, 2002a,b). Plants were watered every day with a spray nozzle to keep the soil slightly humid.

Light regimes

Lisson *et al.* (2000) showed that for Futura 75 in photoperiods less than 14 hours flowering occurs in a minimum constant thermal time, while at longer photoperiods flowering is progressively delayed. Therefore two day–length regimes were established:

1. Short day (SD) = 12 hours, to induce flowering:
2. Long day (LD) = 16.5 hours, to prolong vegetative growth.

Plants in both greenhouses were exposed to natural daylight for 12 hours a day (8 am –8 pm). To avoid etiolation and excessive elongation, artificial light was supplied to reach a photosynthetically active radiation (PAR) of approximately 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level. To avoid damage of the growing points and in order not to hamper the plant's longitudinal growth, the artificial light had to be removed on day 41. Day length extension was performed with incandescent light (Heslop–Harrison and Heslop–Harrison, 1969) for 4.5 hours with Philips 60W light bulbs (R–FR–ratio = 0.6) causing approximately 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR at canopy level. Erroneously, until day 19 these lights were on for 6 instead of 4.5 hours a day, causing a day−length of 18 hours instead of the intended 16.5 hours. However, with respect to the aim of the treatment, keeping the plants vegetative for a prolonged period, no damage was caused.

Treatments

Five different day−length treatments were performed in order to create size ranges of flowering and non−flowering plants:

1. Continuously LD
2. 2 weeks LD, then SD
3. 4 weeks LD, then SD
4. 6 weeks LD, then SD
5. Continuously SD

Plants from 60 pots were used for measurements. Of these pots, 20 were placed under SD and 40 were initially placed under LD the day after sowing. During the experiment the experimental pots were always closely surrounded by border pots to avoid edge effects like branching.

Biocides

After 7 weeks a mild aphid infestation was observed after which the plants were sprayed with Pirimor. To control spider mites, Spindex, a biological insecticide with predatory mite *Phytoseiulus persimili*, was applied.

Harvests

In the continuous LD (1) and continuous SD (5) treatment, plants were harvested seven times on a weekly basis, starting three weeks after sowing. In the transfer treatments (2, 3, and 4) weekly harvesting started one week after the transfer. Per harvest, per treatment, 5–10 plants were harvested. A total number of 178 plants were analysed. Harvested plants were stored in the dark at 4 °C until the measurements were performed and cuts were made. Characteristics of plants and cross−sections were recorded and the remainders of the plants were dried for at least 24 hours at 105 °C to determine plant dry weight (though, without the microscopic sections made).

Temperature and climate control

Set points for automatic temperature control were 20 °C during the 12 hours of natural day light and 15 °C for the remaining 12 hours. Realised average day and night temperatures were 19.2 ± 0.4 °C and 14.5 ± 0.2 °C respectively for SD and 19.4 ± 1.2 °C and 14.8 ± 0.2 °C respectively for LD. Relative humidity was 67% ± 4% for both.

Thermal time

Thermal time, measured in °C · day, is the accumulated temperature above a base temperature. Below this temperature the process under study, development, stops. A mean day temperature is used, with a base temperature of 2 °C (Van der Werf, 1997).

Cross–sections

Initial cross–sections were made at 10 cm above soil level and at ¾ of the length of the first internode. The initial cross–sections were below stubble height which in practice is 15–20 cm (personal communication M. Reinders, Hempflax). Further cuts were made at approximately 10 cm intervals, until no secondary fibres were visible anymore.

Statistical analyses

Statistical analyses of the data ($P < 0.05$) were conducted using GENSTAT® release 9.2. Following tests for normality multiple linear regression analyses were performed. Stepwise addition or subtraction of terms was carried out to define the most suitable model to use in general linear modelling, i.e. the model with the minimum residual mean squares. There were no statistical or biological reasons to test non–linear models. Only plants in which secondary fibres were observed at 10 cm and higher were taken into account.

2.2 Field experiment

The experiment was carried out between 29 April (day 1) and 6 September 2004 (day 130). Stems were taken from a three–replicate randomised field experiment with three sowing densities. Due to unfavourable weather conditions (heavy winds) prior to the intended harvests, part of the crop was damaged. For this reason no yield data on this field experiment were collected. The experiment was repeated in 2005 (Westerhuis *et al.*, 2009a [Chapter 2]). Seeds were sown on 29 April, which is a normal sowing date for fibre hemp in the Netherlands (e.g., Van der Werf, 1994; Struik *et al.*, 2000; Westerhuis *et al.*, 2009b [Chapter 3]), with a precision drill at a depth of approximately 3–4 cm at target plant populations of 120, 240, and 360 plants m^{-2}. Distance between rows was 12.5 cm. No biocides were used. The crop was not irrigated. Normal plants of average size were taken regularly from undisturbed parts of the plots, with at least 1m border rows to avoid edge effects.

Cross–section height

Three cross–sections were made per internode: 2 cm above and 2 cm below the nodes and in the middle of the internode.

3 Results

3.1 Greenhouse experiment

At the first harvest time, when plant height was about 50 cm, in none of the plants secondary fibres were observed (Figure 1). Then, with subsequent harvests or increasing thermal time, the plant height range, as intended, became broader. The upper and lower limit of the size range is indicated by the lines in Figure 1. The height range of heights up to which secondary fibres were present became wider as well, for both flowering and non–flowering plants.

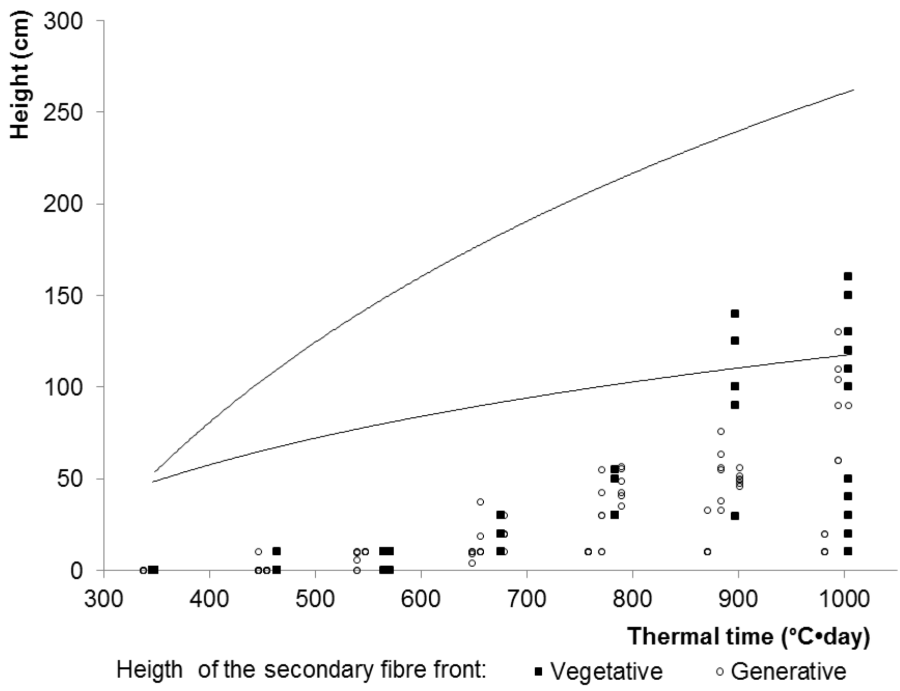


Figure 1. The height of the secondary fibre front against thermal time. The open dots represent flowering plants, the black squares represent non–flowering plants. Due to the experimental design a dot can represent more than one plant. The lines indicate the minimum and maximum plant height in the course of thermal time.

Because of the expected relationship between plant size and the height of the secondary fibre front, the 122 plants in which secondary fibres were observed were further analysed. A single regression line was adequate (Table 1A) to describe the relation between the height of the secondary fibre front and plant weight, where y = –18.3 + 6.1x is valid for both flowering and non–flowering plants (Figure 2). The outcome indicates that when plant dry weight (x) is above 5.5 grams, plants are likely to contain secondary fibres above a 15 cm stubble height.

Regression of the height of the secondary fibre front on plant height showed separate lines for vegetative and flowering plants (Figure 3, Table 1B). In flowering plants the height of the secondary fibre front initially is higher as compared to non–flowering plants of the same height. The lines indicate that flowering plants above 114 cm and non–flowering above 145 cm are likely to contain secondary fibres above a 15 cm stubble height.

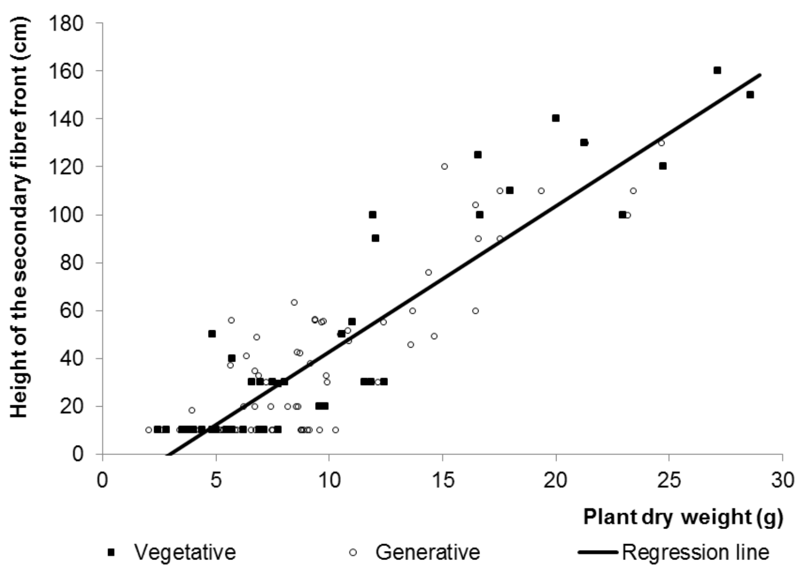


Figure 2. The height of the secondary fibre front plotted against plant dry weight. The open dots represent flowering plants, the black squares represent non–flowering plants. The regression line y = –18.3 + 6.1x is valid for both flowering and non–flowering plants. R^2 = 0.80, n = 122.

Table 1. Analysis of variance table for the multiple linear regression of the height of the secondary fibre front against:

A) plant dry weight
B) plant height

A. Fitted terms ($P < 0.05$)	d.f.	s.s.	m.s.	v.r.	F pr.
1. Dry weight	1	148469.4	148469.4	489.41	< 0.001
Residual	121	36707.1	303.4		
Total	122	185176.5	1517.8		

B. Fitted terms ($P < 0.05$)	d.f.	s.s.	m.s.	v.r.	F pr.
1. Plant height	1	143956.7	143956.7	526.68	< 0.001
2. Flowering	1	6008.5	6008.5	21.98	< 0.001
3. Plant height × flowering	1	2685.3	2685.3	9.82	0.002
Residual	119	32526.0	273.3		
Total	122	185176.5	1517.8		

d.f., degrees of freedom; s.s., sum of squares; m.s., mean sum of squares; v.r., variance ratio; F pr., F probability.

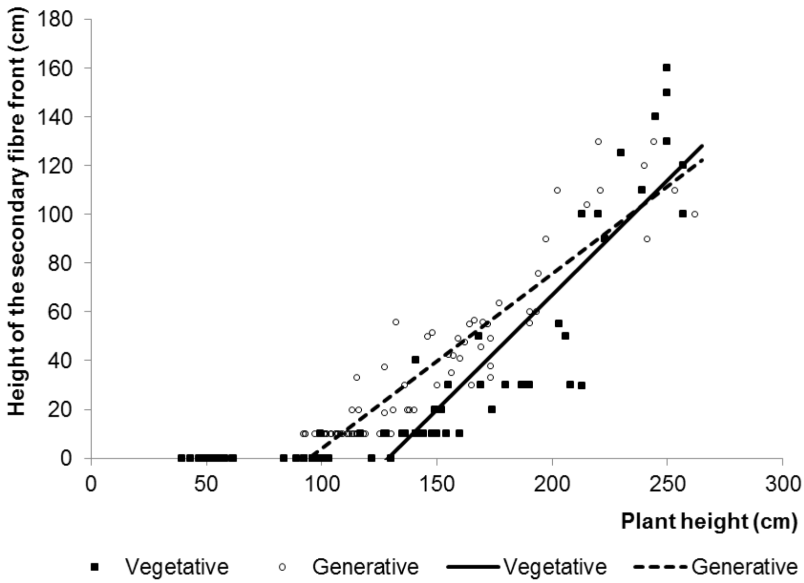


Figure 3. The height of the secondary fibre front plotted against plant height. The open dots represent flowering plants, black squares represent non–flowering plants. The regression lines are different for flowering (broken line; y = –121.0 + 0.94 x; n = 107) and non–flowering plants (drawn line; y = –67.2 + 0.72 x; n = 71). R^2 = 0.82 for the total model (Table 2).

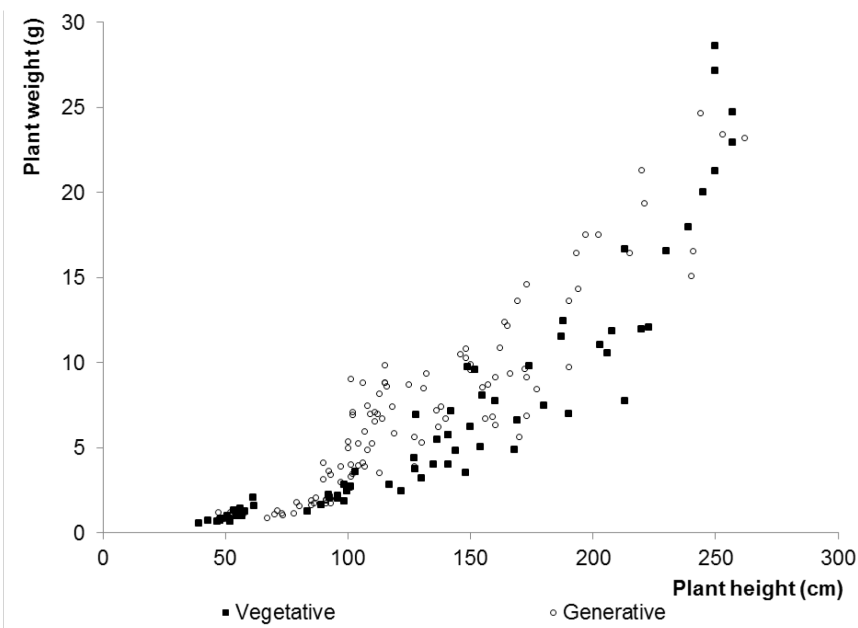


Figure 4. Plant dry weight plotted against plant height. The open dots represent flowering plants, the black squares represent non–flowering plants.

While secondary fibre formation was correlated to both plant weight (Figure 2, Table 1A) and plant height (Figure 3, Table 1B), plant height and weight were obviously correlated, though not linearly (Figure 4). Over a large range of plant heights and weights, flowering plants were found to have a higher plant weight than non–flowering plants of the same height.

3.2 Field experiment

In the field experiment primary fibres were present in all stems that were analysed (Figure 5). With increasing plant height, the distance between the observed primary fibre front and the top of the plants became smaller. In plants above 1.5 m, primary fibres were observed up to approximately 10–15 cm below the top.

Secondary fibres were, with one exception, only observed in plants above 1.3 m and were always observed in plants above 1.5 m. Flowering plants in this experiment were all above 2.1 m, with secondary fibre front heights above 0.8 m.

4 Discussion

As expected, a size range of flowering and non−flowering plants could be created with different day–length treatments and harvest times. Short days triggered flowering, while plants remained shorter. Long days kept the plants vegetative and extending in height. Successive transfer treatments produced flowering plants of increasing height and weight, providing a more–or–less continuous data set for both variables. Almost the full plant height and weight ranges were covered by flowering as well as non–flowering plants (Figures 1–4). With increasing thermal time, the height of the secondary fibre front became increasingly variable for both flowering and non–flowering plants (Figure 1) hence thermal time as such did not explain the height up to which secondary fibres were present in fibre hemp stems.

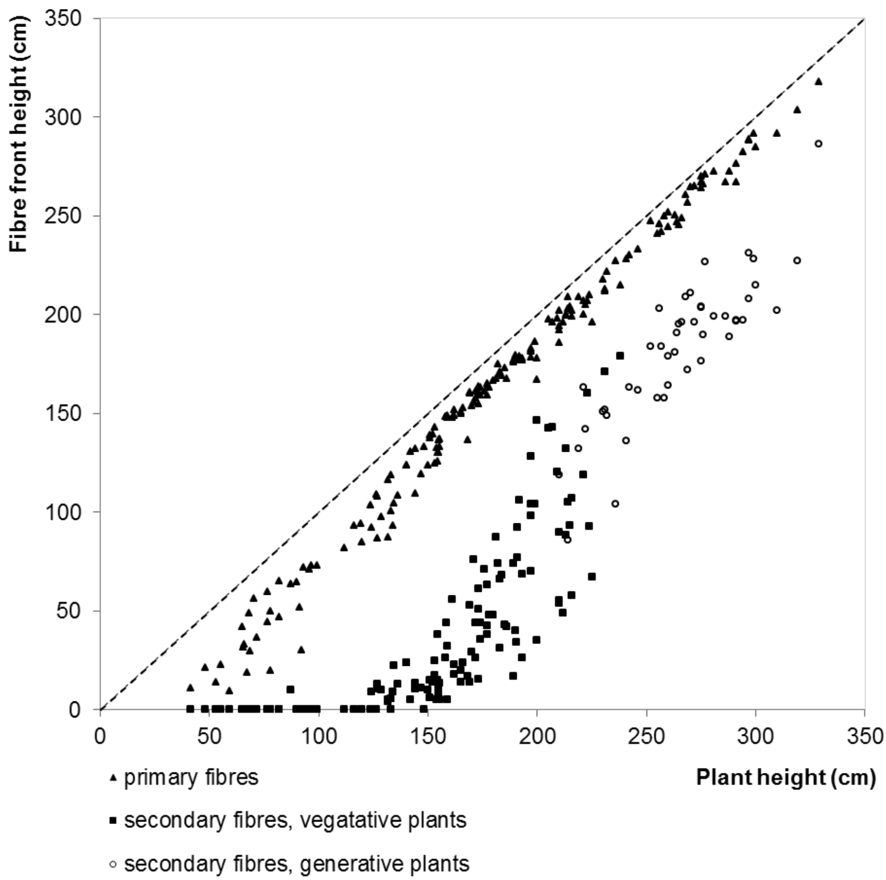


Figure 5. The height of the primary (triangles) and secondary fibre front separately for flowering plants (open circles) and non–flowering plants (black squares) plotted against plant height in 179 plants selected from a field experiment in 2004. The broken line represents the 1:1 line.

4.1 Increasing plant weight causes secondary fibre formation

In the field experiment, flowering plants showed much higher secondary fibre fronts than non–flowering plants. Flowering plants, however, were consistently taller as well (Figure 5). The disentanglement of plant size and phenological stage in the greenhouse experiment revealed that the height up to which secondary fibres are present in fibre hemp stems increases with increasing plant weight (Figure 2) and height (Figure 3).

During early sampling very often no secondary fibres were observed at 10 cm height or higher, comparable to what is shown in Figure 5 for the field experiment.

These samples were not included in the regression analyses as they would lead to non–linearity in the relations (cf. Figures 2 and 3 with Figure 5) .

Although plant weight explains the height of the secondary fibre front in the simplest way with a single regression line that is valid for both flowering and non–flowering plants, providing a biologically nice and simple model, for practical reasons the relation with plant height is more important because primary producers need non–destructive means to know at which height the crop should be harvested to avoid that the unwanted secondary fibres contaminate the valuable primary fibres.

This is more complicated as the regression lines for flowering and non–flowering plants are different. The secondary fibre front is found a little higher in flowering plants when compared to non–flowering plants of the same height. This must be due to the higher weight or momentum of flowering plants as compared to non–flowering plants of the same height (compare Figures 1–4). Consequently, to maximise the length of the stem parts fit for the production of high–quality hemp yarns the crop should be harvested before flowering and before it becomes too tall. Increasing plant weight as a trigger to secondary fibre growth, a form of mechanoperception (Telewski, 2006), was also found in Arabidopsis (Ko *et al.,* 2004) where auxins were identified as the downstream signal transducer.

4.2 A model

Although the change from the vegetative to the generative growing phase as such does not enhance secondary fibre formation higher up along the stems, it can be understood why these phenomena under field conditions often occur roughly at the same time and therefore seemed to be related (Mediavilla *et al.*, 2001a; Schäfer and Honermeier, 2003, 2006). In Figure 6 our conceptual model is drawn.

During the growing season plant height and stem weight increase and consequently the secondary fibre front moves upwards. Possibly the force that is exerted by the stem part above the secondary fibre front is rather constant, as it seems likely that passing a certain threshold value triggers the development of secondary fibres to resist an increased force.

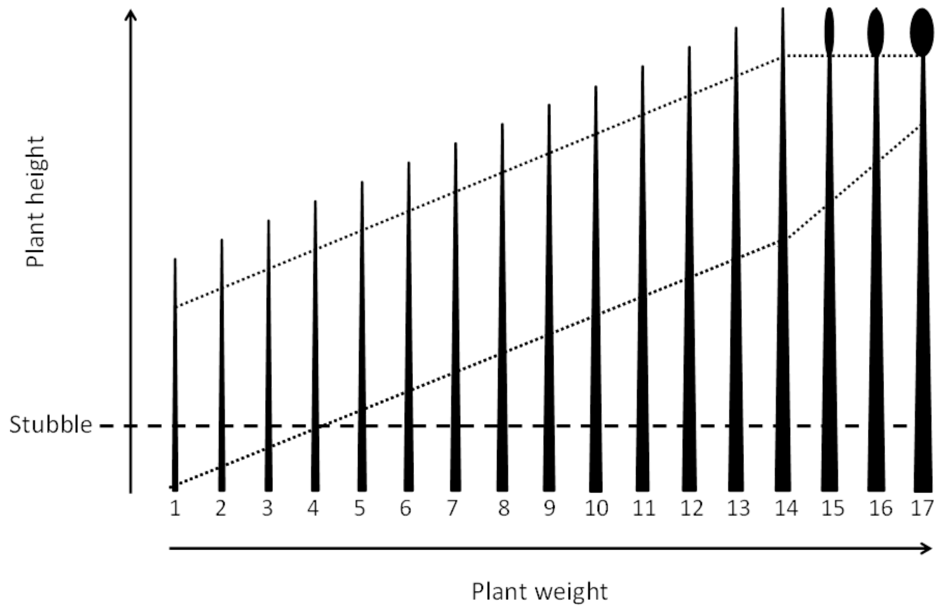


Figure 6. Conceptual model of the harvestable stem part for quality primary fibres in hemp for plants of increasing height, weight, and phenological stage (numbered 1-17 for reference in the text). The stem part that is valuable for textile yarn production, the 'middle' stem part between the dotted lines, moves upwards along the stem. Below the lower line secondary fibres contaminate the valuable primary fibres, above the upper line the developing inflorescences are detrimental for the quality of the primary fibres. Around flowering (stages 14-15) length growth slows down but the weight of the top increases (plants 15–17). An accelerated development of secondary fibres as compared to the length growth, but keeping pace with the increasing weight or momentum could be the result. Consequently, the length of the stem part that is valuable for high–quality yarn spinning becomes shorter around flowering

At the transition from the vegetative to the generative phase length growth slows down (Hoffmann, 1957; De Meijer and Keizer, 1994; Meijer *et al.*, 1995; Schäfer and Honermeier, 2003) and the weight of the top of the plant increases due to the development of the inflorescence and the subsequent filling of the seeds. An accelerated development of secondary fibres as compared to the longitudinal growth, but keeping pace with the increasing weight is hypothesized. Consequently, the length of the stem part that is valuable for high–quality yarn spinning becomes shorter from around flowering onwards.

Plants of increasing height and weight in Figure 6 could represent the same plant at different moments in time, but they can also be considered different plants in a crop at the same time, because with respect to primary (Westerhuis *et al.*, 2009a,b [Chapters 2 and 3]) and secondary fibre development (this chapter), individual plant size is the key factor, not the moment a particular height or weight is reached, or the phenological stage.

The stem part that is valuable for textiles, the stem part between the dotted lines in Figure 6, is located higher up along the stem with increasing plant size. It is supported by a stem base of increasing length and weight, which is unfit for yarn production due to the presence of secondary fibres.

In short plants (Figure 6, plants 1–3) a share of the primary fibres, though fit for yarn spinning, is lost in the stubble, which in practice is 15–20 cm (personal communication M. Reinders, Hempflax). Plants with the secondary fibre front just reaching stubble height (4 in Figure 6) could be considered ideal. Once the secondary fibre front surpasses the stubble height (plants 5–17 in Figure 6) part of the primary fibres are contaminated by secondary fibres. The lower in the plant or the heavier the part of the stem above, the thicker this unwanted fibre layer is likely to be (Van der Werf *et al.*, 1994b; Amaducci *et al.*, 2005, 2008a).

For reasons of simplification, the inflorescences of the plants in Figure 6 have equal lengths for all plants, it could however be expected that this part becomes gradually longer with increasing plant size. However, no data were recorded.

Mediavilla *et al.* (2001) showed in a dioecious variety that the accelerated development of secondary fibres around flowering first takes place in female plants and later in male plants. This makes sense, when the increasing weight is considered the cause of secondary fibre formation. Male plants on average are longer (De Meijer and Keizer, 1994), but the female inflorescences, where the seeds are gradually filled, are likely to have a higher weight. Bócsa and Karus (1998) also state that female plants have a relatively higher secondary fibre content than male plants.

The proposed conceptual model (Figure 6) also implies that homogeneity with respect to plant height is important when growing hemp for textile uses. In stands with plants of different heights the valuable 'middle section' is at variable height, which would cause difficulties in harvesting and processing. The tops of the plants are the comparable parts, not the bottom parts.

4.3 Growing short hemp

According to both Figure 4 (glasshouse) and 5 (field), secondary fibres above stubble height in Futura can be avoided when the crop is cut when plants are around 1.3–1.4 m and not yet flowering. It is likely, however, that this height is different for varieties, as secondary fibre development is reported to be different between varieties (Hoffmann, 1957; Bredemann *et al.*, 1961; Höppner *et al.*, 2004; Amaducci *et al.*, 2008a). Reported differences however, could partially be due to size differences as well. For this reason true varietal differences and differences caused by size differences should be disentangled in future experiments.

A non–flowering crop with a height of 1.3–1.4 m can be grown in about two months when hemp is sown at a normal sowing date in April under Dutch growing conditions (Westerhuis *et al.*, 2009b [Chapter 3]). However, a subsequent second fibre hemp crop, to compensate for the relatively low stem dry matter yield of the short crop (Kamat *et al*., 2002; Liberalato, 2003; Westerhuis *et al.*, 2009b [Chapter 3]), will be a challenge. For the first crop varieties can be chosen based on excellent fibre qualities, for the second crop to be grown late flowering and low secondary fibre content are prerequisites as well. Further the soil has to be prepared into a homogeneous seedbed again and in dry years irrigation might be necessary to achieve even emergence (Westerhuis *et al.*, 2009b [Chapter 3]). Due to the dryer summers and shorter days a second crop seems less realistic in Italy than in The Netherlands. However, even when technically possible, it should be economically sound as well, which is doubtful as yet.

An advantage of a short crop is that the stems could be processed on existing flax (*Linum usitatissimum* L.) processing lines as the limited market for high–quality hemp yarns as yet does not justify the development of specialised hemp scutching and hackling lines (Liberalato, 2003; Ranalli and Venturi, 2004). Such systems are dimensioned for flax ribbons with a length usually between 80 and 120 cm (Vreeke, 1991; Ranalli and Venturi, 2004; Venturi *et al.*, 2007), which is about the length of the useful middle section of the stems in our experiment.

Earlier attempts in Italy to produce smaller hemp plants by stopping their growth with glyphosate at the desired plant height of 1.2 m ('baby hemp') failed due to the environmentally unfriendly production methodology, low yields, low quality, and high costs (Liberalato, 2003; Amaducci, 2005; Venturi *et al.*, 2007; Van der Werf and Turunen, 2008). Westerhuis *et al.* (2009b [Chapter 3]), however, showed that normal amounts of scutched long fibres can be extracted from smaller plants. However, these fibres were not hackled.

It is likely that the early harvested short crop that we need to avoid secondary fibre formation has relatively fine primary fibres as well. Fibres have been reported to be finer with decreasing plant size or conditions that in general cause smaller plants, e.g., earlier harvest or increasing sowing density (Jakobey, 1965; Leupin, 2001; Schäfer and Honermeier, 2003, 2006; Amaducci *et al.*, 2005, 2008). Also with respect to the unwanted lignification of the fibres an early harvest before flowering seems best (Keller *et al.*, 2001).

For future research on this topic it has to be considered that between individual plants of the same height or weight relatively large differences exist in the height of the secondary fibre front (cf. spread of data in Figures 2 and 3). This was not only caused by the fact we measured once every 10 cm stem length; in the field experiment where three cuts were made in every internode a comparable spread was observed (Figure 5). Differences between plants (e.g., weight distribution along the stem, shape of leaves and inflorescences) and, e.g., wind would co-determine the actual forces (momentum) along the stem and thus the mechanoperception (Telewski, 2006) inducing fibre formation. Further, we should keep in mind that countering the forces the plant is subjected to, means countering the fresh weight, not the dry weight we measured hence plant fresh weight should be measured as well. To study the effect of weight on secondary fibre formation in more detail, plant weight could be increased artificially (Ko *et al.*, 2004).

Acknowledgements

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Chapter 6

General discussion
Limited options to manipulate the fibre content in hemp

1 Plant size matters

In this thesis it is shown that within any tested fibre hemp variety, the dry weight of the stems at harvest, and not the factors underlying this dry weight, determine the amounts of bast fibres, wood, and retting losses. In the retted stems the dry matter is split up into fibres and wood in a fixed way, which depends on variety. The options to manipulate this ratio by crop management, if any, are very small and for practical reasons they can be neglected (Westerhuis *et al.*, 2009a,b,c [Chapters 2, 3, and 4]).

The thesis also reveals that the height up to which secondary fibres are present in fibre hemp stems increases with increasing plant weight. A causal relationship between secondary fibre formation and flowering, as suggested in literature, does not exist. However, the secondary fibre front is found a little higher in flowering plants, which must be due to the higher weight or momentum of flowering plants as compared to non–flowering plants of the same height (Chapter 5).

The practical consequence of the above is that when a hemp grower has chosen to produce raw materials for high–quality textile purposes, and has selected a certain variety for reasons beyond the scope of this thesis, his main concern is to avoid that secondary fibre formation, which proceeds upwards along the stem during the growing season keeping pace with the increasing weight, contaminates and devalues his end product. Therefore, crop management should be focused on keeping the plants small enough to avoid secondary fibres above stubble height. Further, a harvest just before flowering is preferable, because the increasing weight of the inflorescences gradually reduces the length of the stem part that is fit for high–quality textile yarn production.

The options to produce plants with the desired size characteristics are manifold. Since sowing density, harvest time, and sowing date do not have an additional effect on the primary fibre content besides the above mentioned indirect effect through stem weight (Westerhuis *et al.*, 2009a,b [Chapters 2 and 3]), any combination of these factors could be chosen to produce the desired crop.

These are the main conclusions of the research, whose objective was to outline the agronomic options for manipulating plant development and crop growth of fibre hemp in order to produce high–quality long textile fibres. In this chapter, the obtained knowledge is summarised, integrated, and considered in a broader perspective.

In earlier publications direct effects of sowing density, sowing date, and harvest time on fibre content or fibre percentage were suggested or claimed. These claims however, were contradictory, incomplete, and weakly substantiated. Most likely the key factor plant size was missed due to focusing on the intended differences between treatments rather than observing and analysing the effects of the treatments: the differences in plant size and the consequences thereof with respect to fibre content.

The split–up of many stem samples into basic compounds and the analysis of the ratios between them turned out to be crucial in elucidating the principles that were not understood before. Instead of looking further forward into the production chain to important fibre quality characteristics such as, e.g., fineness, refinability, homogeneity, and strength, as intended at the very start of the research, a back to basics approach had to be chosen to disclose that sowing density, sowing date, and harvest time only affect the fibre content of hemp indirectly, through their effect on stem weight.

In the next paragraphs the methodology is explained and accounted for. Main lines of the research are summarised and the consequences with respect to growing and processing fibre hemp are considered. Some further thoughts on fibre hemp are presented and finally some goals for further research are set.

2 A different methodology

The strong linear relationships we found between fibre and wood production, between long fibre and total fibre production, and between stem weight before and after retting (Westerhuis *et al.*, 2009a,b,c [Chapters 2, 3, and 4]) could be disclosed by the separate and independent calculation of retting losses and fibre content (Section 2.1) in a very large number of well–defined samples (Sections 2.2 and 2.3) by means of multiple linear regression analyses instead of standard ANOVAs (Section 2.4). In these respects our experiments differ from earlier research on this topic. The fibre extraction method consisting of retting, breaking, and scutching, however, is traditional.

2.1 Disentangling retting losses and fibre percentages

To determine the value of a hemp crop we need to know the quantity and the quality of the fibres. The quantity, the bast fibre yield, can be assessed based on the stem dry matter yield and the fibre percentage (Sankari, 2000). Usually such fibre percentages (Equation 1a) are calculated by dividing the dry weight of the extracted fibres by the dry weight of the stems before retting (e.g., Bredemann, 1922, 1940; Bredemann *et al.*, 1961; Van der Schaaf, 1963; Höppner and Menge–Hartmann, 1994; Van der Werf *et al.*, 1994b; Sankari, 2000; Vetter *et al.*, 2002; Amaducci *et al.*, 2008):

1a. Fibre percentage = 100 × dry weight fibres/dry weight stems before retting

Because merely fibres and wood survive retting (Hann, 2005), all other materials present in hemp stems before retting can be considered as (future) retting losses:

1b. Fibre percentage = 100 × dry weight fibres/dry weight (fibres + wood + retting losses)

This fibre percentage is an inadequate variable to compare crop management factors or combinations thereof and to understand underlying botanical processes, because no distinction is made between the amounts of wood and the materials that are lost during retting; these are considered as a whole. Because we wanted to study these amounts independently, we determined retting losses separately:

1c. Retting loss percentage = 100 × (1 − dry weight retted stems/dry weight stems before retting)

$= 100 \times (1 - \text{dry weight (fibres + wood/)/dry weight (fibres + wood + retting losses)}$

Next we could calculate fibre percentages based on the dry weight of the stems after retting:

1d. Fibre percentage $= 100 \times \text{dry weight fibres/dry weight retted stems}$

$= 100 \times \text{dry weight fibres/dry weight(fibres + wood)}$

or even simpler, calculate or visualise the ratio in which fibres and wood, the remaining components, are produced. The latter has an advantage: interpretation is easier with linear relationships (e.g., stem weight before retting vs. stem weight after retting, wood vs. fibre, long fibre vs. total fibre) than with the curvilinear graphs that result from calculating percentages.

In earlier investigations retting losses were not reported or not in as much detail as needed to calculate the ratio in which fibres and wood were produced. Therefore a proper comparison between the results found in literature with the results we obtained unfortunately is not possible. However, the split–up introduced here improved our insight into fibre production in hemp. Consequently, the background of some of the contrasting results presented in literature so far, at least partially, could be revealed.

2.2 A large number of well–defined samples

Another advantage as compared to earlier publications on this subject is the large number of samples that were analysed. Under controlled conditions and following standard protocols, approximately 1500 samples were fractionated into long fibres, tow, wood, and retting losses.

The use of a Flemish mill for scutching limited the minimum length of the samples to approximately 50 cm and the use of a traditional flax breaker required samples of a certain minimum size and mass. In general, we used 50–stem samples.

Although this might seem to be a rather rough approach, the necessary sample size also guaranteed that the variation within the raw material batches was covered. Besides, the traditional extraction method we used was definitively more fit for the purpose than the frequently used 'Reinfaser' method by Bredemann (1922), which at a first glance, however, might seem to be more 'refined'. As much shorter stem parts can be processed using the method of Bredemann, a more detailed view of the pattern along the stem can be obtained (Bredemann, 1940; Van der Werf, 1994b). It would however not have been possible to distinguish between long fibres and tow, which is of highest importance if one wants to determine whether or not the extracted fibres are fit for yarn production. Further only small samples, usually 3– or 5–stem samples, can be processed (e.g., Bredemann, 1940; Höppner and Menge–Hartmann, 1994; Van der Werf, 1994b; Sankari, 2000) for which it is questionable whether they cover the variation within the batches. Finally, determining the wood weight and retting losses using the methodology of Bredemann (1922) would have been very laborious, taking into account the number of samples and their size. The researchers using the method of Bredemann (1922), e.g., Bredemann (1940), Höppner and Menge–Hartmann (1994), Van der Werf (1994b), and Sankari (2000), did not determine the wood weight of the tested stem parts, for which nevertheless a (time consuming) method is described by Bredemann in the same paper. Unfortunately this makes re–analysis of their results for comparison with our methodology impossible.

2.3 Exclusion of 'underhemp'

Especially in hemp care should be taken to describe the batches of raw materials properly. Very small plants under certain circumstances might survive in the low–light environment under the canopy (Hoffmann, 1957). Their total weight is negligible, long fibres cannot be extracted, and these plants will completely be lost during processing (Van der Schaaf, 1963; Bócsa and Karus, 1998). However, the effect of this 'underhemp' on average plant density, plant height and stem diameter could be large (see, e.g., Westerhuis *et al.*, 2009a [Chapter 2], Table 4). Consequently, reliable measurements could lead to weak conclusions.

For this reason plants shorter than 100 cm were excluded from processing, and plant characteristics were always only measured and recorded on the very plants that were processed. Lisson and Mendham (2000) also discarded the 'severely suppressed' living plants in their hemp trials, however, without reporting the size limit.

2.4 Multiple linear regression analyses instead of standard ANOVAs

During data processing it became apparent that the total amounts of fibre and wood in hemp stems were linearly related (e.g., Westerhuis *et al.*, 2009a [Chapter 2], Figure 4 and Westerhuis *et al.*, 2009c [Chapter 4], Figure 2) and that, because of the large number of samples, multiple linear regression analyses could be used to consider this relationship into more detail.

Multiple linear regression with stepwise addition or subtraction of terms was carried out in GENSTAT. From all possible changes to the model, by adding or dropping an explanatory variable or factor, that one is selected that leads to the largest reduction in the residual error. The model is changed until no further reductions in the residual error are possible.

Likewise the amounts of long fibre and total fibre were analysed (e.g., Westerhuis *et al.*, 2009a [Chapter 2], Figure 8; Westerhuis *et al.*, 2009b [Chapter 3], Figure 3). The difference is that in the first case fibre and wood are independent entities, whereas long fibre and total fibre are not, because the amount of long fibres is part of the total fibre fraction, which means that the explained variance automatically is relatively high. Both applications however, clearly show which factors are important and which factors are not. The relation between stem weight before and after retting was also successfully analysed this way (e.g., Westerhuis *et al.*, 2009b [Chapter 3], Figure 1; Westerhuis *et al.*, 2009c [Chapter 4], Figure 4).

The advantage of using multiple linear regression analyses instead of standard ANOVAs should be stressed here. Standard ANOVAs would have shown the differences in, e.g., fibre percentages between treatments. However, the linear relationship between the amounts of fibres and wood, strikingly visible in all figures, would have been missed, because the weight of the stems had not been taken into account. Multiple linear regression analyses make it possible to distinguish between the effects of treatments on overall stem production on one hand and the ratio in which, e.g., fibres and wood are produced during crop development on the other. As an example: retting losses could be explained much better with multiple linear regression (Westerhuis *et al.*, 2009b,c [Chapters 3 and 4]) as compared to ANOVA (Westerhuis *et al.*, 2009a [Chapter 2]).

3 Retting losses depend on plant size

Retting losses were determined separately because this is necessary to study the ratio in which fibres and wood are produced. The losses as such, however, are interesting as well. They show which part of the dry matter yield is lost in the first step of processing.

The absolute retting losses only slightly increased with increasing stem part weight. Consequently, relative retting losses gradually decrease with increasing weight of the stem part (Figure 1). Assuming an increasing plant weight during the growing season, the retting loss percentage will decrease during the growing season. The cause of the higher weight of a stem part, e.g., lower plant density, earlier sowing date, postponed harvest date, lower stem part, or combinations thereof, is unimportant. The effect is indirectly: through stem weight. Additional direct effects where of no practical significance (Westerhuis *et al.*, 2009b,c [Chapters 3 and 4]).

In Figure 1, the difference between the regression line and the line x = y represents the absolute retting losses. The relationship between the stem part weight before and after retting can be described by a linear regression line $y = ax + b$, with $b < 0$. The intercept b explains the decreasing retting loss percentage with increasing stem weight: the relative effect of b diminishes and the retting loss percentage levels off towards $a(1–a) \times 100\%$, the asymptote of the function.

Lowest relative retting losses thus are found in very tall plants. Such plants however, are unfit for textile purposes (Chapter 1, Section 4.1) hence aiming at low retting losses as such would be a point–less strategy. In case a certain plant size, for

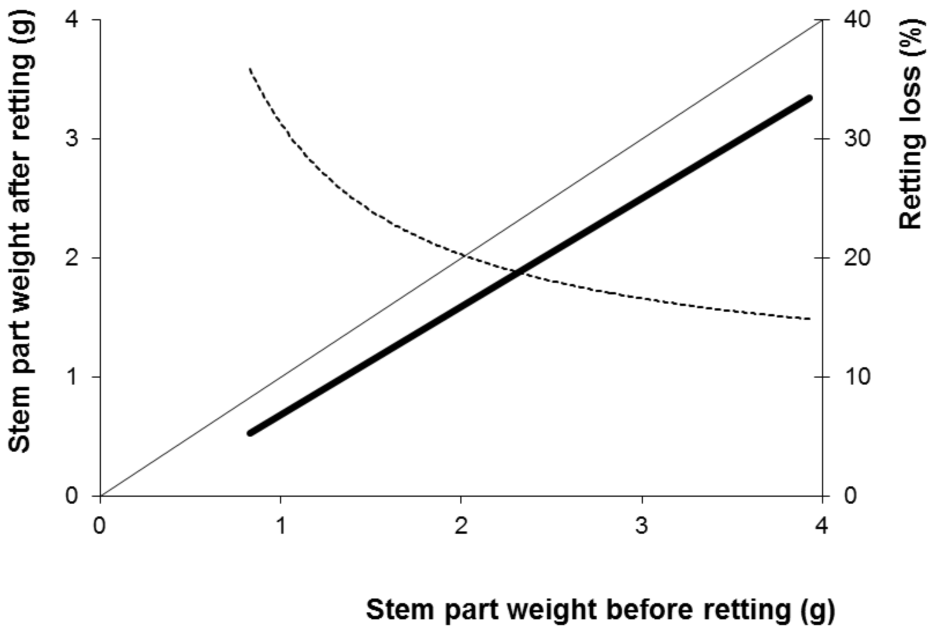

Figure 1. Linear regression line for the stem part weight after retting against the stem part weight before retting. The line, y = 0.91x –0.22 (▬, left Y-axis), with R^2 = 0.98, is derived from Westerhuis *et al.*, 2009b [Chapter 3], Figure 1. The difference between the regression line and the line x = y (—) represents the absolute retting losses. Because the absolute retting losses only slightly increase with increasing stem part weight, the retting loss percentage (---, right Y-axis) gradually decreases with increasing weight of the stem part.

whatever reason, is preferable for textile fibre production, the retting loss percentage must be considered an established fact.

It is questionable whether the intercept in a botanical sense has a meaning. Extrapolation of the regression line towards lower stem part weights, however, could only lead to the conclusion that very small plants will completely be lost during retting, as there are neither fibres nor wood present, which in fact does seem likely.

When stem part weight before retting is allowed as a term in the linear regression models, it accounts for more than 98% of the variance in stem weight after retting (Westerhuis *et al.*, 2009b [Chapter 3], Table 5A; Westerhuis *et al.*, 2009c [Chapter 4], Table 2B), while other factors, though statistically significant, cause differences in slopes or intercepts of the regression lines that are too small to visualise in graphs. Even differences between varieties are very small. Differences in retting losses therefore should not be decisive at all in choosing a variety for a certain purpose, as it only informs us in an indirect way about the size of the plants that were retted.

The use of a standard ANOVA instead of a multiple linear regression analysis would have led to a different interpretation. Stem part factors are not in the regression model in Westerhuis *et al.* (2009b [Chapter 3], Table 5A). A standard ANOVA, however, would have shown differences in retting loss percentages for different stem parts, though the location of the stem part as such is not determinant. Any treatment that would have increased the stem weight would automatically have decreased the relative retting losses.

4 The fibre/wood ratio is in the genes

The ratio in which fibres and wood are produced in fibre hemp is very different for varieties (Section 4.1). However, for all varieties tested a comparable pattern along the stem exists, with highest total fibre/wood ratios in the middle stem part (Section 4.2). Plant size affects the fibre percentage slightly (Section 4.3), but sowing density, sowing date, and harvest time turned out to have only indirect and small effects, through their effects on plant size (Section 4.4).

4.1 Large differences between varieties

To attain a hemp crop with a high fibre content at any site, a variety with an inherently high fibre content should be chosen. Stem dry matter growth is split up into fibres and wood in a fixed way (Westerhuis *et al.*, 2009a,b [Chapters 2 and 3]). Focusing on improving this ratio by crop management, given variety, is a waste of time: it's in the genes (Figure 2).

The differences between varieties are large. The fibre/wood ratio of Beniko approximately doubles the fibre/wood ratio of Tiborszállási. However, this should not necessarily be considered a disqualification of Tiborszállási with respect to textile yarn production. Fibre quality is of highest importance for yarn spinners and Tiborszállási is reported to have finer fibres and less secondary fibres than, e.g., Futura (Amaducci

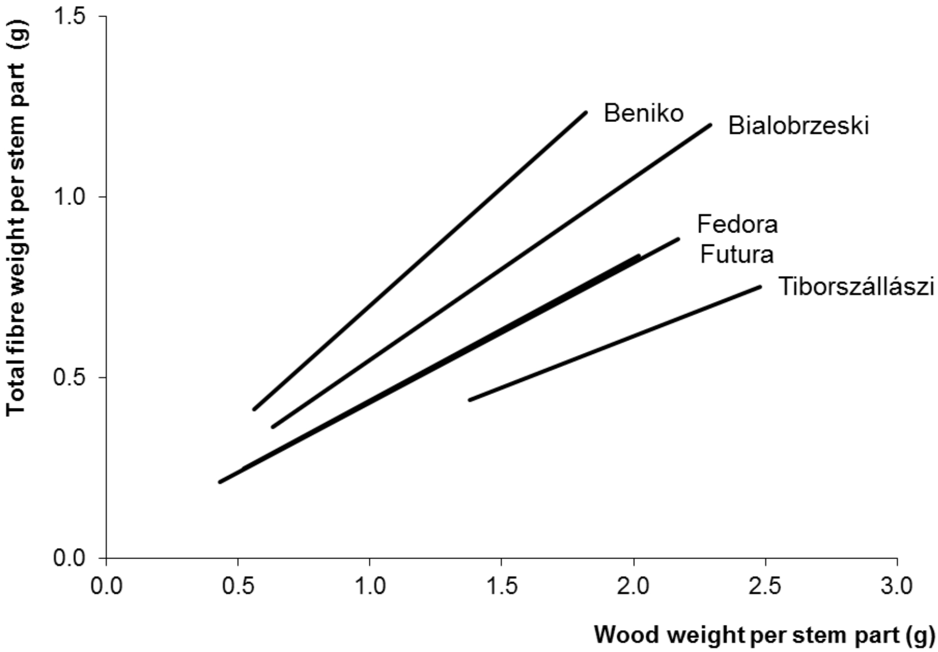

Figure 2. The total weight of the fibres per stem against the wood weight per stem for five varieties. Regression lines for middle stem parts. The figure was derived from Westerhuis *et al.*, 2009c [Chapter 4], Figure 2A.

et al., 2008a). Further, hackling yields or other quality aspects beyond the scope of our experiments could be in favour of Tiborszállási as well.

Between and within experiments (e.g., Vetter *et al.*, 2002) in some cases large differences in fibre percentages are reported within variety. It is likely that these differences are mainly related to differences in the weight of the processed stem parts and would not have been found when our protocol and methodology would have been used.

In case of field retting (e.g., Vetter *et al.*, 2002), differences could also be due to over– or underretting, which could cause deviations from the 'normal' or genetic ratio between the amounts of fibres and wood. The weather dependency of dew retting makes a proper comparison of the results from different treatments or experiments impossible. This is one of the reasons why dew retting is unfit for experiments in which one wants to compare the effect of the treatments before retting (Hoffmann, 1957). Another problem is that retting losses cannot be determined under controlled conditions. Results after dew retting tell us more about the weather history than about the hemp.

Further, the lack of interactions of the fibre/wood ratio with the environment shows good prospects for breeding, as testing this characteristic on only one site can be considered conclusive. (Westerhuis *et al.*, 2009c [Chapter 4]).

4.2 Highest total fibre/wood ratios in the middle parts of the stems

The fibre/wood ratio shows a pattern along the stem, with highest ratios in the middle part of the stem and lower ratios towards both bottom and top. The decreasing fibre percentages (Equation 1a, Section 2.1) reported by Bredemann (1940) and Van der Werf *et al.* (1994b) using the 'Reinfaser' method (Bredemann, 1922), were confirmed with our methodology in practically all treatments in Westerhuis *et al.*, 2009a,b,c [Chapters 2,3, and 4]). Exceptions were three out of twelve varieties from Finland in which no significant differences in total fibre/wood ratios between middle and bottom parts of stems were found (Westerhuis *et al.*, 2009c [Chapter 4]).

An explanation for the lower ratios towards both bottom and top could be that the earliest investments in primary (top) and secondary (bottom) fibre formation are not taken into account because unripe, immature fibres or 'fibre initials' (Chernova and Gorshkova, 2007) are not strong enough yet to survive the chemical (biological) and mechanical stress of processing (Van Dam and Van den Oever, 2006). Cells that eventually will become primary (top) or secondary (bottom) fibres could be lost during retting.

Another reason for the decrease of the fibre/wood ratio towards the stem base could be that a part of the primary or secondary fibre material is lost during breaking and scutching because of sticking to the shives, the woody fragments of the stem core, that remain after fibre extraction. Although at a first glance the shives were practically free of non–woody fibres, the presence of a very small amount of especially the short secondary fibres might have been overlooked. It is impossible, however, that this would fully explain the diminishing fibre/wood ratio towards the stem base.

Based on the experience of several authors however, it is likely that at least a small share of the secondary fibres sticks to the shives during breaking (Dewey and Merrill, 1916; Hoffmann, 1957; Van der Werf *et al.*, 1994; Bócsa and Karus, 1998), while another share ends up in the tow section (Hoffmann, 1957; Bredemann, 1961; Menge–Hartmann and Höppner, 1995) adhered to primary fibres that are lost during scutching. The latter amount however, is not lost in our experiments; it is collected as a part of the total fibre fraction.

Whatever the reason might be, when calculating total fibre/wood ratios, any imperfection in separating fibres and wood in our experiments has a twofold effect on the total fibre/wood ratio. Because the weight of the wood is estimated by subtracting the weight of the fibres from the stem dry weight after retting, both numerator and denominator of the fraction are affected by any incomplete separation. The ratio then decreases, because the numerator is underestimated and the denominator is overestimated.

It is likely that the total fibre/wood ratio is rather stable over a relatively long 'middle section' (Westerhuis *et al.*, 2009a [Chapter 2]). Analyses by Bredemann (1940) and Van der Werf *et al.* (1994b) seem to support this, although the bast fibre content in their experiments was calculated on basis of stem dry matter (i.e. unretted stems), while we used retted stems for analysis. If one would study the total fibre/wood ratio into more detail, e.g., with 10 cm stem parts, our fibre release protocol (Westerhuis *et al.*, 2009a,b,c [Chapters 2,3, and 4]) could be used. However, the Flemish mill, which requires stem parts with a minimum length of approximately 50 cm, should be replaced by a another tool, to remove the shives after breaking. It should not be very difficult to produce such a 'mini–scutcher'. Another option is the methodology chosen by Westerhuis *et al.* (2009a [Chapter 2]), for analysing the bottom part in detail.

4.3 Higher fibre percentages in smaller plants

The relation between the amounts of fibres and wood in hemp stems can be described by a linear regression line $y = ax + b$, with $b > 0$ (Figure 3). The positive effect of the intercept b diminishes with increasing stem part weight hence the fibre percentage (Equation 1d) levels off towards a fibre percentage equivalent to $a/(1+a) \times 100\%$ (Figure 3). Consequently smaller plants show higher fibre percentages (Equation 1d).

A decreasing fibre percentage during the growing season therefore is inevitable, assuming that plant weight increases during the growing season. The argumentation corresponds with the one presented in Section 2 for the retting loss percentage, as in both cases linear regression lines with intercepts are underlying.

The ratio in which fibres and wood are produced, however, does not change when a hemp stem grows hence it is obvious that the (optimum) fibre percentage as such is not an adequate indicator to determine the timing of the harvest as it would lead to a very premature harvest and a very low yield of very small plants.

Whether the intercept b in a botanical sense has a meaning again is questionable. Extrapolation of the regression line towards lower stem part weights, however, would lead to the conclusion that a small plant starts producing fibres before it produces wood or in general that fibre production precedes wood production along the whole stem. As bast fibres develop from phloem and wood from xylem this is in agreement with Aloni (1980) who, with reference to Esau (1965), stated that in the young organs of intact plants the phloem always differentiates before the xylem. When the observed stem grows, beyond a certain minimum weight, when xylem has started to differentiate as well, the increase in dry matter is split up into fibres and wood in a fixed way. The result is a linear relationship between fibre and wood growth with an intercept caused by the earlier start of fibre development as compared to wood production.

Whatever the reason may be, for decision support to primary producers of fibre hemp this is irrelevant, though it might be of interest for botanists. The crop should be harvested when the maximum amount of fibres or long fibres, the choice depends on the intended use of the crop, is produced per unit area, unless quality or economic

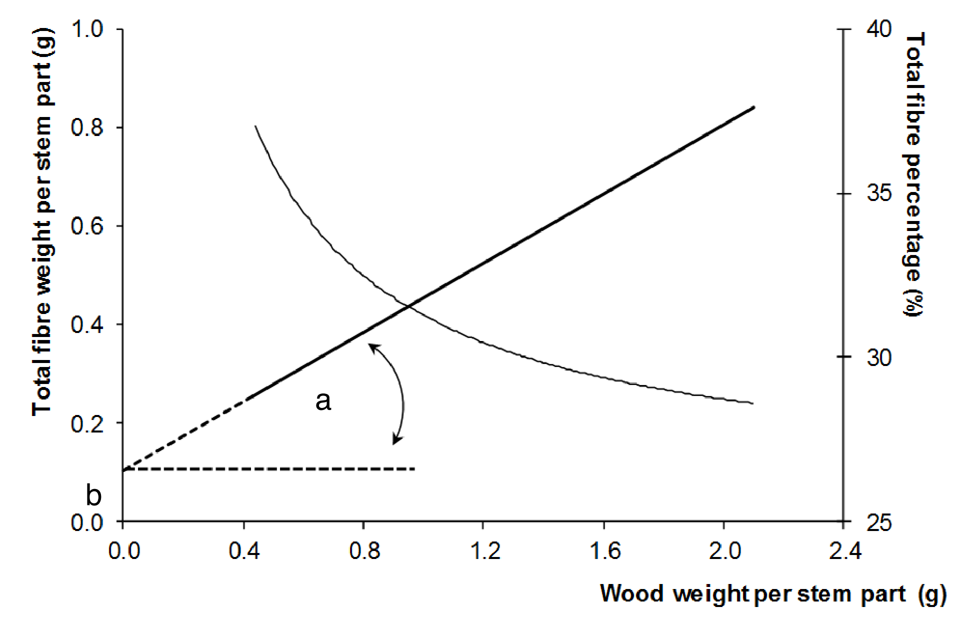

Figure 3. The relation between the amounts of fibres and wood in hemp stem parts can be described by a linear regression line y = *a*x + *b*. The ratio in which fibres and wood are produced (thick line, left Y-axis) does not change when a hemp plant grows. However, the fibre percentage (thin line, right Y-axis) decreases with increasing weight of the stem because the effect of the intercept *b* on the fibre percentage diminishes with increasing weight of the stem. The figure was derived from Westerhuis *et al*. (2009b [Chapter 3], Figure 4B).

aspects beyond the scope of this thesis give rise to other decisions. Important quality characteristics like, e.g., fineness, refine ability, strength or lignin content might show patterns along the stem and probably seasonal patterns as well, which should be taken into account as well when optimising high–quality production.

The decrease of the fibre percentage with increasing stem weight has consequences for breeding experiments aimed at increasing the fibre content. Selecting promising individual plants based on their fibre percentage only, is inadequate. The size of the plant should be taken into account as well. Feaster (1956), Hoffmann (1957), and Bredemann *et al*. (1961) recognized the phenomenon that fibre percentages (Equation 1b) within a variety or breeding line usually decrease with increasing stem weight. Hoffmann (1957) therefore suggested to produce correlation tables and to select only those individuals for further breeding that showed a fibre percentage at least 10% higher than should be expected based on their stem weight. Bredemann *et al.* (1961) used such tables for breeding purposes. According to Hoffmann (1957) such correlation tables should be renewed every year, because climate, soil characteristics, photoperiod etc. would affect the fibre percentage. The results presented in this thesis, however, imply that in case the fibre/wood ratio is used to compare the fibre yield of plants of different sizes for breeding purposes rather than the fibre percentage, updates of the correlation tables for different years or sites are not needed. The fibre/wood ratios seem to be independent of the environmental factors Hoffmann (1957) mentioned.

The slope a or the asymptote $a/(1+a) \times 100\%$, might be useful as standards to compare varieties or breeding lines. The asymptote in fact shows the minimum or basic fibre percentage (Equation 1d) to be expected.

A relatively small set of samples with a relatively large difference in stem part weight would be desirable to derive these characteristics accurately. The required set of samples could for instance be based on sequential harvests, different sowing densities or a combination of these factors, as our experiments show that only the weight of the plant matters, not the factors underlying the weight (Westerhuis *et al.*, 2009a,b [Chapters 2 and 3]).

4.4 No direct effects of sowing density, sowing date and harvest time

Direct effects of sowing density, sowing date and harvest time on the ratio in which any variety produces fibres and wood are absent or minimal. These factors however, affect stem weight and therewith, in an indirect way, the fibre content. To illustrate this: dropping the factor wood weight per stem from the regression models in Westerhuis *et al.* (2009a [Chapter 2], Table 5), makes sowing density and harvest time the main factors explaining the total amount of fibre in a hemp stem, whereas these factors hardly contribute to the explained variance when wood weight per stem is in the model. Sowing density and harvest time determine the weight of the stem part. Stem part weight determined fibre content (Westerhuis *et al.*, 2009a [Chapter 2], Table 4 and Figure 7).

The lack of major sowing density, sowing date, harvest time or site effects could not be due to a lack of variability in plant characteristics at harvest, because a very broad range of raw materials was tested which were not only different in treatment but also in resulting plant size (Westerhuis *et al.*, 2009a,b [Chapters 2 and 3]). Besides the use of a standard ANOVA in this case would have shown effects of the factors mentioned, whereas the factors as such are not determinant; their combined effect on the stem weight is.

It seems unlikely, that any other environmental or agronomic factor would seriously affect the ratio in which any variety produces fibres and wood. Replicates in different years or on different sites obviously were very different with respect to environmental factors such as climate, soil characteristics, photoperiod etc. However, no important year or site effects were found.

Friederich (1964) and Bócsa and Karus (1998) state without experimental details that the fibre content is reduced when relatively high amounts of nitrogen fertiliser are applied. This indeed is to be expected in case our methodology is used, because the individual plant weight in general increases with increasing nitrogen level (Höppner and Menge–Hartmann, 1994; Amaducci *et al.*, 2002; Vetter *et al.*, 2002) and taller plants show a lower fibre percentage (Section 4.3).

4.5 More fibres, more long fibres

The long fibre weight increases linearly with the total fibre weight hence with stem weight (Westerhuis *et al.*, 2009a,b [Chapters 2 and 3]). Although significantly contributing to the regression models, sowing density, sowing date, and harvest time in general were unimportant factors with respect to the long fibre/total fibre ratio. Figures were published to show the absence of practically relevant effects rather than to stress the very small but statistically significant differences (Westerhuis *et al.*, 2009a [Chapter 2], Figure 8C; Westerhuis *et al.*, 2009b [Chapter 3], Figure 4C). The largest effect is shown in Westerhuis *et al.*, 2009b [Chapter 3], Figure 3B: in the postponed sowing date the long fibre/total fibre ratio is slightly lower.

Towards the top of the plant it becomes more difficult to separate fibres and wood due to the presence of unripe fibres and a branched and sometimes sticky inflorescence. Long fibres, often with a greenish colour, were extracted, but (manually) separating tow and wood was too laborious. For the textile industries this part of the hemp stem is of no value (Cappelletto *et al.*, 2001). It could be used for low–value applications, e.g., bio fuels and building and insulation materials (Chapter 1, Section 3.3) for which separation of tow and wood is neither necessary nor economically sound. Besides, with respect to quantity this part contributes very little to the total fibre yield (Bredemann, 1940).

In the bottom 25 cm the long fibre/total fibre ratio is relatively low as compared to the middle section. A more detailed, though unrepeated study from the bottom parts of the stems (Westerhuis *et al.*, 2009a [Chapter 2], Figure 9B) revealed that in plants with an average height of approximately 1.5–2 m (Westerhuis *et al.*, 2009a [Chapter 2], Table 4) the bottom 5 cm contained practically no long fibres. All fibres in this stem part are lost as scutching tow. This can be measured, but it can be seen as well during processing. While scutching the lower end of the lowest stem parts, a net–like fibre structure, approximately 5 cm long, is scutched off at once. The remaining bundles of long fibres hence are shorter than in other stem parts (Chapters 2 and 3).

This seems unimportant for high–quality long–fibre production, because the quality of the fibres that are lost is low and this bottom 5 cm of the stems will be lost in the 15–20 cm (personal communication M. Reinders, Hempflax) stubble anyway. However, the taller hemp grown in Finland showed a lower long fibre/total fibre ratio in the bottom part than the shorter hemp in the Netherlands (Chapter 4). Possibly the bottom section in which no useful long fibres are present is longer in taller plants. Differences between varieties might exist as well hence further experiments are needed.

The highest long fibre/total fibre ratios (Chapters 2 and 3) or fibre percentages (Chapter 4) are found in the 'middle section' of the stem, where irrespective of variety or site around 90% of the extracted fibres are long fibres (Chapter 4, Table 4). The approximately 10% of the fibres that are lost in this part during scutching are primary fibres as can easily be seen from the length of the fibres in the scutching tow.

5 Focus on the 'middle' section

Cappelletto *et al.* (2001) stated that during the heydays of hemp in Italy only these 'middle sections' of the stems were used for textile purposes. Our experiments subscribe this choice, however, growing only middle stem parts, as for now, is not possible. For experimental reasons, Cappelletto *et al.* (2001) defined the top section as the upper quarter, the bottom section as the lowest quarter and the middle section as the intermediate 50% of the stem length. However, from our point of view quality aspects should be decisive. Therefore, we consider the 'middle section' as the part where primary fibres are 'ripe' and extractable and where secondary fibres are absent (Figure 4).

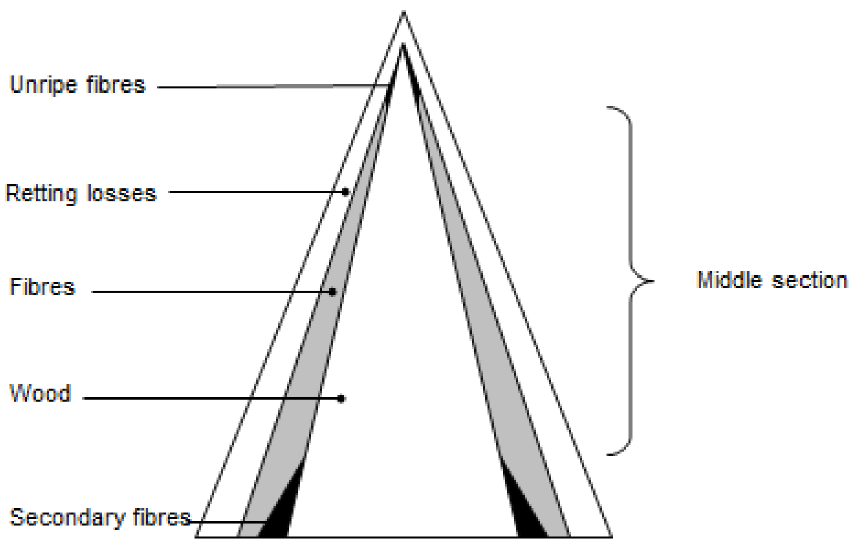

Figure 4. A botanical model of a hemp stem.

5.1 The value of the middle section

A simple, rough assessment of the value of such middle sections can be obtained based on the split–up into fractions we introduced:

1. Based on the dry weight of the middle stem part, the retting losses can be predicted rather precisely as differences between varieties are very small (Section 2).

2. The remaining weight, can be subdivided into primary fibres and wood when the variety is known, because the ratio in which fibres and wood are produced is genetically determined and there are no important deviations to be expected (Section 3.1).

3. Approximately 90% of these primary fibres can be harvested as scutched long fibres, as the subdivision of the total amount of fibres into scutching tow and long fibres, is not very different for varieties (Section 3.5).

The assessment can be performed when the length and weight of the middle section are known hence we need to know the ideal crop height and at which height it should be cut.

5.2 A homogeneous crop

In a crop consisting of identical plants, with exactly the same dimensions and growth stage it would be relatively easy to decide when to harvest, where to cut and how to process. The living nature we work with, however, inevitably shows heterogeneity, whereas optimizing technological processes asks to minimise it as much as possible.

How a hemp crop as homogeneous as possible can be grown is not within the scope of this thesis, though it is likely that, e.g., seeds from homogeneous seed batches should evenly be spread at equal depth in homogeneous seed beds. Dioecious varieties, essentially a mixture of two populations, obviously have an extra complexity with respect to homogeneity (Hoffmann, 1957; Van der Werf and Van der Berg, 1995).

Any deviation from the ideal cut heights is economically unwanted, because it results in a lower yield or a lower quality of the raw materials. In case the lowest cut is too high or the highest cut is too low, valuable long fibres are lost. In case the lowest cut is too low or the highest cut is too high, the raw materials meant for textile fibre processing are contaminated with secondary fibres and inflorescences respectively. A homogeneous crop thus reduces losses of valuable fibres.

Moreover, choosing the right cut height at the bottom part of the stem is more important than at the top because a 1 cm mistake in cut height at the bottom side simply carries more weight (Bredemann, 1940; Mediavilla *et al*., 2001a). Bredemann (1940) therefore emphasised the importance of choosing the right stubble height in relation to yield losses in fibre crops like fibre hemp and nettle.

5.3 Secondary fibre formation: plant size matters

The right stubble height in hemp depends on secondary fibre development. Bast fibres in hemp belong to the sclerenchyma tissue which gives mechanical support to the plants (Kundu, 1942; McDougall *et al.*, 1993; Van Dam and Gorshkova, 2003) and the need for such support increases when plants grow taller and when tops become heavier, due to the development of inflorescences and the filling of the seeds. The development of secondary fibres with increasing plant weight might be considered an example of 'mechanoperception', the perception of mechanical stimuli that keep plants in balance with their physical environment (Ko *et al.,* 2004; Telewski, 2006).

Chapter 5 revealed that the height up to which secondary fibres are present in fibre hemp stems increases with increasing plant weight and that a causal relationship between secondary fibre formation and flowering as such, as suggested in literature (Mediavilla *et al.*, 2001b; Schäfer and Honermeier, 2003, 2006), does not exist. However, the secondary fibre front is found higher in flowering plants when compared to non–flowering plants of the same height, due to the higher weight of these flowering plants. This means that, although flowering as such is not the cause of secondary fibre formation, a harvest before flowering is preferable to optimise the length of the middle section.

The experiments led to an elaboration of the temporal dynamics underlying the model in Figure 4, in which the length development of the 'middle section' is shown (Figure 5).

Plants of increasing height, weight, and phenological stage are drawn. They could represent the same plant at different moments in time, but they can also be

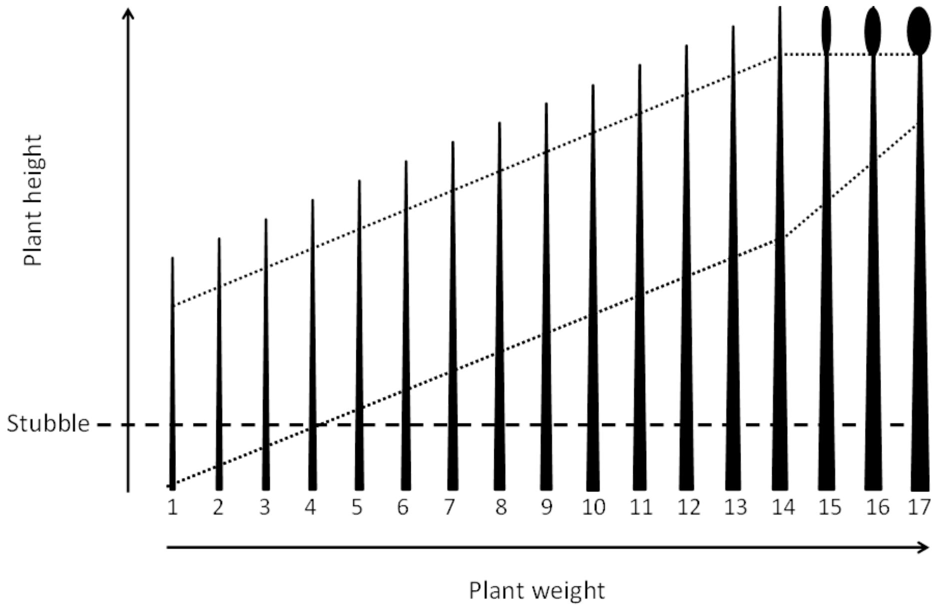

Figure 5. Conceptual model of the harvestable stem part for quality primary fibres in hemp for plants of increasing height, weight, and phenological stage (numbered 1-17 for reference in the text). The stem part that is valuable for textile yarn production, the 'middle' stem part between the dotted lines, moves upwards along the stem. Below the lower line secondary fibres contaminate the valuable primary fibres, above the upper line the developing inflorescences are detrimental for the quality of the primary fibres. Around flowering (stages 14-15) length growth slows down but the weight of the top increases (plants 15–17). An accelerated development of secondary fibres as compared to the length growth, but keeping pace with the increasing weight or momentum could be the result. Consequently, the length of the stem part that is valuable for high–quality yarn spinning becomes shorter around flowering.

considered different plants in a crop at the same time, because with respect to primary and secondary fibre development, the size of the plant is the key factor, not the moment a particular size is reached. The stem part that is valuable for textiles, the stem part between the dotted lines, is located higher up along the stem with increasing plant size. It is supported by a stem base of increasing length and weight, which is unfit for yarn production due to the presence of secondary fibres. When the plant becomes generative, length growth slows down or stops (De Meijer and Keizer, 1994; Meijer *et al.*, 1995; Schäfer and Honermeier, 2003), while the weight of the top of the plant increases, due to the development of a, with respect to fibre production, useless inflorescence. An accelerated development of secondary fibres as compared to the length growth, but keeping pace with the increasing top weight is likely to happen. Consequently, the length of the stem part that is valuable for high–quality yarn spinning becomes shorter around flowering.

Figure 5 also demonstrates that homogeneity with respect to plant height at harvest is important. The tops of the plants are the comparable parts, not the bottom parts. In plants of different heights the valuable 'middle section' is on different heights which inevitably will cause quantitative or qualitative losses (Section 5.2). Figure 5 also shows that dual use (Höppner *et al.*, 2004) for seed and high–quality textile fibres is not an option.

In Italy the presence of secondary fibres in hemp grown for textile purposes is not a main concern (Stefano Amaducci, personal communication). Italian hemp grown for textiles is relatively short at harvest as compared to Dutch fibre hemp which is up to now usually grown for low or medium value applications. In Italy the growing season is shorter, due to the shorter days i.e. earlier flowering and the dryer climate. Length growth is limited under these circumstances. Our experiments show that shorter hemp indeed contains less secondary fibres and that the secondary fibre front is found lower in the plant; mimicking Italian hemp therefore seems wise.

5.4 Short crops are preferable

When a farmer has chosen a certain variety, based on, e.g., agronomic or quality reasons beyond the scope of this thesis, his main concern thus is to avoid that secondary fibre formation, which proceeds upwards along the stem during the growing season, contaminates the valuable primary fibres aimed for textile yarn production. In practice this means that those agronomic choices should be made that keep the stem weight low enough to avoid secondary fibre growth above stubble height. Given variety and plant size, the amounts of fibres, wood, and retting losses then should be considered accomplished facts (Westerhuis *et al.*, 2009a,b,c [Chapters 2, 3, and 4]).

The options to produce plants with the desired size characteristics hence without secondary fibres are manifold. Since sowing density, harvest time, and sowing date do not have an additional effect on the primary fibre content besides the above mentioned indirect effect through stem weight (Westerhuis *et al.*, 2009a,b [Chapters 2, 3]), any combination of these factors, and probably other factors (e.g., fertilisation) as well, could be chosen to produce the desired crop with the desired height.

Possibly the optimal height is different for varieties as secondary fibre development is reported to be different for varieties (Hoffmann, 1957; Bredemann *et al.*, 1961; Höppner *et al.*, 2004; Amaducci *et al.*, 2008a). This thesis however, shows that true varietal differences are likely to be entangled with differences caused by the size of plants, and that these effects should be disentangled to see whether true differences between varieties exist and if so, how large these differences are.

5.5 Processing flax–sized hemp

For economic reasons, processing fibre hemp on existing processing lines for flax as yet seems inevitable. For this reason hemp should be cut in two or more parts before processing or the cultivation technique should be adjusted to grow hemp with the size of flax (Liberalato, 2003; Ranalli and Venturi, 2004; Amaducci, 2005; Esposito and Rondi, 2006; Venturi *et al.*, 2007; Amaducci *et al.*, 2008a).

Despite the negative experience with 'baby hemp' (Liberalato, 2003; Amaducci, 2005; Venturi *et al.*, 2007; Van der Werf and Turunen, 2008) the experiments in this thesis support the second option: hemp with the size of flax. Flax scutching and hackling systems are dimensioned for fibre ribbons with a length usually between 80 and 120 cm (Vreeke, 1991; Ranalli and Venturi, 2004; Venturi *et al.*, 2007). With the right combination of variety and crop management it should be possible to produce hemp with the size of flax or a little longer (Westerhuis *et al.*, 2009b [Chapter 3]) in which secondary fibres are absent (Chapter 5). It is unlikely however, that hemp double the size of flax is free of secondary fibres in the lower of the two sections (Amaducci *et al.,* 2008), although differences between varieties might exist.

The disadvantage of a short crop, however, is the lower yield. To compensate for this two subsequent hemp crops could be grown (Kamat *et al.*, 2002; Liberalato, 2003; Chapter 3), but whether it is possible to grow two subsequent vegetative hemp crops with the desired dimensions (Chapter 5) should be further researched. Whether it is economically sound to grow two subsequent hemp crops is doubtful as well, as there are double costs for seeds and crop management and in dry years for irrigation (Schäfer and Honermeier, 2003; Westerhuis *et al.*, 2009b [Chapter 3]). Another crop after hemp (e.g., Amaducci, 2005), a trap crop (e.g., Timmermans, 2005), a feed crop (e.g., Rowlandson, 1849) or green manure are other options.

6 Future research

In this thesis, steps were made to elucidate the causal relationships between easily measurable characteristics of harvested hemp stems, and the amount and characteristics of the fibres that can be extracted from these stems. Possibilities for improvement, but especially impossibilities, to a higher degree are known, as underlying processes are better understood. Next steps in research could start from here.

6.1 Varietal differences in the length of the middle section

It is likely that the length of the valuable middle section depends on variety for it is known that the ratio primary fibres/secondary fibres is different for varieties. (Hoffmann, 1957; Bredemann *et al.,* 1961; Van der Werf *et al*., 1994b; De Meijer, 1995; Amaducci *et al.*, 2008a). Varieties with desirable primary fibre characteristics therefore should be compared with respect to secondary fibre formation.

6.2 A fibre production model

A basic crop growth and development model to predict total and stem dry matter yield of fibre hemp as a raw material for the Australian newsprint industry was developed by Lisson *et al.* (2000b). To predict the yield of long fibres fit for textile yarn production such a model should be extended with genotypic parameters like critical day length, the ratio in which fibres and wood are produced, and the development of secondary fibres in relation to plant size. When variety, plant density, plant size distribution, and stem dry matter production are known, it should be possible to predict the dry matter distribution over the different components at every height in the crop. On this basis ideal cut heights or optimal harvest times could be calculated.

6.3 Other bast fibre crops

Our analyses showed that in fibre hemp the ratio in which primary fibres and wood are produced is determined by variety, stem part, and stem part weight, and that the effect of sowing density, sowing date, harvest time, and presumably other factors as well is indirect via stem weight. It would be interesting to subject other textile bast fibre crops, e.g., flax (*Linum usitatissimum* L.), stinging nettle (*Urtica dioica* L.) or ramie (*Boehmeria nivea* (L.) Gaudich) to the same methodology and analysis. Possibly similar relatively simple botanical rules or ratios can be found with respect to fibre development.

6.4 Improving hackling yields

The end products of our experiments (Westerhuis *et al.*, 2009a,b,c [Chapter 2,3, and 4]) are bundles of scutched long fibres. During the next step in processing, hackling, these bundles are split up into hackled long fibres and hackling tow. Approximately 60% of the fibre weight is lost during this combing process (Sponner *et al.*, 2005; Tofani, 2006), which is meant to refine and align the long fibres to produce a continuous sliver for spinning. The potential to improve the ± 40% hackling yields seems high. In flax, hackling yields or 'yields of line fibre', are usually 55–65% (Kozlowski *et al.*, 2012).

It could be studied which factors affect the hackled long fibre/scutched long fibre ratio in the valuable 'middle section' of stems. Varietal differences are likely to be present as bundles of scutched long fibres 'feel' very different (e.g., soft versus stiff, aligned versus entangled). Although scutching losses in the middle sections are not very different for varieties (Westerhuis *et al.*, 2009c [Chapter 4]), hackling losses could be.

For further research, a standard protocol should be developed, because it is likely that hackling yields, as for flax (Hann, 2005; Kozlowski *et al.*, 2012) depend on the degree of hackling to which the fibres are subjected (e.g., machine settings). To compare batches properly, processing prior to hackling of course should be standardised as well (Westerhuis *et al.*, 2009a,b,c [Chapter 2,3, and 4]) Improvements could also be made with hackling needles specifically suited for hemp (Tofani, 2006).

6.5 Fibre quality

After hackling it is to a high degree known which amounts of which materials are lost at which stage of processing. However, the quality of the long fibres that will end–up in a yarn is still unknown. Quality measurements before hackling do not seem very significant as one would mainly measure future waste materials. Due to the extremely high losses during hackling (Sponner *et al.*, 2005; Tofani, 2006) it is unlikely that measurements earlier in processing would be reliable predictors of final quality.

After hackling however, quality measurements on especially fibre fineness should be performed. Although a trade–off between strength and fineness is likely to exist (e.g., Schäfer and Honermeier, 2003) and fibres should be strong enough for yarn spinning, it is probably not necessary to improve the strength of the fibres: processing as such seems selective.

To introduce hemp long fibres into the fashion textile sector, fibres should be produced allowing the spinning of yarns between Nm 20 and Nm 40 (Nebel, 1995; Ranalli and Venturi, 2004). Therefore Liberalato (2003) and Ranalli and Venturi (2004) stated that it is necessary to develop hemp varieties with a smaller fibre diameter. That finer yarns can be spun when fibres are finer seems obvious, but it is not only a matter of fibre dimensions. In case a yarn is spun from finer fibres the number of fibres in a cross–section of the yarn is higher than when the same yarn count is spun from coarser fibre, which means that the yarn will be stronger and more even (Allam, 2004). Leupin (2001) showed that differences between varieties exist. It is likely however, that part of the solution will be in the size of the plants again.

When fibre elongation has ceased and cell wall thickening has started, the number of fibre cells in a cross–section of a hemp stem does not change anymore (Gorshkova *et al.*, 2003; Amaducci *et al.*, 2005; Chernova and Gorshkova, 2007). Consequently, the weight increase of the stem after elongation must be the filling of the existing number of primary fibres keeping pace with the increasing wood weight (Westerhuis *et al.*, 2009a,b,c [Chapters 2, 3, and 4]). Consequently, in hemp, like in flax (Hann, 2005), fibres must have a decreasing weight from bottom to top, which likely means finer fibres. Experimental results point that way: fibres are reported to be finer with earlier harvest, decreasing plant size and with increasing sowing density (Jakobey, 1965; Leupin, 2001; Schäfer and Honermeier, 2003, 2006; Amaducci *et al.*, 2008a).

Concluding, the short crop we recommend to avoid secondary fibres will, given variety, contain the finest primary fibres as well.

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Summary

Hemp

Hemp (*Cannabis sativa* L.) is one of the first plants cultivated. It originates from the temperate parts of Asia and it is grown for its bast and wood fibres, its seeds, its oil, and its cannabinoids.

The bast fibres have been used for textiles for about 6000 years, but hemp lost its position as an important textile fibre crop when cheap cotton became available. Consequently, research withdrew from fibre hemp and knowledge and varieties went lost. Growing fibre hemp was even prohibited in many countries for decades due to the association with its narcotic relatives. Nevertheless the crop made a remarkable worldwide comeback in the 1990s, which was among others catalysed by the public concern for the environment and the endeavour towards sustainability.

Fibre hemp is a fast growing, low maintenance crop, which suppresses weeds efficiently and which can be grown organically without major problems. It fits well in the policy of the European Union to combat agricultural surpluses and to support diversification. Besides, growing hemp could broaden the too narrow crop rotations which have increased the incidence of plant diseases, lowered the yields, and enhanced the use of agrochemicals.

Although it can be grown for a multitude of end products and semi manufactures in non–food, food, and feed, economically the most interesting prospects seem to be in the production of long fibres for the finest yarns for fashion textiles. However, at present there is no large–scale production of long fibres that meet the requirements of the yarn spinners, because too little is known about the causal relationships between the primary production process and the quantity and quality of stems and fibres that are fit for the production of fine yarns. The main objective of this thesis is to improve this knowledge.

Primary fibres are valuable, secondary fibres are unwanted

In hemp two types of bast fibres occur, primary and secondary fibres. Both are organised in bundles and the cell walls of both types are enforced with cellulose.

Primary fibre bundles are already present in very young hemp stems. They run longitudinally along the stem from bottom to top and can reach almost the full length of the plants. For spinning high–quality textile hemp yarns only these primary or long fibres (average length of the individual fibre ≈ 20 mm) are valuable and a lot of research effort has been aimed at improving the long fibre content of fibre hemp by optimising crop management. However, many contrasting conclusions were drawn about the effects of, e.g., sowing density, sowing date, harvest time, variety, and site.

Secondary fibres might form when a stem part has reached its maximum length. They are absent in young hemp plants or only present in a thin layer at the stem base. These short fibres (average length of the individual fibre ≈ 2 mm) are too short for spinning and contain too much lignin which is detrimental for the production of fine yarns.

Because it is technically difficult to separate the secondary fibres from the primary fibres during processing from fibre to yarn it should be known how the development of secondary fibres above stubble height (15–20 cm) can be avoided in the raw materials aimed at textile yarn production. Therefore it was the aim of our experiments to elucidate how the amount of primary fibres can be maximised, while at the same time contamination of these valuable fibres with the unwanted secondary fibres is avoided.

Fibre extraction

Before hemp fibres can be used for yarn spinning, the fibre bundles have to be released from the surrounding tissues. The process is similar to linen production from flax (*Linum usitatissimum* L.). We used a traditional fibre–extraction method because of the small size of the samples as compared to industrial processing batches. However, with respect to the procedural steps and the final products, the methods are comparable.

Fibre extraction consisted of warm water retting, drying, breaking and scutching. During retting bacteria degrade all stem substances except the bast fibres and the woody part of the stem. After drying, fibres and wood can be separated in two mechanical steps: breaking and scutching.

Following standard protocols, almost 1500 well–defined hemp stem samples from field experiments in Italy, Finland and the Netherlands were in this way fractionated into long fibres, scutching tow (fibre losses during scutching, mainly primary fibres), wood and retting losses. The effects of plant density, sowing date, harvest time, variety and site were studied. To study the patterns of fibre quantity and quality along the stem, which possibly interact with the other factors that are investigated, different stem parts were analysed. It was necessary anyway to cut the stems, because complete stems cannot be processed with our equipment and processed stem parts need to be of equal length.

Plant weight is the dominant factor

In this thesis it is shown that within any tested variety, the dry weight of the stems at harvest, and not the factors underlying this dry weight, determines the amounts of bast fibres, wood, and retting losses. It is the size of the plant that is important (see Chapters 2, 3, and 4).

Multiple linear regression analyses showed that in the retted stems the dry matter is split up into fibres and wood in a fixed way. The relation between the amounts of fibres (y) and wood (x) in hemp stems can be described by linear regression: $y = ax + b$, with $b > 0$. The positive effect of the intercept (b) diminishes with increasing stem part weight hence the fibre percentage based on the dry weight after retting levels off towards a fibre percentage equivalent to $a/(1+a) \times 100\%$. Consequently smaller plants show higher fibre percentages and a decreasing fibre percentage during the growing season is inevitable, assuming that plant weight increases with time. There is a pattern along the stem with highest fibre/wood ratio's in the middle part of the stem.

The slope *a* in the equation is very different between varieties but practically unaffected by plant density, sowing date, harvest time, and site. It means that in order to attain a hemp crop with a high fibre content at any site, a variety with an inherently high fibre content should be chosen: it's in the genes…

The long fibre weight increased linearly with the total fibre weight (long fibres + scutching tow) hence with stem weight. In the middle part of the stem, which is most valuable for textile yarn production, about 90% of the extracted fibres are long fibres.

In earlier publications direct effects of sowing density, sowing date, and harvest time on fibre content or fibre percentage were suggested or claimed. These claims however, were contradictory, incomplete, and weakly substantiated. Most likely the key factor plant size was missed due to focusing on the intended differences between treatments rather than observing and analysing the effects of the treatments: the differences in plant size and the consequences thereof with respect to fibre content.

The relationship between the stem part weight before (x) and after (y) retting can also be described by linear regression: $y = ax + b$ which results in a decreasing retting loss percentage with increasing stem weight. Assuming an increasing plant weight during the growing season, the retting loss percentage will decrease during the growing season. The cause of the higher weight of a stem part, e.g., lower plant density, an earlier sowing date, a later harvest, a lower stem part, or combinations thereof, is unimportant. It is again the size of the plant that matters.

Microscopic measurements showed that the height up to which secondary fibres are present in the stems also depends on plant weight (Chapter 5). There is no causal relationship between secondary fibre formation and flowering as suggested in literature. However, secondary fibres are found a little higher in flowering plants, which must be due to the higher weight or momentum of flowering plants as compared to non–flowering plants of the same height. This was shown in a greenhouse experiment carried out to disentangle flowering and plant size effects on secondary fibre growth. The short–day response of the plant was used to produce the required range of plant sizes for both flowering and vegetative plants.

Hemp textile crop management

The practical consequence of the above is that when a hemp grower has chosen to produce raw materials for high–quality textile purposes, and has selected a certain variety, his main concern is to avoid that secondary fibre formation, which proceeds upwards along the stem during the growing season keeping pace with the increasing weight, contaminates and devalues his end product. Therefore, crop management should be focused on keeping the plants small enough to avoid secondary fibre formation above stubble height. Further, a harvest just before flowering is preferable, because the increasing weight of the inflorescences gradually reduces the length of the stem part that is fit for high–quality textile yarn production. The options to produce plants with the desired size characteristics are manifold. Since sowing density, harvest time, and sowing date do not have an additional effect on the primary fibre content besides the above mentioned indirect effect through stem weight (Chapters 2 and 3), any combination of these factors could be chosen to produce the desired crop.

Samenvatting

Hennep

Hennep (*Cannabis sativa* L.) is een van de oudste cultuurplanten. Het gewas is afkomstig uit de gematigde streken van Azië en het wordt geteeld voor de bast– en houtvezels, het zaad, de olie en de cannabinoïden.

De bastvezels worden al ongeveer 6000 jaar gebruikt voor het maken van textiel, maar deze toepassing raakte in onbruik toen goedkoop katoen beschikbaar kwam. Bijgevolg werd het vezelhenneponderzoek gestaakt en gingen kennis en rassen verloren. De teelt van vezelhennep werd in vele landen zelfs gedurende tientallen jaren verboden vanwege de geestverruimende verwanten. De vezelhennepteelt is echter terug sinds de negentiger jaren van de vorige eeuw en dat is onder andere het gevolg van het toegenomen milieubewustzijn van de consument en het streven naar duurzaamheid.

Vezelhennep is een snelgroeiend gewas dat gedurende het teeltseizoen weinig aandacht vergt, onkruiden onderdrukt en zonder grote problemen biologisch geteeld kan worden. De teelt past in het beleid van de Europese Unie om landbouwoverschotten te voorkomen en diversificatie te stimuleren. Hennep is bovendien geschikt om de te nauwe vruchtwisseling, die gepaard gaat met een verhoogde ziektedruk, teruglopende opbrengsten en een toenemend gebruik van chemicaliën, te verbreden.

Hoewel hennep geteeld kan worden voor een veelheid aan eindproducten en halffabricaten, lijkt de toepassing van de lange bastvezels in fijne textielgarens economisch gezien de meeste perspectieven te bieden. Er is op dit moment echter nog onvoldoende aanbod van kwaliteitsvezels voor de garenspinners omdat er te weinig bekend is over de relatie tussen de teelt van het gewas enerzijds en de opbrengst en de kwaliteit van stengels en vezels die geschikt zijn voor de productie van fijne garens anderzijds. Het doel van dit proefschrift is de kennis hieromtrent te vergroten.

Primaire vezels zijn waardevol, secundaire vezels zijn ongewenst

In vezelhennep komen twee soorten bastvezels voor, primaire en secundaire vezels. Beide zijn gegroepeerd in bundels en de celwanden van de vezels zijn verstevigd met cellulose.

Primaire vezelbundels zijn al tot vlak onder de top aanwezig in jonge stengels; ze zijn dus bijna even lang als de stengel. Voor het spinnen van hoogwaardige textielgarens zijn alleen die primaire ‘lange’ vezels geschikt (de gemiddelde lengte van de individuele vezel ≈ 20 mm) en er is veel teeltonderzoek gedaan naar de verhogen van het primaire vezelgehalte van de stengels. Dat leidde echter tot tegenstrijdige conclusies met betrekking tot de effecten van bijvoorbeeld zaaidichtheid, zaaitijdstip, oogstmoment, ras en locatie.

Secundaire vezels kunnen worden gevormd als de lengtegroei van een stengeldeel gestopt is. In jonge planten zijn ze nog niet gevormd of alleen aanwezig in een dunne laag aan de stengelbasis. Deze ‘korte’ vezels zijn te kort om te spinnen (de gemiddelde lengte van de individuele vezel ≈ 2 mm) en ze bevatten teveel lignine hetgeen de productie van fijne garens bemoeilijkt.

Omdat het technisch lastig is om tijdens de productie van vezel tot garen de secundaire vezels nog te scheiden van de primaire vezels is het van belang te weten hoe de ontwikkeling van secundaire vezels boven stoppelhoogte (15–20 cm) voorkomen kan worden in hennep die geteeld wordt voor de productie van fijne textielgarens. Onze experimenten waren er daarom op gericht om aan het licht te brengen hoe er zoveel mogelijk primaire vezels geproduceerd kunnen worden zonder dat deze ‘vervuild’ zijn met secundaire vezels.

Vezelextractie

Voordat hennepvezels tot garens gesponnen kunnen worden, moeten ze worden vrijgemaakt uit de omliggende weefsels. Dat gaat op dezelfde wijze als bij de productie van garens voor linnen uit vlas (*Linum usitatissimum* L.). Omdat de experimentele monsters klein waren ten opzichte van industriële partijen hebben we een traditionele verwerkingsmethode gebruikt. De verwerkingsstappen en eindproducten daarvan zijn echter vergelijkbaar met die in de industrie.

De vezelextractie bestond uit warmwaterroten, drogen, brakelen en zwingelen. Tijdens het warmwaterroten breken bacteriën alle materie af, met uitzondering van de bastvezels en het hout. Na het drogen van de stengels kunnen deze in twee mechanische stappen, brakelen en zwingelen, van elkaar worden gescheiden.

De effecten van zaaidichtheid, zaaitijdstip, oogstmoment, ras en locatie werden onderzocht aan bijna 1500 goed beschreven hennepmonsters uit Italië, Finland en Nederland. Alle monsters werden opgesplitst in lange vezels, werk (vezelverlies tijdens het zwingelen, vooral primaire vezels), hout en rootverliezen (zie hoofdstukken 2, 3 en 4). Er werden verschillende stengeldelen met een lengte van 50 cm verwerkt om de eventuele verschillen in kwaliteit en vezelgehalte te kunnen analyseren. Het was in elk geval noodzakelijk om de stengel in stukken te knippen, omdat gehele stengels te lang zijn om te verwerken en omdat de verwerkingsmethode stengels van gelijke lengte vereist.

Het gewicht van de plant is nagenoeg allesbepalend

In dit proefschrift wordt aangetoond dat voor alle onderzochte vezelhenneprassen het drooggewicht van de stengels bij de oogst, en niet de factoren die aan dat gewicht ten grondslag liggen, bepalend is voor de hoeveelheden vezels, hout en rootverliezen (zie hoofdstukken 2, 3 en 4).

Met behulp van meervoudige lineaire regressie werd aangetoond dat in gerote hennepstengels de droge stofverdeling over bastvezels en hout vaststaat. Het verband tussen de hoeveelheid bastvezels (y) en hout (x) kan beschreven worden met lineaire regressie: $y = ax + b$, met $b > 0$. Het positieve effect van b op het vezelpercentage neemt af met toenemend stengelgewicht, dus het vezelpercentage gebaseerd op het drooggewicht van de stengel na roten neemt af en benadert $a/(1+a) \times 100\%$. Bijgevolg hebben kleinere planten een hoger vezelpercentage en neemt het vezelgehalte van henneplanten af tijdens het groeiseizoen, aangenomen dat de plant groeit. In het middeldeel van de stengel is de vezel/hout verhouding het gunstigst.

De richtingscoëfficiënt *a* in de vergelijking is voor elk ras verschillend, maar is praktisch onafhankelijk van de zaaidichtheid, het zaaitijdstip, het oogstmoment of de locatie. Dat betekent dat om een hoger vezelgehalte te verkrijgen een ander ras moet worden gezaaid, een ras met een gunstiger vezel/hout verhouding: het is genetisch bepaald.

Het lange vezelgewicht nam lineair toe met het totale vezelgewicht (lange vezels + werk) en dus met het stengelgewicht. Het voor textiele toepassingen meest waardevolle middendeel van de stengel bevat ongeveer 90% lange vezel.

In eerdere publicaties werd beweerd of gesuggereerd dat zaaidichtheid, zaaidatum en oogstmoment een direct effect op het vezelgehalte zouden hebben. De beweringen waren echter tegenstijdig en niet goed onderbouwd. Waarschijnlijk is de directe relatie met de plantgrootte over het hoofd gezien door vooral te kijken naar de verschillen tussen de behandelingen in plaats van naar de gevolgen van die behandelingen op de plantgrootte en het effect daarvan op het vezelgehalte.

Het verband tussen het gewicht van een stengeldeel voor (x) en na (y) roten kan ook beschreven worden met lineaire regressie: $y = ax + b$. Dat resulteert in een dalend rootverliespercentage bij toenemend stengelgewicht. Gedurende het groeiseizoen zal het rootverlies dus afnemen, aangenomen dat de plant groeit. De reden van het hogere stengelgewicht, bijvoorbeeld een lagere plantdichtheid, vroegere zaai of latere oogst of een combinatie van factoren doet er niet toe.

Het plantgewicht bepaalt ook tot op welke hoogte secundaire vezels aanwezig zijn in een hennepstengel. Dat blijkt uit microscopische waarnemingen. Er is geen causaal verband met de bloei, zoals in de literatuur gesuggereerd wordt. In bloeiende planten worden de secundaire vezels wel hoger in de stengel aangetroffen dan in niet-bloeiende planten, hetgeen te maken moet hebben met het hogere gewicht en het grotere momentum van bloeiende planten in vergelijking tot niet–bloeiende planten met dezelfde stengelhoogte. Dit werd aangetoond in een kasexperiment waarin bloei en plantgrootte werden ontkoppeld door gebruik te maken van de daglengtegevoeligheid van vezelhennep (zie hoofdstuk 5).

Het telen van hennep voor textiel

In praktische zin betekent het voorafgaande dat een hennepteler die hoogwaardige textielvezels wil produceren er vooral voor moet zorgen dat het secundaire vezelfront dat bij toenemend gewicht van de plant steeds hoger in de stengel komt te liggen onder de stoppelhoogte blijft. De planten mogen niet te groot worden omdat de waardevolle textielvezels dan verontreinigd raken met secundaire vezels.

Een oogst voor de bloei is bovendien aan te bevelen omdat het toenemende gewicht en momentum van de bloeiwijze ervoor zorgt dat het deel van de stengel dat geschikt is voor hoogwaardige textielvezels geleidelijk korter wordt.

Aangezien het moment van zaaien, het oogstmoment en de zaaidichtheid geen effect op het primaire vezelgehalte hebben, anders dan het effect via stengelgewicht (zie hoofdstukken 2 en 3) zijn er mogelijkheden te over om een gewas met de gewenste stengellengte te telen.

Nawoord

Toen mij op bijzondere wijze, ik had op een andere onderzoeksfunctie gesolliciteerd, door Paul Struik de mogelijkheid werd geboden promotieonderzoek te gaan doen aan vezelhennep, heb ik die kans met beide handen aangegrepen. Daar heb ik geen moment spijt van gehad, ook niet toen de eerste veldproeven mislukten door regen, hagel en wind. En hoewel ik graag met mensen werk, zijn het ook de dagen alleen op het proefveld, waar ik nog regelmatig aan denk. De rust, de tijd om na te denken.

De voltooiing van dit proefschrift heeft al met al wat langer geduurd dan gepland, maar er zijn ook andere zaken belangrijk en niet alles is te voorzien. Het boek ligt nu voor u en u heeft het al bijna uit, neem ik aan. Een ieder die een bijdrage heeft geleverd aan de totstandkoming ervan, dank ik.

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De meeste experimenten die in dit proefschrift staan beschreven, zijn uitgevoerd in het kader van het door de Europese Unie gesubsidieerde HEMP-SYS-project, waarin universiteiten, onderzoekscentra, landbouworganisaties, producenten, ontwerpers en industrie onder de prima leiding van Stefano Amaducci samenwerkten aan het ontwerpen en ontwikkelen van een textielhenneppproductieketen. De vele deelnemers, in het bijzonder Katri Pahkala, Marco Errani, Jörg Müssig, Hayo van der Werf, Cesare Tofani en Alessandro Zatta, dank ik voor de inspirerende bijeenkomsten, de excursies en de gedeelde kennis. Katri en Stefano dank ik ook voor het beschikbaar stellen van een deel van de in Finland en Italië voor dit project geteelde vezelhennep.

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Het is af,

Het is mooi geweest,

Wim

Curriculum vitae

Willem (Wim) Westerhuis was born on 10 September 1967 in Veendam, The Netherlands. He attended the Rijksscholengemeenschap Winkler Prins in Veendam, from which he graduated in 1985 (Atheneum B) and Wageningen University from which he graduated in 1991 (Plant Sciences). Between 1992 and 2003 he worked among others as a researcher (ATO-DLO), project manager (Ministry of Agriculture) and editor (AgriHolland). Between 2003 and 2009 he conducted the research described in this thesis. Currently he is appointed as project manager at the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb).

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. De Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
- Hemp for textiles

Writing of project proposal (4.5 ECTS)
- Hemp fibre quality

Post-graduate courses (3 ECTS)
- Advanced statistics; PE&RC (2008)
- Ecophysiology pf plants; Functional Ecology, RUG

Laboratory training and working visits (3 ECTS)
- Fibre processing; Gruppa Fibranova (2004)
- Fibre releasing (retting, breaking, scutching), fibre testing, microscopy; AFSG (2004-2007)
- Field trials; MTT Agrifood, Jokioinen, F and DISTA, Bologna, I (2004-2007)

Deficiency, refresh, brush-up courses (0.6 ECTS)
- Insecten en maatschappij lezingencyclus (2007)

Competence strengthening / skills courses (2.9 ECTS)
- Project and time management: PE&RC (2007)
- Scientific writing; PE&RC (2007)

PE&RC Annual meetings, seminars and the PE&RC weekend (0.3 ECTS)
- Quality from soil to healthy people (2006)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)
- HEMP-SYS; EU-project (2003-2006)

International symposia, workshops and conferences (4.2 ECTS)
- HEMP-SYS; Presentations; HEMP-SYS Symposium, Bologna (2004-2006)
- EIHA Conferences; presentations; Cologne and Germany (2005-2007)

Lecturing / supervision of practical's / tutorials (2.4 ECTS)
- Research methods in crop and weed ecology; CWE (2005-2007)
- Plant cell tissue culture; Plant Physiology (2006)
- Population ecology; CWE (2007)

Supervision of MSc students (6 ECTS)
- Fibre development in hemp
- Secondary fibre development in fibre hemp

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