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HEMP FIBRE FOR HIGH-QUALITY APPLICATIONS

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This PhD thesis explores the influence of agronomic and post-harvest practices on hemp fibre extraction and high-tech application.

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ABSTRACT

European hemp fibres are the only natural fibre with an established sustainability certification. Hemp fibre quality is affected by genotype, agronomic techniques, harvest time and retting method. The main objective of this thesis is to outline the agronomic and post-harvest practices for improved fibre extraction and fibre quality, with special attention to multipurpose hemp production delaying the harvest from the flowering stage until seed ripening is complete. Planting density and nitrogen fertilization trials and variety trials were conducted in contrasting environments in Europe. Stems were decorticated following a standardised procedure and longitudinal hemp line for textile and high-added values application was compared with lab-scaled decortication. Impregnated fibre bundle tests were carried out with hemp hackled fibre bundles to compare composites and back-calculated fibre properties between genotypes, harvest times and retting methods. Results of this investigation suggest that i) optimum plant density and nitrogen fertilization are between 90 and 150 plants m^{-2} and 30 and 60 Kg N ha^{-1} respectively; ii) new yellow stemmed varieties are characterized by high decortication efficiency and relative high cleanness of the extracted fibre and iii) long hemp fibre, having properties comparable to those of flax, proved to be suitable for high-tech composites applications.

Keywords: Hemp (*Cannabis sativa*); decorticability; bast fibre content; fibre quality; yellow stem

SOMMARIO

La fibra di canapa europea è l'unica fibra naturale con una certificazione di sostenibilità. La qualità della fibra è influenzata principalmente da genotipo, tecniche agronomiche, epoca di raccolta e metodo di macerazione. L'obiettivo principale della tesi è delineare le pratiche agronomiche e di post-raccolta per migliorare l'estrazione e la qualità della fibra, con particolare attenzione alla produzione di canapa multiuso ritardando la raccolta fino alla maturazione dei semi. Le prove di densità, fertilizzazione azotata e varietali sono state condotte in differenti ambienti Europei. La procedura di decorticazione standardizzata è stata confrontata con la linea longitudinale di estrazione della fibra per applicazioni di alto valore aggiunto. I test su bio-compositi di canapa sono stati effettuati con fibra pettinata per confrontarne le proprietà tra genotipi, epoca di raccolta e metodi di macerazione. I risultati di questa indagine suggeriscono che i) la densità di semina e la concimazione azotata ottimale sono tra 90-150 piante m^{-2} e 30-60 kg N ha^{-1}; ii) le nuove varietà dallo stelo giallo presentano un'alta efficienza di decorticazione e una ridotta contaminazione da canapulo nella fibra, iii) la fibra di canapa, con proprietà comparabili a quelle del lino, si è dimostrata adatta per applicazioni in compositi di alto valore.

Parole chiave: Canapa (*Cannabis sativa*); decorticabilità; fibra corticale; qualità della fibra; stelo giallo

CHAPTER 1

General introduction

General introduction

1. *Cannabis sativa* L.

Cannabis sativa L. belongs to the family Cannabaceae, which comprises two economically important genera, Cannabis and Humulus. *Cannabis s.* is a monotypic genus consisting of a single species (Pillay and Kenny, 2006) that includes a narcotic and a non-narcotic sub-species with both domesticated and ruderal varieties (Small, 2015). The indigenous range of *Cannabis s.* is believed to be in Central Asia (Hillig, 2005); however, no precise area has been identified where the species occurred before it began its association with humans, which happened in the Neolithic (Small, 2015). Domestication of *Cannabis s.*, targeting different products, produced a divergent evolution. *Cannabis s.* might have been introduced into Europe ca. 1500 B.C. by nomadic tribes from Central Asia where it was selected for the strong fibre and for its nutrient seeds; those sub-species are named hemp. Arab traders may have introduced *Cannabis s.* in Africa one to two thousand years ago where it was selected for its psychoactive compounds (Hillig, 2005); this second sub-species are named Cannabis or marijuana. Cultural evolution of *Cannabis s.* use over millennia is the cause of the biological evolution of the plant, resulted in morphological, anatomical, chemical and physiological transformations of the plant; the genus is now distributed worldwide (Small, 2015; Clarke and Merlin, 2016).

2. Hemp cultivation in Europe

Hemp cultivation became widespread in Europe from 500 B.C. and reached its maximum expansion between the 16th and the 18th century as a source of strong fibre for sailing ship equipment (Bouloc, 2013). In Italy, during the first half of the 19th century, hemp cultivated area reached more than 100.000 hectares, second only to Russia, but the quality of Italian hemp fibre was considered the highest (Liberalato, 2013). The decline arrived after the 2nd World War for several reasons: the large-scale production of tropical fibres and particularly of cotton (*Gossypium* ssp), the absence of adequate machinery and the related high labour cost. The availability of cheap cotton and synthetic fibre and the prohibition of hemp cultivation in many countries due to the association with narcotic production reduced drastically the cultivation of hemp (Salentijn *et al.*, 2015). As a result, most of the European hemp genetic diversity was lost and breeding programs, as well as mechanisation, were drastically reduced (Amaducci *et al.*, 2015; Salentijn *et al.*, 2015). Hemp cultivation was uninterrupted mainly in France, for speciality pulp and paper production (Carus *et al*, 2013), and in Eastern Europe, where hemp long fibres were traditionally extracted for textile application due to low labour costs (van der Werf and Turunen, 2008). In the European Union, the ban on industrial hemp was released between 1993 and 1999. Hemp cultivated area increased in the past 20 years to finally reach more than 33,000 ha in 2016. Today, the cultivation area for industrial hemp covers the largest area since the second world war (Carus, 2017). Fibre, in the past the main product of hemp cultivation, nowadays seems to be the limiting factor to a further expansion of hemp cultivation, while seeds used for food destinations and inflorescence for pharmaceutical applications are the main drivers of hemp cultivation growth (Tang *et al.*, 2016; Calzolari *et* *al.*, 2017). For this reason, crop management and processing have to be improved to maximize multipurpose use of the whole plant biomass.

3. Hemp plant

Hemp is a vigorous, annual short-day crop, in which photoperiod and temperature affect plant development and sex expression (Amaducci *et al.*, 2008; Tang *et al.*, 2016). Seed germination is epigeal, after a relatively slow growth period in the first weeks after germination, a period of rapid growth follows in which the crop has high water and nutrients demands (Bócsa and Karus, 1998). The increasing of night-time induces the transition from vegetative to flowering phase. Flowering of a specific variety is adapted to the photoperiod and the microclimatic conditions of its breeding environment; genotypes bred at northern latitudes tend to come into flower readily with shortening days, allowing time for seeds to mature before a killing frost; decreasing the latitude at which the genotypes were selected tend to produce a longer vegetative phase in southern environments (Small, 2015). Naturally, hemp is a dioecious species with sexual dimorphism revealed at the beginning of flowering, which becomes prominent with the proceeding of the generative phase. Male flowers, having a chalice of five sepals and five stamens with bilocular anthers, are arranged in a large terminal panicle composed of numerous axillary racemes. Female flowers are gathered in axillary glomeruli collected in a false spike and compact terminal leaves: each flower is surrounded by a bract, in which the unilocular ovary is found, surmounted by two long pistils. Pollination is anemophilous and male plants die after anthesis, while female plants live three to five weeks longer, until seed ripeness (Clarke and Merlin, 2016). During the reproductive phase, nutrients are mobilized from basal leaves to the top of the plant. Sexual dimorphism is expressed also in plant biometry traits and fibre characteristics, with male plants taller and with thin stems with finer fibre than female individuals (Meijer *et al.*, 1995). Monoecious plants can occur naturally and were the object of intensive breeding programs for improved homogeneity of the crop. Monoecious hemp varieties are considered to be appropriate for double purpose harvest, i.e. stems and seeds (Bocsa and Karus, 1998; Faux *et al.*, 2013). The monoecious state varies from predominantly male to predominantly female extreme phenotype. The ratio between male and female flower, in both monoecious and dioecious varieties, is influenced by genetics and by external factors such as hormonal treatments, photoperiod or nitrogen status (van der Werf and van den Berg, 1995; Faux *et al.*, 2014). The fruit (vulgarly called seed) is a small achene (indehiscent dry fruit) bivalve with a slightly flattened ovoid shape. Hemp seed typically contains over 30% oil and about 25% protein, with considerable amounts of dietary fibre, vitamins and minerals. Hemp seed oil is an exceptionally rich source of the two essential fatty acids linoleic acid (omega-6) and alpha-linoleic acid (omega-3) in a ratio between 2:1 and 3:1, which is considered to be optimal for human health (Callaway, 2004).

4. Hemp fibre

Hemp, as flax (*Linum usitatissimum* L.), is a bast fibre crop as the fibres are contained in the outer layers of the stem. Concentric layers compose the hemp stems; the core part or shives, formed by pith and xylem, is separated by the cambium layer from the bark, formed by phloem, cortex and epidermis (Hernandez, 2007). Fibres are arranged in bundles in the phloem, joined together by the middle lamella, which is composed mainly of pectin, hemicellulose and lignin. Bast fibres can be distinguished in two fibre cell types, primary and secondary fibres. The primary fibres are formed in the apical meristem and they elongate during internode growth. Primary fibres are extremely long (range between 3 and 55 mm) with compact cell walls and small lumen. In contrast, secondary fibres, formed from the secondary meristem, are much thinner, shorter and with higher lignin content than primary fibre cells (Amaducci, 2005; Placet *et al.*, 2014) and for this reason are a cause of undesired coarseness and inhomogeneity (Cappelletto *et al.*, 2001). When present, secondary fibres are present in thin layers at the base of the stem (Keller *et al.*, 2001; Amaducci *et al.*, 2005).

5. Hemp fibre extraction

Primary fibres are the main, and most valuable, product of traditional hemp stem's processing, primarily used in the textile markets. Decortication, the mechanical separation of fibre bundles from hemp stems, is commonly carried out with a series of opposite rolls with a grooved profile that provides the breaking of the internal core and the separation of little woody pieces, named shives, from bast fibre bundles. Decortication is followed by cleaning and subsequent refining, where residuals shives are removed and fibre are mechanically separated from bast fibre bundles. An alternative decortication technique for industrial fibre extraction is based on the use of hammer mills that separate fibre bundles from shives by the impact force of rotating hammers (Saleem *et al.*, 2008; Yan *et al.*, 2013). Several authors have investigated the production of long hemp fibre processed with flax scutching and hackling machines, the so-called "longitudinal line" (Amaducci and Gusovius, 2010), for textile and high-added values application (Amaducci, 2005; Turunen and van der Werf, 2006; Mussig, 2010). However, the possibility to obtain long hemp fibre bundles is limited by the lack of dedicated harvesting machines that can mow hemp stems, lay them on the field in aligned swaths and cut them in 1 meter long portions, so that they can be fed in flax scutching lines (Amaducci and Gusovious, 2010). For this reason, in Europe, hemp stems are mainly processed by 'total fibre' lines (Carus *et al.*, 2013), where the extraction of total fibre content is carried out on disordered stem portions. Short fibres are predominantly used as technical fibres for industrial applications (speciality pulp, bio-composites, insulation): processing technology in industrial fibre application is moving toward injection and compression moulded thermoset plastic materials used for vehicle interior parts (Mussig, 2010). An expansion of the hemp fibre sector is expected, to satisfy the increasing demand of the automotive industry for sustainable bio-composite production. European hemp fibres are in fact the only natural fibre with an established sustainability certification (www.iscc-system.org; Carus, 2017). However, sustainability is only one facet of industry demand; reliable supply, stable and competitive price and homogeneous quality are other industrial requirements that need to be satisfied. The concept of fibre quality depends on the end application of the fibre (Keller *et al.*, 2001; Amaducci and Gusovius, 2010) but stem decorticability and fibre bundles cleanness and refinability, are leading quality parameters in industrial processing. In addition, high fineness of fibre bundles and high tensile strength are required for high-added value applications, i.e. textile and high-tech composites

(Müssig, 2010). Fibre extraction by mechanical decortication and scutching has a strong influence on fibre quality and it is the main cause of damage to the fibre.

Retting method (Placet *et al.*, 2017) as well as agronomic practices (Struik *et al.*, 2000; Amaducci*et al.*, 2002; Amaducci *et al.*, 2008; Westerhuis *et al.*, 2009) and genotype (Sankari, 2000; Jankauskiene *et al.*, 2015) are important determinant of fibre quality, that affect the possibility to use hemp fibre for textile and high-added value applications (Lantbruksuniversitet, 2009).

6. Retting

Commonly, stems are processed after retting to reduce the energy cost and improve decorticability and fibre cleanness, however, decortication of un-retted stem is also suitable for industrial application (Munder *et al.*, 2004). Retting is a biochemical process, in which enzymes produced by microorganisms attach the pectins that glue together fibre cells, aiding the separation of fibre bundles within the bast fibre and of shives from bast fibre (Booth *et al.*, 2004; Akin *et al.*, 2010). Microbiological retting can be carried out in water (water retting) or on the soil (dew retting). In the past, the retting process was carried out in open water basins. Nowadays water retting is considered to have a high environmental impact, due to high water use (Turunen and van der Werf, 2006) and high oxygen demand (BOD) of the waste waters (Keller et al., 2001). The impact of water retting is reduced in the case of controlled warm water retting, where the retting process is optimised with target bacterial inoculum (Di Candilo *et al.*, 2010) and performing retting on un-retted scutched hemp (Amaducci*et al.*, 2008). Dew retting, instead, is carried out on the field, where the stems are left after harvesting, mainly by moulds without the use of water and only relying on rain or air humidity (dew). During dew-retting, pectins that glue fibres together in bundles are degraded by fungi. This process is strictly dependent on the microclimatic condition (Thomsen *et al.*, 2005) and produces un-homogeneous results. The influence of uncontrollable microclimatic conditions on the dew retting process is extremely high: over-retting and under-retting can occur frequently, affecting hemp fibre quality (Jankauskienė et al., 2015). In order to avoid degradation of cellulose, a mixture of selected enzymes or fungi can be spread on the stems (Liu et al., 2017) resulting in a reduced duration of dew retting that is carried out by a standardised microbial population with high pectinolytic and low cellulosic activity. Because of its low cost, dew retting is widespread in Europe (Jankauskiene et al., 2015).

7. Agro-technique

Agronomic factors, like plant density, nitrogen fertilization, harvest time and variety choice affect fibre extraction, yield and quality (Amaducci and Gusovius, 2010). Plant density has an evident effect on plant biometric traits (van der Werf, 1994; Amaducci *et al.*, 2008; Tang *et al.*, 2017). Biometric traits affect harvesting and post-harvest processing, Khan et al. (2010) report an increase of compression load and energy requirement as the diameter of the stem increases, while a decrease in decorticability occurs in thinnest stems (S Amaducci *et al.*, 2002).

Increasing plant population results in shorter and thinner stems and longer basal internodes (Amaducci *et al.*, 2002; Westerhuis *et al.*, 2009). Despite the effect of plant density on stem biometry traits, several authors reported that increasing plant density had a limited effect on stem yield (Struik *et al.*, 2000; Amaducci *et al.*, 2008; Tang *et al.*, 2017). However, high plant density produces finer fibre cells (van der Werf *et al.*, 1995; S Amaducci *et al.*, 2002) and lower presence of lignified secondary bast fibres (van der Werf, 1994; Schäfer and Honermeier, 2006; Amaducci et al., 2008).

Among plant nutrients, nitrogen plays the main role in the formation of hemp yield (van der Werf and van den Berg, 1995; Tang *et al., 2017*), however, the effects of nitrogen fertilization on stem yield are related to soil fertility and are negligible when soil fertility is high (Struik *et al.*, 2000; Tang *et al.*, 2017). Nitrogen fertilization rates between 150 and 240 kg N ha^{-1} gave the highest stem yields (S Amaducci *et al.*, 2002). While bast fibre content in the stem was reduced by nitrogen, with the highest bast fibre yields obtained at between 50 and 150 kg N ha^{-1} (van der Werf and van den Berg, 1995).

8. Hemp breeding in Europe

The historical importance of hemp cultivation in Europe is well reflected by the abundance of cultivars, traditional landraces, and populations that were selected in the main areas of hemp cultivation throughout Europe, resulting in three pool gene: northern and central European, southern European and East Asian ecotypes (Amaducci and Gusovius, 2010; Salentijn *et al.*, 2015).

Before the rediscovery of industrial Hemp in Europe, hemp was cultivated mainly in France for speciality pulp and paper production that is still one of the most important markets for European hemp Fibres, covered mainly by French producers (Carus *et al.*, 2013). Thanks to a stable market (Carus *et al.*, 2013), a continuous and successful breeding program of hemp was feasible in France; for this reason, French varieties are the most spread in the European seed market. France breeding program was focused mostly on three characters: monoicous plants were selected to improve crop homogeneity, high bast fibre content to improve pulp and paper yield and low THC content because of law restriction m (Δ9-THC < 0.2% of inflorescence dry mass, [(EC) No 2860/2000]). Cultivars were selected based on the vegetative period length and bast fibre yield, but the fibre quality traits probably were not influenced (Ranalli, 2004). French verities are monoecious, and therefore particularly suitable for seed production, and descend directly from Fibrimon or from crosses of Fibrimon progeny. Fibrimon is a monoecious cultivar with a high fibre content, selected in Germany between 1951 and 1955 (De Meijer, 1995).

High fibre quality was the target of the breeding program in Eastern Europe, especially in Hungary where hemp long fibres were traditionally extracted for textile application due to low labour costs (van der Werf and Turunen, 2008). Hungarian variety, in general dioecious, were selected from old Italian ecotypes (Ferrara) for improved fibre quality (de Meijer and van Soest, 1992; Amaducci and Gusovius, 2010). The breeding research on hemp in Poland began in 1946 and resulted in first dioecious and then monoecious varieties with improved bast fibre content. The monoecious varieties, Białobrzeskie and Beniko are still registered and cultivated in Europe.

Hemp breeding in Italy was mainly focused on the breeding and maintenance of dioecious landraces like Carmagnola in north Italy and Eletta Campana in the south (Ranalli, 2004).

Breeding for genotypes with improved decorticability may result in a reduction of fibre damages due to mechanical processing and a reduction of costs related to fibre extraction (Salentijn *et al.*, 2015). These objectives seem to be met with the selection of yellow stemmed varieties characterized by reduced nitrogen assimilation resulting in an easier fibre detaching during decortication than conventional varieties (Bouloc, 2013). Yellow lines were generated from a Hungarian variety and were subjected to an intensive breeding program. In The Netherlands were released the dioecious Chamaeleon and the monoecious Markant, Ivory, and Marcello (Toonen *et al.*, 2004; Salentijn *et al.*, 2015). In Italy, the monecious variety Carmaleonte was selected from the breeding of the dioecious variety Chamaeleon and the monoecious variety Carma (Grassi, personal communication). Recently, the new French yellow stemmed monoecious variety, Fibror 79, was released and added to the list of European hemp variety (Thouminot, 2015).

9. Objectives and structure of the thesis

The main objective of this thesis is to outline the agronomic and post-harvest practices for improved fibre extraction and fibre quality, with special attention to multipurpose hemp production delaying the harvest from the flowering stage (traditional harvest for textile destination) until seed ripening is complete.

Chapter 2: Density and nitrogen effect on fibre quality in hemp (*Cannabis sativa* L.)

Sowing density, nitrogen fertilization and harvest time are considered very important factors affecting fibre quality. Stems of Futura 75 from four planting density, four levels of nitrogen fertilization and two harvest times were collected from field trials in four contrasting environments and were decorticated following a standardised procedure. In this chapter is pointed out that optimum agronomic technique for high decortication efficiency and high refined fibre fineness, when the harvest is delayed until seed maturity, were a plant density between 90 and 150 plants m^{-2} and a nitrogen fertilization between 30 and 60 Kg N ha^{-1}.

Chapter 3: Effect of variety on fibre extraction in hemp (*Cannabis sativa L.*)

In Europe varieties were selected from old populations and landraces on the basis of the length of the vegetative period, stem biometry traits and in some cases, seed yield; however, very limited information on fibre quality from dual-purpose harvest is available in literature. In this chapter, six commercial varieties were cultivated in five contrasting environments in Europe and were compared for bast fibre content and decortication efficiency. Results reported in this paper highlight that, in traditional varieties, decortication efficiency and fibre quality were closely linked to the breeding target and in particular that new yellow stemmed varieties were characterized by high decortication efficiency and relative high cleanness of the extracted fibre.

Chapter 4: High-tech composites production from hackled hemp fibre

Hemp fibre quality, and its suitability for high-tech composites production, is affected by genotype, harvest time and retting method. In this study, a longitudinal hemp line for textile and high-added values application were investigated. Impregnated fibre bundle tests were carried out with hemp hackled fibre bundles to compare composites and back-calculated fibre properties between genotypes, harvest times and retting methods, and to compare hemp fibre performance with that of flax fibre. Results, obtained at the industrial level, confirm that yellow stemmed varieties are characterised by higher fibre extraction efficiency than conventional ones during the scutching step. In this study long hemp fibre, having properties comparable to those of flax, proved to be suitable for high-tech composites applications. In particular, these results were underscored for yellow varieties due to the higher decortication efficiency recorded.

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