ETIENNE DE MEIJER

DIVERSITY IN *CANNABIS*

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STELLINGEN

1. De potentiële stengelopbrengst van hennep kan het effectiefst worden vergroot door populaties te importeren die aangepast zijn aan lage breedtegraad.
   Dit proefschrift.

2. Hennephout is een inferieure grondstof voor papierpulp, die ook door veredeling nauwelijks verbeterd kan worden.
   Dit proefschrift.

3. De door Fournier en Paris gebruikte aanduiding 'vezelhennep' (chanvre à fibres) voor hennep met een geringe psychoactieve potentie is niet correct.
   Fournier, G. & M.R. Paris, 1979. Plant Med. Phytother. 13: 116-121.
   Fournier, G. & M. Paris, 1982. Ann. Fals. Exp. Chim. 75: 7-13.
   Dit proefschrift.

4. De door Small & Cronquist maatschappelijk en juridisch gewenst geachte taxonomische indeling van *Cannabis sativa* L., op basis van het veronderstelde doel waarvoor planten geteeld worden en hun veronderstelde psychoactieve potentie, is van een zelfde orde als de taxonomische onderscheiding van 'soepgroenten' en 'saladeachtigen' binnen *Allium cepa* L. (ui).
   Small, E. & A. Cronquist, 1976. Taxon 25: 405-435.

5. Het is in het geval van *Cannabis* eenvoudiger om een mogelijk te grote genenbankcollectie te beheren dan om gefundeerd te besluiten welke herkomsten wél, en welke niet tot een 'core collectie' moeten gaan behoren.

6. Het imago van Nederlandse landbouwproducten is meer gebaat bij een terugkeer naar oude productiemethoden dan bij verdergaande innovatie.

7. In een krimpende overheidsorganisatie is geboortejaar de belangrijkste verklarende variabele voor het perspectief van de medewerkers.

8. De duurzame samenleving vangt niet aan voor de fossiele brandstoffen opgemaakt zijn.

9. Een beroep wat in 1950 nog niet bestond is misbaar.

10. Er is geen zinvolle definitie van 'hard drugs' te bedenken waar alcohol niet onder zou vallen.
    Erik van Ree, Volkskrant 8 oktober, 1994.

11. Het geluk van de een lijkt op dat van de ander, maar ieder ongeluk heeft zijn eigen bijzonder karakter.
    Leo N. Tolstoi, Anna Karenina.

Stellingen behorend bij het proefschrift "Diversity in *Cannabis*" door Etienne de Meijer, te verdedigen op 22 december 1994 te Wageningen.

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Etienne de Meijer

CONTENTS

1.	General introduction	1
2.	The CPRO *Cannabis* germplasm collection	4
3.	Variation of *Cannabis* for phenological development and stem elongation in relation to stem production	14
4.	Evaluation of current methods to estimate pulp yield of hemp	30
5.	Variation of *Cannabis* with reference to stem quality for paper pulp production	44
6.	Characterization of *Cannabis* accessions with regard to cannabinoid content in relation to other plant characters	60
7.	Evaluation and verification of resistance to *Meloidogyne hapla* Chitwood in a *Cannabis* germplasm collection	78
8.	Multivariate patterns of diversity in *Cannabis*	90
9.	General discussion	110
	References	116
	Summary	123
	Samenvatting	127
	Curriculum vitae	131
	Appendix 1	
	Appendix 2	

CHAPTER 1

GENERAL INTRODUCTION

IMMEDIATE CAUSE OF THE PRESENT STUDY

In an effort to reduce the overproduction of a too limited number of arable food crops, several research programs focusing on industrial crops have been initiated recently in several countries in Northwest Europe (e.g., Hennink *et al.*, 1994). The 'Dutch hemp research program' investigated from 1990 to 1994 the feasibility of hemp as an arable crop for the Netherlands and as a raw material for paper pulp production. In this context, breeding, agronomy, plant pathology, mechanization, processing and economics were subject of a comprehensive study, the overall results of which have been summarized by van Berlo (1993).

Fibre hemp cultivation has a long history in the Netherlands (Hoogendoorn, 1993) although it has never been of great importance. The crop disappeared at the beginning of the 20th century (de Jonge, 1944). Two previous attempts to re-introduce fibre hemp have been reported (de Jonge, 1944; Friederich, 1960).

Breeding of fibre hemp in the Netherlands seems unprecedented. In the framework of the present research program, it was considered necessary to investigate the prospects of breeding directed at increased stem yield potential, improved stem quality for pulp production, disease resistance and low psychoactive potency. A collection of *Cannabis* germplasm, which was presumed to cover the variation within the genus, was therefore established and evaluated at the DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO, Wageningen). This thesis reports on the composition of the collection and its evaluation for traits relevant to the introduction of hemp in crop rotations in the Netherlands and its utilization as a raw material for paper pulp.

The present study shows strong analogy with recent evaluations of kenaf germplasm (*Hibiscus cannabinus* L.), a bark fibre crop traditionally used for rope and textile production, and since the 1950s under investigation as a source of pulp fibre in the USA and Australia (Adamson & Bagby, 1975; Wood, 1990).

THE STATE OF KNOWLEDGE ON *CANNABIS* DIVERSITY PRIOR TO THIS RESEARCH

Domesticated *Cannabis* has long been cultivated as a dual purpose crop, producing bark fibres to be used for manufacturing ropes, textiles and specialty paper, as well as seeds for the extraction of edible oil

and technical oil for paint and varnish. Other domesticated strains are used for the production of hashish and marijuana. Features so far evaluated in agronomic research concern: stem yield (e.g., Friederich, 1960; Cristea *et al.*, 1973); bark fibre yield (e.g., Friederich, 1960; Demkin & Bondarenko, 1965); bark fibre quality criteria such as tensile strength (e.g., Baksheeva *et al.*, 1980), flexibility (I. Bócsa, pers. commun., 1990), fineness (Kerékgyártó & Nagy, 1962) and the ratio of secondary to primary fibre-cells (Tarakan, 1969; Horkay, 1982); seed yield (e.g., Demkin & Bondarenko, 1965; Cristea *et al.*, 1973); seed oil content (Getmanov, 1966) and cannabinoid profiles (e.g., Fournier & Paris, 1979; Murari *et al.*, 1983; Gorshkova *et al.*, 1988). Some references focus on crop protection against the fungi *Botrytis cinerea* Pers. ex Fr. (grey mold), *Sclerotinia sclerotiorum* (Lib.) de Bary (stem canker), *Phytium debaryanum* auct. non R. Hesse (causing seedling damping-off) and *Fusarium oxysporum* Schlechtend. (fusarium wilt); the insects *Grapholitha delineana* Walk. (hemp leaf roller), *Psylliodes attenuata* Koch (hop flea beetle), *Ostrinia nubilalis* (Hübner) (corn borer) and the parasitic weeds *Orobanche ramosa* L. (broomrape) and *Cuscuta europaea* L. (dodder). Varietal differences in reactions to these pests and diseases were rarely reported. Morphological traits (Quimby *et al.*, 1973; Small *et al.*, 1976; Anderson, 1980; Verzár-Petri *et al.*, 1981), anatomical traits (Anderson, 1974), cannabinoid profiles (Small & Beckstead, 1973) and electrophoretic patterns of seed proteins (Lawi-Berger *et al.*, 1982; Yumaguzina *et al.*, 1979a,b) have been observed in biosystematic oriented research.

In the past decades, numerous, mainly Central European and Russian, references, reported on trials in which the performance of fibre cultivars was tested in combination with various cultural treatments: e.g., the application of fertilizers, growth regulators, chemical defoliants and pesticides, and factors such as sowing depth, row width, plant density, soil types and preceding crops in the rotation. For fibre strains, the expression of the traditional agronomic characters in continental climates, and the effects of growth factors, are hence quite well documented. Reports on large-scale germplasm screenings, primarily aimed at the identification of useful breeding material, are rare (e.g., Virovets *et al.*, 1987). In biosystematic research, some very large collections of *Cannabis* germplasm were evaluated (Anderson, 1980; Small & Beckstead, 1973; Small *et al.*, 1976), comprising domesticated drug and fibre strains as well as undomesticated populations, but the traits involved were of little relevance to the present goal of cellulose production in the Netherlands.

Prior to this research program, knowledge on agronomic performance with some relevance to paper pulp production in the maritime climate of the Netherlands, was restricted to a small number of older and largely replaced fibre strains tested by Friederich (1960). The

performance with regard to stem yield and quality of some currently available fibre strains has been studied by van der Werf (1994) in the context of the present program.
The agronomic performance of a representative collection of *Cannabis* germplasm remained to be evaluated under temperate maritime conditions. Some aspects were specific for the present program and supplementary to previous hemp research. In contrast to the traditional use of fibre hemp, this program was directed at the utilization of whole stalks, including the woody core, for pulp production (du Bois, 1982). Woody core characters have not been subject of large-scale germplasm evaluations so far but were considered important for the present one. Also omitted in previous studies, but considered relevant to local crop rotations in the Netherlands, were the host reactions to soil pathogens.

OBJECTIVES OF THE PRESENT STUDY

The practical work consisted of the establishment, maintenance and evaluation of a representative *Cannabis* germplasm collection. This study explores, on the basis of diversity among and occasionally within accessions, the prospects for the breeding of improved cultivars. The main objectives were:

* The identification of relevant agronomic traits to be evaluated and the assessment of evaluation methods.
* The characterization of accessions with respect to agronomic, morphological and genetic traits.
* The study of the stability of, and mutual relations between traits.
* A practical classification of diversity in *Cannabis*.

OUTLINE OF THE THESIS

The established germplasm collection is presented in Chapter 2, with origin and pedigree of accessions specified. Chapter 3 describes the variation for the presumed yield-related characters, stem length and phenological development, and seeks to verify the relation with stem dry matter production. In Chapter 4 some methods are evaluated for the estimation of potential pulp recovery of hemp stem samples. Variation in stem quality parameters is presented in Chapter 5. In Chapter 6, accessions are classified according to cannabinoid profiles and the relation between chemical and non-chemical traits is investigated. The evaluation of resistance to the root-knot nematode *Meloidogyne hapla* Chitwood in a seedling test and the verification of observed varietal differences in a field trial are described in Chapter 7. In Chapter 8, the observed traits are considered in a multivariate way. Variation patterns are related to passport data and electrophoretic patterns of seed proteins. In the final Chapter 9, implications with regard to cultivation and breeding are discussed and some issues that remain to be studied are identified.

Revised and updated version of:

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CHAPTER 2

THE CPRO *CANNABIS* GERMPLASM COLLECTION

ABSTRACT

The CPRO *Cannabis* germplasm collection was established between 1988 and 1993. In its final state it comprises ca. 200 accessions. In this chapter, origin and pedigree of accessions, and maintenance of the collection are described.

Key words: cultivars, germplasm collection, origin, pedigree

INTRODUCTION

Classification of Cannabis

There is no general agreement on the infrageneric taxonomic treatment of *Cannabis*. Schultes *et al.* (1974) distinguished three species within the genus: *C. sativa* L., *C. indica* Lam. and *C. ruderalis* Janischevsky. Other authors (e.g., Hoffmann, 1961) referred to these taxa only at subspecific level within *C. sativa*. Small & Cronquist (1976) divided the single species *C. sativa* in the two subspecies *sativa* and *indica* each consisting of a domesticated and a wild variety. Within the subspecies *sativa* these were called var. *sativa* and var. *spontanea*, and within the subspecies *indica*, var. *indica* and var. *kafiristanica*, respectively. Taxa are usually discriminated on morphological, and physiological grounds, but Small & Cronquist based their key partly on the presumed psychoactive effects and the purpose and state of domestication. All strains within the genus *Cannabis* intercross readily and produce fertile hybrids (Schultes *et al.*, 1974). The pattern of variation within the genus is continuous (Small *et al.*, 1976). The haploid chromosome number is 10. In their natural state all strains are diploid (Purseglove, 1974).

Various non-biosystematic classifications are employed for *Cannabis*. Examples are a chemotype classification (using cannabinoid contents), an ecotype classification (based on adaptation latitude) and a subdivision according to the type of utilization (fibre and seed hemp versus drug strains). Opinions about the most preferable system to classify *Cannabis* are beyond the scope of this chapter. Terms derived from several systems will be used to specify accessions in the next sections.

Domestication and geographical distribution of the genus Cannabis

The centre of origin of *Cannabis* is supposed to be in Central Asia.

According to Sharma (1979), truly or nearly wild populations occur still in the Himalayas. Since ancient times cultivation has taken place in Asia and Europe (Purseglove, 1974). The genus also occurs widely in Africa (Haney & Bazzaz, 1970). In post-Columbian times it was introduced into North and South America (Dempsey, 1975). Persisting naturalized populations occur in Central Europe and the former Soviet Union (Hoffmann, 1961), the Midwestern USA (Haney & Bazzaz, 1970) and Canada (Clarke, 1981). The domestication status ranges from truly wild to naturalized, landrace, breeders material (mutants, inbred populations) and cross-bred cultivar (up to hybrid F_1 cultivar).

Until the 1950s, fibre hemp strains were usually derived from indigenous landraces by selection (Hoffmann, 1961). Well-performing exotic strains were frequently imported and adapted to local conditions or used for cross-breeding. Between 1940 and 1960 breeding in many countries was aimed at monoecious cultivars (Dempsey, 1975). Heterosis breeding became important in Hungary in the late 1950s. Hoffmann (1961) reported fibre hemp breeding in the former Soviet Union, Italy, Hungary, former Yugoslavia, Rumania, Germany, Sweden and the USA. Tschaneff (1959) reported breeding in Bulgaria. Dempsey (1975) listed 18 institutes engaged in hemp research: six in the former USSR, six in Eastern Europe, four in Western Europe and two in East Asia. Nowadays, fibre hemp breeding is continued to some extent in the Ukraine, Hungary, Poland, Rumania, France and China. The OECD schemes for the varietal certification of seed moving in international trade (Anonymous, 1991a) list a total of 18 European fibre cultivars registered in five countries: Italy (5), France (7), Hungary (4), Rumania (1) and former Czechoslovakia (1). At least 9 cultivars are registered and currently cultivated in the former USSR. Two cultivars are presently registered in Poland. No information was traced on Chinese cultivars.

Production of *Cannabis* drugs takes place in Afghanistan, Colombia, India, Jamaica, Lebanon, Mexico, Morocco, Nepal, Thailand and the USA (Cherniak, 1982). Since the 1980s domestic production of marijuana (female inflorescences) is regularly reported in the Netherlands. Drug type landraces are maintained in many of the traditional marijuana and hashish (resin) producing countries. Crosses between imported landraces from Afghanistan, Thailand, India and Nepal formed the basis for domestic drug cultivars in the USA (Clarke, 1981). Many of these hybrids, and some selected pure strains are presently commercialized in the Netherlands. Private companies publish seed lists with up to 20 drug strains for either indoor or outdoor cultivation.

Cannabis germplasm is stored in only a few genebanks. Collections occur mainly in connection with recently abandoned or current fibre hemp breeding. The largest collection, at the Vavilov Institute (St.

Petersburg, Russia), contains ca. 400 accessions (Lemeshev *et al.*, 1994), the Hungarian genebank stores about 70 accessions. Collections of up to 20 accessions are preserved in genebanks in Germany, Turkey and Japan. Some botanical gardens maintain a few accessions. Compared to other crops the available number of accessions is quite limited, and accessions are generally poorly documented.

Maintenance of Cannabis *germplasm*

Cannabis populations are easily affected by environmental conditions. Changes in cannabinoid profiles (Hakim *et al.*, 1986) and plant habit (Tschaneff, 1959) after a few generations under altered conditions have been reported. Special caution during multiplication is required in order to preserve specific properties. For commercial cultivars, selection for a desired phenotype usually takes place during outdoor multiplication. To avoid introgression, a distance of at least one km between multiplication fields is assured for monoecious cultivars in the Ukraine and two km for dioecious cultivars in Hungary. Multiplication of exotic accessions should be organized indoors to allow seed development on every female plant even late in the year. Doing so, one can oppose natural selection in favour of early-flowering genotypes (drift). Seed collections should be stored under optimal conditions in order to reduce the number of rejuvenation cycles.

THE CPRO *CANNABIS* COLLECTION, MAINTENANCE AND DESCRIPTION OF ACCESSIONS

Seed samples were acquired between 1988 and 1993 from genebanks, breeding and research institutes, seed companies and botanical gardens (Appendix 1). A few samples were taken from bird-seed and from marijuana.

Except for some current fibre cultivars, of which seeds were sufficiently available, and hybrid F_1 varieties with segregating progeny, samples were multiplied in pollen-isolated greenhouse compartments to obtain sufficient seed amounts for evaluation trials. No conscious selection took place during multiplications. About 150 seedlings per accession were planted in pots. An average number of 50 male and 50 female plants survived until maturity. Some generations were the progeny of lower numbers of plants due to the supply of small quantities of seed which sometimes had poor viability. Seed yields varied widely and ranged from 5 to 600 g, with an average of ca. 100 g. Late-flowering accessions generally yielded the smallest seed amounts. Seeds used for experiments were stored in paper bags in a dark coldroom at 4 °C and relative air humidity of 30%. Under such conditions seed moisture content reached an equilibrium of ca. 6%.

In order to identify optimal long term storage conditions an experiment was initiated in which subsamples from one seed lot were kept under various combinations of tem-

perature and seed moisture content. Germination tests at three month intervals were carried out to monitor the decline of seed viability. Anticipating the final results (unpublished, not included in this thesis), about 20 units of ca. 250 seeds each were stored per accession, with 4.5% moisture content at -20 °C in vacuum sealed bags.

Passport data and storage data were entered in the database management system 'Genis PCC8', an application of 'Oracle' (Hazekamp & van Hintum, 1989).

By mid-1994, in its final state, the collection contained ca. 200 accessions. According to status and purpose of domestication these can be grouped as fibre cultivars (68 accessions), fibre landraces (50), drug strains (28), ornamental cultivars (1) and wild or naturalized populations (16). Ca. 40 accessions remained *a priori* unspecified. Figs. 1, 2 and 3 show some examples of the diversity in the collection. Passport data are summarized in Appendix 1. Information on pedigree, provided in the next sections, could be traced for the breeder's cultivars in Appendix 1 only.

French fibre cultivars

The collection includes the monoecious cultivars Futura 77, Fibrimon 24, Fibrimon 56, Fedrina 74, Felina 34, Ferimon 12 and Fedora 19 which are currently cultivated for paper production in France. 'Fibrimon' is the patented name for a monoecious cross-bred cultivar with high fibre content. It was bred in Germany between 1951 and 1955 (Bredemann *et al.*, 1961). The parental populations were: inbred material derived from monoecious plants spontaneously occurring in 'Havelländische' or 'Schurigs' hemp which was again a selection from Russian origin (Hoffmann, 1961); dioecious selections with very high fibre content from Germany and dioecious late-flowering landraces from Italy and Turkey. 'Fibrimon' was transferred to France in the 1950s where further selections, named Fibrimon 21, 24 and 56, were made with diverging days of maturity. In general, the higher the numbers added to the names of French cultivars the later they flower. Other French cvs. were selected directly from 'Fibrimon' or from cross progenies of 'Fibrimon' and several exotic cvs. (J.P. Mathieu, pers. commun., 1992). In 1965 about 150 fibre hemp strains were evaluated for quality, productivity and future availability. The intention of this screening was to select parents for the production of hybrid F_1 cultivars (heterosis breeding) without domestic maintenance of the parental populations. Selected strains were crossed with 'Fibrimon'. The idea of heterosis breeding was, however, abandoned and the best performing hybrids were backcrossed with 'Fibrimon' and stabilized to new true-breeding monoecious cultivars. Three of these, 'Fedora 19', 'Felina 34' and 'Fedrina 74' are currently cultivated. Their dioecious parents were 'JuS 9',

'Kompolti' and 'Fibridia', respectively. 'Fibridia' is described in detail by Bredemann *et al.* (1961). 'Futura 77' was selected for a somewhat later day of flowering from the same hybrid offspring as 'Fedrina 74'. The more recent cultivar 'Ferimon 12' is an early maturing selection from 'Fibrimon 21', it is especially used for seed production.

No new crosses have been made since the late 1970s. The main objective since then was selection for low psychoactive potency within the existing cultivars.

Hungarian fibre cultivars

In the Balkan area, landraces were replaced in the 1950s by cultivars of which Fleischmann hemp or cv. Kompolti was the most important one (Hoffmann, 1961). 'Kompolti' has been selected from Italian hemp. Cv. Kompolti Hyper Elite is a further selection of 'Kompolti' with increased fibre content. 'Kompolti Sárgaszárú' is a cross between a spontaneous yellow stemmed mutant from Germany and 'Kompolti' (Bócsa, 1969). Commercial cultivars from Hungary are generally dioecious. It is the only country where heterosis breeding of hemp became important. This resulted in F_1 hybrid cultivars like 'Kompolti Hybrid TC', a three way cross hybrid in which two selections from Chinese origin, 'Kinai Kétlaki' (dioecious) and 'Kinai monoecious', and 'Kompolti' are combined (I. Bócsa, pers. commun., 1990). Other hybrids are 'Uniko-B', 'B-7' and 'Kinai Uniszex'. 'Uniko-B' is the F_2 of a hybrid F_1 of 'Kompolti' x 'Fibrimon' (Bócsa, 1966). 'B-7' is the hybrid F_1 of 'Kinai Kétlaki' x 'Kompolti'. 'Kinai Uniszex' is the unisexual female F_1 of the cross 'Kinai Kétlaki' x 'Kinai monoecious'. Unisexual generations like these are used as female parent for the production of three way cross hybrids and can be considered as an analogue for male sterility since they allow large-scale hybrid seed production with limited labour.

Rumanian fibre cultivars

Cv. Fibramulta 151 is an improved selection of cv. ICAR 42-118 which is a cross progeny of Italian and Rumanian strains (Hoffmann, 1961). Cv. Lovrin 110 was released in 1981, as a replacement for cv. Fibramulta 151. It was bred by selection among family groups from the Bulgarian cultivar Silistra (Paraschivoiu, 1982) which is also part of the collection ('Silistrenski').

Polish fibre cultivars

The collection comprises three Polish cultivars. The dioecious cultivar LKCSD was selected between 1945 and 1955 from imported 'Schurigs' hemp. In 1968 it was replaced by the monoecious cultivar Bialobrzeskie (R. Kozlowski, pers. commun., 1992). 'Bialobrzeskie' is a cross combination of four European strains: ((('LKCSD' x 'Kompolti') x 'Bredemann 18') x 'Fibrimon 24'), (B. Jaranowska, pers. commun., 1992). The most recent cultivar Beniko is

registered in 1986. It is a cross combination of 'Fibrimon 24' and 'Fibrimon 21' (B. Jaranowska, pers. commun., 1992).

Fibre cultivars from the former USSR

In the former USSR the areas for hemp cultivation were divided according to latitude into northern, central, and southern regions, each with a set of adapted landraces and cultivars (ecotypes). Northern hemp is of no practical use at present, it was grown by peasants before the revolution. This group is represented in the collection by two landraces from the Kirov region (883289, 883290) and one from the Novosibirsk region (921217). The group of central Russian hemp is represented in the collection by a number of Russian and Ukrainian cultivars. Southern Russian hemp comprises strains from the southern Ukraine, southern Russia, the Caucasian region, the Asiatic republics and Far Eastern hemp. Many cultivars were received under names specifying details like geographic origin, ecotype (yuzhnaya = southern) and the monoecious character (odnodomnaya).

Central Russian fibre cultivars

This group is represented by landraces from the Orlov, Altaij, Transcarpathian and Mari region, and the cultivars Odnodomnaja Bernburga, Bernburgskaya and SOU. Cv. Odnodomnaja Bernburga or Bernburgskaya is a monoecious cultivar which was originally produced in Germany in the 1940s under the name 'Bernburger einhäusigen' (Hoffman, 1961).

Southern Russian fibre cultivars

According to Hoffmann (1961), late maturing Italian strains and local Caucasian strains were crossed to obtain improved southern Russian cultivars. Series of southern Russian cultivars in the collection are indicated 'Krasnodarskaya', 'Dneprovskaja', 'JuS' or 'YuS', 'JSO' or 'YuSO', and 'USO'. Cv. Krasnodarskaya is the standard dioecious cultivar of the Caucasus region. Cvs. Krasnodarskaya 35 and Krasnodarskaya 56 are probably improved selections. Cv. Juznaya Odnovremenno Sozrevajuscaya 1 (abbreviated 'JSO1' or 'YuSO1') is a simultaneously maturing monoecious cultivar referred to at least since 1965 (Demkin & Bondarenko, 1965). Cvs. JSO4, JSO14, JSO16 and JSO19 are possibly improved selections from 'JSO1', but may be newly made crosses as well. Cv. Zolotosskaja 11 (abbreviated 'USO-11') and 'Zolotosskaja 13' are probably synonyms for 'Zolotonoshskaya 11' and 'Zolotonoshskaya 13', respectively. Both cultivars are monoecious. The latter was released in 1986 in the north Caucasus region. It was produced by selection in the progeny from a cross between 'JSO 16' and 'Dneprovskaya Odnodomnaya 6' (Orlov *et al.*, 1987).

Fig. 1. Monoecious fibre cultivar, crop height up to 4m.

Drug strains

The drug strains in the collection are poorly documented. 'Skunk' is a hybrid between indigenous strains from Afghanistan, Mexico and Colombia. The hybridization work took place in 1976 in California, USA. Ten years of inbreeding and selection of the progeny resulted in a consistent true-breeding cultivar (D.P. Watson, pers. commun., 1990). 'Skunk' is currently commercialized for indoor cultivation in the Netherlands. 'Rjaf' was selected from a hashish type drug strain from Afghanistan, and is presently used for indoor cultivation at northern latitudes (D.P. Watson, pers. commun., 1990). The term 'Nederwiet', assigned to some accessions, indicates any drug strain either indoor or outdoor cultivated in the Netherlands. At present 'Nederwiet' comprises a number of so called *indica* x

Fig. 2. Ornamental cultivar (accession 910914) at the Agricultural Research Institute in Kompolt, Hungary.

Fig. 3. Naturalized hemp in a roadside vegetation in Kiev, Ukraine.

sativa hybrids and pure strains of both taxa. Breeders of drug strains consider plants from the Afghanistan region, with wide leaflets, compact habit and relatively early maturation, as type examples for the taxon *C. indica*. These strains are traditionally used for resin (hashish) production. Strains from Colombia, Thailand, South Africa and Mexico, with narrow leaflets, slender habit and later maturation represent from this point of view the taxon *C. sativa* and are traditionally used for marijuana production.

Ornamental cultivar

The ornamental cultivar in the collection, indicated as var. *globosa* cv. Panorama (Fig. 2) is a cross between a globular-shaped dwarf mutant which spontaneously occurred in a Lebanese strain, and the monoecious fibre cultivar Fibrimon (I. Bócsa, pers. commun., 1990).

CLOSING REMARKS

A considerable mutual relatedness seems to exist among the fibre cultivars in the collection. Especially Italian and German strains have directly been the basis of, or have been used as breeding parent for many of these cultivars. Also the ornamental cultivar, the group of central European naturalized populations (var. *spontanea* and ssp. *ruderalis*), and the fibre landraces seem closely related to the modern fibre cultivars. The pure drug strains are probably not so closely interrelated due to geographic isolation. Close relatedness between drug and fibre strains seems unlikely.

Fibre cultivars grouped under the name Kentucky hemp which were cultivated until the mid 1950s in the USA are still lacking in the collection. Hoffmann (1961) mentioned four US cultivars which were hybrids of imported Chinese and Italian strains. It is doubtful whether viable germplasm of these cultivars still exists. Despite current cultivation of hemp in China, only very few Chinese accessions could be acquired.

In its final state, the collection seems sufficiently representative for investigating diversity in the genus *Cannabis*.

Based upon:

Meijer, E.P.M. de & L.C.P. Keizer, 1994. Variation of *Cannabis* for phenological development and stem elongation in relation to stem production. Field Crops Research 00: 00-00 (*in press*).

CHAPTER 3

VARIATION OF *CANNABIS* FOR PHENOLOGICAL DEVELOPMENT AND STEM ELONGATION IN RELATION TO STEM PRODUCTION

ABSTRACT

The variation of *Cannabis* in phenological development and in stem elongation was studied in relation to stem production. In two field evaluations, one including 98 and the other 75 accessions, large variation was found for day of anthesis and day of seed maturity. A higher latitude of origin of accessions was associated with earlier anthesis and seed maturity. The phenological pattern proved to be stable over years.

Stem elongation in the evaluation trial of 98 accessions was characterized by the slope coefficient, inflexion point, and upper asymptote of a sigmoid curve fitted to periodical measurements of stem length. Significant differences among accessions were found for each of these curve parameters. Stem elongation was less stable than the phenological pattern over years. The stem elongation parameters were significantly correlated with the day numbers of anthesis and seed maturity. Early-flowering accessions were shorter than late ones. The influence of phenological development on stem elongation however was only evident for relatively early-flowering accessions. Stem elongation continued generally after the onset of anthesis. The proportion of the stem formed in the generative stage was larger the earlier accessions started to flower. Very late-flowering accessions reached the ultimate length in the vegetative stage.

In the evaluation trial consisting of 75 accessions, phenological development and final stem length were observed in relation to stem dry matter production. Day of anthesis and final stem length were strongly and positively related to stem yield. Some very late-flowering fibre hemp landraces exceeded by far the standard fibre cultivars in stem yield. Late-flowering drug strains were less persistent than late fibre strains in a dense crop situation.

It was concluded that in an efficient crop growth system, seed reproduction and stem production should occur in separate geographic areas, i.e. seed reproduction at lower latitude and stem production at higher latitude. Breeding can contribute to yield potential by improving the persistency of cultivars. There seem also possibilities to select for more efficient stem dry matter accumulation and dry matter partitioning.

Key words: anthesis, maturity, origin latitude, persistency, stem elongation, stem production

INTRODUCTION

Prior to the breeding for improved yield potential, it is a first essential to assess the production, and features related to it, of a wide range of accessions covering the variation within the genus *Cannabis*.

Yield of hemp, being a raw material for textile and cordage, is generally expressed as the amount of bark fibre produced per unit area (Huhnke *et al.*, 1951; Bredemann *et al.*, 1961; Hoffmann, 1961). Seed also is often considered part of the economic yield (Huhnke *et al.*, 1951; Allavena, 1967). In the present research program the suitability of both the woody core and the bark of the stems as potential raw materials for paper pulp was investigated and seeds were not considered a valuable by-product. Therefore the economic yield of a hemp crop was defined as the yield of stem dry matter. A similar approach was followed by Higgins & White (1970) and Muchow (1979) for kenaf (*Hibiscus cannabinus* L.) as a source of paper pulp.

Evaluating a large germplasm collection by means of direct assessment of stem yield is problematic as it requires large experimental plots and, throughout the growing season, a standardized plant density. Lack of sufficient amounts of seed and an uncontrollable degree of self-thinning obstruct the proper realization of such experiments. The presence of indirect measures for stem yield would therefore facilitate large-scale evaluations.

For kenaf, up to 90% of the variance in stem yield could be accounted for by plant density and mean stem length (Muchow, 1979). Stem length of single plants was also considered a reliable selection criterion for straw yield in hemp (Hoffmann, 1961). This implies, at a given plant density, a close relationship between stem length and stem dry matter production.

Phenological events such as seedling emergence, anthesis and seed maturity demarcate developmental stages of the life cycle. Flowering of *Cannabis* is induced by short days (Fleischmann, 1938; Huhnke *et al.*, 1951; Bredemann *et al.*, 1957; Dempsey, 1975) and is genetically controlled (Hoffmann, 1961). The critical photoperiod for induction of flowering increases with latitude of adaptation. Many authors describe changes in phenological development when adapted populations are transferred to other latitudes. Cultivation at a lower latitude results in earlier, and cultivation at higher latitude in delayed anthesis (Fleischmann, 1938; Huhnke *et al.*, 1951; Bredemann *et al.*, 1957; Hoffmann, 1961). The resulting differences in duration of the life cycle probably have an effect on stem production.

The present study is aimed primarily at the assessment of expected yield-related features for *Cannabis*, i.e. stem length and phenological development, and their mutual relationship. Secondly, it seeks to verify the supposed relation between stem production, and both

stem length and phenological development.

MATERIALS AND METHODS

A large part of the CPRO *Cannabis* collection (Chapter 2; Appendix 1) was tested in two experiments. Accessions tested in experiment 1 originated from areas between 28 and 58 degrees northern latitude in Europe and Asia. For experiment 2, accessions were used with origin latitudes between 19 and 58 degrees, including two from the southern hemisphere. The origins of the tested accessions represented the range of latitudes with former or current fibre hemp cultivation (Huhnke *et al.*, 1951).

Experiment 1: the evaluation of phenological development and stem elongation

Exploratory investigations of phenological development and stem elongation had been carried out in 1988 and 1989 using two complementary subsets of accessions. In 1990, 98 accessions, including the previously tested subsets, were evaluated. Sowing date was 10 April (day 100 in the year). Accessions were grown in two replicates using a randomized block design with plots of 4.5 x 0.75 m^2. Each plot contained three rows, 25 cm apart. Shortly after germination, plots were thinned to an average density of about 35 plants per m^2.

Observations per plot were:

* Seedling emergence: day when 50% of the expected number of seedlings were visible.
* Anthesis: day when 50% of the plants, irrespective of sex, had a visible inflorescence.
* Seed maturity: day when the first achenes of 50% of the female and/or hermaphrodite plants were resistant to compressing.
* Stem length of an initial number of 60 labelled plants, measured at 2-week intervals during the entire growing season.

A logistic equation (I) was fitted to the periodical measurements of stem length of 15 successive (neighbouring) male plants that survived until anthesis, and 15 successive female plants that survived until seed maturity.

$$y = C/(1 + e^{(-B(x-M))}) \qquad (I)$$

where:

y stem length at day x (cm)
B slope coefficient (day^{-1})
M curve inflexion point (day)
C upper asymptote of stem length (cm)

Curve parameters B, M and C were estimated using Genstat 5 statistical package (Payne & Lane, 1987).

Experiment 2: the evaluation of phenological development, final stem length and stem dry matter production.

In 1993, 75 accessions, including 10 accessions of the 1990 trial, were evaluated. Sowing date was 14 April (day 104). The same experimental set-up was used as for the 1990 trial, but after emergence, plots were now thinned to a density of exactly 60 plants per m^2 which corresponds better with fibre hemp cultivation in practice. The phenological events seedling emergence, anthesis and seed maturity were determined as previously. Additional observations per plot were:

* Stem length of 30 male and 30 female plants, at the end of the life cycle, i.e. at seed maturity.
* Stem dry matter production of a 1 x 0.75 m^2 subplot at seed maturity.
* Plant density at seed maturity, using the same subplot as used for the estimation of stem production.

RESULTS

Phenological development

The 98 accessions evaluated in 1990 ranged from day 140 to 260 for anthesis and from day 190 to 337 for seed maturity. The 75 tested in 1993 ranged from day 130 to 270 for anthesis and from day 193 to 335 for seed maturity. The days of anthesis and seed maturity of the individual accessions, corrected for the year effect (procedure Chapter 8), are presented in Appendix 2. Despite large differences between the summers of 1990 and 1993, the phenological patterns were stable over years (Table 1).

The influence of latitude of adaptation on the phenological development is demonstrated in Fig. 1 for 67 accessions of known geographical origin tested in 1990. Emergence was comparable for all accessions. Anthesis and seed maturity were negatively related to the latitude of origin. According to the fitted regression line, accessions adapted to the Netherlands (52° latitude) started flowering at day 175. Accessions introduced from

Table 1. Coefficients of correlation between years for accession mean days of anthesis and seed maturity.

	30 accessions 1988-1990	39 accessions 1989-1990	9 accessions 1990-1993
Anthesis	0.85**	0.93**	0.87**
Seed maturity	0.74**	0.92**	0.93**

** significant at p = 0.01

higher latitudes flowered earlier and those from lower latitudes later. As the two latest accessions failed to develop viable seeds due to frost, approximated days of seed maturity are given for these in Fig. 1. Accessions deviated from the expectations based on latitude of origin somewhat more for anthesis than for seed maturity. The coefficients of correlation for the two traits with latitude of origin were -0.73 and -0.83, respectively ($N = 67$; $p = 0.01$). The relation between adaptation latitude and phenological development was similar in the 1993 trial where the coefficient of correlation was -0.75 for both anthesis and seed maturity (based on 42 accessions; $p = 0.01$).

Stem elongation

The symmetric sigmoid growth curve satisfactorily described stem elongation of *Cannabis* plants. The percentage of variance accounted for generally ranged between 95 and 100%, irrespective of accession. Considerable variation was found for individual plant estimates of B, M and C within plots. Stem elongation parameters in 1990, and final stem length in 1993, were therefore expressed on a plot basis using mean values for male and female plants separately. The accession means for the elongation parameters of male and female plants were highly correlated ($r \geq 0.85$ for B, M and C in 1990, $N = 93$, $p = 0.01$; $r = 0.86$ for final stem length in 1993, $N = 74$,

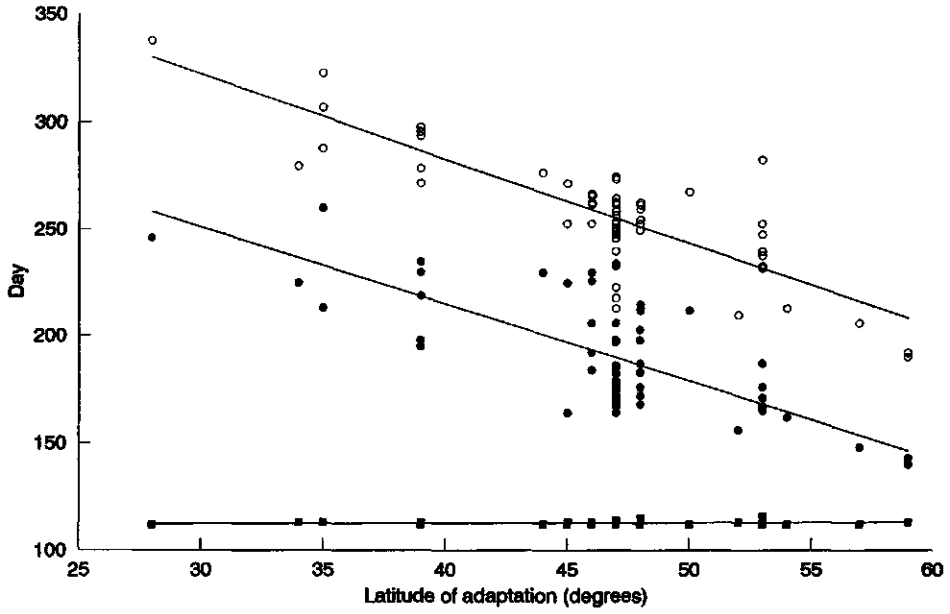


Fig. 1. Seedling emergence (squares), anthesis (solid circles) and seed maturity (open circles) in 1990 in relation to the latitude of adaptation of accessions.

Table 2. Anova tables based on plot means for the stem elongation parameters B, M and C of 94 accessions tested in 1990 and for final stem length of 73 accessions tested in 1993.

Source of variation	1990				1993	
		Mean squares				Mean squares
	d.f.	B [a]	M [a]	C [a]	d.f.	Final length
Block	1	2.3	58.6	6090	1	33436
Accessions	93	2.3***	44.1***	2798***	72	2590***
Residual	93	0.4	12.3	523	70	445
Total	187				143	

*** significant at p=0.001; [a] see text for explanation

p=0.01). On average, male plants exceeded females in final stem length (C) by about 40 cm in 1990 and 20 cm in 1993. Since the tested collection comprised a number of monoecious cultivars in which male plants were rare, the results and discussions below are restricted to female and/or hermaphrodite plants. The individual accession means for the final length of female plants, corrected for the year effect, are presented in Appendix 2.

The analyses of variance presented in Table 2 are for the 1990 trial based on 94 accessions; two accessions with exceptionally large residuals were excluded, as well as two uniform and very short-stemmed accessions. For the 1993 trial, Table 2 is based on 73 accessions; one uniform short-stemmed accession was excluded and one accession did not complete its life cycle due to a total premature loss

Table 3. Coefficients of correlation between years for accession mean stem elongation parameters.

Stem elongation parameters [a]	30 accessions 1988-1990	39 accessions 1989-1990	9 accessions 1990-1993
B	0.47**	0.17	-
M	0.26	0.72**	-
C	0.39*	0.87**	0.77* [b]

*, ** significant at p=0.05 and p=0.01, respectively; [a] see text for explanation; [b] based on the correlation between the calculated parameter C in 1990 and the measured final length in 1993

Table 4. Spearman rank correlations between accession means for stem elongation parameters and the days of anthesis and seed maturity, respectively.

Stem elongation parameters [a]	Anthesis	Seed maturity
B	-0.77**	-0.65**
M	0.71**	0.64**
C	0.54**	0.41**

N = 98; ** significant at $p = 0.01$; [a] see text for explanation

of plants. 'Accessions' was significant ($p < 0.001$) for each of the parameters and final length. Accession means in 1990 ranged from 0.056 to 0.1253 (day^{-1}) for B ($LSD_{0.05} = 0.012$), from day 144 to 188 for M ($LSD_{0.05} = 7$), and from 63 to 360 cm for C ($LSD_{0.05} = 46$). In 1993 the accession means varied for final stem length from 98 to 364 cm ($LSD_{0.05} = 42$).

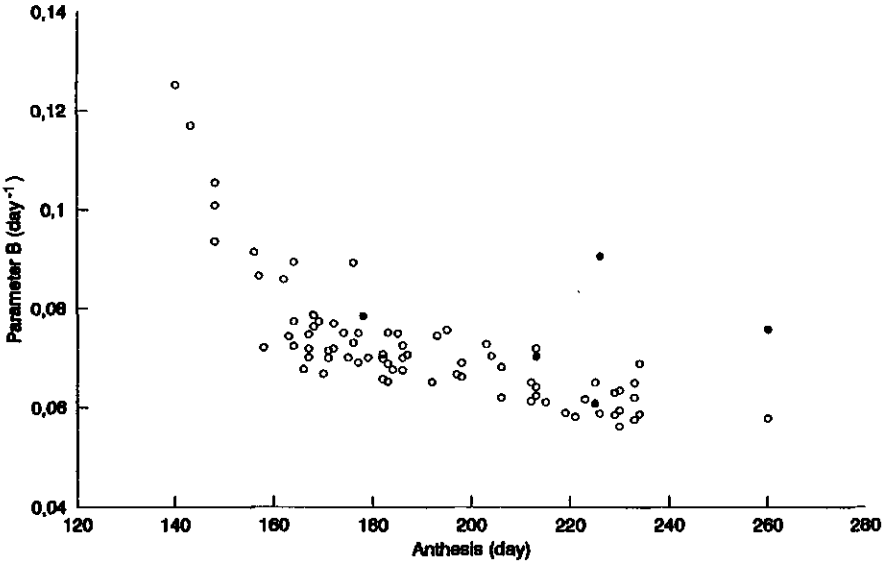
The year means of the accessions were less well mutually correlated for stem elongation parameters than for the days of anthesis and seed maturity (Tables 1 and 3).

The influence of phenological development on stem elongation

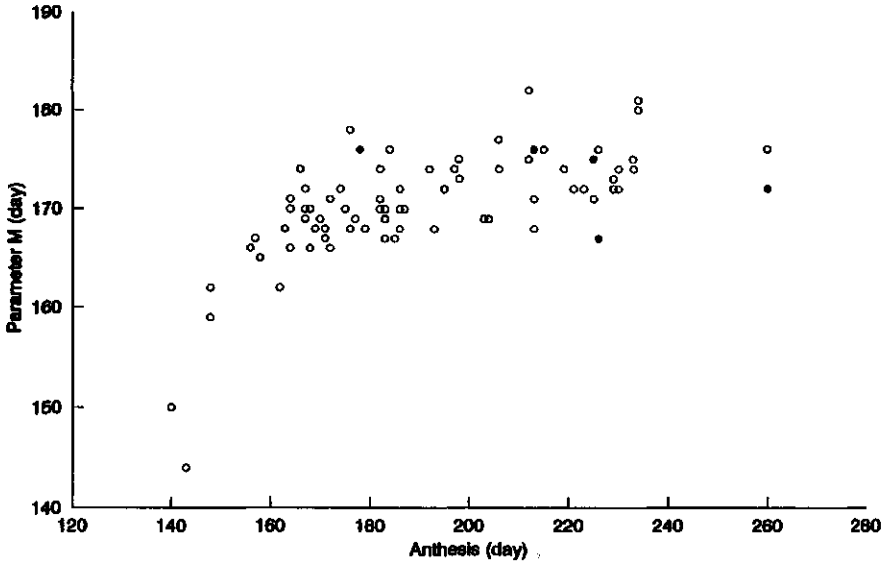
Table 4 shows high correlations between stem elongation parameters and phenological characters. Days of anthesis and seed maturity were more closely related to B and M than to C. As a larger part of the variation in elongation parameters was accounted for by anthesis than by seed maturity, the former was further used as a phenological index to investigate the relation between phenological development, and stem elongation and production.

In Fig. 2 the stem elongation parameters of 82 accessions tested in 1990 are plotted against day of anthesis. The crop type of each accession, i.e. fibre or drug, is indicated to test the assumption that variation in stem elongation parameters among accessions with similar phenological development is associated with the purpose of domestication. Sixteen accessions, either undomesticated or of unknown crop type were excluded. Slope parameter B decreased, and the curve inflexion point M increased with later anthesis. The wide range for anthesis resulted, apart from the two first flowering accessions, in relatively small differences in final stem length (C). A similar small effect of day of anthesis on final length was found in 1993, again with the exception of very early-flowering accessions. So in general, the relation between phenological development and stem elongation was strong for early-flowering accessions (anthesis $\leq$ day 175), and became less pronounced for later-flowering ones. Fig. 2 indicates that fibre strains generally had lower B

(a)


0,14
0,12
0,1
0,08
0,06
0,04
Parameter B (day-1)
120
140
160
180
200
220
240
260
280
Anthesis (day)

(b)


190
180
170
160
150
140
Parameter M (day)
120
140
160
180
200
220
240
260
280
Anthesis (day)

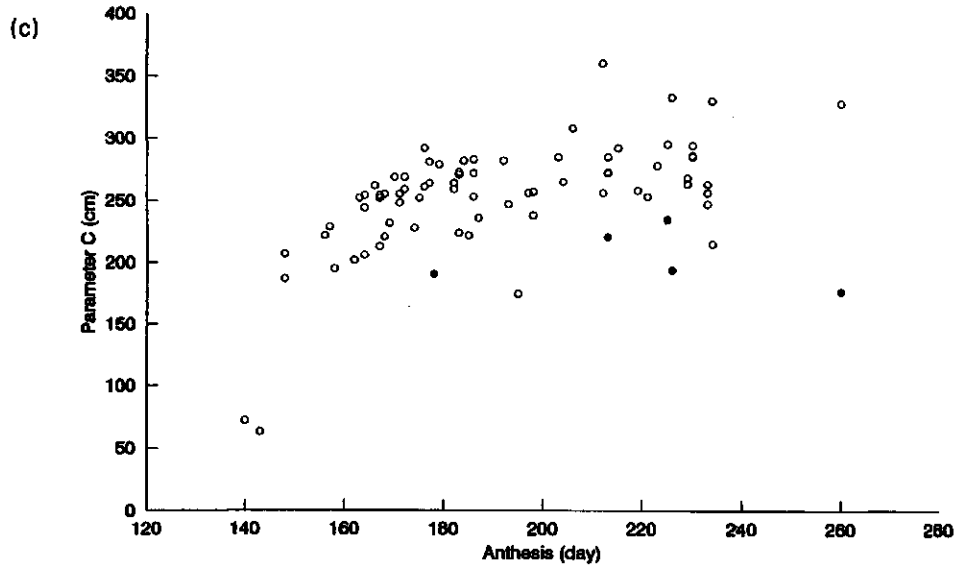


Fig. 2. Mean values of stem elongation parameters B (a), M (b) and C (c), respectively, for 82 accessions plotted against day of anthesis. The performance of drug strains is indicated by solid symbols.

values (i.e. faster growth) and mostly a higher final stem length (C), than drug strains with similar phenological development.

Fig. 3 shows stem elongation curves for five hypothetical fibre strains differing in anthesis. The curves were obtained by substituting in equation I, average B, M and C values for various times of anthesis. Below the inflexion point the curves are almost overlapping. Curves of strains with relatively early anthesis show that small differences in anthesis have a large effect on the upper asymptote. The opposite is true for curves of relatively late-flowering strains.

Fig. 3 illustrates that the day of anthesis is not associated with a fixed position on the stem elongation curve. The degree of stem elongation before and after the day of anthesis was investigated for the accessions tested in 1990. Length at anthesis was calculated for each accession by substituting the observed day of anthesis as well as the estimated elongation parameters in equation I. Length at seed maturity was calculated likewise. Relative stem lengths at anthesis and at seed maturity were for each accession expressed as a proportion of the estimated final length (C). The relative length at anthesis ranged among accessions from 20 to 100% of the final length and clearly depended on the day of anthesis (Fig. 4). A greater part of the stem length was

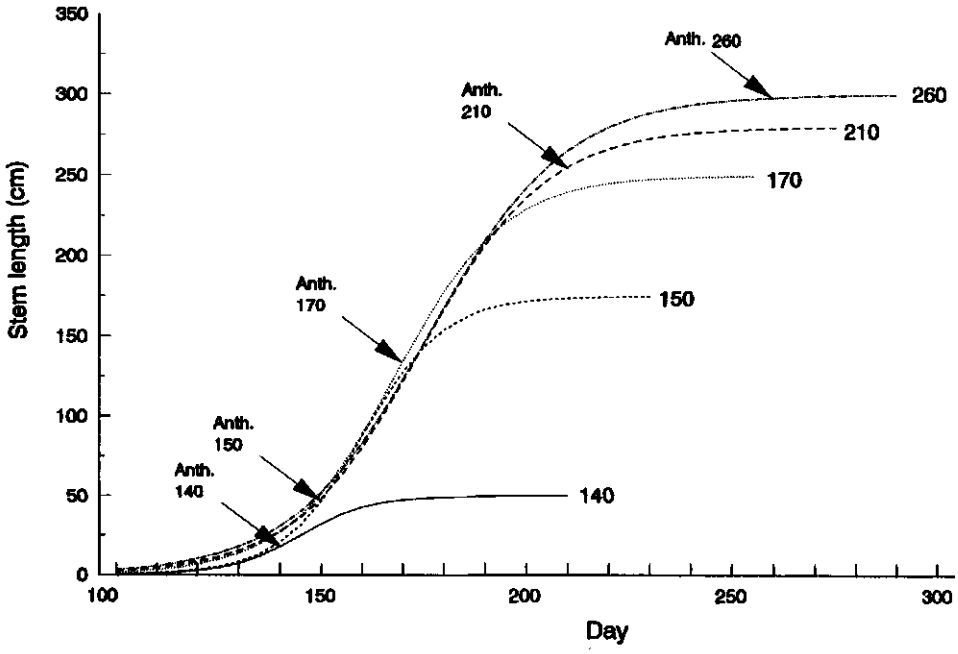


Fig. 3. Stem elongation curves for hypothetical fibre strains differing in day of anthesis.

formed after anthesis the earlier accessions started to flower. Elongation stopped at seed maturity.

Phenological development and stem length in relation to stem production.

Significant differences ($p<0.001$) between accessions were found for the phenological traits and stem length and stem production in the 1993 trial (Table 5). Also, the remaining plant density at seed maturity differed significantly among accessions (Table 5). Not surprisingly, it tended to decrease with later day of seed maturity. At similar day of seed maturity the strains of the drug type tended to have lost more plants than had the fibre strains (Fig.5).

Figs. 6a,b show the relations between stem production, and day of anthesis and final stem length, respectively. Effects of plant losses were minimized by taking into account only accessions with comparable plant density at seed maturity (i.e. 33 accessions with final densities between 40 and 50 plants/m^2). Accession means for the day of anthesis and stem production were rather strongly related ($r_{spearman}=0.81$, $p=0.01$), and so were accession means for stem length and stem production ($r_{spearman}=0.73$, $p=0.01$). The relation between day of anthesis and final stem length was somewhat weaker ($r_{spearman}=0.65$, $p=0.01$), and in accordance with the results

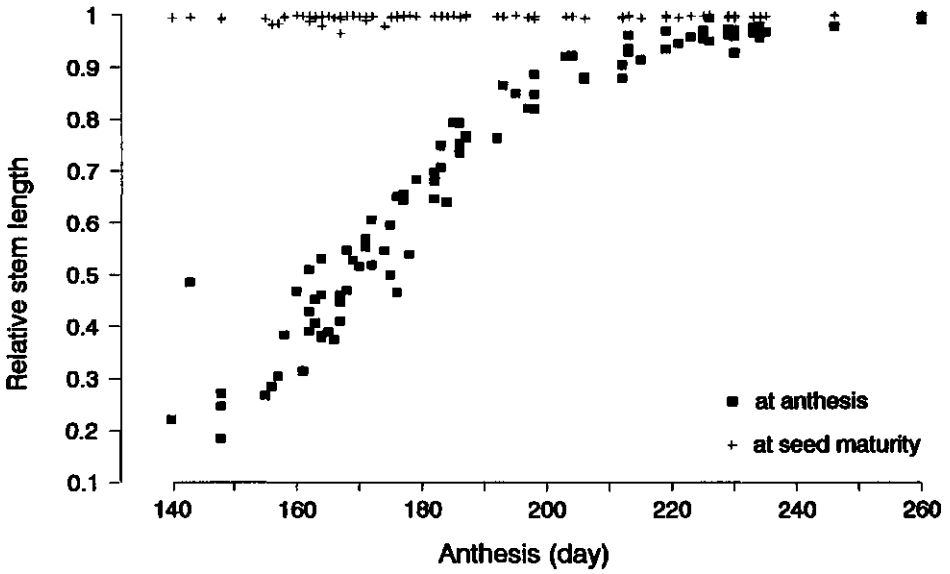


Fig. 4. Accession means for relative stem length at anthesis and at seed maturity, plotted against the day of anthesis.

Table 5. $LSD_{0.05}$ and range for the accession means of various traits in the 1993 evaluation trial. The table is based on 74 accessions.

Traits	$LSD_{0.05}$	Range
Seedling emergence (day no.) [a]	0.75	112 - 115
Anthesis (day no.) [a]	18.5	130 - 270
Seed maturity (day no.) [a]	19.8	193 - 335
Stem length ♂ (cm)	45	102 - 410
Stem length ♀ (cm)	42	98 - 364
Plant density at seed maturity (pl/m^2) [b]	18	10 - 60
Stem dry matter production at seed maturity (g/m^2)	572	119 -2443

[a] sowing took place at day no. 104; [b] the plant density was shortly after emergence thinned to 60 plants per m^2

of the 1990 trial, it was only evident for the relatively early-flowering accessions.

DISCUSSION

Phenological differences among accessions from the same origin (Fig. 1) may partly be due to breeding, e.g., those among the accessions from 48° latitude. These accessions are French cultivars that differed in anthesis and in seed maturity up to 47 and 12 days, respectively. The early cultivars were selected for both seed and fibre production whereas the late ones were solely bred for fibre production (J.P. Mathieu, pers. commun., 1992).

The stability over years of the phenological development of accessions (Table 1) indicates a high heritability. However, phenological patterns of introduced accessions cannot be expected to remain stable after (domestic) reproduction in the field, since *Cannabis* populations adapt rapidly to changed conditions (von Lucke, 1925; Tschaneff, 1959; Dempsey, 1975). Therefore, maintenance of a certain phenological pattern of *Cannabis* populations requires outdoor seed reproduction at the appropriate latitude to avoid rapid genetic shift.

The higher stability over years for phenological characters than for stem elongation parameters (Tables 1 and 3) is due to the fact that anthesis of an accession at a given

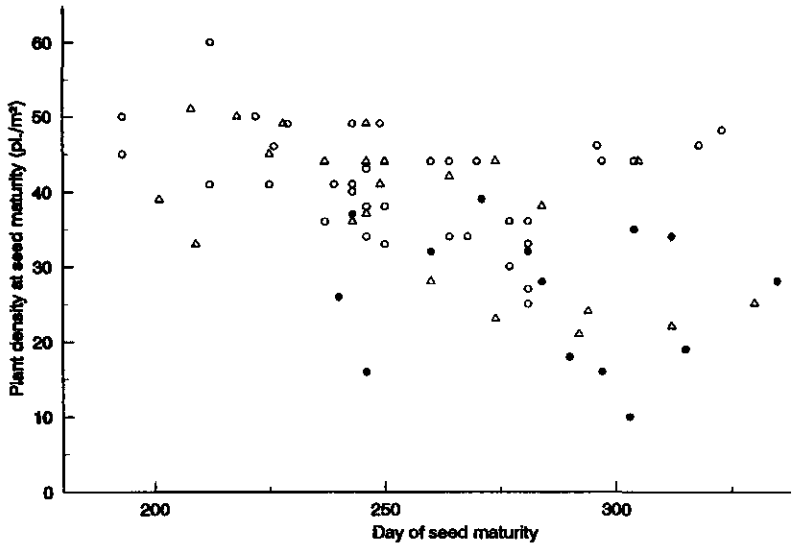


Fig. 5. The relation between plant density at seed maturity and the day of seed maturity, for 74 accessions tested in 1993. The performance of drug strains is indicated by solid circles, that of fibre strains by open circles and the triangles represent either undomesticated accessions or accessions of unknown crop type.

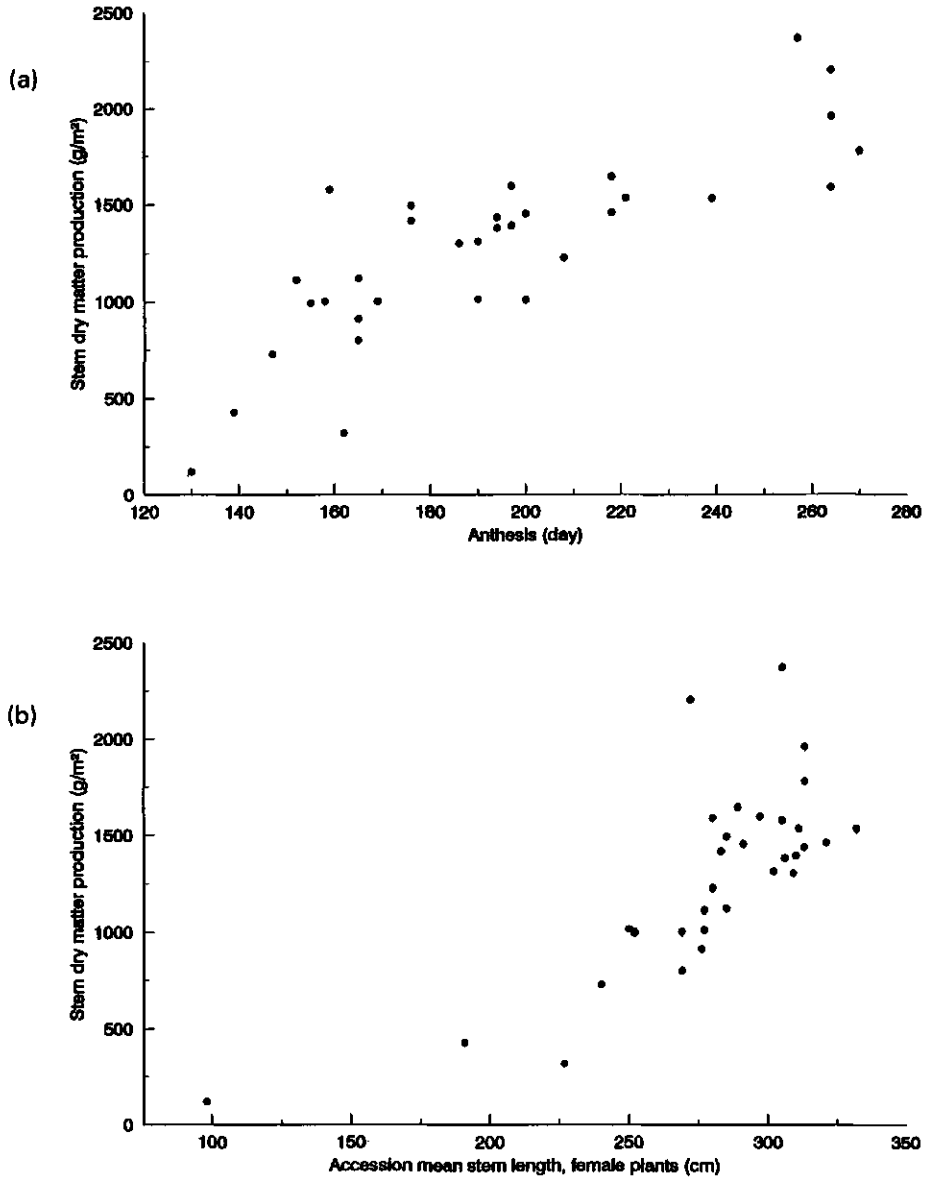


Fig. 6. The relations between the accession means for day of anthesis and stem dry matter production (a), and for stem length of female plants and stem dry matter production (b). The performance of 33 accessions which had a similar plant density at seed maturity is represented.

site is primarily day length dependent, while stem elongation is affected by more variable factors such as temperature, radiation and supply of water and nutrients.

The relation between phenological development and stem elongation was strong for early-flowering accessions (anthesis ≤ day 175), and less pronounced for later-flowering ones (Fig. 2). As day 175 is approximately the time of anthesis of accessions adapted to the latitude of the Netherlands (Fig. 1), more variation with regard to stem elongation is introduced by using adapted accessions from higher than from lower latitude.

Stem elongation of late-flowering accessions occurred almost entirely in the vegetative stage (Fig. 4). This implies that little dry matter is invested in reproductive organs before maximum stem length is reached. A more favourable dry matter distribution, besides a longer growing season, seems therefore an advantage of late-flowering accessions.

Among accessions with similar life cycle there were significant differences in plant density at seed maturity (Fig. 5 and Table 5). Persistency as such may be considered as a yield as well as a stem quality-related factor, that should not be ignored in hemp breeding. Poor persistency seems to be a disadvantage of the use of drug strains in the breeding for increased yield potential.

Day of anthesis and final stem length are positively related to the yield potential of accessions (Figs. 6a,b). Yield improvement by breeding for late anthesis seems however a redundant effort since lateness can more easily be obtained and preserved by organizing seed reproduction at low latitude for the purpose of stem production at higher latitude. With regard to stem yield, breeding should focus on the factors that determine persistency, like plant vigour, susceptibility to diseases, and the genetic homogeneity of populations.

The stem dry matter yields of the 1993 experiment, as presented in Fig. 6, should be regarded with some reservations due to the lack of sufficient buffer rows. It is likely that the productivity of late-flowering accessions was somewhat overestimated as plots with these accessions were frequently neighboured by empty plots late in the season. However, the yield levels of three accessions, i.e. the Hungarian cultivars Kompolti Sargászáru and Kompolti Hybrid TC, and the Japanese landrace Kozuhara zairai, recently tested under semi-practical conditions in the Netherlands, appeared quite acceptable in the 1993 trial. The respective yields were 928, 1403 and 1592 g/m^2 of stem dry matter, which corresponded well with their performances in growth analysis trials (van der Werf, 1994).

Three Korean landraces (accessions 901161, 901162 and 901163) produced 1964, 2375 and 2209 g of stem dry matter per m^2, respectively. Two of them outyielded significantly ($LSD_{0.05}$ = 572 g/m^2, Table 5) the equally late-flowering and long-stemmed landrace Kozuhara zairai which was so far the most productive population tested in the Netherlands. These differences in the efficiency of utilizing the life cycle for stem dry matter accumulation and in the amount of dry matter partitioned per unit of stem length, indicate an additional opportunity for breeding.

CONCLUSIONS

Phenological development of *Cannabis* accessions is primarily determined by their origin, i.e. latitude of adaptation. A low latitude of adaptation is associated with late anthesis and seed maturity under Dutch growing conditions. The time of anthesis is not associated with a fixed position on the stem elongation curve. Stem elongation proceeds less during the generative stage the later accessions start to flower. When economic yield is defined as stem yield, late-flowering strains are therefore favourable since they invest little dry matter in reproductive organs.

Day of anthesis and final stem length are useful indicators for stem yield potential.

Seed reproduction at low latitude for the purpose of stem production at high latitude is the most effective way of increasing yield. Breeding can contribute to yield potential by improving the persistency of cultivars. Also there seem possibilities to select for a more efficient stem dry matter accumulation and dry matter partitioning.

Based upon:

Meijer, E.P.M. de & H.M.G. van der Werf, 1994. Evaluation of current methods to estimate pulp yield of hemp. Industrial Crops and Products 2: 111-120.

CHAPTER 4

EVALUATION OF CURRENT METHODS TO ESTIMATE PULP YIELD OF HEMP

ABSTRACT

Large-scale evaluation of hemp stems from field trials requires a rapid method for the characterization of stem quality. The large differences between bark and woody core in anatomical and chemical properties, make a quantification of these two fractions of primary importance for quality assessment. This chapter evaluates accuracy and power of discernment of current procedures for the analysis of the composition of hemp stems.

To reduce the amount of plant material to be analyzed it was investigated if the contents of bark, bark fibre and woody core in the stem could be estimated in a representative stem segment (according to Arnoux *et al.*, 1969). Analysis of segments, however gave less accurate information on stem composition than analysis of entire stems.

The recovery of the bark and woody core in the pulping process is referred to as the pulp yield of the two fractions. Two procedures for the estimation of potential pulp yield, one common in fibre hemp breeding (Bredemann method) the other in paper pulp technology (TAPPI method 212), were compared. The Bredemann method determines the content of the economically important bark fibres, and approximates the content of woody core. The TAPPI method determines, separately, the solubility of ground bark and woody core as a measure for yield of these stem fractions in the pulping process. The two methods show similarity since a boiling NaOH solution is used for fibre extraction in the first method as well as for dissolving ground components in the latter. Results of the simple and less laborious Bredemann method were compared with those of the TAPPI method, which are readily interpretable in pulp technology, and also with an intermediate method. The latter determines the initial content of woody core and subjects the separated bark tissue to the Bredemann fibre extraction procedure. The initial content of woody core of the stem (intermediate method) correlated well with the content of insoluble woody core (TAPPI). The measurements of bark fibre content (Bredemann) were quite similar to those of the content of insoluble bark components (TAPPI).

Key words: bark, fibre content, methods, pulp yield, stem quality, woody core

INTRODUCTION

Bark and woody core of hemp stems possess distinct anatomical and chemical properties (see Chapter 5, Table 1). The fact that fibres of the woody core are very short has a negative effect on paper strength (Wood, 1981). Bark fibres are much longer, making them a suitable raw material for a range of high quality paper grades. Due to its low *α*-cellulose content the potential utilization of the woody core seems restricted to mechanical pulps, whereas the bark of hemp is already used in chemical pulps for specialty papers (Bosia, 1975; Triolo, 1980). Only limited variation in chemical properties of bark and woody core among hemp cultivars was reported by Bedetti *et al.* (1979) and Triolo (1980). Therefore the large mutual differences between bark and woody core make a quantification of the two mass fractions of primary importance for characterizing stem quality. The recovery of each fraction in the pulping process is referred to as pulp yield.

A classical method for estimating the bark fibre content of stems, still used in hemp breeding, was developed by Bredemann (1922). Bark fibres are extracted after boiling dry stems in a 2% NaOH solution, non-fibrous bark elements are removed by rinsing. The woody core is partially degraded during this treatment. The initial content of woody core is approximated by multiplying the fraction of partially degraded wood with a correction factor for woody core losses. Entire stems are used since the proportion of bark fibre and woody core changes with height in the stem.

For breeding purposes Arnoux *et al.* (1969) suggested a modification of the Bredemann method. They proposed using a representative stem segment to reduce the amount of stem material to be handled; the segment between 30 and 40% of the total length above soil was shown to meet this requirement. Bark and wood tissues of the segment are separated mechanically and subsequently the fibre content of the bark is determined according to Bredemann (1922). A combined method based on Bredemann (1922) and Arnoux *et al.* (1969) is used in the present Dutch breeding research, it comprises boiling of the mentioned segment in a 2% NaOH solution, extraction of bark fibres, and determination of the contents of bark fibres and partially degraded woody core in the segment. This method does not require a lot of time and is easy to apply as it requires only a small amount of stem material and no grinding.

Raw materials for pulp and paper production are generally evaluated using standard procedures of the Technical Association of the Pulp and Paper Industry (TAPPI). Procedure 212 om-88 (Anonymous, 1991b) determines 'one percent sodium hydroxide solubility of wood and pulp', being an estimate of pulp yield of source material. The procedure requires grinding of air dried stem samples or separated bark and

Table 1. Treatments of the field trial.

Treatment code	Accession	Plant density (plants/m^2)	
		17 May	16 Sept
CP90	CPRO-accession no. 883213	90	83
TC10	Kompolti Hybrid TC	10	10
TC30	Kompolti Hybrid TC	30	30
TC90	Kompolti Hybrid TC	90	81
TC270	Kompolti Hybrid TC	270	158
HE90	Kompolti Hyper Elite	90	86

wood followed by extraction of solubles in a boiling solution of NaOH. The residue, expressed as proportion of the dry weight of the original sample, is a measure of pulp yield. This procedure is successfully applied to annual crops like hemp and kenaf (e.g., Nieschlag *et al.*, 1960). The TAPPI procedure is more laborious but gives more relevant data, i.e. standardized parameters for the pulp industry. This method is mainly used in agronomic and technological research when low numbers of samples are to be analyzed.

The TAPPI method was considered as a reference method. The objective of this study was to compare the respective methods of Bredemann/Arnoux and TAPPI for determination of stem fractions and potential pulp yield. In addition to these methods, a third protocol was followed to test some possible improvements of the Bredemann/Arnoux method.

MATERIAL AND METHODS

Plant material

Stems were obtained from a plant density trial with two Hungarian fibre hemp cultivars ('Kompolti Hybrid TC' and 'Kompolti Hyper Elite') and one Japanese landrace (CPRO-accession no. 883213). The experiment was carried out on a clay soil near Wageningen, and had a completely randomized block design with four replications. Plot size was 6 x 14 m^2, date of sowing was 19 April 1991. As a result of self-thinning the highest plant densities decreased during the growing season. Treatments of the field trial are summarized in Table 1. Stems were collected from the field trial on 16 September for two experiments:

* A study of the intra-stem variation for contents of bark tissue, bark fibre and woody core, using 18 separate stems of each of the treatments CP90, TC90, and HE90, from one block.

* A comparison of three methods for the estimation of pulp yield, using random samples of stems or segments of each plot of all treatments.

Intra-stem variation in contents of bark tissue, bark fibre and woody core

Intra-stem variation was investigated to test the conclusion of Arnoux *et al.* (1969), that the composition of the stem segment between 30 and 40% of the stem height resembles that of the entire stem. Each of 54 stems was divided into ten segments, each representing 10% of the entire stem length.
The segments were numbered from 1 (base) to 10 (top). Segments were dried at 105 °C for 12 h, weighed, and boiled in a solution of 2% NaOH for 10 min. The bark was manually separated from the woody core. The still intact woody core was rinsed with water, dried at 105 °C for 12 h and weighed. Bark fibres were released by further boiling of bark tissue in 2% NaOH for one h, followed by rinsing, drying at 105 °C for 12 h and weighing. The contents of bark fibre and woody core of each segment were expressed as percentages of segment dry weight. The contents of the entire stem, i.e. weighed means, were calculated from the corresponding segment data.

Comparison of methods

Stems or segments were analyzed according to the following methods:

Method 1: According to the combined procedures of Bredemann (1922) and Arnoux *et al.* (1969) a sample of ten segments taken between 30 and 40% of the total stem length was boiled for 2 h in a 2% NaOH solution. After rinsing, drying and weighing of extracted bark fibres and remaining wood, the following pulp yield parameters were calculated:

* By1 (bark yield 1), mass fraction of bark fibre in segment dry matter (%).
* Wy1 (wood yield 1), mass fraction of partially degraded wood in segment dry matter (%).

Method 2: A flax breaker consisting of seven pairs of fluted cylinders was used to separate a sample of twenty air dry stems into bark and wood. Stems were processed three to five times through the machine, to break the wood into fragments of less than 2 cm. Shaking of tangled bark mass removed most of the wood. Remaining wood was removed by hand. The bark was boiled for 2 h in a 2% NaOH solution. Rinsing, drying and weighing of extracted bark fibres and drying and weighing of the wood fraction allowed calculation of the following parameters:

* By2 (bark yield 2), mass fraction of bark fibre in stem dry matter (%).

* Bl2 (bark loss 2), mass fraction of non-fibrous bark components in stem dry matter (%).
* Wy2 (wood yield 2), mass fraction of untreated wood in stem dry matter (%).

Method 3: A sample of twenty stems was separated in bark and wood as described for method 2. Each fraction was ground in a Wiley type mill equipped with a 0.5-mm round hole screen. Conform TAPPI procedure 212 om-88, solubility of the ground samples in 1% NaOH was determined. The following parameters were calculated:

* By3 (bark yield 3), mass fraction of insoluble bark components in stem dry matter (%).
* Bl3 (bark loss 3), mass fraction of soluble bark components in stem dry matter (%).
* Wy3 (wood yield 3), mass fraction of insoluble wood components in stem dry matter (%).
* Wl3 (wood loss 3), mass fraction of soluble wood components in stem dry matter (%).

RESULTS

Intra-stem variation in contents of bark tissue, bark fibre and woody core

The histograms of Figs. 1 and 2 show the weighed means of the segment contents of bark fibre and woody core, calculated on the basis of 18 stems per accession. The distribution of the mass fraction of bark fibre in the stem appears to be specific for accessions. The two Hungarian cultivars had the highest bark fibre contents in the segments 2, 3 and 4, and showed a decrease towards the top. In the landrace 883213 the proportion of bark fibre did not clearly change with the height in the stem. None of the segment contents of this accession deviated strongly from the stem average. The mean content of segments no. 5 was about equal to the mean content of entire stems for each accession.

The pattern of distribution of the woody core fraction in the stem was similar for the three accessions. Consequently also the bark tissue, being the complementary stem fraction, had a similar distribution for the three accessions. The woody core contents in the lower 5 segments were equal to or exceeded the average content. There was a gradual decrease of woody core content from the sixth segment towards the top. The content of segments no. 3 resembled best the mean content of entire stems. This applied for woody core as well as for its complement, i.e., bark tissue.

Usually, the contents of bark fibre and woody core in the lower segments (nos. 2 to 6) of individual plants correlated well with the contents of the corresponding entire stems (Table 2). In 'Kompolti Hyper Elite', however, the correlations for bark fibre content were relatively weak. This seems mainly due to one plant with a deviating relation between stem and segment content (Fig. 3).

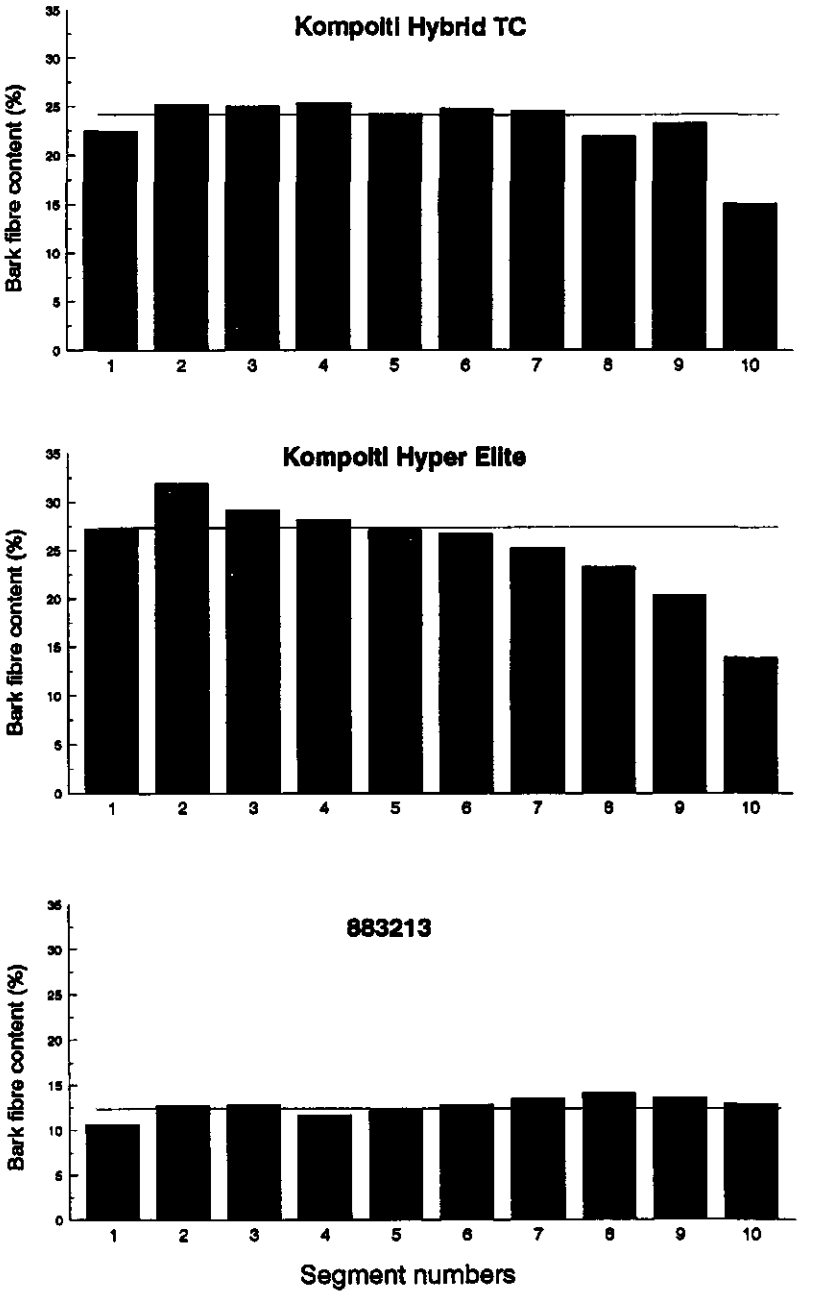


Fig. 1. The distribution of bark fibre in the stem for the three accessions. The histogram bars indicate the weighed mean content of bark fibre in segments 1 (base) to 10 (top) calculated on the basis of 18 stems per accession. The horizontal line indicates the weighed mean content of the 18 entire stems.

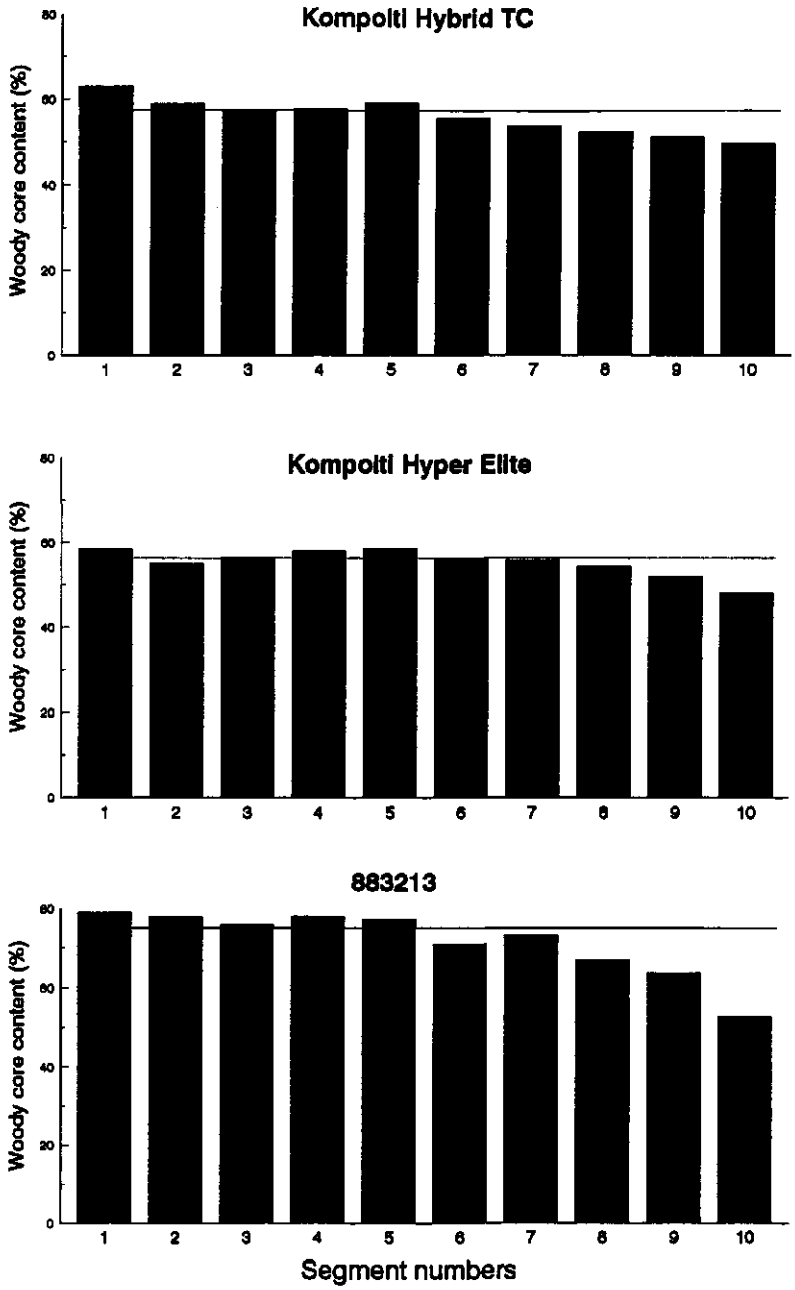


Fig. 2. The distribution of the woody core in the stem for the three accessions. The histogram bars indicate the weighed mean content of woody core in segments 1 (base) to 10 (top) calculated on the basis of 18 stems per accession. The horizontal line indicates the weighed mean content of the 18 entire stems.

Table 2. Coefficients of correlation between the entire stem and various stem segments for the contents of bark fibre and woody core, calculated for 18 plants per accession. Segments are numbered 1 to 10 from base to top.

Segment no.	Kompolti Hybrid TC		Kompolti Hyper Elite		883213	
	Bark fibre	Woody core	Bark fibre	Woody core	Bark fibre	Woody core
1	.96**	.87**	.80**	.76**	.80**	.77**
2	.98**	.91**	.75**	.93**	.91**	.88**
3	.98**	.96**	.72**	.93**	.97**	.98**
4	.97**	.91**	.74**	.90**	.96**	.98**
5	.96**	.88**	.48*	.87**	.92**	.96**
6	.97**	.74**	.64**	.87**	.91**	.77**
7	.93**	.82**	.53**	.76**	.92**	.81**
8	.88**	.78**	.35	.79**	.59**	.86**
9	.47*	.24	.36	.76**	.72**	.94**
10	.85**	.63**	.18	.54*	.48*	.79**

*,** significant at $p = 0.05$ and $p = 0.01$, respectively

In Fig. 3 the bark fibre content of entire stems is plotted against that of corresponding segments no. 4. A *t*-test revealed a significant difference between the slopes of the linear regression lines of the Kompolti cultivars. The regression coefficients of accession 883213 did not differ significantly from those of the two Kompolti cultivars. Intercept and slope of the regression lines differed significantly from 0 and 1, respectively, for 'Kompolti Hybrid TC' and 883213, but not for 'Kompolti Hyper Elite'. This seems again mainly the effect of one plant with a deviating relation between stem and segment content within 'Kompolti Hyper Elite'. For bark fibre, the content of the segment deviated from the content of the entire stem, in an accession-dependent way.

Fig. 4 presents in a similar way the relation between woody core content of entire stems and that of corresponding segments no. 4. *t*-Tests revealed no significant differences between the regression coefficients of accessions, but intercept and slope of each of the lines differed significantly from 0 and 1, respectively. At stem core contents below 60%, the content of the segment was inferior to that of the entire stem, whereas at stem core contents above 60%, the content of the segment exceeded that of the entire stem. So the woody core content of segment 4 deviated systematically from the true stem values, but not in an accession-dependent way.

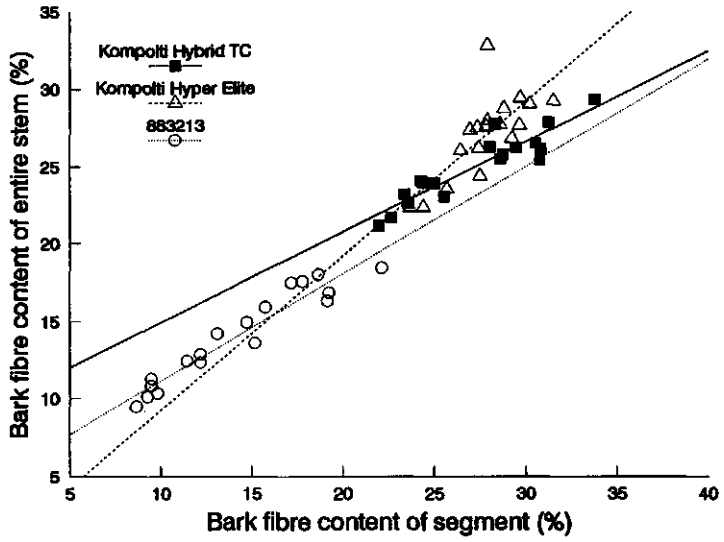


Fig. 3. Relation between bark fibre contents of entire stems and the corresponding stem segments between 30 and 40% stem height. Data of 18 stems per accession.

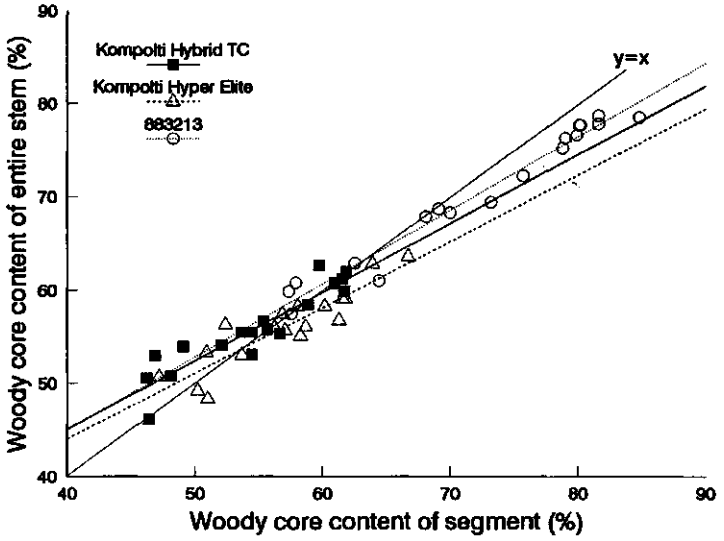


Fig. 4. Relation between woody core contents of entire stems and the corresponding stem segments between 30 and 40% stem height. Data of 18 stems per accession.

Table 3. Coefficients of correlation (r) between parameters of the three methods. Correlations were calculated on the basis of 23 individual plots, one replication was missing for treatment TC30.

	Parameters	r
Bark yield	By1-By3	.94**
	By2-By3	.99**
Wood yield	Wy1-Wy3	.78**
	Wy2-Wy3	.98**
Bark losses	Bl2-Bl3	.75**

** significant at p=0.01

Comparison of methods

Estimates for similar parameters obtained by methods 1 and 2 should be, if not equal to, at least highly correlated with the parameters of reference method 3 (TAPPI). This was the case for bark yield and wood yield obtained by method 2 (Table 3). Estimated bark losses by method 2 correlated less with those of the reference. Correlations between parameters of method 1 and method 3 were lower than correlations between parameters of methods 2 and 3.

The efficiency of the methods to distinguish treatment differences in the field trial for bark yield (By1, By2,By3), wood yield (Wy1,Wy2, Wy3), bark losses (Bl2,Bl3) and for wood losses (Wl3), was evaluated by analysis of variance. Results are presented in Table 4. Standard

Table 4. Treatment means for various measures of pulp yield and pulp loss. Per column, means showing a common letter are not different at p=0.05. Treatment codes are explained in Table 2.

Treatment code	Parameters								
	By1	By2	By3	Wy1	Wy2	Wy3	Bl2	Bl3	Wl3
CP90	17.08 a	13.43 a	13.65 a	68.72 a	78.97 a	55.80 a	7.60 a	7.38 a	23.18 a
TC10	26.55 b	24.15 b	24.02 b	62.25 b	66.75 b	45.05 bc	9.10 b	9.23 b	21.70 b
TC30	26.74 b	25.19 bc	25.06 bc	59.73 bc	65.54 bc	45.89 b	9.27 b	9.40 b	19.65 c
TC90	26.23 b	26.48 c	25.37 c	57.45 c	64.15 d	44.42 bc	9.37 b	10.48 c	19.73 c
TC270	25.90 b	24.77 b	24.73 bc	58.02 c	64.63 cd	43.92 c	10.60 c	10.65 c	20.70 bc
HE90	28.52 c	30.08 d	28.33 d	58.22 c	59.87 e	40.55 d	10.05 bc	11.80 d	19.33 c
Means	25.19	24.01	23.50	60.71	66.68	45.94	9.32	9.83	20.73
SED	0.85	0.74	0.66	1.79	0.62	0.75	0.55	0.45	0.65

Table 5. Split-plot anova tables for methods to determine bark yield (By1,By2,By3) and woody core yield (Wy1,Wy2,Wy3), respectively.

Source of variation		Bark yield		Wood yield	
	d.f.	M.s.	F prob.	M.s.	F prob.
Block.plot stratum					
Treatment	5	287.0	<0.001	328.0	<0.001
Residual	14(1)	1.4		2.4	
Block.Plot.*Units* stratum					
Method	2	18.1	<0.001	2733.5	<0.001
Method x Treatment	10	3.6	0.002	9.8	0.002
Residual	34(2)	1.0		2.6	
Total	68(3)				

errors of differences of means (SED) for By1 and Wy1 were larger than those for the related parameters obtained by methods 2 and 3. SEDs of related parameters of method 2 and method 3 were of comparable size. Power of discernment of method 1 was therefore less than that of method 2 and 3, whereas there were no obvious differences in discernment between methods 2 and 3. Each of the methods distinguished differences for bark yield between different accessions at equal plant density (treatments CP90, TC90, HE90). For wood yield, method 1 unlike methods 2 and 3, did not discriminate between TC90 and HE90. Methods 2 and 3 also performed better than method 1 in revealing subtle differences caused by plant density (treatments TC10, TC30, TC90, TC270).

The mean values of bark yield parameters By2 and By3, presented in Table 4 were not significantly different, the mean of By1 differed slightly but significantly from the means of By2 and By3 ($LSD_{0.05}$ = 0.59). The means of wood yield parameters varied widely and significantly ($LSD_{0.05}$ = 0.94). Means of bark loss parameters Bl2 and Bl3 differed significantly, but the difference was only small ($LSD_{0.05}$ = 0.35).

Methods for determining bark yield and wood yield were mutually compared by a split-plot analysis of variance (Table 5). For bark yield, significant effects were found for the main factors 'method' and 'treatment' and also a small but significant effect for the interaction 'method x treatment'. Since method 3 was assumed to give the most realistic parameters for pulp yield, the 'method x treatment' interaction can be attributed to an overestimation of the bark yield of accession 883213

(treatment CP90) by method 1, as can be derived from Table 4.

For wood yield, significant effects were found for the main factors 'method' and 'treatment' and also a small but significant effect for the interaction 'method x treatment'. The interaction was due to overestimation of the wood yield of treatments TC10 and HE90 by method 1 (see Table 4).

DISCUSSION

The results do not support the conclusion of Arnoux *et al.* (1969) that the average content of bark fibre and woody core of the stem can be estimated in segment no. 4. For bark fibre, the content of segment 4 proved to deviate from the content of the entire stem, in an accession-dependent way. This accession-dependent relation between contents of segment and stem is due to differences between accessions in distribution of the bark fibre fraction (Fig. 1). The woody core content of segment 4 deviated systematically, but not in an accession-dependent way, from the true stem values. For estimating the stem average woody core and bark tissue content of composite stem samples, segments no. 3 appeared more suitable than segments 4 (Fig. 2). The bark fibre content in segments no. 5 resembled most the average bark fibre content of composite stem samples (Fig. 1).

The relatively low correlation between bark yield estimated by method 1 and by the reference method, and the 'treatment x method' interaction for bark yield, is obviously due to the inaccurate and accession-dependent estimation of the entire stem content on the basis of segment no. 4 (used in method 1) as was demonstrated in Fig. 3. The relatively low correlation between wood yield estimated by method 1 and by the reference method, and the 'method x treatment' interaction for wood yield, cannot be attributed to an accession-dependent relation between the composition of segments and that of entire stems (Fig. 4). In this case the interaction is probably due to the treatment-dependent degradation of the unground wood (according to method 1). Factors affecting the progress of degradation of wood by NaOH may be accession-specific, such as diameter and density of the wood tissue, or be induced by plant-density which strongly affects stem diameter (Tarakan, 1969). Effects of these factors are eliminated by grinding in method 3, and avoided in method 2 by leaving the wood untreated by NaOH.

The values of bark yield parameters of method 2 and the reference method were about equal (Table 4), indicating that the bark fibre content is an accurate measure for the content of insoluble bark components. The wood yield parameters of method 2 and the reference method showed a strong correlation which allows an indirect derivation of the content of insoluble wood components on the basis of

the content of untreated woody core (Table 3).

CONCLUSIONS

The assessment of potential pulp yield by using a stem segment (method 1, based on the combined methods of Bredemann (1922) and Arnoux *et al.* (1969)) is less accurate than the TAPPI 212 method applied to separated and ground bark and woody core of the entire stem. There are two drawbacks to method 1: Contents of bark fibre and woody core of the stem segment used to deviate systematically from the contents of the entire stem (for bark fibre even in an accession-dependent way); the degradation of the unground woody core is only partial and depends on accession / plant-density treatments.

For the purpose of germplasm screening and selection, the practical advantages of handling unground stem segments (method 1) instead of separated, ground bark and woody core of entire stems are evident. For estimating the stem average woody core and bark tissue content of composite stem samples by means of segments, the segments no. 3 appear most suitable. The stem average bark fibre content of composite stem samples, can best be estimated in segment no. 5.

For estimating potential bark pulp yield of individual plants, the accuracy of method 1 can be improved by using accession-specific regression coefficients for calculating the content of bark fibre of the entire stem on the basis of segment contents.

For estimating potential wood pulp yield of individual stems, the accuracy of method 1 can be improved by reducing the degradation of the woody core of the segment to a minimum. This can be achieved by manually removing the woody core after boiling the segment in 2% NaOH for not more than 10 min, followed by further extraction of the bark fibres. The content of intact woody core of the entire stem can be calculated on the basis of the content of the segment using average (accession-independent) linear regression coefficients.

The bark fibre content of the stem (Bredemann) and the content of insoluble bark components determined by TAPPI procedure 212 have similar values. The content of untreated woody core correlates strongly with the content of insoluble woody core components determined by TAPPI procedure 212).

Mechanical separation of entire stems into bark and woody core, followed by measurement of 1% NaOH solubility of the ground tissues (TAPPI 212) seems most appropriate for trials in which accurate and more detailed information (including information on bark and wood losses) is required. This method, however, is much more time consuming and involves higher costs.

Based upon:

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CHAPTER 5

VARIATION OF *CANNABIS* WITH REFERENCE TO STEM QUALITY FOR PAPER PULP PRODUCTION

ABSTRACT

Ca. 160 accessions were evaluated for stem quality characteristics. Stems from field trials in two years were analyzed. The mass fractions of woody core, bark and primary and secondary bark fibres in the stem dry weight, and the dimensions of the wood fibres were considered most relevant for the evaluation of *Cannabis* germplasm as a raw material for paper pulp.

The accession means for the woody core fraction ranged from 50% in fibre cultivars to 78% in wild populations, drug strains and fibre landraces. Consequently, the bark tissue, being the complementary stem fraction, ranged from 50% down to 22%. The ranges of the accession means were 8 to 27% and 0 to 14% for the fractions of primary and secondary bark fibres, respectively. The total bark fibre fraction ranged from 9 to 34%. Fibre cultivars had strongly increased fractions of primary and secondary bark fibres in comparison to wild populations, drug strains and fibre landraces. The levels of the assessed stem fractions were stable over years.

Significant differences in wood fibre length among accessions were only detected in one of the two trials. The accession means in this trial ranged from 433 to 613 μm. For wood fibre width the accession means ranged from 24 to 37 μm and from 25 to 41 μm in the two trials, respectively. The stability of wood fibre dimensions over years was low. Within accessions, the wood fibres of male plants were usually somewhat shorter and wider than those of females. Apart from this difference between sexes there was little variation for wood fibre dimensions among individual plants within accessions.

Breeding for improved woody core quality is considered not very promising. The best way for genetic improvement of hemp pulping quality is continuous selection for increased bark fibre content, which implies a reduction of the woody core fraction.

Key words: bark, bark fibre, stem quality, woody core, wood fibre

INTRODUCTION

The present chapter describes the evaluation of *Cannabis* germplasm for relevant stem quality characteristics. Various terms are applied for describing stem quality of dicotyledonous annuals. In accordance

with Esau (1965), Adamson & Bagby (1975), Bedetti *et al.* (1979) and Wood (1981) the term 'bark' will be employed here to indicate all stem tissue outside the vascular cambium. The constituent fibres of the bark will be referred to as 'bark fibres'. These are often indicated as 'bast fibres' by authors reporting on crops like hemp, kenaf, roselle and jute (e.g., Nieschlag *et al.*, 1960; Esau, 1965; Anderson, 1974; Adamson & Bagby, 1975; Wood, 1981; Catling & Grayson, 1982; Wood, 1990). Some authors distinguish primary and secondary bark fibres (Kundu, 1942; Senchenko & Tarakan, 1970; Horkay, 1982). The term 'woody core' will be applied to indicate as a unit all tissue inside the vascular cambium conform to Bosia (1975) and Bedetti *et al.* (1979). The constituent fibres are called 'wood fibres' in accordance with Esau (1965) and Catling & Grayson (1982).

Traditionally, fibre hemp is grown for the production of the total fraction of primary and secondary bark fibres, being a raw material for textile and cordage. The present research in the Netherlands focuses on the utilization of entire stems. Bark and woody core of dicotyledons possess distinct properties; these are for *Cannabis* summarized in Table 1.

α-Cellulose and total cellulose (i.e. α-cellulose + amorphous cellulose + hemicellulose) contents are positively related to the yields of bleached and unbleached pulps, respectively (Wood, 1981). A low lignin content is desirable as it requires less chemicals and shorter cooking times during pulping (Wood, 1981). The determination of chemical characters was omitted in the present evaluation. Bedetti *et al.* (1979) and Triolo (1980) reported only small differences among French and Italian fibre hemp cultivars for the contents of α-cellulose, hemicellulose and lignin measured in the bark as well as in the woody core. Fibre length is important for its effect on the total length available for bounding (Horn, 1973). According to Wood (1981), a length of 3 mm provides optimal paper strength. The ratio of fibre length to fibre wall thickness is positively related to paper strength (Horn, 1973). Fibres with large lumina and thin walls are desired as they give strong and flexible paper (Wood, 1981). Due to its low α-cellulose content the potential utilization of the woody core of hemp seems restricted to mechanical pulps (Bosia, 1975; Triolo, 1980), whereas the bark is already presently used in high quality chemical pulps.

As the properties of the bark meet in general the requirements for manufacturing high-quality pulp, the present evaluation was restricted for bark to the assessment of the mass fractions of the entire tissue and its constituent primary and secondary bark fibres. The fraction of bark fibres in the stem is a direct measure for the potential recovery of bark pulp in the pulping process (Chapter 4). Horkay (1982) found that as a result of selection for increased bark

Table 1. Chemical and anatomical properties of bark and woody core of *Cannabis* stems.

Properties	Bark	Woody core
α-Cellulose content (%)	62 *(b)* 64 *(d)* 60-72 *(h)*	37 *(b)* 38 *(d)* 36-41 *(h)*
Hemicellulose content (%)	15 *(b)* 13 *(d)* 11-19 *(h)*	35 *(b)* 30 *(d)* 31-37 *(h)*
Lignin content (%)	4.1 *(b)* 4.3 *(d)* 2.3-4.7 *(h)*	20 *(d)* 19-21 *(h)*
Fibre length (mm) *)	(1)-9-(34) *(c)* 25 *(d)* (10)-40-(100) *(e)* 2; 13 *(f)* 2-20 *(g)*	0.26-0.44 *(a)* 0.55 *(b,d,e)* 0.57 *(g)* 0.30 *(f)*
Fibre width (μm) *)	(16)-30-(67) *(c)* 25 *(d)* 18-25 *(e)* 17; 34 *(f)* 21 *(g)*	14-18 *(a)* 25 *(b,d)* 27 *(g)* 15 *(f)*
Fibre wall thickness (μm)	-	0.7-3.4 *(a)*
Fibre lumen width (μm)	-	17 *(b)*

Single values represent averages, ranges are notated as minimum-maximum or as (minimum)-average-(maximum). Data sources are: *(a)* Anderson, 1974; *(b)* Bosia, 1975; *(c)* Catling & Grayson, 1982; *(d)* Gilabert Pérez, 1983; *(e)* Heuser, 1927; *(f)* Kundu, 1942; *(g)* Nieschlag et al., 1960; *(h)* Triolo, 1980). *) Most authors seem to present pooled data for the two types of bark fibre. Kundu *(f)* reports explicitly lengths of 2 and 13 mm, and widths of 17 and 34 μm for the secondary and primary fibres, respectively.

fibre content, both the primary and secondary fibre cells had increased in number though the latter to a greater extent. This affected textile quality negatively since secondary fibres are less strong than the pri-

mary ones (Senchenko & Tarakan, 1970), and too short and coarse (I. Bócsa, pers. commun., 1990). The effect of the increased ratio of secondary to primary fibres on pulping quality of the bark is not clear from the reviewed references.

The short hemp wood fibres cause low paper strength. The present evaluation included therefore, besides the assessment of the mass fraction of the woody core, also the estimation of length and width of its constituent fibres. The mass fraction of the woody core is not equal to, but correlates strongly with the recovery of woody core pulp in the pulping process (Chapter 4). The current germplasm evaluation for wood fibre dimensions is analogous to recent evaluations in kenaf where significant varietal differences were found for mean wood fibre length ranging up to 935 μm, whereas the current commercial cultivars have wood fibre lengths of 600-700 μm (Wood, 1990).

MATERIALS AND METHODS

Cultivation of plant material

Ninety-seven accessions of the CPRO *Cannabis* collection (Chapter 2) were tested in 1990, and 75 in 1993. Ten reference accessions were included in both trials. Accessions were grown in two replicates using a randomized block design with plots of 4.5 x 0.75 m^2. Each plot contained three rows, 25 cm apart. Sowing dates were 10 April in 1990 and 14 April in 1993. Shortly after germination plots were thinned to an average density of about 35 plants per m^2 in 1990 and 60 in 1993. Stem samples of each plot for quality assessment were collected at the day of seed maturity, being defined as the day when the first achenes of 50% of the female and/or hermaphrodite plants were resistant to compressing.

Estimation of the mass fractions of woody core, bark, and primary and secondary bark fibre

The estimations were restricted to female (and/or hermaphrodite) plants. At the moment of sampling, i.e. at initial seed maturity, female stems were still in good condition whereas the bark of male stems was often decayed which hampered the separation of primary and secondary bark layers, and the accurate assessment of various mass fractions as a percentage of the original stem dry weight. Preliminary research had shown that, for the total mass fractions of bark fibre, the accession means for male and female plants were similar and highly correlated ($r = 0.92$, $N = 93$, $p = 0.01$). Furthermore, the sampling was restricted to stems in the diameter range between 8 and 11 mm, measured at 35 cm height. This was to reduce the effect of variation in stem diameter on the fraction estimates, as reported by Senchenko & Tarakan (1970). Differences in stem composition would otherwise partly be due to plant density variation. Stem diameter of hemp is reported to be

Table 2. Coefficients of correlation between stem diameter and various stem mass fractions for three accessions. The calculations are based on 18 stems per accession. The distribution of stem diameter was taken equal for the three accessions within the range from 4 to 14 mm.

Accessions	Mass fractions			
	Woody core	Total bark fibre	Primary bark fibre	Secondary bark fibre
Kompolti Hybrid TC	0.68**	-0.56*	-0.81**	0.85**
Kompolti Hyper Elite	0.12	0.25	-0.71**	0.89**
883213	0.95**	-0.87**	-0.93**	0.73**

*,** significant at p = 0.05 and p = 0.01, respectively

strongly affected by plant density (Tarakan, 1969), a factor that cannot be standardized in the course of the growing season. A preliminary study confirmed the expected relation between stem diameter and stem composition, and showed that it was for some fractions even accession-dependent (Table 2).

Stems of five female plants were collected per plot in the 1990 trial, and stems of 10 in the 1993 trial. Composite subsamples, consisting of the segments between 20 and 25% of the total stem height were taken from these initial samples. They were dried at 70 °C for 48 h and weighed. After boiling for 10 min in 2% NaOH, the primary and the secondary bark layer were lifted one by one from the stem segments with dissecting needles (van der Werf *et al.*, 1994). The remaining, still intact, woody core was dried and weighed. The separated bark tissues were once more boiled in NaOH for one h, non-fibrous elements were then removed by rinsing on a sieve, the remaining fibre strands were dried and weighed. The mass fractions of primary and secondary bark fibre and woody core were expressed as percentages of the segment dry weight. The total fraction of bark fibre was calculated as the sum of the primary and secondary fibre fractions. The fraction of bark was calculated as being the complement of the woody core.

Estimation of wood fibre dimensions

Four wood samples were taken per plot, two from male and two from female plants in the stem diameter range from 10 to 15 mm, at 35 cm height. Samples were taken at a standard height since preliminary research had shown a systematic variation in wood fibre length along the vertical direction of the stem (Fig. 1), such variation did not exist in the horizontal direction.

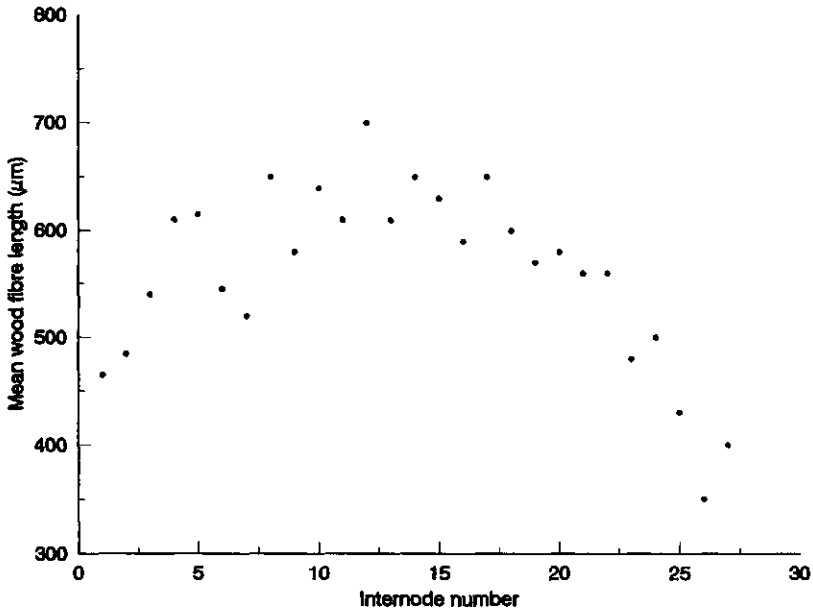


Fig. 1. Mean wood fibre length measured in subsequent internodes of one hemp stem. Internodes are numbered ascending from base to top.

A woody core section of ca. 5 cm long was taken from the central part of the internode situated at 5% stem height. The four wood samples of each plot were treated separately. Radially cut wood chips were macerated for 20 h at room temperature in a mixture consisting, according to Jeffrey's method (Dodd, 1986), of 43 g CrO_3, 100 ml HNO_3 (65%) and 1 l H_2O. Then the maceration fluid was removed and twice replaced by distilled water and once by ethanol, successively. In ethanol, the still cohesive tissue was shaken to obtain a suspension of single cells. A few drops of the suspension were transferred to a microscopic slide using a pipette and the cells were spread with dissecting needles. The ethanol was evaporated by gently heating the slide, and replaced by melted Kaiser's glycerol gelatin (Merck). After adding glass cover slips the glycerol gelatin coagulated at room temperature. The thus prepared slides could be kept in good condition for a few months. Length and width of 40 intact fibre cells per slide were measured using a Zeiss Axioskop microscope connected with a Sony CCD video camera. The video signal was digitized by a Data Translation frame grabber (DT-2255) in a Macintosh IIfx computer. The length and largest width of the projected fibres were manually indicated by mouse clicks. The processing was controlled by TCL image software. Each intact fibre within one projected image (covering ca. 2.5 x 2.5 mm^2 of the slide) was measured.

On average 3 to 4 images per slide were sufficient to obtain the required 40 measurements. Afterwards the measurements were multiplied by a calibration factor (ca. 5) to obtain the true dimensions. The ratio length/width was calculated per individual fibre. Subsequently, the means and median values for fibre length, width and their ratio were calculated per slide to characterize fibre dimensions of the corresponding plant.

RESULTS

The fractions of woody core, bark, and primary and secondary bark fibre

In both years, significant variation among accessions was found for the mass fractions of woody core, bark, and primary, secondary and total bark fibre in the analyzed stem segments of female plants (Table 3).

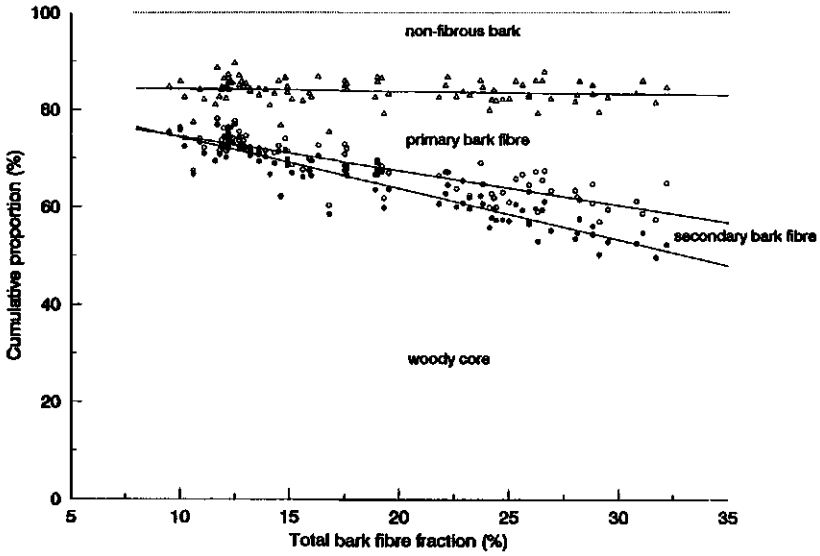
Figs. 2a and b show how the stem composition relates to the total bark fibre fraction, being the main quality criterion for fibre hemp breeding up to now. On increasing total bark fibre fraction, the woody core decreased, and consequently the bark fraction increased. The fractions of secondary and primary bark fibre increased both. The total of woody core and bark fibre remained constant and so did the fraction of non-fibrous bark. Mass fractions of bark fibre from 15 up to 34%, were found in fibre cultivars of which those from Hungary had the highest contents. The smallest mass fractions of bark fibre were found in

Table 3. $LSD_{0.05}$ (%), range (%), grand mean (%) and F-values for accession means for various mass fractions. Data are based on 93 accessions tested in 1990, and 74 in 1993.

Mass fraction	Year	$LSD_{0.05}$	Range	Mean	F-value
Woody core	1990	7.5	49.6 - 77.2	65.2	7.64***
	1993	8.9	49.9 - 77.7	69.4	3.63***
Bark	1990	7.5	22.8 - 50.4	34.8	7.64***
	1993	8.9	22.3 - 50.1	30.6	3.63***
Primary bark fibre	1990	3.6	8.4 - 27.2	15.3	12.19***
	1993	3.2	8.8 - 22.3	13.7	7.18***
Secondary bark fibre	1990	2.4	0.0 - 12.6	3.2	9.73***
	1993	2.0	0.1 - 14.3	3.3	16.55***
Total bark fibre	1990	2.8	9.5 - 32.2	18.5	42.76***
	1993	2.8	9.0 - 34.2	16.9	29.19***

*** significant at $p = 0.001$

(a)



(b)

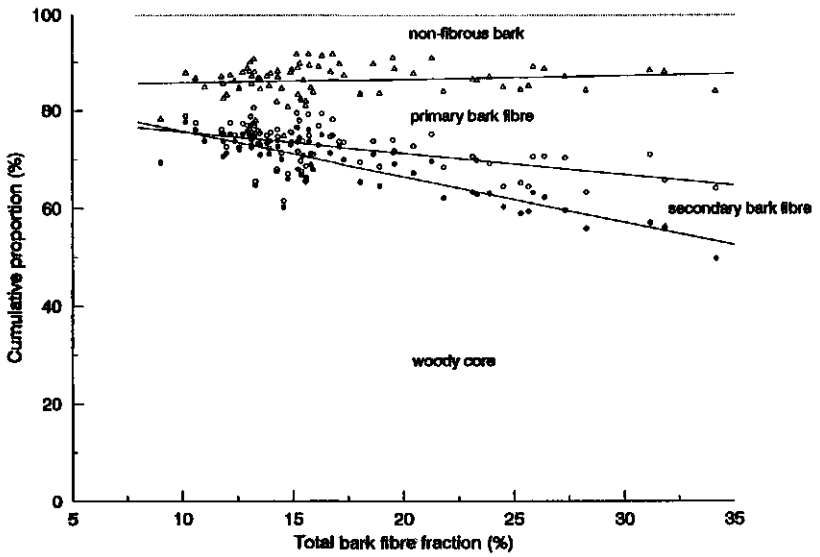


Fig. 2. The accession mean stem composition in relation to the fraction of total bark fibre for 92 accessions tested in 1990 (a) and 74 accessions tested in 1993 (b).

Table 4. Anova tables based on plot means for the stem fractions of six reference accessions tested in two years. The data for four of the initial ten reference accessions were incomplete over the two years. For the secondary bark fibre fraction the anova was based on log transformed data.

Source of variation		Mean squares			
	d.f.	Woody core	Secondary bark fibre	Primary bark fibre	Total bark fibre
Year.block stratum					
Year	1	163.6	4.68	5.2	7.5
Residual	2	41.4	1.02	3.3	4.1
Year.block.accessions stratum					
Accession	9	202.8**	8.06***	49.0***	219.3***
Year x accession	5(4)	21.5	0.47*	1.9	0.6
Residual	13(5)	39.9	0.15	4.2	3.0
Total	30(9)				

*,**,*** significant at p=0.05, p=0.01 and p=0.001, respectively

drug strains, wild and naturalized populations and also in fibre landraces. These accessions are represented by the concentration of data points between 9 and 15% bark fibre. The accession means for the stem fractions, corrected for the year effect (procedure Chapter 8), are presented in Appendix 2.

Means of six reference accessions for each of the fractions correlated strongly over years. The coefficients of correlation were 0.90 (p= 0.05), 0.99 (p=0.01), 0.96 (p= 0.01) and 0.99 (p=0.01) for the woody core, total, primary and secondary bark fibre fraction, respectively. Despite large differences between the summers of 1990 and 1993, none of the traits was significantly affected by 'year'. The interaction term 'year x accession' was significant for the fraction of secondary bark fibre only (Table 4).

Estimation of wood fibre dimensions

The characterization of fibre dimensions of individual plants by mean or median values of the 40 fibre measurements yielded usually similar results. This indicates that fibre dimensions were normal distributed within the woody core section of individual stems. Preference was however given to the use of median values as these are not affected by occasionally occurring extremely deviating individual fibres. The use of plant median values resulted for

Table 5. $LSD_{0.05}$, range, grand mean and F-values for accession means for wood fibre dimensions. Data are based on 97 accessions tested in 1990, and 74 in 1993.

Dimensions	Year	$LSD_{0.05}$	Range	Mean	F-value
Fibre length (μm)	1990	n.s.	479 - 604	534	1.21
	1993	58	433 - 613	531	2.57***
Fibre width (μm)	1990	7	24 - 37	31	1.43*
	1993	7	25 - 41	33	1.94**
Ratio length/width	1990	n.s.	14 - 26	18	1.38
	1993	4	13 - 23	17	1.91**

*,**,*** significant at p = 0.05, p = 0.01 and p = 0.001, respectively

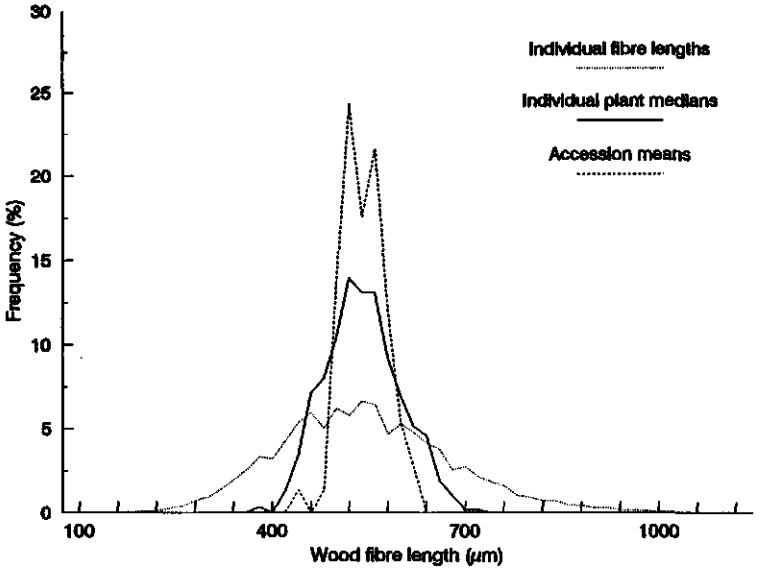
the reference accessions in somewhat higher correlations between years than the use of plant mean values.

Accession means for wood fibre dimensions of male and female plants were significantly (p = 0.01) correlated. In the 1990 trial (N = 88) the coefficients of correlation between sexes, for length, width and the ratio length/width, were 0.56, 0.66 and 0.69; in 1993 (N = 74) the respective values were 0.59, 0.67 and 0.62. The factor 'sex' affected highly significantly the wood fibre dimensions, although the differences between medians of male and female plants were only small, they were strongly consistent. In both years, the accession means for median wood fibre length of the male plants were on average 20 μm smaller than those for the females, whereas the accession means for plant median wood fibre width of the males were on average 2.5 μm larger.

Accession means (male and female plant medians averaged) differed significantly for fibre length, width and the ratio length/width in 1993. In the 1990 experiment, only the varietal differences for fibre width were significant (Table 5). The ranges for accession mean fibre dimensions were narrow. Fibre length and width were hardly interrelated, coefficients of correlation between the accession means for length and width were -0.25 in 1990 (N = 97, p = 0.05) and -0.17 in 1993 (N = 74, n.s.). The accession means for the wood fibre dimensions, corrected for the year effect, are presented in Appendix 2.

The lengths of the total of individual fibres measured in the 1993 experiment ranged from 200 to 1000 μm. The frequency distribution for median wood fibre length of the pooled individual plants was only 200 μm wider than that of the pooled accession means (Fig. 3a). This indicates that the variation for

(a)



(b)

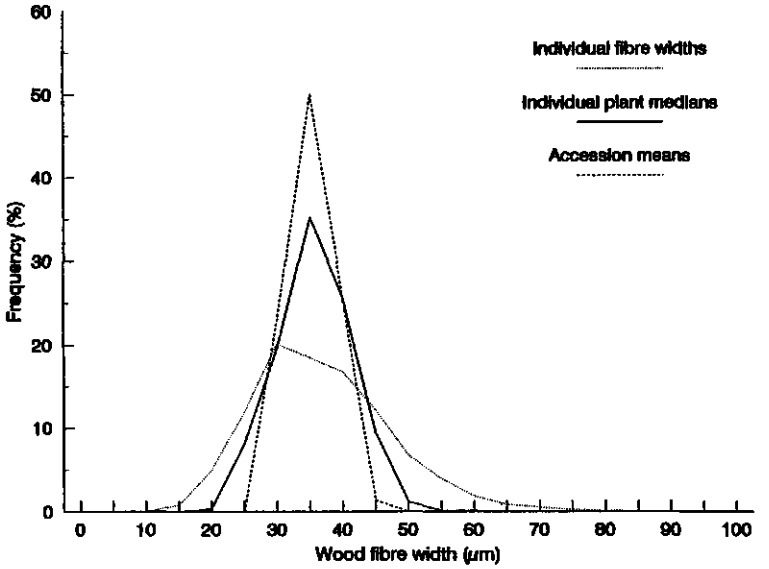


Fig. 3. Frequency distributions for wood fibre length (a) and width (b) in the 1993 trial, at the level of individual fibres (N = 23480, 520 missing values), at the level of individual plants (N = 587, 13 missing values) and at the level of accessions (N = 74, one missing value).

Table 6. Anova tables based on plot means for the wood fibre dimensions of nine reference accessions tested in two years. The data for one of the initial ten reference accessions were incomplete over the two years.

Source of variation		Mean squares		
	d.f.	Length	Width	Length/width
Year.block stratum				
Year	1	4491*	100.3	63.5
Residual	2	246	88.4	37.0
Year.block.accessions stratum				
Accession	9	3734*	35.3*	10.2
Year x accession	8(1)	871	10.4	4.3
Residual	16(2)	1077	10.7	5.8
Total	36(3)			

* significant at $p = 0.05$

wood fibre length within accessions, between individual plants, is generally very limited. The findings of the 1990 trial were comparable. The distribution for fibre width at the individual plant level was only 20 μm wider than that at the accession level in both years' experiments, which again indicates little variation within accessions (Fig. 3b).

Means of nine reference accessions for wood fibre dimensions correlated rather poorly over years. Coefficients of correlation were 0.70 ($p = 0.05$), 0.59 (n.s.) and 0.52 (n.s.) for length, width, and the ratio length/width, respectively. Only fibre length was significantly affected by 'year'. None of the traits showed a significant year x accession interaction (Table 6).

Strong relations between wood fibre dimensions and other stem traits were absent (Table 7). Fibre length was for instance not related to plant height. There seemed to be a weak relation between stem composition and wood fibre width. The strongest correlation was that between the mass fraction of secondary bark fibre and wood fibre width which indicates that fibre cultivars tend to have somewhat wider wood fibres than wild populations and drug strains.

DISCUSSION

Fig. 2 illustrates what hemp breeding in the 20th century has achieved with respect to stem composition. A fraction of 10 to 15% bark fibre can be considered 'natural' for

Table 7. Coefficients of correlation between accession means for wood fibre dimensions and for other traits in 1990 (92 accessions) and in 1993 (74 accessions).

Other traits	Wood fibre dimensions					
	Length		Width		Length/width	
	1990	1993	1990	1993	1990	1993
Plant height	0.09	0.12	0.28**	0.24*	-0.15	-0.21
Woody core fraction	0.01	0.18	-0.35**	-0.07	0.29**	0.19
Primary bark fibre fraction	-0.03	-0.29*	0.30**	0.14	-0.26*	-0.28*
Secondary bark fibre fraction	-0.02	-0.20	0.42**	0.30**	-0.35**	-0.38**
Total bark fibre fraction	-0.03	-0.27*	0.37**	0.24*	-0.32**	-0.35**

*, ** significant at $p = 0.05$ and $p = 0.01$, respectively

Cannabis, as can be derived from the abundance of data points within this range representing the performance of drug strains, undomesticated populations and fibre landraces. Such fibre contents were the starting point for breeders until the 1950s (e.g., Feaster, 1956). The bark tissue fraction in the stem has been increased from 25 up to 47%, and the fibre content of the bark was increased from 40 to 68%. The increase of fibre within the bark was relatively stronger for the secondary (from practically 0 to 17%) than for the primary ones (from 40 to 51%). This agrees with Horkay (1982) who reported that on increasing the fibre content both the primary and secondary fibre cells increase in number though the latter to a greater extent.

The relation between the composition of stem segments and that of entire stems has been studied in Chapter 4 and by van der Werf *et al.* (1994). The average woody core and bark tissue fraction, of composite samples of entire stems are about equal to those of the analyzed composite samples of stem segments. The total and the secondary bark fibre fractions of entire stems may deviate somewhat in an accession-dependent way from those measured in the used segments.

The factor 'year' affected hardly the levels of the tested stem quality traits, although the summers of

1990 and 1993 were quite different. The ranking order of accessions for stem mass fractions proved to be very stable over years which indicates a high heritability for stem composition. The opposite applies for the measured wood fibre dimensions. This, in combination with the limited variation within and between accessions, makes breeding for improved wood quality not very promising. More knowledge of the genotypic contribution to differences in wood fibre dimensions can be obtained by testing clones (cuttings) from distinct individual plants. The present results are in contrast with those of recent germplasm evaluations of kenaf. Wood (1990) concluded that the considerable variation in wood fibre length among kenaf accessions offered the promise that fibre length of current cultivars could be increased substantially by breeding. The variation for wood fibre dimensions within *Cannabis* accessions, appears even more limited since it is partly due to the contrast between male and female plants.

The best way for genetic improvement of hemp pulping quality is continuous selection for increased bark fibre content, which implies a reduction of the woody core fraction. This seems very well possible since there is a considerable variation between individual plants in stem composition, even within the best fibre cultivars at present (Chapter 4, Figs. 3 and 4). A consideration should however be that a certain minimal wood fraction might be necessary to provide sufficient strength to the stem and persistency to the crop (Hoffmann, 1961).

The present results are in contradiction with Anderson (1974) who reported that *Cannabis indica* has shorter and wider wood fibres than *C. sativa*. Average wood fibre length and width of three drug type accessions, received explicitly under the name *C. indica*, were 572 and 29 μm, respectively. The average wood fibre length and width of 105 fibre strains, generally classified as *C. sativa*, were 534 and 31 μm, respectively.

Fibre cultivars of *Cannabis* tend to have somewhat wider wood fibres than accessions with low bark fibre content. Adamson & Bagby (1975) reported relatively short wood fibres for kenaf fibre cultivars and suggested a causal relation between this feature and selection for easy decortication. It is uncertain if such a relation applies for *Cannabis* wood fibre width as well. Wider wood fibres as a natural reaction to the replacement of woody core by bark was not reported earlier.

CONCLUSIONS

There is considerable variation among accessions for the distribution of the mass fractions of woody core, bark and primary and secondary bark fibres. Stem composition with respect to these mass fractions is stable over years and clearly related to the state and purpose of domestication.

The variation for wood fibre dimensions among and within accessions is limited. Wood fibre dimensions are not strongly related to any other trait.

Breeding for increased wood fibre length is not very promising.

The best way for genetic improvement of hemp pulping quality is a continued selection for increased bark fibre content, which implies a reduction of the woody core fraction.

Revised and updated version of:

Meijer, E.P.M. de, H.J. van der Kamp & F.A. van Eeuwijk, 1992. Characterisation of *Cannabis* accessions with regard to cannabinoid content in relation to other plant characters. Euphytica 62: 187-200.

CHAPTER 6

CHARACTERIZATION OF *CANNABIS* ACCESSIONS WITH REGARD TO CANNABINOID CONTENT IN RELATION TO OTHER PLANT CHARACTERS

ABSTRACT

Accessions were evaluated for contents of the major cannabinoids delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), in relation to non-chemical plant characters. Considerable variation within and among accessions was found for cannabinoid contents. Within accessions, THC content was less variable than CBD content. The accession mean cannabinoid contents were strongly affected by the year of cultivation, but THC content was more stable than CBD content. There were no strict relationships between the observed chemical and non-chemical traits. It was demonstrated that, although uncommon, some accessions combine a high bark fibre content and a considerable psychoactive potency. On the accession average level, relatively wide leaflets and slow phenological development were weakly associated with a stronger psychoactive potency.

Key words: chemical phenotype, cannabinoids

INTRODUCTION

The presence of psychoactive compounds in *Cannabis* has attracted much scientific attention in the past decades. Several theories have been proposed for the biological function of the cannabinoid containing resin for the plant itself. A relation was suggested with antibiotic activity (Krejci, 1970) and drought and heat tolerance (Schultes, 1970). Warm, dry and windy conditions were believed to induce higher densities of resin glands where the biosynthesis of the cannabinoids takes place. Murari *et al.* (1983) estimated higher contents of cannabinoids in the same populations when grown in a continental climate than in a maritime climate.

The presence of psychoactive compounds is considered an important reason for the decline of fibre hemp cultivation in the course of the 20th century (Bredemann *et al.*, 1957; Dempsey, 1975). Due to legislation, hemp cultivation is now prohibited or restricted in many countries. Current fibre hemp breeding in the former USSR (e.g., Virovets *et al.*, 1987; Gorskhova *et al.*, 1988) and France (J.P. Mathieu, pers. commun., 1992) is aimed at

Table 1. Chemical phenotype classifications according to Small & Beckstead (1973) and Fournier & Paris (1979).

Phenotypes	[THC](%)	[CBD](%)	[THC]/[CBD]
Small & Beckstead			
Drug	>0.3 (in both sexes)	<0.5 (in both sexes)	-
Intermediate	>0.3 (in females)	>0.5 (in both sexes)	-
Non-drug	<0.3 (in females)	>0.5 (in both sexes)	-
Fournier & Paris			
Fibre hemp	<0.5	>0.5	<1
Resinous *Cannabis*	>0.5	<0.5	>1

negligible psychoactivity.

For the breeding of suitable hemp cultivars for cellulose production in the Netherlands, various *Cannabis* strains other than fibre cultivars, may be useful for providing desired characters such as a long vegetative growth (Chapter 3), and resistance to soil borne diseases (Chapter 7). However, the psychoactivity of newly introduced accessions and cross-bred progenies should be considered. Association of cannabinoid contents and easily visible plant characters would allow an indirect chemical characterization of accessions, whereas close linkage of cannabinoid contents and other agronomic characters would hamper breeding. The relation between chemical and non-chemical traits was therefore evaluated.

Classification of Cannabis *accessions on the basis of cannabinoid composition*

The concentration of cannabinoids in the dry matter of inflorescence leaves is the most direct measure to classify *Cannabis* according to psychoactive potency. Of the major cannabinoids, delta-9-tetrahydrocannabinol (THC) is generally accepted to cause the psychoactive effects of *Cannabis* preparations. The concentration of THC is often presented in connection with that of the other major cannabinoid, cannabidiol (CBD), and sometimes in connection with that of minor cannabinoids such as cannabinol (CBN). Fetterman *et al.* (1971) characterized a plant- or population chemical phenotype by the cannabinoid ratio: ([THC] + [CBN])/[CBD]. If this ratio exceeded 1, plants were classified as 'drug phenotype', otherwise as 'fibre phenotype'. Small & Beckstead (1973) distinguished chemical phenotypes using the combination of THC and CBD content. Plants with less than 0.3% THC were considered to possess no psychoactive potency. This value was therefore used to discriminate the phenotypes: 'drug', 'intermediate' and 'non-drug' (Table 1). Fournier & Paris (1979)

reported a tolerated maximum content of 0.5% THC for fibre hemp in France and recognized 'fibre hemp' and 'resinous *Cannabis*' on the basis of this threshold (Table 1). In the former USSR, 0.2% THC was the maximum tolerated content in fibre hemp, and since the 1970s breeders aim successfully at cultivars completely lacking THC (V.G. Virovets, pers. commun., 1991).

Prediction of psychoactive potency on the basis of non-chemical features

Several criteria have been proposed to recognize *Cannabis* plants or populations with strong psychoactive potency by visual means. Bredemann *et al.* (1957) divided domesticated *Cannabis* in three not sharply demarcated groups, morphologically and physiologically differing as a result of domestication and adaptation to climatic conditions: 'fibre hemp' (long, unbranched plants with poor seed production), 'oil seed hemp' (short, early maturing plants with rich seed production) and 'drug hemp' (short, strongly branched plants with small dark green leaves). Small *et al.* (1976) defined three classes of plants on the basis of cannabinoid content, and scored the plants for many attributes. They concluded that when plants were grown under standardized conditions, plants of the 'northern low-intoxicant type' could be discriminated from those of the 'southern high-intoxicant type' by means of multivariate analysis of many attributes but that groups could not be identified by practical visual means. A promising morphological character to select for drug content according to Small *et al.* (1976) is the resin gland density, which was supposed to increase with higher psychoactive potency. According to Turner *et al.* (1978) however, there is no correlation between gland density and cannabinoid content. Positive results with regard to the predictive value of gland density were reported by Gorskhova *et al.* (1988) and Petri *et al.* (1988). Nonetheless the relationship between gland density and THC content did not eliminate the necessity of a quantitative chemical characterization in selection programs in Hungary (Petri *et al.*, 1988). Also in the former USSR preference is still given to a direct estimation of cannabinoids by means of chromatography (V.G. Virovets, pers. commun., 1991).

In systematic keys, achene characters like size, marbling of the perianth and presence or absence of a conspicuous abscission layer are often used to discriminate taxa within the genus *Cannabis* (e.g., Schultes *et al.*, 1974; Small & Cronquist, 1976). Achene characters may hence be expected to show some relation to cannabinoid content, because of the presumed differences in psychoactive potency among the commonly discriminated taxa.

MATERIALS AND METHODS

Cultivation of plants and assessment of non-chemical traits

Field trials were carried out in 1989, 1990 and 1993. In the preliminary study of 1989, 32 accessions were grown in unreplicated plots of 4.5 x 0.75 m^2. Each plot contained three rows, 25 cm apart. The average plant density was ca. 35 plants per m^2. In 1990, 97 accessions were grown in two replicates of a randomized block design, with plots of the same size and plant density as in the 1989 experiment. In 1993, 75 accessions were grown in two replicates of a randomized block design, again with the same plot size but now in a plant density of 60 plants per m^2. Field trials were carried out at CPRO (52° N latitude) on a sandy soil rich in organic matter.

Traits observed for the non-chemical characterization in 1990 are listed in Table 2. The determination of resin gland density was considered too elaborate, and the prospects too uncertain to be part of the evaluation. Seeds for sowing were used to score achene characters (traits 3,4,5,6,9 and 10). Other traits were determined during the field experiment, except stem quality (16,17) which was examined afterwards. Traits 1 to 6 are multiple state characters, traits 7 to 23 are continuous.

Sampling and sample treatment for the chemical characterization

Samples were, in accordance with Small *et al.* (1976), taken at initial seed maturity as defined in Table 2 for trait 13. The number of plants considered to be a good representation for an experimental plot varies widely among references. Small & Beckstead (1973) sampled six individual plants, three males and three females, per population, and analyzed them separately. Avico & Zuccaro (1984) recommended a sample size of 50 individual plants per population to be analyzed separately. The European Community procedure to determine THC content in fibre hemp cultivars demands a sample size of 500 individual plants, to be analyzed as a composite sample (Anonymous, 1986). Because of this range of sample sizes in quoted references, the interplant variation for cannabinoid content and the minimal sample size were subject of the 1989 preliminary experiment. As a result (see next section), the cannabinoid content of a composite sample of 20 plants was considered to provide a reliable approximation of the average content of a small plot of plants. Sampling was restricted to female and/or hermaphrodite plants since male plants are not commonly used for drug production.

Cannabinoid profiles are generally expressed as mass fractions in the dry matter of the leafy parts of inflorescences (Quimby *et al.*, 1973;

Hemphill *et al.*, 1980; Avico & Zuccaro, 1984). In the present experiment the upper 30 cm of the main stem inflorescence was collected for analysis.

El Kheir *et al.* (1986) identified light, high temperature and high oxygen content as the main agents for deterioration of THC during sample storage. In the present study the plant tops were left intact and dried in the dark in cotton bags at 35 °C during one week and stored afterwards in the dark at 15 °C and 15% relative humidity until further processing. Shortly before chemical analysis the dried inflorescences were crushed and sieved in order to remove seeds and straw. The leaf material was ground in a Retch centrifugal mill over a 0.2 mm sieve to ensure complete extraction of the cannabinoids. Leaf powder samples were stored at room temperature in the dark until gas chromatography (GC) analysis was performed. Preliminary observations demonstrated that cannabinoid contents did not change in such leaf powders, when stored during six weeks in the dark, even at a temperature of 40 °C.

Chemical analysis

Five ml of hexane containing 0.2 mg/ml of squalane as internal standard was added to 100 mg of leaf powder in a glass culture tube. The suspension was placed in an ultrasonic bath for 20 min and then centrifuged. The hexane layer was decanted and the pellet was extracted once more with 5 ml of hexane. Of the combined decanted extracts 1 μl was injected into a gas chromatograph without derivatisation.

Analyses were performed on a Varian 3700 gas chromatograph with autosampler 8000 equipped with a split-splitless injector, a flame ionisation detector and a fused silica column (25 m x 0.25 mm I.D.) coated with CP Sil 5 CB. GC was carried out under the following conditions: carrier gas (helium) flow rate 0.8 ml/min; split 25 ml/min; injector and detector temperature 280 °C; oven temperature program: 5 min isothermal at 240 °C, then increasing with 10 °C/min to 280 °C. Peaks were integrated by Nelson 2600 chromatography software. Peak areas of cannabinoids were compared with the peak area of the internal standard. A factor of 1.15 was applied for the correction of relative detector response. The structure of the detected cannabinoids was confirmed by gas chromatography - mass spectrometry (GC-MS).

Twenty leaf powder samples of 5 g were placed at 103 °C for 3 h in order to determine dry weights. The average moisture content of the 20 samples (10.9%, S.D. ± 0.45) was used to calculate cannabinoid contents on the basis of leaf powder dry weight. Cannabinoid contents were presented as the sum of the neutral compound and the corresponding carboxylic acid (conform Small & Cronquist, 1976).

Table 2. Non-chemical traits observed in 1990 in connection with cannabinoid contents.

No.	Description
1	Leaflet width, nine classes: very narrow to overlapping margins, general impression of main stem leaves.
2	Presence of monoecious plants, two classes: hermaphrodite plants not, or commonly present.
3	Achene abcission layer, five classes: always absent to always and conspicuously present.
4	Achene marbling of perianth, five classes: not marbled to conspicuously marbled.
5	Achene colour of testa, three classes: grey, some grey and some brown, brown.
6	Achene shape of apex, three classes: blunt to acuminate.
7	Leaflet index, length/width ratio of central leaflet of primary leaf at fourth stem node. Average of five plants.
8	Percentage of male plants, counted among 50 flowering plants.
9	Achene length (mm), average length of 20 seeds.
10	Thousand seed weight (g).
11	Day no. of seedling emergence.
12	Day no. of anthesis, 50% of the plants, irrespective of sex, with visible inflorescences.
13	Day no. of initial seed maturity, first achenes of 50% of the female and/or hermaphrodite plants resistant to compressing.
14	Days from seedling emergence to anthesis, trait 12 minus trait 11.
15	Days from anthesis to initial seed maturity, trait 13 minus trait 12.
16	Total bark fibre mass fraction in stem dry matter, average of 10 mature stems of female plants.
17	(Partly degraded) woody core mass fraction in stem dry matter, average of 10 mature stems of female plants.
18	Stem elongation parameter B (day^{-1}), average of 15 female plants (Chapter 3).
19	Stem elongation parameter M (days), average of 15 female plants (Chapter 3).
20	Stem elongation parameter C (cm), average of 15 female plants (Chapter 3).
21	(Measure for) internode length; stem length from soil level to fifth stem node (cm), average of 10 mature female plants.
22	Stem diameter at 30 cm stem height (mm), average of 10 mature female plants.
23	Percentage of plants with stem lesions caused by *Botrytis cinerea*, counted among 50 mature plants.

Classification of accessions according to chemical phenotype

Accessions were characterized by their THC and CBD content and THC/CBD ratio. The content of the cannabinoid CBN was ignored since preliminary observations revealed only very low CBN contents ranging between 0.01 and 0.07%.For demarcating the phenotype groups, the threshold of 0.5% for both THC and CBD was chosen, conform Fournier & Paris (1979). It appeared necessary to recognize a third group which

Table 3. Criteria used for the classification of accessions according to cannabinoid profile.

Phenotype	[THC](%)	[CBD](%)	[THC]/[CBD]
Non-drug	<0.5	≥0.5	<1
Intermediate	≥0.5	≥0.5	-
Drug	≥0.5	<0.5	>1

partly matched the criteria for Fournier & Paris's phenotype 'fibre hemp' and partly those of their phenotype 'resinous *Cannabis*'. The terms 'non-drug', 'intermediate' and 'drug' were employed to indicate the distinguished groups (Table 3).

Statistical methods for assessing the relationships between chemical and non-chemical characters

In order to assess relationships between chemical and non-chemical characters, statistical procedures conform Small *et al.* (1976) were applied to the collected data. Cluster analyses were carried out using a similarity coefficient combining information of the discrete as well as the continuous characters as described by Gower (1971). Cluster algorithms involved were nearest neighbour, farthest neighbour, and average linkage. A multiple discriminant analysis was performed using the continuous characters and leaflet width (trait 1), the only ordinal character with an empirical distribution behaving reasonably normal. Continuous characters were always standardized, and in some cases transformed to achieve normality. A discriminant analysis was applied for which the prior probabilities for the groups were equal, thus correcting for the different sizes of the three groups of phenotypes as defined in Table 3. Additionally an unweighed discriminant analysis was performed in which prior probabilities were dependent on group size, to investigate the consequences of weighing. Furthermore, a number of principal component analyses, weighed and unweighed, was carried out to investigate whether the most important principal component scores would reveal the predefined groups. Finally, analyses of variance were carried out for each of the continuous characters and the multiple state character leaflet width. For the remaining nominal and ordinal characters, Chi-square tests on interaction between categories and phenotypes were performed.

RESULTS AND DISCUSSION

Interplant variation

Fig. 1 shows a considerable variation among female plants within accessions for chemical phenotype

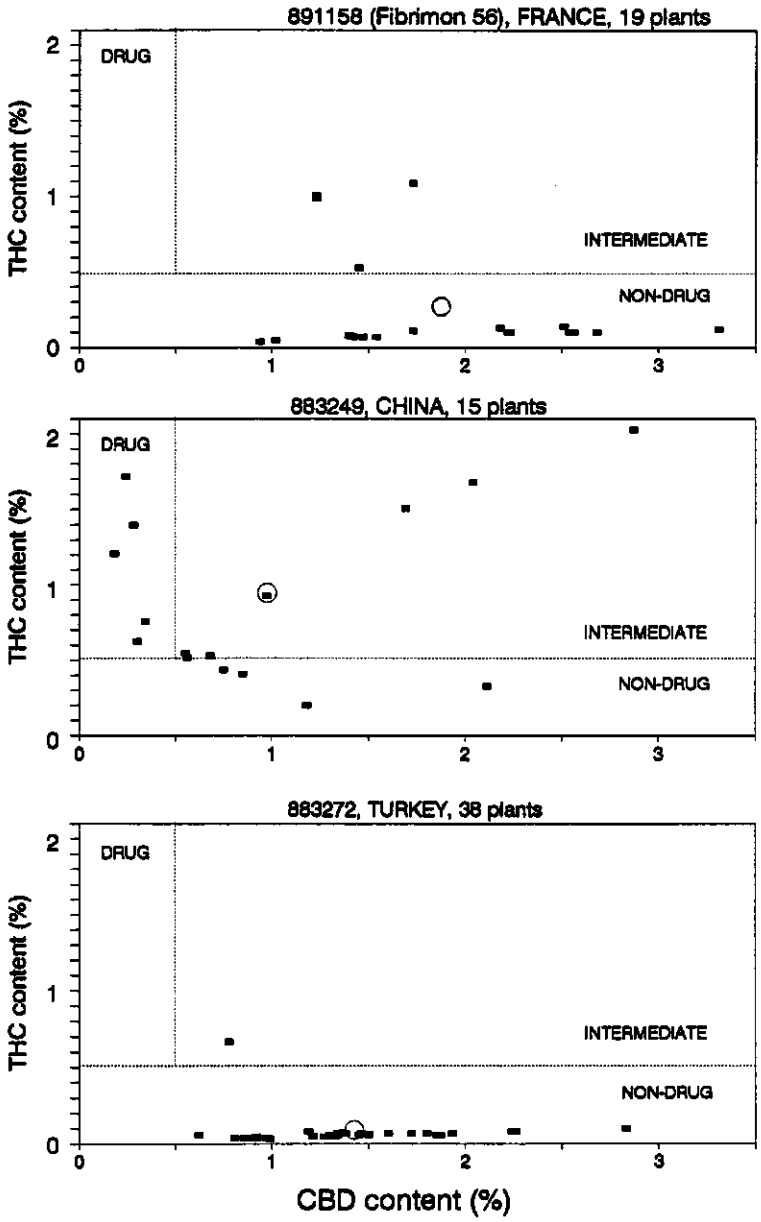


Fig. 1. THC content versus CBD content of individual plants belonging to three accessions (square symbols). The areas of the distinguished phenotypes are indicated in the diagrams. The circles represent the mean contents for THC and CBD.

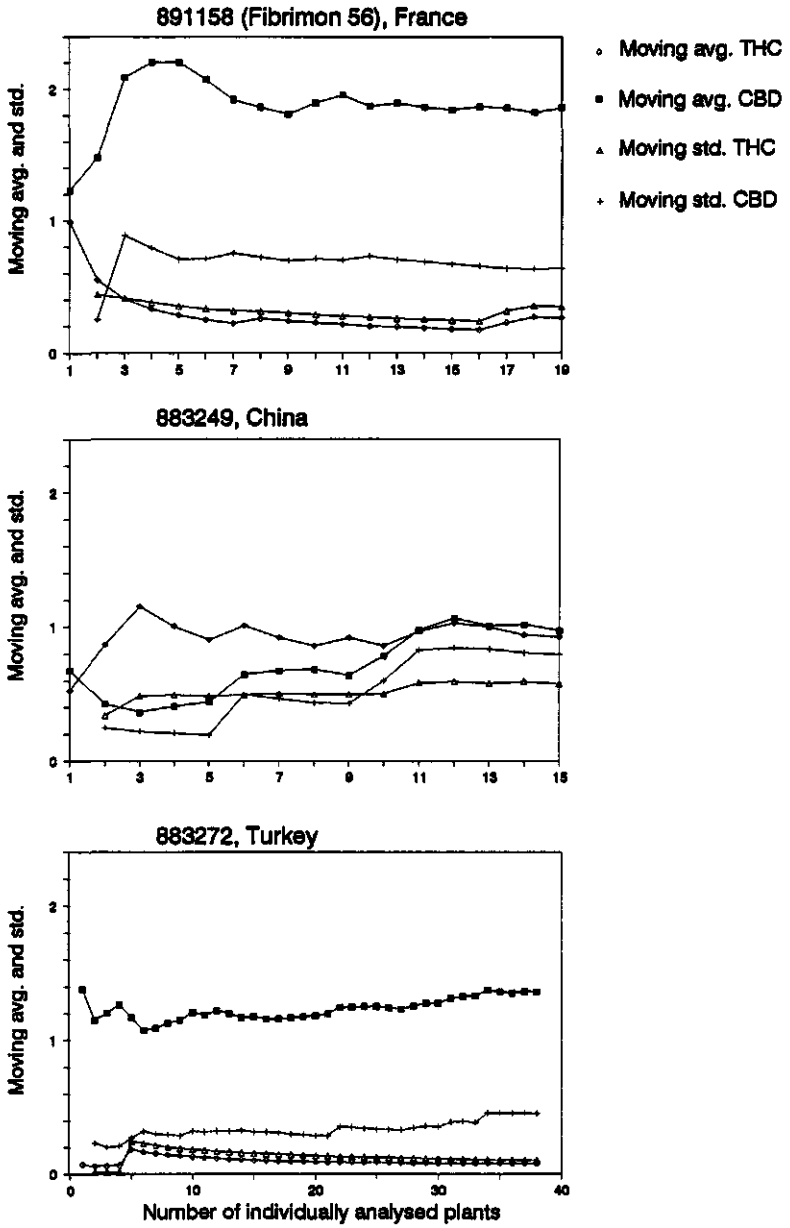


Fig. 2. Moving average and moving standard deviation of THC and CBD content versus the number of analyzed plants taken into account, for three accessions.

in the preliminary 1989 field experiment. Individual plants belonging to different phenotype groups occurred in each of the three tested accessions, even in the commercial cultivar Fibrimon 56 (891158). A very wide range of THC and CBD contents was found in the Chinese accession 883249. The interplant variation for CBD content appeared larger than that for THC content. Comparable heterogeneity for THC content was reported by Avico & Zuccaro (1984) for Italian fibre cultivars and by Fournier & Paris (1979) for French fibre cultivars. Only a very limited interplant variation could be detected for CBN content. Within the three accessions represented in Fig. 1, CBN content ranged from 0.00 to 0.07, 0.00 to 0.09 and 0.01 to 0.05%, respectively.

Moving average of THC and CBD content became more or less stable at a number of 10 to 15 analyzed plants per plot (Fig. 2). The content of a bulk sample composed of 20 plants was hence considered to be a safe estimate for the average content per plot as used in the present experiments.

Consistency of chemical phenotype

A subset of 32 accessions was grown as well in the 1989 preliminary experiment as in the 1990 evaluation trial. Although in both years the summers were relatively warm and dry for Dutch standards, there were considerable differences between the cannabinoid profiles in the two years (Fig. 3). The 1990 CBD contents were almost systematically lower than those in 1989. Eight of 32 accessions were classified as different phenotypes in different years. CBD content appeared to be less conservative than THC content. The coefficients of correlation between 1989 and 1990 were 0.76 ($p=0.01$), 0.20 (n.s.) and 0.66 ($p=0.01$) for accession mean THC content, CBD content and the THC/CBD ratio, respectively. The lower stability of CBD compared to THC was confirmed later with nine accessions tested in 1990 as well as in 1993, for which the coefficients of correlation between years were 0.84 ($p=0.01$), 0.48 (n.s.) and 0.99 ($p=0.01$), for accession mean THC content, CBD content and the THC/CBD ratio, respectively. In the cold summer of 1993, reference accessions had much lower cannabinoid contents than they had in 1990, but the cannabinoid ratios were not strongly affected by the factor 'year'.

Chemical characterization of accessions

There were significant ($p=0.001$) differences among accessions for cannabinoid contents and ratio in 1990 as well as in 1993. The ranges in the two years were 0.07-1.77% ($LSD_{0.05}=0.42$) and 0.01-1.38% ($LSD_{0.05}=0.20$) for THC content; 0.22-2.19% ($LSD_{0.05}=0.52$) and 0.05-1.36% ($LSD_{0.05}=0.20$) for CBD content; 0.06-7.46 ($LSD_{0.05}=1.10$) and 0.02-24.63 ($LSD_{0.05}=2.04$) for the cannabinoid ratio. The means for

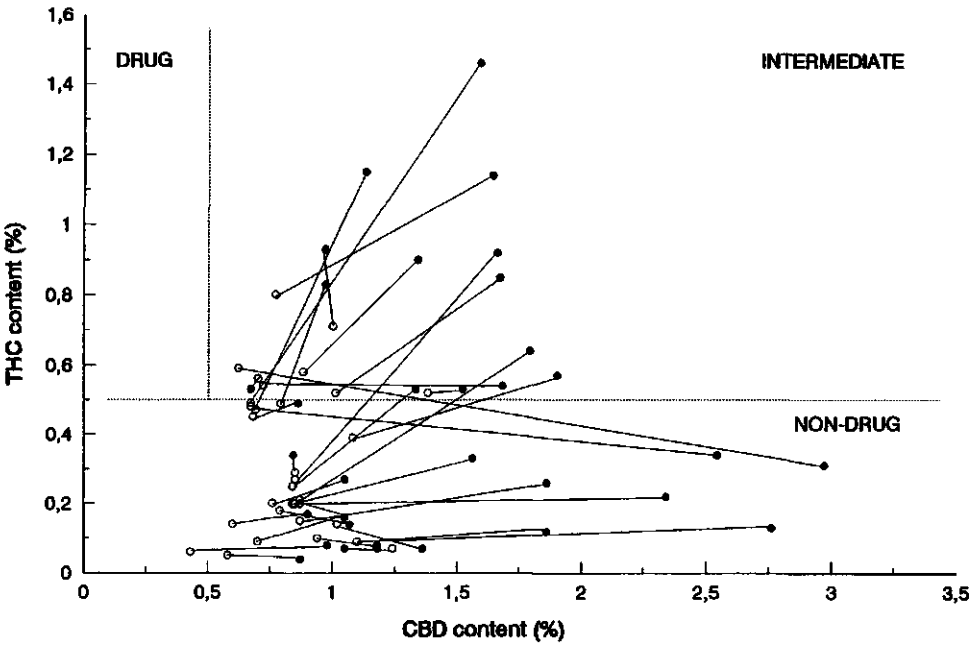


Fig. 3. Mean chemical phenotypes of 32 accessions in two years. Each line connects two symbols representing the performance of one accession. The solid symbols indicate the 1989, and the open symbols the 1990 position in the THC versus CBD diagram.

the individual accessions, corrected for the year effect (procedure Chapter 8), are presented in Appendix 2.

Fig. 4 shows the classification according to chemical phenotype for the accessions tested in 1990. There was a continuous pattern of variation for the contents of THC and CBD. The non-drug group (62 accessions) comprised fibre landraces from Central Russia, Turkey and Hungary, and fibre cultivars from Central Russia, France, former Czechoslovakia, Italy, Poland, Rumania and Hungary. Also weedy populations of Central European origin belonged to this group. The intermediate group (27 accessions) comprised fibre landraces from Hungary and Turkey, Hungarian hybrid F_1 fibre cultivars with one parent rich in THC, and fibre cultivars from Rumania, Italy, France and southern Russia. Also included were accessions from China, Turkey, Afghanistan and Nepal, not used for fibre production and with considerable contents of THC but with too high CBD content to meet the criteria of the drug phenotype. The drug phenotype group (8 accessions) included a marijuana strain like

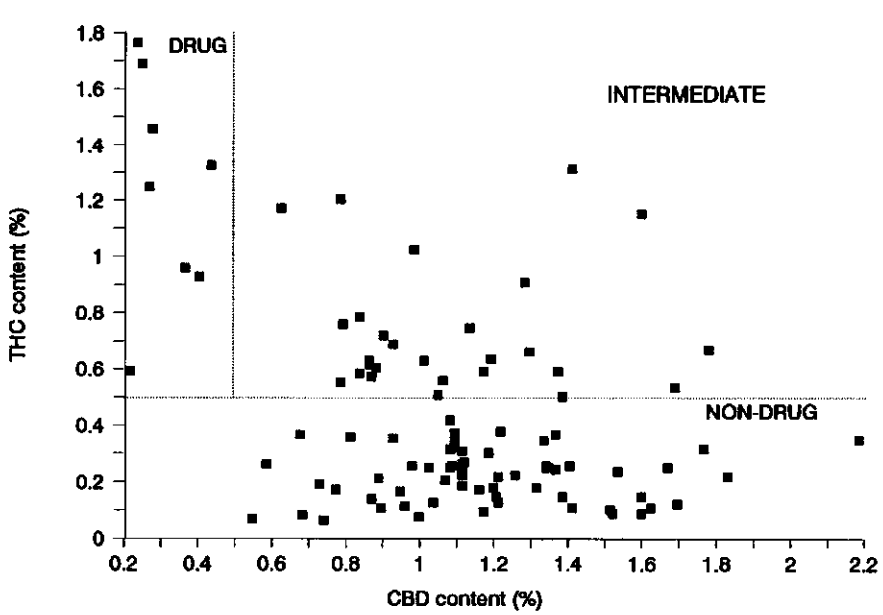


Fig. 4. Mean THC content versus mean CBD content of 97 accessions tested in 1990. The areas of the distinguished chemical phenotypes are demarcated.

'Skunk 1' (883270) and 'Nederwiet' (883260), which is a group indication for any drug strain grown in the Netherlands, and accessions from Lebanon (891194) and Afghanistan (891383). Also selections from Hungary (originally from China), 'Kinai Uniszex' (891342) and 'Kinai Kétlaki' (883046), which are used as parents for the production of hybrid F_1 fibre cultivars belonged to this group. Remarkable was the presence in the drug phenotype group of an accession which was sold as bird-seed in a Spanish supermarket (891348).

The order of magnitude of the measured contents of THC and CBD in well defined fibre cultivars corresponded rather well with the contents reported for these cultivars by Small & Beckstead (1973), Avico & Zuccaro (1984) and Petri *et al.* (1988). THC contents measured in the drug strains in the field trials were much lower than those occurring in indoors produced marijuana from these same strains. Due to breeding, and the cultivation of seedless cuttings ('sinsemilla', see Clarke, 1981) of superior individual plants, very high THC contents of over 10% and relatively low CBD contents (less than 1%) do nowadays occur in indoors produced marijuana in the Netherlands. There are no references reporting such extremely high contents of THC in outdoor grown *Cannabis* crops raised from seed. Brenneisen & Kessler (1987) however measured THC contents of up to 5.7% in

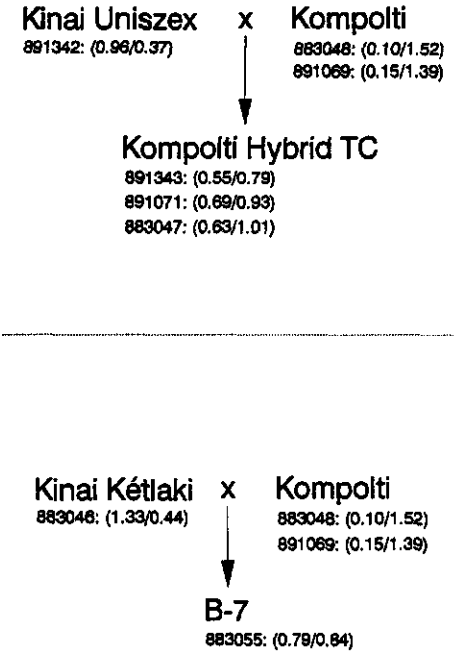


Fig. 5. Schemes showing the descent of two Hungarian hybrid cultivars (I. Bócsa, pers. commun., 1990). THC/CBD contents of the corresponding accessions are indicated in parenthesis.

marijuana of outdoor grown, widely spaced drug strain plants in Switzerland. Probably the psychoactive potency of drug strains is suppressed when plants are grown outdoors in a dense crop as in the present experiments.

The 1990 evaluation experiment included as well Hungarian F_1 hybrid cultivars as their parental populations. THC and CBD contents of the hybrid F_1 progenies resembled the means of the contents of the parental populations, indicating an intermediate inheritance of these characters at the population level (Fig. 5).

Relationships between chemical and non-chemical characters

The relationships between chemical and non-chemical characters were few and loose, and could best be described in terms of univariate statistics. Cluster analyses, of whatever type, did not reveal any form of group presence, nor did principal component analyses based on various subsets of the variables. This was in accordance with the findings of Small *et al.* (1976). Multiple discriminant analyses, weighed or unweighed, did not identify linear combinations of traits which performed substantially better in allocating accessions to their groups than did the best discriminating individual traits. This is in contradiction with the results of Small *et al.* (1976) who identified a linear combination of 23 characters which discriminated satisfactory, whereas each of the constituents of that linear combination had little discriminating power. Two groups of traits were identified with significant F-values for the phenotype factor (non-drug, intermediate, drug), and therefore possessing some discriminating power: the leaflet traits (1 and 7) and the group of phenological traits (12,13,14 and 19) (Table 4). Within both groups, traits had absolute mutual correlation coefficients all above 0.65. Between the groups little correlation existed, which was also the fact for most of the other

Table 4. Mean values of discriminating traits and standard errors for the means (S.E.) per chemical phenotype group. Traits are explained in Table 2.

	Chemical phenotype groups					
	Non-drug		Inter-mediate		Drug	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Leaflet traits						
1 Leaflet width	3.66	0.17	5.33	0.33	7.38	0.39
7 Leaflet index	4.34	0.05	4.01	0.09	3.82	0.16
Phenological traits						
12 Anthesis	184	3.5	199	5.2	220	6.1
13 Seed maturity	249	3.2	267	4.0	279	3.7
14 Vegetative stage	71	3.5	88	5.3	107	6.3
19 Parameter M	169	0.8	171	0.7	175	2.0

correlations between traits. No complementarity of variables was found for discriminating power. A representation of the results on the relationship between chemical characters and leaflet width (trait 1) is given by histograms in Fig. 6. Although leaflet width was the best discriminating character according to the size of the F-value, there was still considerable overlap between the phenotypes, no clearcut discrimination was possible. The relation between chemical phenotype and leaflet width must be regarded with some reservation as it may be biassed by the fact that the 1990 field trial comprised *indica* type drug strains only, and no *sativa* drug strains, which combine high THC content with morphological characteristics similar to non-drug *sativas* (E. Rosenthal, pers. commun., 1992). Indeed, the 1993 field trial included one drug strain (910972), explicitly received as *C. sativa*, which belonged evidently to the drug phenotype on the basis of its cannabinoid profile and had very slender leaflets.

Chi-square tests for the association of phenotype with certain categories of nominal and ordinal variables were significant. However it was not possible to identify the phenotypes by exclusive occurrence in particular categories.

Fig. 7 shows that the contents of the two main products of domesticated *Cannabis*, i.e. THC and bark fibre, were not strongly interrelated. Within the range of the diagram, every combination of the two contents appeared to be possible. There were likewise no obvious relations between chemical characters and

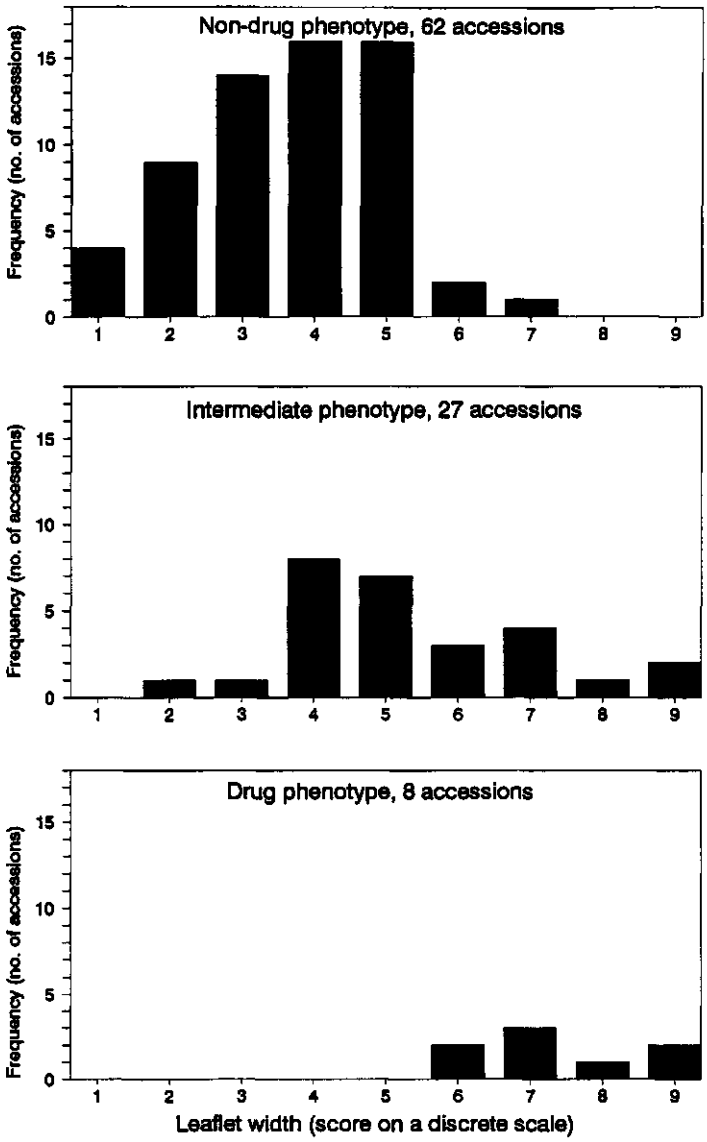


Fig. 6. The distribution of accessions over nine classes of leaflet width (1 = very narrow; 9 = wide with overlapping margins), per chemical phenotype group.

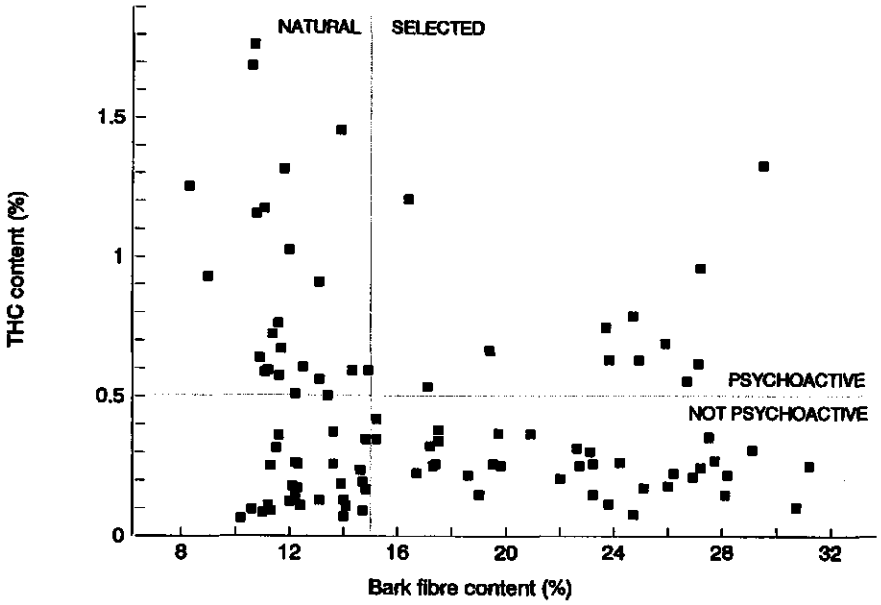


Fig. 7. THC content versus bark fibre content for 97 accessions tested in 1990. The vertical dotted line separates cultivars selected for high fibre content from accessions with natural fibre contents. The horizontal dotted line separates accessions with psychoactive potency from those with too limited THC content to be psychoactive.

agronomic characters such as plant height (trait 20) and susceptibility to *Botrytis cinerea* Pers. ex Fr. (trait 23).

CONCLUSIONS

Variation for contents of THC and CBD within and among accessions is considerable. Plants belonging to different phenotypic groups can easily be found within accessions. There are good prospects for selection for either high or low psychoactive potency. The inheritance of THC and CBD content at the level of populations appears to be intermediate.

The mean chemical phenotype of accessions is not very consistent over years, fluctuations in CBD content are larger than those in THC content.

Cannabinoid contents are not strongly associated with other agronomic traits. Average psychoactive potency of accessions can be predicted roughly on the basis of leaflet width and phenological development, however, strict relations do not exist. Morphological descriptors like achene characters, internode length and stem diameter possess no discriminating value for the chemical phenotype. Selection for a desired cannabinoid profile requires direct analytical methods.

Based upon:

Meijer, E.P.M. de, 1993. Evaluation and verification of resistance to *Meloidogyne hapla* Chitwood in a *Cannabis* germplasm collection. Euphytica 71: 49-56.

CHAPTER 7

EVALUATION AND VERIFICATION OF RESISTANCE TO *MELOIDOGYNE HAPLA* CHITWOOD IN A *CANNABIS* GERMPLASM COLLECTION

ABSTRACT

A large part of the *Cannabis* collection was evaluated for resistance to the root-knot nematode *Meloidogyne hapla* Chitwood in a seedling test. After inoculation with a larval suspension, significant variation was found for the number of galls and egg masses on the seedling roots. These parameters were considered as estimates for nematode infection and larval multiplication, respectively. A subset of the tested accessions was grown on a naturally infested arable field to study the relation between the test results and host characteristics in the field. The ranking order of accessions for the number of galls in the seedling test agreed well with that for the number of root galls on juvenile plants in the field. Therefore the test provides a useful indication for nematode infection. The number of egg masses in the seedling test and the population density of *Meloidogyne* measured in post-treatment soil samples of the field trial, showed a comparable ranking of accessions. Differences between accessions in the field were, however, smaller and not statistically significant.

Key words: Meloidogyne hapla, resistance, root-knot nematode

INTRODUCTION

An increase of root-knot nematodes (*Meloidogyne* spp.) is expected on lighter soils in the Netherlands due to the increase of dicotyledons in crop rotations and a decrease in the use of soil disinfectants. As hemp for paper pulp was intended to be grown in an area with sandy soils and narrow crop rotations dominated by potatoes and sugar beet, being a poor host for *Meloidogyne* spp. was an important precondition for a successful introduction.

Breeding hemp for resistance to soil pathogens seems unprecedented. Hemp is generally considered to be a self-compatible crop. Continuous cropping for 5 to 10 years was common in the former USSR and had no apparent effect on yield (V.G. Virovets, pers. commun., 1991). In France, cultivation currently occurs for two or three consecutive years (J.P. Mathieu, pers. commun., 1992). In the Netherlands,

uninterrupted cultivation of fibre hemp was practice until the 19th century (van Hall, 1828; Snellen, 1853). Investigations focusing on the effect of hemp on nematode populations seem therefore more opportune for the introduction of hemp in rotations of susceptible crops, than those dealing with nematode damage caused to the hemp crop itself.

Kok & Coenen (1994) reported that *Cannabis* is a poor host for *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley. The host status of *Cannabis* for *Meloidogyne hapla* Chitwood was first reported by Norton (1966). Recent preliminary research in the Netherlands indicated that, in comparison to other crops, some tested fibre hemp cultivars were moderate hosts for this nematode. Better hosts were lettuce, sugar beet and pea (A. Termoshuizen, pers. commun., 1990), and chicory and lupin (M. Doorgeest, pers. commun., 1988) whereas nematode multiplication was less on sunflower, Brussels sprouts and flax (M. Doorgeest, pers. commun., 1988). The present chapter explores the variation within the genus *Cannabis* in host reactions to *M. hapla*.

Testing for Meloidogyne *resistance*

The method applied at CPRO for testing resistance to *Meloidogyne* spp. is based on a mass screening method for resistance to sugar beet nematode (*Heterodera schachtii* Schmidt) (Lubberts & Toxopeus, 1982). The adapted protocol was used for testing reactions to *Meloidogyne* spp. in cucurbits, tomato and crucifers (Boukema *et al.*, 1983; den Nijs & Hofman, 1983). According to this method, seedlings of the tested crops are inoculated with a larval suspension. Incubation lasts for a period of one generation cycle of the nematode (6 to 8 weeks) after which the roots of the plantlets are examined. The numbers of root galls and egg masses are used to classify according to resistance.

This method was used to evaluate *M. hapla* resistance in the CPRO *Cannabis* collection (Chapter 2). Two additional tests were carried out. For a small subset of accessions the relation between the evaluation parameters and larval multiplication under test conditions was investigated. A number of individual plants with exceptionally low numbers of galls and egg masses in the evaluation trial were tested once again to verify resistance and to obtain resistant genotypes for breeding purposes.

The relation between seedling test results and resistance in the field

Evaluation results are only applicable in practice when a strong relation exists with infection and larval reproduction under conditions of cultivation of the crop involved. According to Boukema *et al.* (1984) the results of a seedling test for screening resistance to *M. incognita* (Kofoid & White) Chitwood in *Cucumis* spp. agreed well with larval multiplication

under commercial glasshouse conditions. In this chapter such a relation is studied for *Cannabis* and *M. hapla* in a field trial. Effects of crop cultivars on *Meloidogyne* population density can be estimated by the infestation in soil samples collected after the growing season (post-treatment samples). Infestation is either estimated by extraction of second-stage juveniles or by bioassays with susceptible host plants (Barker *et al.*, 1985). Both methods were applied to soil samples from a trial with *Cannabis* accessions with distinct evaluation results, grown on a field with a natural *M. hapla* infestation.

MATERIALS AND METHODS

Testing for Meloidogyne *resistance*

Evaluation of the CPRO *Cannabis* collection

Pre-germinated seeds of 148 *Cannabis* accessions and a susceptible standard (lettuce, *Lactuca sativa* L., cv. Norden) were planted in open-ended PVC-tubes (1.5 x 2 x 12 = 36 cm^3), containing moist silversand. These were placed in closed asbestos containers, each one containing 148 tubes with *Cannabis* accessions and 20 with lettuce. The test comprised a total of 25 containers, each being a replication of a randomized block design. Containers were placed in a growth chamber at 20 °C and 90 to 100% RH. A large-meshed gauze was installed above each container to support the densely spaced plantlets. Plants were illuminated 20 h per day to prevent flowering, but despite this long photoperiod, some male plants still flowered. Two weeks after planting, each plantlet was inoculated with a mean number of 150 prehatched second stage *M. hapla* juveniles suspended in 2 ml water using a veterinary syringe (inoculum 'HP1', a sample of a natural population collected ca. 1985 in Maasbree, the Netherlands). Seven weeks after inoculation root systems were washed free of sand on a sieve. Before examination, egg masses were stained for 10 min in a Phloxine B solution (150 mg/l). Observed parameters per plant were: number of galls, number of egg masses and root fresh weight. Calculated parameters were: number of galls per g root weight, number of egg masses per g root weight and mean number of egg masses per gall.

The parameter values of the lettuce plants were used to estimate 'container' (replication) effects, and to test for homogeneity of infection within containers. Anova revealed significant differences ($p<0.001$) among containers for each evaluation parameter, but infection was homogeneous within containers, so there was no need to exclude replications.

Larval multiplication under test conditions

Four distinct accessions were selected on the basis of geographic origin and domestication history. For

these accessions, evaluation parameters were determined without staining the egg masses with Phloxine B. After the assessment of evaluation parameters the second generation larvae were hatched by placing each root separately on a nematode filter covering a water layer in a closed petri-dish. Hatching occurred at 20 °C, in the dark at maximal RH, over a three week period. The water was weekly refreshed after decanting the larvae containing water which was stored at 4 °C. The pooled larvae production per root was estimated by counting three subsamples taken from the combined and well-stirred effluents.

Testing clones of plants with low numbers of root galls and egg masses

Twenty-five individual plants with exceptionally low numbers of galls and egg masses on normally developed roots were retained during the collection evaluation. Clones consisting of five cuttings were made from each of these plants and from each of five plants of cv. Kompolti Hybrid TC which was used as a standard. Rooted cuttings were planted in cylindrical tubes (diameter = 4.5 cm, height = 15 cm, volume = 240 cm^3), filled with moist silversand, and placed on separate trays in a growth chamber at 20 °C and 90 to 100% RH. The 30 genotypes were tested as clones in a randomized block design with each cutting as one of five replications. Two weeks after planting, each cutting was inoculated with 600 larvae. Seven weeks after inoculation, the evaluation parameters were once more determined.

The relation between seedling test results and resistance in the field

Six accessions covering the range of variation observed in the collection evaluation were grown on a naturally infested sandy soil in the southeast of the Netherlands in 1992. Crops in the preceding four years had been pearl onions, scorzonera, potatoes and sugar beet, successively. *M. hapla* was the predominant *Meloidogyne* species, but *M. chitwoodi* also occurred. An exploratory sampling shortly before sowing showed on average 100 free-living *Meloidogyne* larvae per 100 cm^3 of soil. The trial had a randomized block design with four replicates and plots of 8 x 6 = 48 m^2. It was sown on 8 May 1992, at a seeding rate of 160 vital seeds per m^2 and a row distance of 13.3 cm. For all accessions, plant density decreased in the course of the season from 130 plants/m^2 shortly after emergence to about 85 on 7 September.

The side root formation and the degree of infection by *Meloidogyne* were assessed on 29 July and 7 September using ten soil cores (length = 20 cm, diameter = 4.8 cm, volume = 400 cm^3) per plot. These were collected with a soil sampler exactly in the middle between rows. Soil was washed from the roots and per core the root

fresh weight and the number of galls were determined. The number of galls per gram root weight was calculated.

The post-treatment *Meloidogyne* infestation was estimated in soil collected at harvest i.e. on 16 November 1992, and once again on 22 January 1993. Per plot, composite soil samples of five litres were collected, consisting of about 120 cores taken with a soil sampler of 1.3 cm width and to a depth of 25 cm. Each composite sample was gently mixed and split into ten subsamples of 400 cm^3 for testing infestation using bioassays (Zondervan, 1987) and two of 100 cm^3 for nematode extraction. Bioassays were carried out with lettuce cv. Norden and included planting of four-week-old lettuce plants in each pot containing a 400 cm^3 subsample of soil. Pots from one block in the field trial were randomly placed in one block in a greenhouse. After six weeks the galls on the root systems of the lettuce plants were counted.

For counting free-living juveniles of *Meloidogyne* spp., nematodes were extracted using an Oostenbrink elutriator ('s Jacob & van Bezooijen, 1984). The larvae were collected on a set of 0.045 mm sieves, transferred to a nematode filter and hatched into a water layer over one night. The number of *Meloidogyne* larvae extracted from 100 cm^3 soil was estimated by counting larvae in three subsamples taken from the well-stirred decanted water layer.

RESULTS AND DISCUSSION

Testing for Meloidogyne *resistance*

Evaluation of the CPRO *Cannabis* collection

The analyses of variance, and the discussion in this section are restricted to 123 accessions. Excluded were three accessions with large residuals, and 22 accessions represented with less than 15 surviving plants (replications). 'Accessions' affected significantly the variation for each evaluation parameter (Table 1).

Table 1. Anova tables of the evaluation of 123 *Cannabis* accessions for various parameters. (1 = no. of galls; 2 = no. of egg masses; 3 = root fresh weight; 4 = no. of galls per g root weight; 5 = no. of egg masses per g root weight; 6 = no. of egg masses per gall).

Source of variation		Mean squares					
	d.f.	1	2	3	4	5	6
Container	24	2596	1794	.0755	88574	66118	.666
Accession	122	467***	308***	.0208***	20386***	14067***	.191***
Residual	2450	128	77	.0043	5910	3303	.049

*** significant at p=0.001.

Table 2. $LSD_{0.05}$, range and grand mean for the accession means of various evaluation parameters (based on 123 accessions).

Parameter	$LSD_{0.05}$	Range	Mean
No. of galls	6.3	10.7 - 34.9	23.6
No. of egg masses	4.9	4.0 - 21.9	12.7
Root weight (g)	0.04	0.09 - 0.26	0.18
Galls per g root weight	42.6	67.7 - 222.2	140.2
Egg masses per g root weight	31.9	21.9 - 151.2	75.1
Egg masses per gall	0.12	0.22 - 0.68	0.51

More variation among accessions was found for the number of egg masses than for the number of galls. Due to significant differences among accessions in root fresh weight, the numbers of galls and egg masses needed to be expressed per unit of root weight (replacing parameters 1 and 2 by parameters 4 and 5, respectively). This avoids selection in favour of genotypes with small root systems.

A summary of the observed variation is presented in Table 2. The numbers of galls and egg masses per unit root weight are presented for the individual accessions in Appendix 2. The lowest numbers were

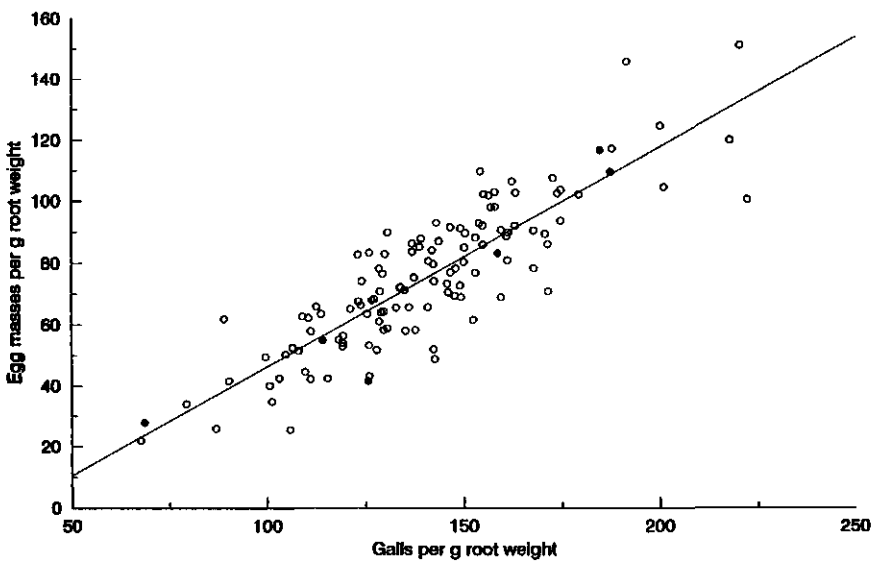


Fig. 1. The relationship between the densities of egg masses and galls (123 accession means). The performance of six accessions used for verification of resistance in the field trial is indicated by solid symbols.

Table 3. Coefficients of correlation between the second generation larvae production and the numbers of galls and egg masses per root system, respectively.

Accession [a]	No. of plants	Correlation coefficients	
		Galls-larvae	Egg masses-larvae
883140	14	.54*	.71**
883289	22	.75**	.89**
891343	21	.68**	.92**
891383	23	.52*	.60**
Overall	80	.60**	.83**

*, ** significant at p=0.05 and p=0.01, respectively; [a] see Appendix 1 for passport data

observed in the Hungarian fibre cultivar Kompolti Sargászáru, in the wild Nepalese population 891193 and the drug strain 891196 both obtained from the same accession donor, and in the accessions 880973 and 891240 both obtained from the Madrid botanical garden (see Appendix 1 for passport data of accessions). The highest densities were found in a group of closely related French fibre cultivars and in Russian cultivars and landraces.

Fig. 1 shows the observed variation for numbers of galls and egg masses, both expressed per unit root fresh weight. Although significant differences between accessions existed for the ratio of egg masses to galls (Tables 1, 2), there was a strong correlation between the two traits (r=0.86; N=123; p=0.01).

Larval multiplication under test conditions

For four accessions, the second generation larvae production was related to the densities of galls and egg masses (Table 3). Due to its stronger correlation with the number of larvae, the number of egg masses was considered to be a better indicator for larval multiplication than the number of galls.

There were no significant differences among the four tested accessions in numbers of galls and egg masses, in larvae production and in the ratio larvae to egg masses (Table 4). The ratio larvae to galls differed significantly, i.e. was more specific for accessions, indicating again that larval multiplication is predicted best by numbers of egg masses.

Table 4. Accession means for numbers of galls, egg masses and second generation larvae and the ratios of larvae to galls and larvae to egg masses. For the larvae/gall ratio, means showing a common letter are not different at p = 0.05.

Accessions	No. of galls	No. of egg masses	No. of larvae	Larvae/ gall	Larvae/ egg mass
891343	25.5	13.5	3264	117 ab	224
891383	24.7	13.6	3305	138 bc	246
883140	29.0	14.0	2329	81 a	180
883289	24.5	17.0	4520	171 c	258
$LSD_{0.05}$	-	-	-	48	-

Testing clones of plants with low numbers of root galls and egg masses

Test results of 25 clones derived from plants with very low numbers of galls and egg masses in the collection evaluation are presented in Table 5. Numbers of galls and egg masses obtained with clones of the standard cultivar were much lower than those obtained with seedlings (see Table 2). This difference can hardly be attributed to the lower inoculation rate, i.e. 2.5 versus 4.2 larvae/cm^3 soil, respectively. In the secondary roots of cuttings, infection is probably more hampered than in the primary roots of seedlings.

Table 5. Results of the testing of cuttings of 25 selected plants and 5 plants of cv. Kompolti Hybrid TC (standard). Results of the standard and the rejected clones are presented as ranges of clone means.

	Clone no.	Source accession	Clone means	
			Galls/ g root wt.	Egg masses/ g root wt.
Retained clones	5	891193	0.0	0.0
	7	891001	0.5	0.0
	14	891196	4.0	0.0
	12	891193	4.8	0.8
Standard clones (N = 5)			2.5 - 5.3	0.9 - 1.7
Rejected clones (N = 21)			1.5 - 49.2	0.4 - 33.3

Four clones consistently with low values for both evaluation parameters were retained for breeding purposes. These best performing clones were however not significantly better than clones of the moderately susceptible standard cultivar. The majority of the clones performed worse than those of the standard. Probably these plants were not properly inoculated in the collection evaluation. The retained clones number 5 and number 12 originated both from accession 891193. The mean result of this accession was the lowest in the collection evaluation. Together with accession 891196 (source of retained clone number 14) it was obtained from one donor address (Appendix 1).

The relation between seedling test results and resistance in the field

Infection in the field trial was estimated by the number of galls per unit hemp root weight. On 29 June, 7 weeks after sowing there were still no galls visible. Gall counts for root samples taken on 7 September, at the end of the vegetative stage, are presented in Table 6. The ranking order of accessions for the number of galls per gram root fresh weight agreed rather well with that in the collection evaluation. There were significant differences between accessions, the relative differences in the field trial were even larger than those in the seedling test. The power of discernment of the field trial was however smaller than that of the seedling test.

Post-treatment infestation in the field trial as measured by the number of galls in the lettuce bioassay and

Table 6. Accession mean number of galls per g *Cannabis* root fresh weight in the seedling test and in the field trial. Per column, means showing a common letter are not different at $p = 0.05$.

Accessions	Galls per g root fresh weight	
	Seedling test	Field trial (7 September)
Kompolti Sargászáru	69 a	7.5 a
Kompolti Hybrid TC	113 b	13.8 a
883213	126 bc	10.1 a
Kompolti Hyper Elite	159 cd	24.5 ab
Fedrina 74	185 d	49.2 c
Futura 77	187 d	33.4 bc
$LSD_{0.05}$	42	17.7

Table 7. Accession means for the numbers of egg masses per g root fresh weight in the seedling test and estimates of post-treatment field infestation, i.e. mean numbers of galls per lettuce root system in the bioassay and mean numbers of extracted free-living larvae, determined with soil samples collected on 16 November and 22 January. Per column, means showing a common letter are not different at $p = 0.05$.

Accessions	Seedling test	Field trial (16 November)		Field trial (22 January)	
	Egg masses/ g root wt.	Bioass. No. of galls	Extracted larvae in 100 cm^3	Bioass. No. of galls	Extracted larvae in 100 cm^3
K. Sargászáru	28 a	130	56 a	96	56
883213	41 a	132	82 ab	90	79
K. Hybrid TC	55 ab	203	108 abc	133	130
K. Hyper Elite	83 bc	244	138 bc	123	101
Futura 77	110 cd	191	88 ab	139	113
Fedrina 74	117 d	196	185 c	130	158
$LSD_{0.05}$	31	-	79	-	-

by the number of extracted free-living *Meloidogyne* juveniles is presented in Table 7. The bioassay did not reveal significant differences among accessions using soil collected at either of the two dates. Accessions only differed significantly for the number of extracted free-living larvae for soil collected on 16 November. The ranking order of accessions for the measures for *Meloidogyne* infestation in the field agreed rather well with that for the density of egg masses in the seedling test. Differences between accessions in the seedling test were not proportionally reflected in the field. This might be due to various abiotic factors, the absence of antagonistic organisms in the seedling test, and differences between the *M. hapla* inoculum used in the test and the *M. hapla* population in the field.

Since an average number of 100 free-living *Meloidogyne* larvae per 100 cm^3 soil was extracted from soil collected shortly before sowing (pre-treatment), it can be concluded that the population was not strongly altered by cultivation of *Cannabis* in general. Cv. Kompolti Sargászáru seems to have decreased and cv. Fedrina 74 seems to have increased the population somewhat (Table 7).

CONCLUSIONS

The used seedling test shows significant variation among *Cannabis* accessions in number of galls and egg masses per unit root fresh weight developed in reaction to inoculation

with *Meloidogyne hapla* larvae. The number of egg masses discriminates more between accessions and is a better measure for larval reproduction.

The number of galls in the seedling test reflects the potential degree of infection of *Cannabis* in a naturally infested field, both with respect to ranking orders and relative differences between accessions. The power of discernment of a field trial is smaller than that of the seedling test.

The ranking order of accessions for the multiplication rate of *M. hapla* in the field agrees rather well with that for the number of egg masses in the seedling test. Relative differences between accessions are however much smaller in the field than in the seedling test and not statistically significant.

The population density of *M. hapla* in the field is not strongly affected by cultivation of *Cannabis*.

Selection of genotypes for breeding *Cannabis* strains with increased resistance to *M. hapla*, by means of the described seedling test, is a realistic possibility.

Based upon:

Meijer, E.P.M. de & L.C.P. Keizer, 1994. Multivariate patterns of diversity in *Cannabis*. Submitted to Genetic Resources and Crop Evolution.

CHAPTER 8

MULTIVARIATE PATTERNS OF DIVERSITY IN *CANNABIS*

ABSTRACT

This chapter is directed at diversity for the various, previously described agronomic and morphological traits considered together. For the interpretation of diversity patterns, accessions were grouped on the basis of the presumed purpose and status of domestication resulting in the recognition of four 'plant-use groups': fibre cultivars, fibre landraces, drug strains, and truly wild or naturalized populations. Principal component analysis showed that stem quality, phenological development, psychoactive potency and host reactions to root-knot nematodes explained most of the total variation among accessions. The plant-use groups were reasonably well separated in the principal component space. Discriminant analysis showed that the *a priori* defined plant-use groups could be discriminated quite well on the basis of linear combinations of all agronomic and morphological traits. The contents of bark fibre and cannabinoids had the highest grouping value. Re-allocations concerned mainly old fibre cultivars which were placed in the group of fibre landraces due to a low content of bark fibre.

A genetic characterization of accessions was based on electrophoretic patterns of seed proteins. Isoelectric focusing patterns of bulk seed extracts showed variation for the presence of six out of 45 protein bands in the pH range from 4.75 to 6.40. Banding patterns were however independent from any grouping based on origin or agronomic and morphological traits and did not reflect expected common ancestry of accessions.

Key words: agronomic traits, electrophoresis, seed proteins

INTRODUCTION

Cannabis variation was previously described in a univariate way for traits related to stem yield (Chapter 3), stem quality (Chapter 5), psychoactive potency (Chapter 6) and resistance to the root-knot nematode *Meloidogyne hapla* Chitwood (Chapter 7). Also a number of morphological traits, used for discriminating taxa in *Cannabis*, were observed to establish if a relation exists between current taxon classifications and groupings of agronomical interest. The present study compares the various agronomic and morphological traits with regard to stability, it investigates trait associations, and establishes multivariate patterns of diversity. The study seeks to relate agronomic and morphological diversity both to groupings of accessions pre-defined

on the basis of passport data and to groupings genetically characterized on the basis of electrophoretic seed protein patterns.

Classification of Cannabis

The interpretation of diversity patterns requires an *a priori* classification (labelling) of accessions. Apart from taxon name, possible criteria for the labelling of accessions are geographic origin, domestication status and the type of plant-utilization.

There is no general agreement on the infrageneric taxonomic treatment of *Cannabis*. Small & Cronquist (1976) recognized one complex species *C. sativa* L. with two subspecies, i.e. *sativa* and *indica* each of these comprising two botanical varieties. Their key to subspecies and varieties is however based on taxonomically unacceptable criteria such as the domestication status, the purpose for which plants are cultivated and their psychoactivity. Others treated the genus as polytypic and discriminated the species *C. sativa* L., *C. indica* Lam. and *C. ruderalis* Janischevsky (Schultes *et al.*, 1974; Emboden, 1974; Anderson, 1980; Emboden 1981). The key to the species of Schultes *et al.* (1974) uses plant height, degree of branching and achene morphology. Anderson (1980) added leaf morphology to discriminate *C. sativa*, *C. indica* and *C. ruderalis*. The subspecies of *C. sativa*, ssp. *sativa* (not psychoactive) and ssp. *indica* (psychoactive) of Small & Cronquist (1976) do not correspond with the species *C. sativa* and *C. indica* of Schultes *et al.* (1974), Emboden (1974) and Anderson (1980) since the latter authors circumscribe these taxa on morphological grounds, irrespective of the psychoactive potency. Growers and breeders of drug strains generally adhere to a polytypic genus concept and distinguish, among the domesticated drug strains, both *C. indica* with wide leaflets, compact habit and early maturation and *C. sativa* with narrow leaflets, slender habit and late maturation (e.g., Cherniak, 1982).

Agronomic and morphological traits

The agronomic traits 5,6,7 and 9 to 20 of Table 1 are relevant to the introduction of hemp in Dutch field crop rotations and its utilization for paper pulp. Practical implications have been described in Chapter 6 for traits related to psychoactive potency (5,6,7), In Chapter 3 for traits related to stem yield (9,10,11), for quality-related traits (12 to 18) in Chapters 4 and 5 and for host reactions to *Meloidogyne hapla* (19,20) in Chapter 7. Some of the agronomic traits have been used for taxon delimitation as well. Anderson (1974) for example discriminated *C. sativa* and *C. indica* on the basis of wood characteristics such as fibre length and width (12,13,14). Small & Cronquist (1976) used 'intoxicant potential' (5,6,7) as criterion for discriminating *C. sativa* ssp. *sativa* and ssp. *indica*. Plant size (11) was

Table 1. Codes and descriptions for observed traits

No.	Code	Description
1	ACHtsw	Thousand seed weight (g).
2	ACHlen	Achene length (mm), average length of 20 well developed seeds.
3	ACHabs	Achene abscission layer, 5 classes: always absent to always and conspicuously present.
4	ACHmar	Achene marbling of perianth, 5 classes: not marbled to conspicuously marbled.
5	THCcon	THC content in leaf dry matter of mature female inflorescences (%).
6	CBDcon	CBD content in leaf dry matter of mature female inflorescences (%).
7	CANrat	Cannabinoid ratio, (THC content)/(CBD content).
8	LEAind	Leaflet index, length/width ratio of central leaflet of primary leaf at fourth stem node.
9	ANTday	Day no. of anthesis, 50% of the plants, irrespective of sex, with visible inflorescences.
10	MATday	Day no. of initial seed maturity, first achenes of 50% of female and/or hermaphrodite plants resistant to compressing.
11	PLAhei	Height of mature female plants (cm).
12	FIBlen	Wood fibre length (μm).
13	FIBwid	Wood fibre width (μm).
14	FIBrat	Wood fibre length/width ratio.
15	WOOcor	Woody core mass fraction in stem dry matter (%).
16	SECfib	Secondary bark fibre mass fraction in stem dry matter (%).
17	PRIfib	Primary bark fibre mass fraction in stem dry matter (%).
18	TOTfib	Total bark fibre mass fraction in stem dry matter (%).
19	HAPgal	Number of *Meloidogyne hapla* root galls per g root fresh weight.
20	HAPegg	Number of *M. hapla* egg masses per g root fresh weight.

employed for species delimitation by Schultes *et al.* (1974) and Anderson (1980).

Morphological traits were selected for their presumed diagnostic value for the recognition of taxa. The morphology of achenes (1,2,3, 4), being related to natural dispersal mechanisms, is rather conservative and enables the recognition of wild (or naturalized) and domesticated taxa (Vavilov, 1926; Emboden, 1974; Schultes *et al.*, 1974; Small & Cronquist, 1976). Anderson (1980) employed a leaflet index (8) for species discrimination.

Electrophoretic characterization of Cannabis

Electrophoretic variation for seed proteins is generally considered to reflect genetic variation (e.g., Vaughan, 1983; Gepts, 1990). Yumaguzina *et al.* (1979a,b) discriminated successfully central and southern Russian *Cannabis* ecotypes on the basis of seed protein patterns. Lawi-

Berger *et al.* (1982) compared seed protein patterns of drug and fibre strains from varying geographic origin. Although variation was found, the patterns were neither related to psychoactive potency, nor to origin.

MATERIALS AND METHODS

A priori *grouping of accessions*

The taxon name under which accessions were received did not provide unambiguous information on their identity. Many accessions were received merely under the genus name. If an infrageneric name was assigned, fibre strains were received as *C. sativa* and drug strains as *C. sativa* or *C. indica* or as hybrids between these. Accessions collected in the wild in eastern Europe were received as *C. sativa* ssp. *ruderalis* or *C. sativa* var. *spontanea*. The latter name was used by Vavilov (1926) as a synonym for *C. ruderalis* Janischevsky.

The available keys (Schultes *et al.*, 1974; Small & Cronquist, 1976; Anderson, 1980) did not enable a tentative taxon determination. Under the high plant densities in the field trials the branching of accessions received as *C. indica* was not expressed whereas the plant height of accessions named *C. indica*, *C. sativa* ssp. *ruderalis* or *C. sativa* var. *spontanea* exceeded by far the sizes given by Schultes *et al.* (1974) for *C. indica* and *C. ruderalis*, respectively. None of the fibre strains would have been classified as *C. sativa* on the basis of leaflet length to width ratios according to the criteria of Anderson (1980).

Geographic origin and adaptation latitude were known attributes for the majority of the fibre strains and the wild populations. Drug strains were often used for indoor cultivation and were hence not adapted to the natural conditions of either their primary or secondary region of origin.

Finally, labelling based on a combination of population status (breeder's cultivar; landrace; wild or naturalized population) and the purpose for which accessions were developed (drug or fibre production) seemed most adequate and least artificial. One of the following 'plant-use labels' could be assigned to 162 accessions out of the total collection of 206.

F: fibre strains, named breeder's cultivars (68 accessions)
f: fibre strains, landraces (50)
d: drug strains (28)
w: truly wild and/or naturalized populations (16)

There was insufficient information to separate landraces and breeder's cultivars among drug strains. Group w consisted of naturalized populations from central European origin and a few accessions collected in the wild in Nepal where truly wild or nearly wild populations probably still occur (Sharma, 1979). The 44 accessions that remained ungrouped comprised, besides unspecified accessions, one ornamen-

tal cultivar and some bird-seed samples.

Assessment of agronomic and morphological traits

Of the total of 206 accessions, including some presumed duplicates, 160 were evaluated for the traits 1 to 18, and 132 for all traits listed in Table 1. Traits 3 and 4 were scored on an ordinal scale, other traits were continuous variables. Seed lots from greenhouse multiplications, and for commercial cultivars from original shipments, were used for the unreplicated observation of achene characters (1 to 4). Two field trials were carried out for the assessment of traits 5 to 18. Ninety-seven accessions were tested in 1990 and 75 in 1993. Ten reference accessions, representing the range of variation for most traits, were included in both trials. The trials were carried out at CPRO (52° N latitude) on a sandy soil rich in organic matter. Accessions were grown in two replicates using a randomized block design with plots of 4.5 x 0.75 m^2. Each plot contained three rows, 25 cm apart. Sowing dates were 10 April in 1990 and 14 April in 1993. Shortly after emergence plots were thinned to a density of 35 plants per m^2 in 1990 and 60 in 1993. Methods for the assessment of cannabinoid contents are described in Chapter 6. The assessment of stem quality is described in Chapter 5 and that of stem yield-related traits in Chapter 3. Host reactions to *Meloidogyne hapla* were assessed in a seedling test (Chapter 7).

Treatment of agronomic and morphological data

The plot mean scores of the reference accessions for traits 5 to 18 were used to test the effect of 'year' and the 'year x accession' interaction. Correlations between years were calculated on the basis of reference accession means. Linear regression coefficients for the relation between the scores in two years were also calculated on the basis of the means of the reference set. These coefficients were used to adjust per trait the mean accession scores obtained in 1993 to a fictitious 1990 level. Negative adjusted values for traits 5,6,9,11 and 16 were avoided by forcing the intercept at the origin. For 160 accessions, labelled F,f,d,w or *, the adjusted (1993) and measured (1990) accession means for traits 5 to 18 were combined in one matrix, together with the measured means for traits 1,2,3,4,19 and 20. This matrix (Appendix 2) was used to test in a univariate way the plant-use group factor and to calculate mutual correlations between traits.

Multivariate patterns of variation were established by performing principal component analysis and discriminant analysis on the standardized data matrix. Principal component analysis was performed to identify the traits that contributed most to the total variation among accessions. Accession scores on the first two axes were used to investigate if groups could be identified *a posteriori* in the principal component space.

Discriminant analysis was performed to verify the *a priori* plant-use group classification, and to identify the most discriminative traits. Principal coordinate analysis was performed to calculate Mahalanobis distances between group centroids. Overall group differences were tested by multiple analysis of variance (manova). Calculations and tests were performed using Genstat 5 statistical package (Payne & Lane, 1987).

Electrophoresis

The technique of isoelectric focusing in immobilized pH gradient (IEF-IPG) was applied as it gives a fine resolution of large numbers of proteins (van den Berg, 1991).

Bulk samples were made for 150 accessions, each consisting of 50 achenes. For some accessions, achenes from more multiplication cycles were included separately. Various subsamples taken from a few seed lots were analyzed to test the reproducibility of the procedure. After weighing samples, achenes were crushed in plastic test tubes. Four µl extraction buffer per mg seed weight and a spoon tip of silver sand were added. The extraction buffer consisted of 9 M urea, 2% (v/v) Triton X-100, 2% (v/v) ß-mercaptoethanol and 2% (v/v) Pharmalyte 3-10. Homogenization was achieved by grinding for one min using a homogenizer connected to a drilling machine. The mixture was incubated at room temperature for 30 min and centrifuged for 10 min (7000 rpm). Supernatants were stored at -20 °C until further use. Protein concentrations were determined according to Ramagli & Rodriguez (1985), the extracts were further diluted with extraction buffer to obtain standardized protein contents of 5 mg/ml. Proteins were separated on a horizontal electrophoresis system (Multiphor II, Pharmacia). All separations were performed at 14.5 °C on precast gels with a linear pH gradient from 4 to 7. Before use the gels were rehydrated in 8 M urea, 0.5% (v/v) Triton X-100, 0.15% (w/v) dithiotreithol, 0.5% (v/v) Pharmalyte 3-10, 20 mM acetic acid and a few grains of Orange G. Quantities of 2 µl of the extracts were applied at the anodic side of the gels, pI markers were loaded as external standards on the outer lanes. The running conditions were 300 V, 1 mA, 5 W for 2:30 h (650 Vh), followed by 3500 V, 1 mA, 5 W for 5 h (16000 Vh). The proteins were fixed by incubation overnight in a 10% TCA solution. General silver staining was performed according to Heukeshoven & Dernick (1985).

Two subsamples of each extract were separated on different gels. The protein patterns were evaluated visually by scoring the absence and presence of the variable bands. Per extract the scores were based on the two replications considered together.

Table 2. Coefficients of correlation between years for reference accession means, and F-values for the effect of 'year', 'accession' and the 'year x accession' interaction term.

Trait		Nacc.	r_{years}	F_{year}	$F_{accession}$	$F_{year \times acc}$
5	THCcon[1)]	9	0.84**	143.75**	56.84***	3.41*
6	CBDcon[1)]	9	0.48	172.10**	47.69***	5.16**
7	CANrat[1)]	9	0.99**	0.36	103.04***	2.62*
8	LEAind	10	0.79**	2.25	17.80***	2.57*
9	ANTday	9	0.87**	9.08	76.56***	5.75***
10	MATday	9	0.93**	0.00	58.71***	2.17
11	PLAhei	9	0.77*	7.82	49.31***	7.31***
12	FIBlen	9	0.70*	18.29*	3.47*	0.81
13	FIBwid	9	0.59	1.13	3.28*	0.97
14	FIBrat	9	0.52	1.72	1.75	0.74
15	WOOcor	6	0.90*	3.95	5.81**	0.62
16	SECfib[1)]	6	0.99**	4.60	52.81***	3.05*
17	PRIfib	6	0.96**	1.58	11.67***	0.46
18	TOTfib	6	0.99**	1.85	72.63***	0.18

*,**,*** significant at p=0.05, p=0.01 and p=0.001, respectively; [1)] log transformed data were used for anova

RESULTS

Agronomic and morphological traits

Stability of traits

Table 2 presents measures for the stability of traits over years. Low correlations between years were found for CBD content and wood fibre dimensions and high correlations for stem fractions, phenological events and the cannabinoid ratio. Cannabinoid contents, but not their ratio, and wood fibre length were significantly affected by 'year'. Reference accessions had much lower cannabinoid contents in the cold summer of 1993 than in the hot summer of 1990. Except the wood fibre dimensions all traits were much stronger affected by 'accession' than by 'year'. The 'year x accession' interaction term was significant for day of anthesis, stem height, cannabinoid contents, leaflet index and the fraction of secondary bark fibre.

Variation and association of traits

Accession effects on the separate agronomic traits as shown in previous chapters were highly significant for all traits, except the wood fibre dimensions. Table 3 presents univariate statistics for the predefined plant-use groups. Each trait showed significant differences between at least certain combinations of the plant-use groups.

Table 4 presents coefficients of correlation between traits as a measure for trait association. The strongest correlations ($r>0.6$) were not un-expected. The bark fibre and

Table 3. Univariate F-values for the plant-use group factor, group mean values and standard errors for the means. Per trait, means showing a common letter are not different at p=0.05. Group codes are explained in the text.

Trait	F-value	Plant-use groups							
		F(N=63)		f(N=38)		d(N=18)		w(N=11)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1 ACHtsw (g)	20.13***	17.70 c	0.33	18.58 c	0.58	14.36 b	0.80	11.24 a	1.28
2 ACHlen (mm)	25.50***	4.42 c	0.03	4.30 b	0.05	3.97 a	0.06	3.77 a	0.11
3 ACHabc	5.32**	2.45 a	0.22	2.47 a	0.27	2.56 a	0.35	4.64 b	0.65
4 ACHmar	4.28**	3.68 a	0.22	3.95 a	0.25	4.22 a	0.46	5.73 b	0.73
5 THCcon (%)	45.53***	0.35 a	0.04	0.47 a	0.07	1.62 b	0.18	0.19 a	0.10
6 CBDcon (%)	10.04***	1.12 b	0.04	1.03 b	0.06	0.61 a	0.10	1.07 b	0.12
7 CANrat	27.20***	0.43 a	0.07	0.59 a	0.12	3.70 b	0.82	0.45 a	0.17
8 LEAind	10.37***	4.28 b	0.04	4.08 a	0.08	3.92 a	0.14	4.75 c	0.07
9 ANTday (day)	6.67***	191.5 a	3.7	182.5 a	4.8	218.2 b	6.5	179.7 a	11.4
10 MATday (day)	8.24***	253.7 a	2.6	250.1 a	5.2	287.8 b	6.5	251.1 a	15.0
11 PLAhei (cm)	7.47***	261.9 b	3.9	238.4 a	8.7	226.8 a	7.9	213.6 a	13.3
12 FIBlen (μm)	8.58***	534.2 a	2.9	534.7 a	3.8	560.2 b	6.7	563.6 b	11.5
13 FIBwid (μm)	8.37***	32.11 c	0.42	30.45 b	0.55	29.11 ab	0.92	27.36 a	1.05
14 FIBrat	16.41***	17.13 a	0.24	18.24 b	0.38	20.60 c	0.91	21.85 c	1.12
15 WOOcor (%)	27.30***	60.4 a	0.8	69.7 c	0.7	66.4 b	0.9	67.1 bc	1.0
16 SECfib (%)	42.15***	5.03 b	0.32	1.46 a	0.14	1.11 a	0.23	1.26 a	0.28
17 PRIfib (%)	36.75***	18.19 b	0.54	11.94 a	0.38	12.29 a	0.48	11.74 a	0.73
18 TOTfib (%)	56.79***	23.07 b	0.71	13.53 a	0.43	13.14 a	0.42	13.04 a	0.74
19 HAPgal (g^{-1})	16.00***	143.4 a	3.0	142.3 a	4.8	139.4 a	8.3	217.2 b	29.0
20 HAPegg (g^{-1})	14.04***	77.8 a	2.5	79.9 a	4.0	71.6 a	7.1	139.1 b	27.0

,* significant at p=0.01 and p=0.001, respectively

Table 4. Coefficients of correlation between traits. Coefficients in italics are significant at p=0.05, those in bold face at p=0.01. Correlations with traits 19 and 20 were calculated on the basis of 132-135 accessions, all other correlations were based on 157-163 accessions.

Trait																			
1 ACHtsw																			
2 ACHlen	**.74**																		
3 ACHabc	**-.38**	**-.30**																	
4 ACHmar	**-.33**	**-.40**	**.46**																
5 THCcon	.04	*-.19*	.07	.01															
6 CBDcon	.11	**.23**	.01	.05	**-.38**														
7 CANrat	.10	**-.21**	-.06	.06	**.83**	**-.47**													
8 LEAind	**.25**	**-.11**	.15	.01	**-.35**	.06	*.17*												
9 ANTday	.13	.01	*-.18*	*-.17*	**.45**	**-.13**	**.35**	**-.32**											
10 MATday	.12	.00	*-.19*	-.12	**.50**	**-.10**	**.37**	**-.33**	**.89**										
11 PLAhei	**.36**	**.37**	-.09	-.13	.02	.09	**-.06**	*-.18*	**.41**	**.36**									
12 FIBlen	**-.12**	*-.18*	.04	.09	**.30**	**-.09**	**.27**	**-.21**	**.41**	**.40**	.09								
13 FIBwid	*.17*	*.18*	.07	**-.03**	*-.17*	.02	**-.14**	**-.06**	**.04**	.00	**.23**	**.24**							
14 FIBrat	**-.24**	**-.26**	.13	.12	**.27**	**-.06**	**.25**	**-.04**	*.17*	.14	*.16*	**.63**	**-.85**						
15 WOOcor	.15	**-.04**	.04	.03	.10	.04	.06	*.17*	*-.20*	**-.09**	**-.28**	.05	**-.21**	*.20*					
16 SECfib	.10	**.24**	.02	**-.07**	**-.27**	.07	**-.23**	.13	.08	**-.05**	**.38**	**-.13**	**.38**	**-.38**	**-.74**				
17 PRIfib	.05	*.17*	.00	.12	*.19*	.01	**-.15**	*.18*	.04	**-.07**	**.37**	*-.18*	**.23**	**-.30**	**-.84**	**.68**			
18 TOTfib	.01	**.22**	.00	**-.09**	**-.25**	.05	**-.21**	*.18*	.06	.06	**.39**	*.18*	**.33**	**-.37**	**-.87**	**.87**	**.95**		
19 HAPgal	**-.46**	**-.35**	**.34**	*.20*	*.21*	.05	.07	*.17*	**-.32**	**-.38**	**-.34**	**-.11**	**.14**	**.08**	**.02**	**.11**	**.06**	**.10**	
20 HAPegg	**-.42**	**-.29**	**.37**	*.17*	*.19*	.07	.05	.13	**-.32**	**-.41**	**-.31**	**.01**	*.20*	*.18*	**.02**	**.12**	**.06**	**.09**	**.88**
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

woody core mass fractions were highly intercorrelated since they are more or less complementary stem components (Chapter 5). Association is also common between days of anthesis and seed maturity and achene weight and length. Mathematically expected was the correlation of separate traits and their ratio (THCcon and CBDcon with CANrat; FIBlen and FIBwid with FIBrat). The correlation between HAPgal and HAPegg, measures for *M. hapla* infection and multiplication, respectively, reflects a logical relationship. Other correlations were often significant, but weak.

Multivariate patterns of diversity

Results of principal component analysis are presented in Table 5. The percentages of total variation among accessions accounted for by the first three principal components was 59.0%. Interpretation of the results did not require rotation to achieve more simple structure. Stem quality traits had the highest loadings on the first principal component. Phenological development, psychoactive potency and host reactions to *M. hapla* had the strongest correlations with the second one.

Fig. 1 shows the scores on the first and second principal component axes for all accessions without missing data for the 20 traits. Although there was a continuous spectrum of accessions in the principal component space most plant-use groups showed a certain cohesion. Accessions pre-defined as fibre cultivars were separated by the first principal component axis on the basis of stem quality, drug strains along the second axis on the basis of late phenological development, stronger psychoactivity and poor host suitability for *M. hapla*. The naturalized accessions were placed in the lower left quadrant on the basis of early phenological development, low psychoactive potency and good host suitability for *M. hapla*. One accession, also labelled w, appeared quite remote in the upper left quadrant. It was a Nepalese accession, collected in the wild, with a late phenological development, poor host suitability for *M. hapla* and a relatively strong psychoactive potency. Fibre landraces occupied an intermediate area in the PCA plot. Further labelling of accessions according to geographic origin revealed two tendencies. The fibre cultivars ordered from left to right in the diagram (sorted by increasing bark fibre content mainly) were successively obtained from Ukraine/Russia, Poland/France and Hungary. An increasing second principal component score was associated with decreasing origin latitude indicating the slower phenological development of accessions adapted to lower latitudes (Chapter 3).

The discriminant analysis was initially based on a complete data set including 111 accessions, designated *a priori* to one of the groups F, f, d or w. Three discriminant functions were calculated for the four groups. The first two functions explained about 84% of the varia-

Table 5. Percentages of total variation among accessions explained by the first three principal components, and the principal component loadings for the six most important traits. Per principal component the traits are sorted according to decreasing loading.

	Principal component					
	1		2		3	
Variation explained (%)	25.7		19.0		14.3	
Principal component loadings	TOTfib	.405	MATday	.397	WOOcor	-.381
	SECfib	.380	ANTday	.380	ACHtsw	-.356
	PRIfib	.359	THCcon	.351	ACHlen	-.299
	WOOcor	-.331	HAPgal	-.336	PRIfib	.262
	FIBrat	-.303	HAPegg	-.321	FIBlen	.262
	FIBwid	.276	CANrat	.275	TOTfib	.261

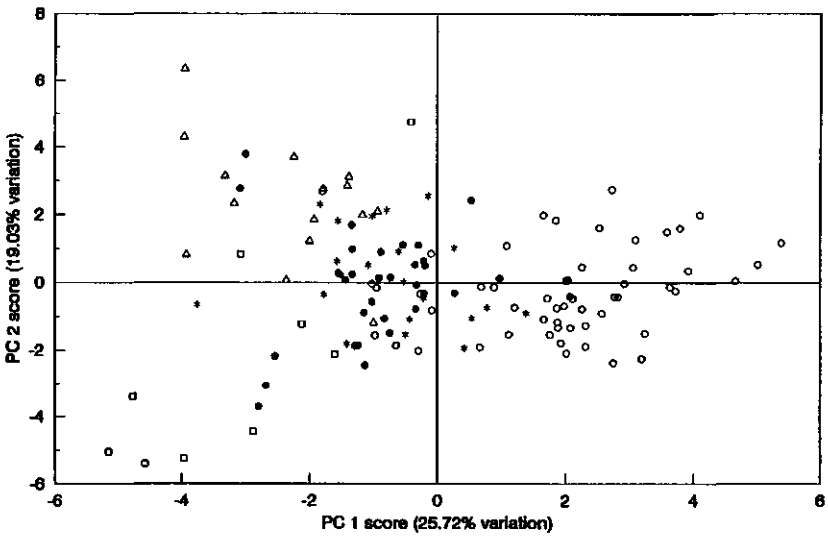


Fig. 1. Scores of 132 accessions on the first two principal component axes. Predefined fibre cultivars are indicated by circles, fibre landraces by solid circles, drug strains by triangles and wild or naturalized populations by squares. Asterisks represent *a priori* unspecified accessions.

Table 6. Coefficients of correlation between the original traits and the first two discriminant functions. The coefficients are ranked 1 to 20 according to decreasing absolute size. In brackets, the percentages of variation among groups explained by the discriminant functions.

Trait	Function 1 (59.4%)		Function 2 (24.8%)	
1 ACHtsw	0.49	6	-0.05	19
2 ACHlen	0.64	4	-0.06	18
3 ACHabc	-0.25	15	0.21	13
4 ACHmar	-0.25	16	0.14	14
5 THCcon	-0.47	7	-0.72	1
6 CBDcon	0.27	14	0.40	3
7 CANrat	-0.44	9	-0.58	2
8 LEAind	0.15	19	0.38	4
9 ANTday	-0.13	20	-0.37	6
10 MATday	-0.28	13	-0.35	7
11 PLAhei	0.41	12	-0.14	15
12 FIBlen	-0.42	10	0.04	20
13 FIBwid	0.41	11	-0.08	17
14 FIBrat	-0.57	5	0.10	16
15 WOOcor	-0.47	8	0.30	9
16 SECfib	0.72	2	-0.21	12
17 PRIfib	0.69	3	-0.25	10
18 TOTfib	0.77	1	-0.25	11
19 HAPgal	-0.24	17	0.34	8
20 HAPegg	-0.21	18	0.38	5

tion among groups. The importance of the original traits for group discrimination is indicated by their correlations with these functions (Table 6). Bark fibre contents, achene size and wood fibre ratio had the highest grouping value for the first function, successively. Cannabinoid contents, and to a lesser extent leaflet index, host reactions to *M. hapla* and phenological development were important for the second one.

Fig. 2 shows that the first two discriminant functions enable a clear-cut separation of the predefined groups F, d and w, whereas groups F and f show a mutual overlap. The discriminant scores of two fibre landraces from Korea (901162 and 901163) were strongly deviating from the f group due to an extremely low bark fibre content. Discriminant scores of presumed duplicates were quite similar.

Mahalanobis distances between group centroids are presented in Table 7. Overall, the probability for differences among centroids was significant (Approximate F-test, $p < 0.01$). Mutual differences between the centroids of groups F, d and w were the largest ones and of almost equal size. The f centroid occupied an intermediate position between these three and approached most the F centroid.

Verification of the original grouping of accessions was based

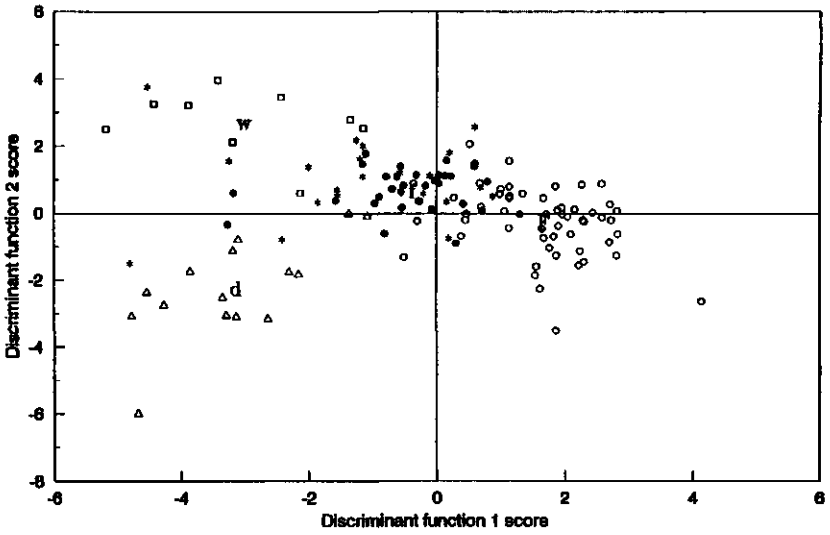


Fig. 2. Discriminant scores of 132 accessions on the first and second axes. Predefined fibre cultivars are indicated by circles (centroid F), fibre landraces by solid circles (centroid f), drug strains by triangles (centroid d) and wild or naturalized populations by squares (centroid w). Asterisks represent *a priori* unspecified accessions.

on the Mahalanobis distances between individual accessions and the four group centroids. Accessions were (re)allocated to the group with the nearest centroid. According to this criterion 15 out of 111 accessions (14%) were assigned to another group. Twenty-one unspecified accessions without missing data were also allocated. To (re)allocate 28 accessions not tested for host reactions to *M. hapla* (19,20), the procedure was repeated using traits 1 to 18 only. Table 8 summarizes for 160 accessions the original grouping and the allocation on the basis of discriminant scores. The proportion of reallocations was 22/128 (17%), mainly due to transfer of old named fibre cultivars to the fibre landrace group owing to low bark fibre content. Of the *a priori* ungrouped accessions, the Hungarian ornamental cultivar Panorama (910914) was allocated to

Table 7. Squared Mahalanobis distances between group mean discriminant scores (centroids). Group codes are explained in the text.

Group	F	f	d	w
F	-			
f	2.90	-		
d	5.21	4.32	-	
w	5.70	4.59	5.20	-

Table 8. Accession numbers per plant-use group according to the original grouping and to the discriminant scores (new allocation). For 132 accessions the new allocation is based on 20 traits, and for 28 accessions on 18 traits. Group codes are explained in the text, the subset of *a priori* unspecified accessions is coded *.

Original grouping	New allocation				
	F	f	d	w	Total
F	50	11	0	0	61
f	1	33	3	1	38
d	0	2	16	0	18
w	2	2	0	7	11
*	4	23	3	2	32
Total	57	71	22	10	160

group d. Two bird-seed samples, 883262 and 891348, were placed into groups F and f, respectively, confirming that bird-seed is a by-product of fibre hemp.

Electrophoretic patterns of seed proteins

Electrophoretic patterns of seed proteins were studied in the pH interval from 4.75 to 6.40. Beyond this range bands were insufficiently focused (anodic side), or too densely stained (cathodic side). Ca. 45 bands could be detected, variation among accessions was identified for band presence at pH 4.93, 5.08, 5.47, 5.55, 5.85 and 6.25 (Fig. 3). Polymorphism at pH 5.55 was not evaluated as a decision on band presence was often doubtful.

Different extracts made from the same seed lot used to give similar patterns, indicating reproducibility. Patterns proved to be stable over generations, also when extracts from original seed shipments were compared with those obtained from greenhouse multiplications. However, extracts from seed lots presumed to be duplicates gave sometimes different patterns.

Table 9 presents the patterns found and their distribution over accessions and plant-use groups. Variable bands were more often absent than present. Abundantly present variable bands were those at pH 5.47 and pH 5.85. Individual bands or banding pattern were not exclusive for any of the plant-use groups or taxa and were not geographically dependent.

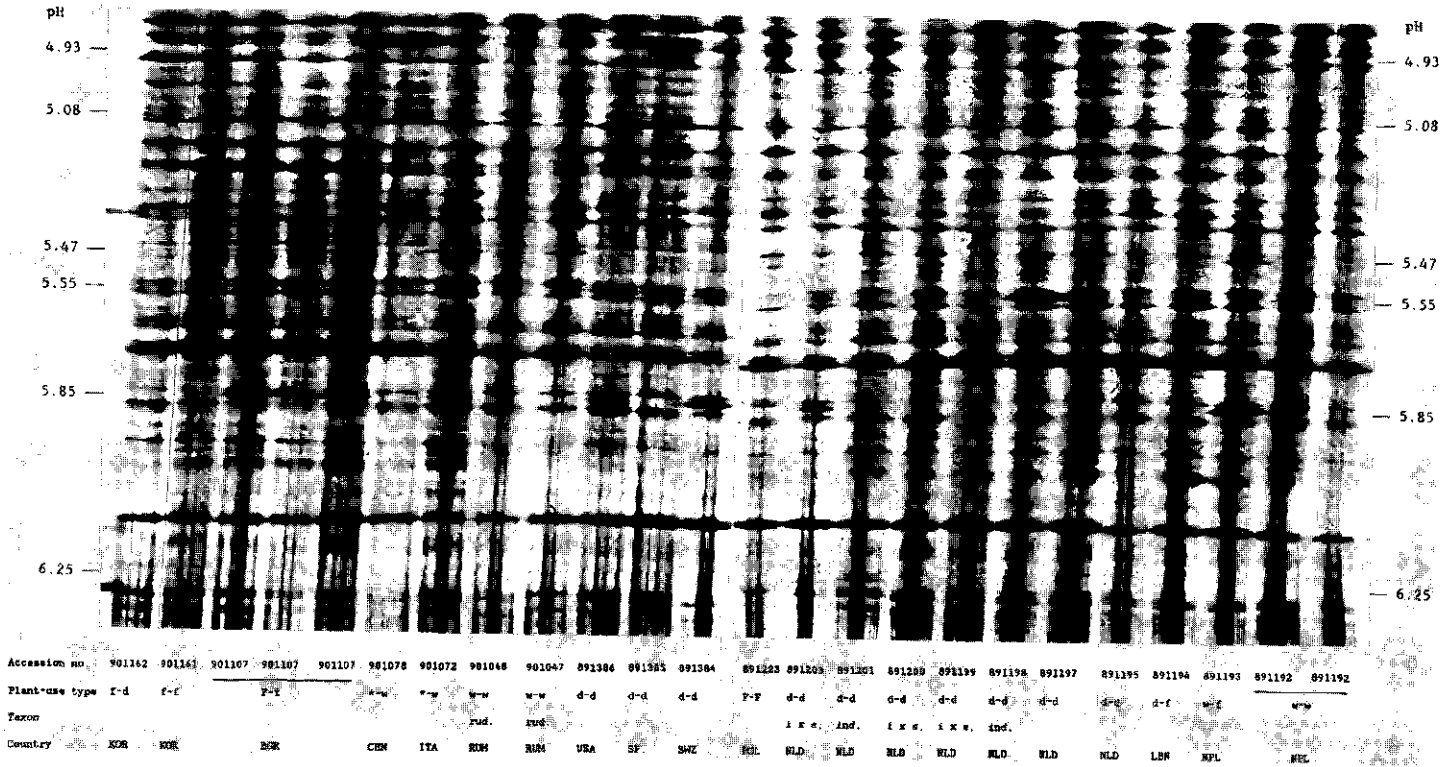


Fig. 3. IEF-IPG patterns of seed extracts of divergent *Cannabis* accessions. Different extracts made from the same seed lot are underlined in the lane legend. Lane legends mention accession no., plant-use type (*a priori-a posteriori*; see text for explanation), taxon (rud. = *ruderalis*; ind. = *indica*; i x s. = *indica* x *sativa* hybrid) and country of origin (KOR = Korea; BGR = Bulgaria; CHN- = China; ITA = Italy; RUM = Rumania; USA = United States; SF. = Southern Africa; SWZ = Swaziland; POL = Poland; NLD = Netherlands; LBN = Lebanon; NPL = Nepal).

Table 9. The distribution of banding patterns over accessions and plant-use groups. The banding patterns are based on five variable bands (presence = 1; absence = 0). Codes for plant-use groups are explained in the text.

Variable band pI					Nos. of accessions per group				Total no. of accessions
4.93	5.08	5.47	5.85	6.25	F	f	d	w	
0	0	0	0	0	25	21	8	4	58
1	0	0	0	0	2	0	0	1	3
0	1	0	0	0	0	2	0	0	2
0	0	1	0	0	4	3	2	4	13
0	0	0	1	0	16	25	5	1	48
0	0	0	0	1	0	0	1	0	1
0	1	1	0	0	0	0	1	0	1
0	0	1	1	0	4	11	0	1	16
0	0	0	1	1	0	2	1	1	4
1	0	1	1	0	1	0	0	0	1

DISCUSSION

The procedure used to correct accession mean scores for year effects is most justifiable for conservative, probably highly heritable traits, i.e. traits with high correlations between years, stronger accession effects than year effects, and insignificant 'year x accession' interactions. Table 2 shows that these criteria were well met by the various stem mass fractions and the day of seed maturity. The procedure was most doubtful for CBD content.

The F-values in Table 3 are an indication for the grouping value of separate traits. No trait enabled the unambiguous recognition of fibre landraces. Plant height, wood fibre ratio and stem composition however were helpful for the discrimination of fibre cultivars. Cannabinoid contents and phenological development could be used to discriminate drug strains. Wild and naturalized accessions differed in achene characters and host reactions to *M. hapla*. It is remarkable that the wood fibre dimensions possessed discriminating power whereas their F-values for ungrouped accessions were hardly significant (Chapter 5). Obviously, plant-use groups differ slightly but consistently for wood fibre dimensions.

The less self-evident correlations between traits in Table 4 were generally weak, indicating that strict linkages are absent. Many trait associations are man-made rather than natural. Associations with traits not subjected to conscious selection, such as wood fibre dimensions, *M. hapla* host reactions and leaflet index, reflect natural relations. Selection of fibre strains has clearly enhanced the association between large achenes, long stems, high bark fibre contents and low psychoactive

potency. The association of these features with somewhat wider wood fibres seems an indirect result of selection. The association of high THC content, low CBD content and high cannabinoid ratio with low leaflet index, slow phenological development and low (i.e. natural) bark fibre content matches with descriptions for *C. indica* drug strains. The association of these features with relatively slender wood fibres disagrees with the findings of Anderson (1974). Low stature is associated with a complex of 'wild' achene characteristics (small, marbled, constricted abscission layer) which is expected on the basis of descriptions for *C. ruderalis*. The association of these features with a relatively good host-suitability for *M. hapla* was not reported earlier.

Some individual accessions illustrated that most associations were indeed not strict. The only drug strain which was received as *C. sativa* (910972) had all features in common with other drug strains except an unusually high leaflet index (5.06). Two fibre cultivars from Hungary, Kinai Uniszex (891342) and Kinai Kétlaki (883046) had high bark fibre contents (26.6 and 31.7%, respectively) as well as relatively high THC contents (1.0 and 1.3%, respectively).

Principal component analysis showed that CBD content, achene size and leaflet index were of little importance for explaining the total variation among accessions. However, discriminant analysis showed that they had considerable value for discriminating plant-use groups. Due to its instability, CBD content seems only suitable for such purpose when measured in standardized trials and considered in combination with THC content. Achene size and leaflet index enable a tentative recognition of groups of agronomical interest. The low percentages of reallocation indicated that the *a priori* assigned group labels indeed predict roughly the agronomic characteristics of accessions.

Grouping of accessions based on seed protein patterns was independent from groupings based on passport data or experimental data. This agrees with findings of Lawi-Berger *et al.* (1982) also obtained with bulk seed extracts, and disagrees with those of Yumaguzina *et al.* (1979a,b). No support was found for the assumption in Chapter 2 that fibre cultivars and landraces, and naturalized populations are closely interrelated whereas these groups were thought to be more distinct from drug strains. Expected genetic similarity was often not confirmed, for example, not even within series of fibre cultivars probably derived from a common source population. A close relationship, between the female parent Kinai Kétlaki (883046) and its F_1 hybrid offspring, the cultivar B-7 (883055), was however confirmed by a common rare band at pH 4.93.

It seems unlikely that banding patterns of accessions were affected by environmental factors acting during seed multiplication since they proved stable over generations.

Environmental factors affect mainly quantitative parameters of seed proteins (Gepts, 1990). The use of bulk samples, instead of single achenes, can have obstructed the identification of the products of less frequent alleles or gene combinations as these will be present in insufficient concentration in a bulk sample extract (Wills *et al.*, 1979 cited by Vaughan, 1983). The effect of this can indeed be that subtle differences between accessions have been overlooked. Differences between accessions are possibly quantitative rather than qualitative and should, instead of dichotomously scored, be expressed in terms of band frequencies calculated on the basis of single seed patterns. Still, such an approach would likely yield a quite similar clustering of accessions. Apart from methodological considerations, the use of seed proteins as genetic markers might be doubtful, since according to Gepts (1990), they have only limited coverage of the genome. Furthermore, storage proteins have never been a subject of conscious selection in *Cannabis*. Hence, genetic diversity for important agronomic and morphological traits may not be reflected in protein banding patterns. The fact that presumed duplicates and cultivars within the same series sometimes showed different patterns can, apart from administrative mistakes, be due to introgression or genetic drift. Founding events are especially likely to occur when very few plants are selected for cultivar improvement or seed multiplication.

CHAPTER 9

GENERAL DISCUSSION

INTRODUCTION

This chapter considers the results of the preceding research in relation to the initial objectives (Chapter 1). Each of the Chapters 3,5,6 and 7 has been supplied with a discussion of the results on a specific aspect of *Cannabis* variation relevant to the purpose of arable paper pulp production in the Netherlands, whereas in Chapter 8 the variation for all observed traits in mutual connection has been discussed. The present chapter identifies topics that remain to be investigated and focuses on some implications of the previous results for direct cultivation and breeding. Due to the growing interest in other products and by-products appearing in the course of the hemp research project, the discussion will not refer exclusively to the purpose of paper pulp production.

RELEVANT TOPICS FOR ADDITIONAL EVALUATIONS

Additional topics, becoming opportune in the case of actual hemp breeding and cultivation in the Netherlands, concern mainly reactions to pathogens. The introduction of new crops into the narrow rotations of the Netherlands requires the evaluation of host reactions to soil pathogens, especially to those with wide host ranges (Kok & Coenen, 1994). Research of Kok *et al.* (1994) indicated that an evaluation of the *Cannabis* collection for resistance to the root-lesion nematode *Pratylenchus penetrans* Cobb, comparable to the study aimed at resistance to *Meloidogyne hapla* Chitwood (Chapter 7), might be useful.

In the course of the research program, the fungus *Botrytis cinerea* Pers. ex Fr. appeared to cause abundant lethal stem lesions in hemp field crops. Tentative observations (unpublished) indicated variation in susceptibility among accessions. Differences among current fibre cultivars in *Botrytis* incidence were also reported for southern England (Cromack, 1994). A more detailed evaluation seems justified.

The genetic characterization of accessions based on electrophoretic patterns of seed proteins was unsatisfactory as it did not reflect expected common ancestry of accessions (Chapter 8). Attempts using RFLP or RAPD techniques may be more successful in enhancing knowledge on genetic relationships for actual breeding (A.J. Gibbs, pers. commun., 1994).

IMPLICATIONS FOR CULTIVATION

The performances of individual accessions are summarized in Appendix 2. Commercial cultivars, readily available for large-scale cultivation, form only a small minority in the evaluated collection. All agronomic traits are phenotypic, hence the character states reported in Appendix 2 hold for the Netherlands only, also they depend on the year of cultivation and cultural practices. Relative differences among accessions are much more stable.

The stem yield potential at a certain latitude is a.o. determined by the phenological development (Chapter 3; van der Werf, 1994) and consequently by the latitude of adaptation of accessions (Chapter 3). Out of the current fibre cultivars, Kompolti (883048) and Kompolti Hybrid TC (883047, 891071, 891343) from Hungary had the slowest phenological development. The stem production of extremely late-flowering fibre landraces such as the accessions 901161, 901162 and 901163 from Korea was much higher than that of the best yielding fibre cultivars, but these strains have low contents of bark fibre. Phenological observations appear also useful to identify accessions with strong generative development and early maturation, suited for seed production. The presumed relation with seed yield however remains to be verified.

Mass fractions of woody core and primary, secondary and total bark fibre in the stem, being measures for the potential recovery of distinct fractions in the pulping process (Chapters 4 and 5) are the most important parameters for stem quality. Under standardized plant density they seem hardly susceptible to the year of cultivation (Chapter 5). The Hungarian fibre cultivars Uniko B (883045), Kinai Kétlaki (883046), Kompolti (883048), Kompolti Sargászáru (883049) and Kompolti Hyper Elite (910915) possess the best quality for the purpose of pulp production due to their superior total bark fibre content. Also the Polish cultivars Bialobrzeskie (891223, 921019) and Beniko (921040) contain high amounts of bark fibre. The length of the woody core fibres showed too limited variation to be considered in the selection of optimal cultivars for direct cultivation (Chapter 5). The evaluated stem mass fractions are helpful in identifying suitable strains for textile production as well. For such purpose the total bark fibre content and the proportion of secondary bark fibres are important criteria (Horkay, 1982). Although the latter proportion seems quite systematically related to the total bark fibre content (Chapter 5), some variation among fibre cultivars can be observed. For example, 'Kompolti Hyper Elite' (910915), 'Uniko B' (883045) and 'Beniko' (921040) have about equal total bark fibre contents (30.2-30.9%) whereas their proportions of secondary bark fibre are 11.4, 8.6 and 7.8%, respectively. This makes 'Beniko' and 'Uniko B' more suitable

for textile production than 'Kompolti Hyper Elite'.

Cannabinoid profiles are strongly affected by the year of cultivation (Chapter 6) and are expected to depend strongly on location as well. The climate of the Netherlands is generally unfavourable for the accumulation of cannabinoids in field grown *Cannabis*. It is unlikely that crops of any of the current fibre cultivars will develop significant psychoactivity. The collected data on accession mean cannabinoid profiles may be useful for the identification of suitable genotypes for the production of THC or CBD, for example for medical purposes (Mikuriya, 1973; Grinspoon & Bakalar, 1993; Clarke & Pate, 1994). High THC contents and relatively low CBD contents were found in accessions such as 891385 or 910972. Reverse profiles with high CBD contents and little THC were found in the accessions 921017 and 921018. Optimized production of purified THC or CBD for pharmaceutical purposes should be organized indoors, using cuttings of superior female plants selected from appropriate accessions. Although cultivars with high bark fibre content as well as considerable THC content do occur in the collection, e.g., 'Kinai Kétlaki' (883046) and 'Kinai Uniszex' (891342), it does not seem profitable to produce both components by means of a dual purpose field crop.

Cultivation of the relatively resistant fibre cultivar Kompolti Sargászáru (883049) can be recommended for soils infested with *Meloidogyne hapla* (Chapter 7).

IMPLICATIONS FOR BREEDING

Indications for the prospects of *Cannabis* breeding can be based on the diversity for separate traits within and among accessions, the degree of genetic control of traits (heritability) and the nature of mutual associations of traits. Variation for separate traits, among and occasionally within accessions, has been discussed extensively in the Chapters 3,5,6 and 7. Chapter 8 compared the stability of traits, indicating the degree of genetic control, and investigated trait associations.

The conclusion of Chapter 3, that an increase of stem yield potential can most effectively be achieved by organizing seed reproduction at much lower latitude than the latitude of cultivation, leaves a limited contribution to breeding. At a given phenological pattern, breeding efforts should be directed at the improvement of persistency of cultivars in dense crops and the increase of the efficiency of stem dry matter accumulation. Variation among accessions in the latter features has indeed been noticed (Chapter 3).

Breeding for improved woody core quality seemed not very promising (Chapter 4). Continued selection in the best fibre cultivars at present, for increased bark fibre content and consequently for a reduction of the woody core fraction

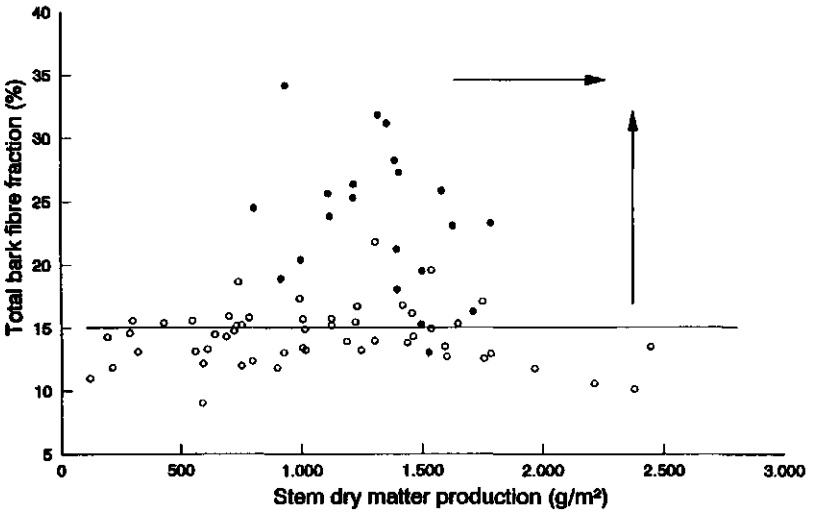


Fig. 1. The total bark fibre mass fraction in the stem versus the stem dry matter production. Means are presented for 74 accessions tested simultaneously in 1993. The performance of named fibre cultivars is indicated by solid symbols, open symbols represent accessions belonging to other plant-use groups (see Chapter 8) and *a priori* unspecified accessions. The arrows indicate breeding strategies which are explained in the text.

is considered the most convenient strategy for the improvement of pulping quality. Technological research should reveal to what extent the coinciding increase of the proportion of secondary bark fibre can be accepted. There seem certain prospects to affect the ratio of secondary to primary bark fibre, as is indicated by the variation among fibre cultivars with similarly high total bark fibre contents (previous section).

Fig. 1 shows the absence of any natural relationship between stem dry matter production and total bark fibre content at the accession mean level, although such a relationship might be present within populations. The named fibre cultivars combine bark fibre contents exceeding 15% (artificial) with moderate stem yields. Accessions with natural fibre contents (10-15%) have a wide range of stem yields, which is according to Chapter 3, strongly related to their respective origin latitudes. The tested fibre cultivars were obtained from latitudes ranging between 44 and 53°. There appear to be two extreme strategies to obtain better cultivars, indicated by arrows in Fig. 1:

* Selection for increased fibre content within the best yielding populations, meanwhile maintaining the late-flowering character.

* Adaptation of existing fibre cultivars to low latitude, resulting in delayed anthesis, meanwhile maintaining the high bark fibre content.

The second strategy is possible, provided that there is still sufficient variation in photoperiod response within the original cultivars. It is most attractive as it requires only a few repeated multiplication cycles performed outdoors at low latitude under a mild selection in favour of genotypes with high bark fibre content.

Diverse combinations of cannabinoid profiles and other economic character states occur among readily available accessions (Appendix 2) or can be established by breeding. Since plants with less than 0.3% (Small & Beckstead, 1973) or 0.5% (Fournier & Paris, 1979) THC can be considered to possess no psychoactive potency, there seems little need for efforts aimed at a further reduction of THC content in the current fibre cultivars (Chapter 6). The same applies for the breeding of cultivars for dual purpose utilization, producing cellulose as well as cannabinoids in considerable amounts (previous section). The variation in cannabinoid profiles (Appendix 2) will be more pronounced at the level of individual plants (Chapter 6). Selection within accessions with desired mean profiles will easily yield female genotypes suited for making clones for the purposeful production of one of the major cannabinoids.

The suitability of seedling inoculation for estimating resistance to *Meloidogyne hapla* was confirmed in Chapter 7. The fibre cultivar Kompolti Sargászáru (883049) was identified as relatively resistant, and some highly resistant individual plants were retained from accessions with poor agronomic properties. Breeding prospects were experimentally explored by Hennink & Dieleman (1994). They used 'Kompolti Sargászáru' for further selection and tested crosses between resistant genotypes and the susceptible fibre cultivar Kompolti Hyper Elite (910915). The mass selection of 'Kompolti Sargászáru' showed little progress which was supposed to be due to a limited genetic variation. The crosses of resistant clones and the susceptible cultivar yielded offsprings with intermediate resistance. The use of the resistant sources as recurrent parent was considered promising for the increase of resistance in agriculturally acceptable cultivars.

Chapter 8 showed an absence of strong associations between the observed traits. This indicates that in general, breeding will not be hampered by strict linkages, i.e. that probably any desired combination of character states within the observed ranges can be established through breeding.

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SUMMARY

In an effort to reduce the overproduction of a too limited number of arable food crops, several research programs focusing on industrial crops have recently been initiated in the Netherlands. The 'Hemp research program' investigated from 1990 to 1994, the feasibility of hemp as an arable crop and as a raw material for paper pulp. In this context, breeding, agronomy, plant pathology, mechanization, processing and economics were the subject of a comprehensive study.

This thesis reports on a part of the breeding research. A collection of *Cannabis* germplasm, covering variation within the genus, was established and evaluated at the DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO, Wageningen). Stem production, stem quality, psychoactive potency and resistance to soil pathogens were considered relevant criteria for the introduction of hemp in crop rotations in the Netherlands and its utilization as a raw material for paper pulp. Prospects for the breeding of improved cultivars were based on the diversity for these traits, their stability, and mutual relations.

In its final state the established germplasm collection comprised ca. 200 accessions (entries, populations), including fibre strains, drug strains, an ornamental cultivar and truly wild or naturalized populations (Chapter 2). According to consulted references, considerable mutual relatedness seemed to exist among fibre cultivars. Italian and German strains, especially, have directly been the basis of, or have been used as breeding parents for many of the present cultivars. Also, the central European naturalized populations and the fibre landraces seemed quite closely related to modern fibre cultivars. The drug strains were expected to be more distinct. The collection was considered to be sufficiently representative for investigating diversity in *Cannabis*.

Traits related to stem production, stem quality and psychoactive potency were observed for ca. 160 accessions in field trials, or determined afterwards with field grown materials. Trials were carried out at CPRO (52° N latitude). Accessions were grown in small plots in two replicates, in high plant density to obtain results that are readily interpretable for fibre hemp cultivation. Host reactions to the root-knot nematode *Meloidogyne hapla* Chitwood were assessed for ca. 150 accessions in a seedling test, and verified in a field trial for a subset of accessions.

Variation in phenological development and in stem elongation was studied in relation to stem production (Chapter 3). Large variation among accessions was found for the day of anthesis (ranging from approximately 10 May to 30 September) and consequently for the day of seed maturity. A higher latitude of origin was associated with earlier

anthesis and seed maturity ($r = -0.75$). The phenological pattern was stable over years. Stem elongation was characterized by the parameters of a sigmoid curve fitted to periodical measurements of stem length. Significant differences among accessions were found for these parameters. The final stem length ranged from 60 to 360 cm. Stem elongation was less stable over years than the phenological pattern. Day of anthesis and final stem length were strongly and positively related to stem dry matter production ($r > 0.7$). Some very late-flowering fibre landraces, with low bark fibre contents, produced up to 2200 g of stem dry matter per m^2 and exceeded the fibre cultivars (producing 800 to 1750 g/m^2) in stem yield. Late-flowering drug strains were less persistent in a dense crop than late fibre strains. It was concluded that in an efficient crop growth system, seed reproduction and stem production should occur in separate geographic areas. Seed reproduction (adaptation) should be organized at lower latitude to obtain late-flowering, high yielding cultivars for stem production at higher latitude. At a given phenological pattern, breeding can contribute to yield potential by increasing both the persistency of cultivars and the efficiency of stem dry matter accumulation.

The large differences between bark tissue and woody core in anatomical and chemical properties, make a quantification of these two fractions important for stem quality assessment. The bark is the most valuable component for pulp production. Accuracy and power of discernment of current procedures for the analysis of the composition of hemp stems were evaluated (Chapter 4). A method using stem segments, intended to reduce the amount of material to be handled, gave somewhat less accurate, but still usable information, than analysis of entire stems. The relation was studied between the simple estimation of the fractions of bark fibre and woody core, commonly applied in fibre hemp breeding, and the assessment of potential pulp recovery according to a more laborious standard method of the pulp and paper industry. The recovery of bark in the pulping process (pulp yield) is accurately predicted by the fraction of bark fibre. The woody core recovered as pulp is a fixed fraction (ca. 69%) of the intact woody core.

Variation among accessions was assessed for the woody core fraction in the stem dry weight, which ranged from 50 to 78% (Chapter 5). The fraction of bark, being the complementary tissue, ranged hence from 22 to 50%. The fraction of primary bark fibre in the stem varied from 8 to 27%, the secondary bark fibre from 0 to 14%, and the total bark fibre from 9 to 34%. The fibre cultivars had strongly increased fractions of bark tissue and primary and secondary bark fibre, and reduced fractions of woody core in comparison to wild populations, drug strains and fibre landraces. The levels of the assessed stem fractions

were stable over years. Since woody core fibres of hemp are on average too short (550 μm according to references) to produce high quality paper pulp, the evaluation was directed at detecting variation for wood fibre dimensions as well. Accession means for wood fibre length ranged only from 433 to 613 μm. Also the variation within accessions was limited. Breeding for improved woody core quality is considered not very promising. The best strategy for genetic improvement of pulping quality seems a continued selection for increased bark fibre content in fibre cultivars, which implies a reduction of the woody core fraction.

A simple possibility to increase bark fibre yield for pulp production is indicated in Chapter 9. It consists of the adaptation of current, well performing fibre cultivars to low latitude, meanwhile maintaining the high bark fibre content by a mild selection. The resulting delay in phenological development is expected to increase the potential bark fibre yield.

Large variation among accessions was found for the contents of the major cannabinoids delta-9-tetrahydrocannabinol (THC; 0.01 to 1.77% in the female inflorescence dry matter) and cannabidiol (CBD; 0.05 to 2.19%) (Chapter 6). THC is the psychoactive compound in *Cannabis*. Within accessions, THC content was less variable than CBD content. Both contents were strongly affected by the year of cultivation, but THC content was more stable than CBD content. Accessions were classified into the phenotypes 'drug', 'intermediate' and 'non-drug' on the basis of cannabinoid profiles. There were no strict relationships between the cannabinoid profiles and non-chemical traits. A few accessions combined for example a considerable psychoactive potency and a high content of bark fibre. On the accession average level, relatively wide leaflets and slow phenological development were weakly associated with a stronger psychoactive potency. There seems little need for efforts aimed at a further reduction of THC content in the current fibre cultivars. The collection comprised a number of accessions, suitable for the selection of genotypes for the production of either THC or CBD.

Inoculation of *Cannabis* seedlings with a larval suspension of the root-knot nematode *Meloidogyne hapla*, resulted in significant variation for the number of galls and egg masses on the seedling roots (Chapter 7). These parameters were considered as estimates for nematode infection and larval multiplication, respectively. A subset of the tested accessions was grown on a naturally infested arable field to verify the relation between the test results and host reactions in the field. The ranking order of accessions for the seedling test parameters agreed satisfactory with that for nematode infection and multiplication in the field. The collection comprised one relatively resistant fibre cultivar with good agronomic properties which seemed a useful source for further

selection. Some highly resistant individual plants retained from accessions with poor agronomic properties were expected to be useful for cross-breeding.

The diversity for the observed agronomic and morphological traits considered together was studied in Chapter 8. Mutual associations among traits were weak, indicating that many combinations of character states within the observed ranges can be established through breeding. For the interpretation of diversity patterns, accessions were grouped *a priori* on the basis of the presumed purpose and status of domestication resulting in the recognition of four 'plant-use groups': fibre cultivars, fibre landraces, drug strains, and truly wild or naturalized populations. Plant-use groups could be discriminated quite well on the basis of experimental observations. Contents of bark fibre and cannabinoids were most discriminative.

A genetic characterization of accessions was additionally attempted on the basis of electrophoretic patterns of seed proteins. Although reproducible variation in banding patterns was found among accessions, the results were quite unsatisfactory as they did not reflect expected common ancestry. Furthermore, the banding patterns were independent from any grouping based on origin or agronomic and morphological traits.

Individual accessions suitable for cultivation and breeding are identified in Chapter 9. Issues requiring further evaluation in case of actual hemp breeding and cultivation in the Netherlands are, resistance to the root-lesion nematode *Pratylenchus penetrans* Cobb and the fungus *Botrytis cinerea* Pers. ex Fr. (grey mold). Also genetic relationships among accessions need further clarification.

SAMENVATTING

Naar aanleiding van de overproductie van een te beperkt aantal akkerbouwgewassen zijn in Nederland diverse onderzoekprogramma's gestart, gericht op de introductie van alternatieve industriële gewassen. Het 'hennep onderzoekprogramma' onderzocht van 1990 tot 1994 de haalbaarheid van hennepteelt voor de productie van papierpulpgrondstof. In dit verband zijn veredeling, teelt, mechanisatie en verwerking en plantenziektekundige en economische aspecten bestudeerd.

Dit proefschrift behandelt als onderdeel van het veredelingsonderzoek, *de evaluatie van een gevarieerde* collectie van *Cannabis* accessies (herkomsten, populaties) die gedurende de onderzoeksperiode werd opgebouwd bij het DLO-Centrum voor Plantenveredelings- en Reproductieonderzoek (CPRO-DLO, Wageningen). Stengelproductie, stengelkwaliteit, psychoactieve potentie en resistentie tegen bodempathogenen werden relevante eigenschappen geacht voor de introductie van hennep in Nederlandse gewasrotaties en voor het gebruik als pulpgrondstof. Perspectieven voor het kweken van verbeterde rassen werden gebaseerd op de aangetroffen diversiteit voor deze eigenschappen, hun stabiliteit en hun onderlinge relaties.

De opgebouwde collectie omvatte uiteindelijk ongeveer 200 accessies waaronder materiaal gebruikt voor vezelproductie, voor productie van hasjisch en marihuana, een siercultivar, en echt wilde of verwilderde populaties (Hoofdstuk 2). Volgens de literatuur bestaat er een behoorlijke onderlinge verwantschap tussen vezelcultivars. Vooral Italiaanse en Duitse populaties vormen de directe basis voor, of waren kruisingsouder van veel van de huidige cultivars. Ook verwilderde populaties uit centraal Europa en landrassen van vezelhennep lijken verwant met moderne vezelcultivars. Van de typen gebruikt voor drugproductie werd verwacht dat ze genetisch vrij sterk zouden afwijken van voornoemde groepen. De opgebouwde *collectie werd als voldoende* representatief beschouwd voor het onderzoeken van de diversiteit binnen het genus *Cannabis*.

Parameters gerelateerd aan stengelproductie, stengelkwaliteit en psychoactieve potentie zijn voor ongeveer 160 accessies vastgesteld in veldexperimenten of achteraf bepaald aan de hand van plantmateriaal uit deze proeven. De experimenten werden uitgevoerd op het CPRO (52° Noorderbreedte). Accessies werden geteeld op kleine veldjes in twee herhalingen, in hoge plantdichtheid om resultaten te verkrijgen die interpreteerbaar zijn voor de praktijkteelt van vezelhennep. Waardplantreakties voor het wortelknobbelaaltje *Meloidogyne hapla* Chitwood werden voor ongeveer 150 accessies vastgesteld in een zaailingentoets en voor een subset van accessies geverifieerd in een veldexperiment.

Variatie in fenologische ontwikkeling en stengellengtegroei werd bestudeerd in relatie tot stengelproductie (Hoofdstuk 3). Accessies varieerden sterk m.b.t. bloeidatum (van 10 mei tot 30 september) en als gevolg daarvan ook m.b.t. de datum van zaadrijpheid. Een hogere oorsprongsbreedtegraad ging samen met vroegere bloei en rijpheid ($r=-0.75$). Het fenologisch patroon was stabiel over jaren. Stengellengtegroei werd gekarakteriseerd door de parameters van een op periodieke lengtemetingen gebaseerde S-curve. Accessies verschilden significant voor deze parameters. De uiteindelijke stengellengte varieerde van 60 tot 360 cm. Lengtegroei bleek minder stabiel over jaren dan het fenologisch patroon. Bloeidatum en uiteindelijke stengellengte waren beide positief gecorreleerd met stengeldrogestofproductie ($r>0.7$). Enkele zeer laat bloeiende vezellandrassen, met lage bastvezelgehaltes, produceerden tot 2200 g/m^2 aan stengeldrogestof en overtroffen daarmee de vezelcultivars die van 800 tot 1750 g/m^2 opbrachten. Laat bloeiende drugtypen waren in een dichte gewassituatie minder standvastig dan vezeltypen. Geconcludeerd is dat in een efficiënt productiesysteem, zaadreproductie en stengelproductie plaats moeten vinden in afzonderlijke gebieden. De zaadreproductie (adaptatie) zou georganiseerd moeten worden op een lage breedtegraad, om laat bloeiende cultivars te verkrijgen die op hogere breedtegraad een hoge stengelopbrengst geven. Bij een gegeven fenologisch patroon kan veredeling bijdragen aan de potentiële opbrengst door zowel de standvastigheid, als de efficiëntie van de stengeldrogestofaccumulatie te verbeteren.

De grote anatomische en chemische verschillen tussen het bastweefsel en de houtkern van de hennepstengel maken een kwantificering van deze twee fracties belangrijk voor de bepaling van de stengelkwaliteit. De bast is het meest waardevol voor papierpulpproductie. De nauwkeurigheid en het onderscheidingsvermogen van gangbare procedures voor de bepaling van de samenstelling van hennepstengels zijn vergeleken (Hoofdstuk 4). Een methode die, om de hoeveelheid te analyseren materiaal te beperken, uitgaat van stengelsegmenten, gaf bruikbare maar iets minder nauwkeurige informatie, dan de analyse van gehele stengels. De relatie is onderzocht tussen enerzijds de vrij eenvoudig vast te stellen fracties aan bastvezel en houtkern in de stengel (deze worden gangbaar bepaald bij de selectie van vezelhennep), en anderzijds de potentiële pulpopbrengst bepaald volgens een meer bewerkelijk protocol van de pulp- en papierindustrie. De opbrengst aan bast in het verpulpingsproces bleek nagenoeg gelijk aan de bastvezelfractie. De houtkern pulpopbrengst is een vast gedeelte (ca. 69%) van de intacte houtkern.

De houtkernfractie varieerde tussen accessies van 50 tot 78% in de stengeldrogestof (Hoofdstuk 5). De bastfractie, het complementaire weefsel in de stengel, varieerde dus

van 22 tot 50%. De fractie aan primaire bastvezel in de stengel lag tussen 8 en 27%, die van secundaire bastvezel tussen 0 en 14%, en de totale bastvezelfractie tussen 9 en 34%. Vezelcultivars hadden grotere fracties bastweefsel en primaire en secundaire bastvezel, en een gereduceerd aandeel aan houtkern, in vergelijking met wilde populaties, drugtypen en vezellandrassen. De niveaus van de stengelfracties waren stabiel over jaren. Omdat de houtkernvezels van hennep gemiddeld te kort zijn (550 μm volgens de literatuur) om een goede kwaliteit pulp te produceren, was de evaluatie tevens gericht op het opsporen van variatie voor kernvezelafmetingen. De accessiegemiddelden voor kernvezellengte varieerden slechts van 433 tot 613 μm. Ook binnen accessies was de variatie gering. De beste strategie voor de genetische verbetering van de stengelkwaliteit voor pulpproductie is een verdere selectie ten gunste van een hoog bastvezelgehalte in vezelcultivars. Dit impliceert tegelijk een afname van de houtkernfractie.

Een eenvoudige strategie om de bastvezelopbrengst te verhogen is aangegeven in Hoofdstuk 9. Deze bestaat uit de aanpassing van de beste, gangbare vezelcultivars aan een lage breedtegraad. Het hoge bastvezelgehalte moet onderwijl door een lichte selectie behouden blijven. Naar verwachting zal door de vertraagde fenologische ontwikkeling de potentiële stengelopbrengst, en daarmee de bastvezelopbrengst toenemen.

Grote verschillen tussen accessies werden aangetoond voor de gehaltes aan de cannabinoïden delta-9-tetrahydrocannabinol (THC; variërend van 0.01 tot 1.77% in de droge stof van vrouwelijke bloeiwijzen) en cannabidiol (CBD; 0.05-2.19%) (Hoofdstuk 6). THC is verantwoordelijk voor de psychoactieve werking van *Cannabis* preparaten. Binnen accessies was het THC gehalte minder variabel dan het CBD gehalte. Beide gehalten werden sterk beïnvloed door het teeltjaar waarbij het THC gehalte stabieler bleek dan het CBD gehalte. Accessies werden ingedeeld in de fenotypen 'drug', 'intermediate' en 'non-drug' op basis van cannabinoïd profielen. Er zijn geen strikte verbanden aangetoond tussen cannabinoïd profielen en de niet-chemische eigenschappen. Afwijkend van het gebruikelijke patroon hadden enkele accessies bijvoorbeeld zowel een behoorlijke psychoactieve potentie als een hoog bastvezelgehalte. Op het gemiddelde niveau van accessies waren relatief brede blaadjes en een trage fenologische ontwikkeling zwak geassocieerd met een sterkere psychoactieve potentie. Er bestaat weinig aanleiding voor inspanningen gericht op een verdere verlaging van het THC gehalte in de huidige vezelcultivars. De collectie omvatte een aantal accessies die geschikt lijken als bron voor selectie van genotypen voor doelbewuste productie van THC of CBD.

Gestandaardiseerde inoculatie van *Cannabis* kiemplanten met larven van het wortelknobbelaaltje

Meloidogyne hapla resulteerde in significante variatie voor de aantallen knobbels en eimassa's op de wortels (Hoofdstuk 7). Deze parameters werden beschouwd als maat voor respectievelijk aaltjesinfectie en aaltjesvermeerdering. Een subset van de getoetste accessies werd geteeld op bouwland met een natuurlijke *M. hapla* besmetting om de relatie te bestuderen tussen de toetsresultaten en de waardplantreacties onder praktijkcondities. De rangorde van accessies voor de toetsparameters stemde behoorlijk overeen met die voor aaltjesinfectie en -vermeerdering in het veld. De collectie bevat een relatief resistente vezelcultivar met goede overige landbouwkundige eigenschappen welke geschikt lijkt als bron voor selectie op verhoogde resistentie. Enkele zeer resistente individuele planten die zijn geselecteerd uit landbouwkundig onbruikbare accessies werden nuttig geacht als geniteur voor kruisingen.

De diversiteit voor alle landbouwkundige en morfologische eigenschappen gezamenlijk is beschreven in Hoofdstuk 8. Onderlinge samenhang tussen eigenschappen was in het algemeen zwak, hetgeen aangeeft dat veel verschillende combinaties van eigenschappen, binnen de gevonden variatiebreedtes, door veredeling tot stand gebracht kunnen worden. Voor de interpretatie van diversiteit werden accessies gegroepeerd op grond van de status en het doel van hun domesticatie. Dit resulteerde in de onderscheiding van vier zogenaamde plant-use groepen: vezelcultivars, vezellandrassen, drugtypen, en echt wilde of verwilderde populaties. Deze vooraf ingedeelde groepen konden redelijk goed onderscheiden worden op basis van experimentele waarnemingen. Bastvezel- en cannabinoïd gehalten waren de belangrijkste onderscheidende kenmerken. Als aanvulling hierop werd geprobeerd de accessies genetisch te karakteriseren op basis van elektroforesepatronen van zaadeiwitten. Alhoewel reproduceerbare variatie in bandjespatronen tussen accessies werd gevonden waren de resultaten onbevredigend omdat te verwachten gemeenschappelijke afstamming niet weerspiegeld werd. Bovendien waren de patronen onafhankelijk van enigerlei classificatie op basis van oorsprongsgegevens, of landbouwkundige en morfologische waarnemingen.

In Hoofdstuk 9 worden individuele accessies aangewezen die voor veredeling en teelt geschikt lijken. Onderwerpen die verder onderzocht moeten worden in geval van daadwerkelijke akkerbouwmatige hennepteelt in Nederland en een daarop gerichte veredeling zijn resistentie tegen het wortellesieaaltje (*Pratylenchus penetrans* Cobb) en de grauwe schimmel (*Botrytis cinerea* Pers. ex Fr.). Ook de genetische relaties tussen accessies dienen verder opgehelderd te worden.

CURRICULUM VITAE

Etienne Petrus Maria de Meijer werd op 3 april 1959 geboren te Zuiddorpe (Zeeuws-Vlaanderen). Hij behaalde het HAVO-diploma en het VWO-diploma aan de scholengemeenschap St. Eloy te Oostburg. In 1986 studeerde hij af aan de Landbouwhogeschool te Wageningen in de richting landbouwplantenteelt. Van september 1987 tot februari 1994 was hij werkzaam als onderzoeker van potentiële akkerbouwgewassen bij het Centrum voor Genetische Bronnen Nederland (CGN) en het latere DLO-Centrum voor Plantenveredelings- en Reproductieonderzoek (CPRO) te Wageningen. Een deel van het daar uitgevoerde onderzoek is beschreven in dit proefschrift.

Appendix 1. Passport data of the CPRO *Cannabis* collection, see legend below for explanation.

ACCESS.	ADDR.	PARALLEL No.	P	TAXON	CULTIVAR	OTHER NAME	COU	DISTRICT/LOCATION	CROP
880816	UKZUZ	No. 880016 I.S. '87-'88	B		Rastislavicke		CSK		F
880817	AABGTH	No. 1154 I.S. '87					BRD		
880823	INRASU		B		Futura 77		FRA		F
880824	INRASU		B		Fibrimon 24		FRA		F
880825	INRASU		B		Fedrina 74		FRA		F
880826	INRASU		B		Felina 34		FRA		F
880827	INRASU		B		Ferimon 12		FRA		F
880828	INRASU		B		Fibrimon 56		FRA		F
880884	BERBAM	No. 468 I.S. '87					DDR		
880888	BRNOHB	No. 289 I.S. '88					CSK		
880973	MADRJB	No. 139 I.S. '88					ESP		
883038	BBA	BGRC 16216	B		Eletta Campana		ITA		F
883039	BBA	BGRC 16217	B		Krasnodarskaya		SUN		F
883040	BBA	BGRC 16218	B		Superfibra		ITA		F
883041	BBA	BGRC 16825	B		Fibrimon 56		FRA		F
883042	BBA	BGRC 28089	B		Fibrimon		BRD		F
883043	TAPRCA		B		Fibrimon		HUN	Kompolt	F
883044	TAPRCA		B		Szegedi-9		HUN	Kompolt	F
883045	TAPRCA		B		Uniko-B F_1		HUN	Kompolt	F
883046	TAPRCA		B		Kinai Kétlaki		HUN	Kompolt	F
883047	TAPRCA		B		Kompolti Hybrid TC		HUN	Kompolt	F
883048	TAPRCA		B		Kompolti		HUN	Kompolt	F
883049	TAPRCA		B		Kompolti Sargászáru		HUN	Kompolt	F
883050	TAPRCA	RCA3000023	L		Nyiregyházài-B-TF		HUN		F
883051	TAPRCA	RCA3000001	L			Leveleki-A-TF	HUN		F
883052	TAPRCA	RCA3000002	L			Nagyi-A-TF	HUN		F
883053	TAPRCA	RCA3000003	L			Cacsályi-A-TF	HUN		F
883054	TAPRCA	RCA3000004	L			Nyiregyházài-D-TF	HUN		F
883055	TAPRCA	RCA3000008	B		B-7		HUN	Kompolt	F
883056	TAPRCA	RCA3000016	L			Mezónagymihályi-A-TF	HUN		F
883057	TAPRCA	RCA3000018	L			Csengeri-A-TF	HUN		F
883058	TAPRCA	RCA3000020	L			Kisszekeresi-A-TF	HUN		F
883063	ROUEJB	No. 196 I.S. '87					FRA		
883065	INRASU		B		Fedora 19		FRA		F
883066	INRASU		B		Futura 77		FRA		F
883067	INRASU		B		Fibrimon 56		FRA		F
883080	ZIGUK	CAN 16/17					CSK		F
883081	ZIGUK	CAN 21/86					RUM		F
883082	ZIGUK	CAN 17/81					HUN		F
883083	ZIGUK	CAN 19/87					ITA		F
883110	BUDAHB	No. 959	W	var. *spontanea*			HUN	Dombóvàr	
883113	BUDAHB	No. 956	W	var. *spontanea*			HUN		
883114	BUDAHB	No. 955	W	var. *spontanea*			HUN		
883140	HALLBG	No. 3532	W				DDR		
883141	HALLBG	No. 997	W	var. *spontanea*			DDR		
883154	IASIGB		W	ssp. *ruderalis*			RUM		
883161	DNIHBU	No. 455 I.S. '85-'88					SUN		
883172	ICCPT		B		Secuieni 1		RUM		F
883173	ICCPT		B		Lovrin 110		RUM		F
883174	ICCPT		B		Fibramulta 151		RUM		F
883213	TSUPRS	No. 00035444	L		Kozuhara zairai		JPN	Shiga prefecture	F

Appendix 1. continued

ACCESS.	ADDR.	PARALLEL No.	P	TAXON	CULTIVAR	OTHER NAME	COU	DISTRICT/LOCATION	CROP
883247	VIR	WIR 48	L				SUN	Orlov region	F
883248	VIR	WIR 100	L				SUN	Altaij territory	F
883249	VIR	WIR 184	B				CHN		F
883250	VIR	WIR 214	B		Odnodomnaja Bernburga		SUN		F
883251	VIR	WIR 236	B				SUN		F
883260	CGN					Nederwiet	NLD		D
883262	CGN						ESP		B
883270	AMSRS		B	*indica x sativa*	Skunk 1		USA		D
883271	AMSRS		B	*indica*	Rjaf 1		AFG		D
883272	VDHAVR		L			Kenevir Tokumu	TUR		F
883289	VIR	WIR 315	L				SUN	Kirov region	F
883290	VIR	WIR 313	L				SUN	Kirov region	F
883291	VIR	WIR 75	B		SOU		SUN		F
883292	VIR	WIR 199	L				SUN	Ukraine	F
883293	VIR	WIR 429	B		Juznaja Odnovremenno Sozrevajuscaja 1 (JSO 1)		*SUN*		F
883294	VIR	WIR 106	B		Juznaja Krasnodarskaja		SUN	Krasnodar territory	F
891001	AABGTH	No. 1040 I.S. '88-'89					BRD		
891046	TAPRCA	RCA3000040 or 41	L			Komoroi-A-TF	HUN		F
891047	TAPRCA	RCA3000042	L			Penyigei-B-TF	HUN		F
891048	TAPRCA	RCA3000051	L			Apagyi-A-TF	HUN		F
891049	TAPRCA	RCA3000044	L			Mezónagymihályi-B-TF	HUN		F
891051	TAPRCA	RCA3000054	L			Debreceni-C-TF	HUN		F
891054	TAPRCA	RCA3000062	L			Ajkai-A-TF	HUN		F
891055	TAPRCA	RCA3000066	L			Polgári-A-TF	HUN		F
891056	TAPRCA	RCA3000039	L			Csárdaszállási-A-TF	HUN		F
891057	TAPRCA		L				HUN		
891058	TAPRCA	RCA3000063	L			Dormándi-A-TF	HUN		F
891059	TAPRCA	RCA3000038	L			Orosi-A-TF	HUN		F
891060	TAPRCA	RCA3000045	L			Geleji-A-TF	HUN		F
891061	TAPRCA	RCA3000046 or 47	L			Napkori-A-TF	HUN		F
891062	TAPRCA	RCA3000024	L			Penyigei-A-TF	HUN		F
891063	TAPRCA	RCA3000027 or 28	L			Nagykállói-C-TF	HUN		F
891064	TAPRCA	RCA3000015	L			Nagykállói-B-TF	HUN		F
891065	TAPRCA	RCA3000011	B		Szegedi 9		HUN		F
891066	TAPRCA	RCA3000059	L			Ujfehértói-A-TF	HUN		F
891067	TAPRCA	RCA3000061	L			Nagyecsedi-D-TF 8	HUN		F
891068	TAPRCA	RCA3000068				Csehslovák-A-TF	CSK		F
891069	TAPRCA	RCA3000070	B		Kompolti		HUN		F
891070	TAPRCA	RCA3000069	B		Uniko-B		HUN		F
891071	TAPRCA	RCA3000071	B		Kompolti Hybrid TC		HUN		F
891088	ARARI	TR31595	L				TUR		F
891090	ARARI	TR48445	L				TUR		
891092	ARARI	TR37061	L				TUR		
891093	ARARI	TR37297	L				TUR		
891094	ARARI	TR42015	L				TUR		
891131	ROSTBG	No. 114 I.S. '87					DDR		
891137	BRNOHC						CSK		
891158	ZELDER		B		Fibrimon 56		FRA		F
891186	VIR	WIR 558	B		Zolotosskaja (USO 11)		SUN		F
891187	VIR	WIR 557	B		Zolotosskaja (USO 13)		SUN		F

Appendix 1. continued

ACCESS.	ADDR.	PARALLEL No.	P	TAXON	CULTIVAR	OTHER NAME	COU	DISTRICT/LOCATION	CROP
891191	PASSIO	620E-7	W				NPL	Kalopani	
891192	PASSIO	620E-4	W				NPL	Kalopani	
891193	PASSIO	620E-10	W				NPL	Dana	
891194	PASSIO	600E	L				LBN		D
891195	PASSIO	640E				Nederwiet	NLD		D
891196	PASSIO	630E				Nederwiet	NLD		D
891197	PASSIO	450E-1				Nederwiet	NLD		D
891198	PASSIO	7E-1		*indica*		Nederwiet	NLD		D
891199	PASSIO	420E	B	*indica* x *sativa*	Skunk		NLD		D
891200	PASSIO	470-D	B	*indica* x *sativa*	Four Way		NLD		D
891201	PASSIO	570E-1	B	*indica*	Afghaan		NLD		D
891203	PASSIO	610E	B	*indica* x *sativa*	Thai/Skunk		NLD		D
891204	PASSIO	470E	B	*indica* x *sativa*	Four Way-F$_1$		NLD		D
891223	BYIHAR		B		Bialobrzeskie		POL		F
891228	VIR	WIR 499	B		JSO 14		SUN		F
891229	VIR	WIR 500	B		JSO 16		SUN		F
891240	MADRJB						ESP		
891285	WARPAH						POL		F
891286	WARPAH						POL		F
891287	WARPAH						POL		F
891288	WARPAH						POL		F
891326	VIR	WIR 547	B		Dneprovskaja Odnodomnaja 6		SUN	Ukraine	F
891327	VIR	WIR 349	L				SUN	Mari ASSR	F
891328	VIR	WIR 135	L				SUN	Transcarpathian Ukraine	F
891329	VIR	WIR 460	B		JSO 4		SUN	Krasnodar	F
891330	VIR	WIR 510	B		Dneprovskaja 4		SUN	Ukraine	F
891331	VIR	WIR 501	B		JSO 19		SUN	Ukraine	F
891332	VIR	WIR 433	B		Krasnodarskaja 35		SUN	Ukraine	F
891333	VIR	WIR 508	B		Krasnodarskaja 56		SUN	Ukraine	F
891342	GATE		B		Kinai Uniszex		HUN		F
891343	GATE		B		Kompolti Hybrid TC		HUN		F
891348	CGN						ESP		B
891383	SCHOEN						AFG		D
891384	SCHOEN						SWZ		D
891385	SCHOEN						SF.		D
891386	SCHOEN						USA	Hawaii	D
901047	IASIGB		W	ssp. *ruderalis*			RUM		
901048	IASIGB		W	ssp. *ruderalis*			RUM		
901072	ZIGUK	CAN 24/89					ITA		
901078	SHANBG	No. 243 I.S. '90					CHN		
901107	SADOPR	No. 152 I.S. '88-'91	B		Silistrenski		BGR		F
901161	YEONG		L			daema	KOR	Andong	F
901162	YEONG		L			daema	KOR	Bonghwa	F
901163	YEONG		L			daema	KOR	Milyang	F
910914	GATE		B	var. *globosa*	Panorama		HUN	Kompolt	O
910915	GATE		B		Kompolti Hyper Elite		HUN	Kompolt	F
910972	AMSRS	71.3		*sativa*			NLD		D
921017	BBA	BGRC 52092	L				TUR		
921018	BBA	BGRC 52137	L				TUR		
921019	INFIBR		B		Bialobrzeskie		POL		F
921020	INFIBR		B		LKCSD		POL		F

Appendix 1. continued

ACCESS.	ADDR.	PARALLEL No.	P	TAXON	CULTIVAR	OTHER NAME	COU	DISTRICT/LOCATION	CROP
921040	INFIBR		B		Beniko		POL		F
921049	VIR	WIR 21					YUG		
921050	VIR	WIR 112					ITA		
921051	VIR	WIR 175					CHN		
921052	VIR	WIR 408					HUN		
921053	VIR	WIR 496	B		JuS 12		SUN		F
921054	VIR	WIR 562	B		Dneprovskaja 84		SUN		F
921106	TSUPRS	No. 508 I.S. '92					JPN		
921119	LINZBG	BVAL 903279	L				KOR		
921122	AMSRS						CHN	Island Hunan	F
921168	PRAGGR		B		Rastislavicke		CSK		F
921198	AMSRS						CHN	Island Hunan	F
921199	INDUV	A-2					AFG	Mazar-i-Sharif	D
921200	INDUV	A-3					AFG		D
921201	INDUV	C-338	W				CHN		
921203	INDUV	No. 20 coll. E. Small	W				CAN		
921204	INDUV	No. 150 coll. E. Small	B		Ramo		BRD		F
921205	INDUV	H-3					USA	Hawaii	D
921206	INDUV	H-316	W	*ruderalis*			HUN		
921207	INDUV	I-1	L				IND	N.W. Himalayas	F
921208	INDUV	No. 152 coll. E. Small					JPN		
921209	INDUV	No. 66 coll. E. Small					JAM		D
921210	INDUV	WIR 11	L				YUG		F
921211	INDUV	WIR 22					YUG		F
921212	INDUV	WIR 29					YUG		F
921213	INDUV	WIR 57					ESP		F
921214	INDUV	WIR 58					SUN	Far East	F
921215	INDUV	WIR 125					SUN		F
921216	INDUV	WIR 126	L				SUN	Ukr., Mukachevsky distr.	F
921217	INDUV	WIR 142	L				SUN	Novosibirsk region	F
921218	INDUV	WIR 185					CHN	Shan-Va	F
921219	INDUV	WIR 205					SUN	Ukraine	F
921220	INDUV	WIR 220	B		Bernburgskaya 219354		DDR		F
921221	INDUV	WIR 252	B		Bernburgskaya		DDR		F
921222	INDUV	WIR 311					SUN	Ukr., S. Cherkasskaya	F
921223	INDUV	WIR 354					SUN	Russia, Chuvash ASSR	F
921224	INDUV	WIR 356					SUN	Russia, Mari ASSR	F
921225	INDUV	WIR 391	B		JuS 6		SUN	Central Russia	F
921226	INDUV	WIR 405					SUN	S. Archonskaya	F
921227	INDUV	WIR 447	B		Fibrimon		HUN		F
921228	INDUV	WIR 499	B		JSO 14		SUN		F
921229	INDUV	WIR 523	B				SUN		F
921230	INDUV	M-1					MEX		D
921231	INDUV	No. 24 coll. E. Small					MEX		D
921232	INDUV	No. 289 coll. E. Small					MEX		D
921233	INDUV	N-1	W				NPL		D
921234	INDUV	No. 235 coll. E. Small					ZIM		D
921235	INDUV	SA-2					SF.		D
921236	INDUV	No. 63 coll. E. Small					SLE		D
921237	INDUV	Th-2					THA		
921238	INDUV	Tr-1					TUR		F

Appendix 1. continued

ACCESS.	ADDR.	PARALLEL No.	P	TAXON	CULTIVAR	OTHER NAME	COU	DISTRICT/LOCATION	CROP
921239	INDUV	U-2					UGA	Mbale district	D
921240	INDUV	No. 25 coll. E. Small	W				USA	Minnesota	
931036	ROOTS					Red Dawn	SUN		

Appendix 1. legend

ACCESS.: Unique number identifying every accession, the first two digits indicate the year of receipt.

ADDR.: Address code of donor (listed below in alphabetical order).

AABGTH	Botanischer Garten der Technischen Hochschule, Aachen, Germany
AMSRS	Research Seeds, Amsterdam, Netherlands
ANGEJB	Faculte Mixte de Medecine et de Pharmacie, Angers, France
ARARI	Agricultural Research and Introduction Centre, Izmir, Turkey
BBA	Institut für Pflanzenbau und Pflanzenzüchtung der Bundesforschungsanstalt für Landwirtschaft, Braunschweig-Völkenrode, Germany
BERBAM	Bereich Botanik und Arboretum des Museums der Humboldt-Universität, Berlin, Germany
BRATBG	Botanical Garden of Natural Science, Faculty of Komensky University, Bratislava, Czechoslovakia
BRNOHB	Hortus Botanicus, Universitatis Purkynianae, Brno, Czechoslovakia
BRNOHC	Hortus Centralis, Cultura Herb. Medic., Facultas Medica, Universitas Purkyniana, Brno, Czechoslovakia
BUDAHB	Hortus Botanicus, Instituti Plantarum Medicinalium, Budakalasz, Hungary
BYIHAR	Ogrod Botaniczny, Instytut Hodowli i Aklimatyzacji Roslin, Bydgoszcz, Poland
CGN	Centre for Genetic Resources the Netherlands, Wageningen, Netherlands
DIJOJB	Jardin Botanique de la Ville, Dijon Cedex, France
DNIHBU	Hortus Botanicus Universitatis, Dniepropetrowsk, Ukraine
GATE	Godolloi Agrartudomanyi Egyetem Kutatointezete, Agricultural Research Institute, Kompolt, Hungary
HALLBG	Botanischer Garten der Martin-Luther-Universität (Sect. Bio-Wissenschaften), Halle-Saale, Germany
IASIGB	Gardina Botanica a Universitatii "Al.I.Cuza", Iasi, Rumania
ICCPT	I.C.C.P.T. - Fundulea, 8264- Fundulea Jud., Calarasi, Rumania
INDUV	Indiana University, Bloomington, USA
INFIBR	Institute of Natural Fibres, Poznan, Poland
INRASU	INRA, Ministere de l'Agriculture, Domaine Pluridisciplinaire du Magneraud, Surgeres, France
LINZBG	Botanischer Garten und Arboretum der Stadt Linz, Linz, Austria
MADRJB	Real Jardin Botanico, Csic, Madrid, Spain
PASSIO	Dutch Passion Seed Company, Amsterdam, Netherlands
PEKIHB	Hortus Botanicus Pekinensis, Institutum Botanicum Sinicae, Peking, China
PRAGGR	Res. Inst. of Pl. Production, Div. of Genetics and Pl. Breeding Methods, Praha, Czechoslovakia
ROOTS	Roots, zaden & planten, Rotterdam, Netherlands
ROSTBG	Botanischer Garten der Wilhelm-Pieck-Universität, Rostock, Germany
ROUEJB	Jardin Botanique de la Ville de Rouen, Rouen, France
SADOPR	Institute of Introduction and Plant Resources 'K. Malkov', Sadovo, Bulgaria
SCHOEN	Fa. Schoenmakers, Lent, Netherlands
SHANBG	Shanghai Botanic Garden, Long Wu Lu, Shanghai, China
TAPRCA	Research Centre for Agrobotany I.P.P.Q., Sect. for Plant Introduction and Gene Bank, Tapioszele, Hungary
TSUPRS	Tsukuba Medicinal Plant Research Station, National Inst. of Hygienic Sciences, Tsubaku city, Japan
UKZUZ	The Central Checking and Testing Institute of Agriculture, Praha, Czechoslovakia
VDHAVR	Kweekbedrijf D.J. van der Have B.V., Rilland, Netherlands
VIR	N.I. Vavilov All-Union Institute of Plant Industry, St Petersburg, Russia

Appendix 1. legend continued

	WARPAH Polska Akademia Hauk, Ogrod Botanczny (Bot. Gard. of the Pol. Acad. of Science), Warszawa, Poland YEONG Yeongnam Crops Exp. Station, R.D.A. Milyang - 1085 Naidong, Korea ZELDER Kweekbedrijf Zelder B.V., Ottersum, Netherlands ZIGUK Institut für Genetik und Kulturpflanzenforschung, Gatersleben, Germany
PARALLEL No.:	Original registration number in donor collection. ('I.S.' is abbreviation for 'Index Seminum'; 'Collection E. Small' numbers refer to Small & Beckstead, 1979)
P:	Population status (B=Breeder's cultivar, L=Landrace, W=Wild or naturalized).
TAXON:	Botanical infrageneric name (if explicitly provided by donor).
CULTIVAR:	Cultivar name (information provided by donor).
OTHER NAME:	Any other indication of accession apart from taxon name or cultivar name (information provided by donor).
COU:	Code of country where accession was obtained from by donor. (AFG=Afghanistan; BGR=Bulgaria; BRD=former Federal Republic of Germany; CAN=Canada; CHN=China; CSK=former Republic of Czechoslovakia, DDR=former German Democratic Republic; ESP=Spain; FRA=France; HUN=Hungary; IND=India; ITA=Italy; JAM=Jamaica; JPN=Japan; KOR=Republic of Korea; LBN=Lebanon; MEX=Mexico; NLD=Netherlands; NPL=Nepal; POL=Poland; RUM=Rumania; SF.=Southern Africa; SLE=Sierra Leone; SUN=former Union of Soviet Soc. Rep.; SWZ=Swaziland; THA=Thailand; TUR=Turkey; UGA=Uganda; USA=United States; ZIM=Zimbabwe)
DISTRICT/LOCATION:	Region where accession is adapted to.
CROP:	Type of utilization of domesticated accessions (B=bird-seed sample; D=drug strain; F=fibre hemp; O=ornamental cultivar).

Appendix 2. Summary of compiled evaluation data, see legend below for explanation.

a	b	c	c'	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
880816	50	F	F	19.8	4.5	1	3	0.22	1.83	0.12	4.25	212	268	256	556	32	17.6	67.8	4.2	13.4	17.6	110.1	50.5
880817	*	*	F	17.2	4.5	1	3	0.35	2.18	0.16	4.60	162	243	212	545	31	17.6	71.7	2.3	12.5	14.8	119.2	55.0
880823	48	F	F	17.4	4.5	7	3	0.15	1.20	0.12	5.15	212	260	360	538	32	17.8	**59.5**	**5.7**	**17.1**	**22.4**	191.8	113.2
880824	48	F	F	16.8	4.7	1	3	0.26	1.34	0.20	4.50	203	250	285	526	33	16.3	57.8	4.9	19.3	24.2	149.6	85.3
880825	48	F	F	17.0	4.6	1	3	0.25	1.67	0.17	4.25	176	263	261	546	32	18.0	60.0	3.6	18.9	22.6	155.9	89.6
880826	48	F	F	16.2	4.5	1	3	0.15	1.59	0.08	4.60	187	253	236	515	28	18.5	60.6	5.2	20.1	25.3	144.1	94.6
880827	48	F	F	15.4	4.7	1	3	0.17	1.16	0.15	4.40	172	253	269	534	28	19.1	57.1	3.9	21.1	25.0	142.4	87.5
880828	48	F	F	16.4	4.5	3	3	0.30	1.18	0.26	4.20	183	255	224	487	30	16.8	59.6	2.3	20.9	23.2	147.7	74.8
880884	*	*	f	17.2	4.3	3	5	0.38	1.21	0.33	4.70	162	258	266	535	35	16.1	67.7	1.4	17.6	19.0	126.8	77.1
880888	*	*	f	15.2	4.5	7	5	0.34	1.09	0.31	4.20	164	237	221	564	30	19.3	63.6	3.0	15.9	18.9	159.2	98.6
880973	*	*	f	18.2	4.2	1	5	**0.84**	**0.84**	**0.84**	**4.62**	**152**	**259**	**263**	**559**	**34**	**17.5**	**69.4**	**0.8**	**11.4**	**12.2**	103.9	36.9
883038	44	F	F	20.0	4.7	1	3	0.63	0.86	0.74	3.80	230	277	286	507	30	17.4	59.6	2.2	22.1	24.3	122.9	55.1
883039	45	F	F	23.8	4.5	1	1	1.21	0.78	1.59	3.70	225	272	295	485	32	15.1	67.2	1.1	18.1	19.2	135.1	75.0
883040	44	F	F	16.8	4.6	3	3	0.37	1.36	0.29	4.45	229	284	268	554	35	15.6	59.8	2.6	20.6	23.2	150.1	79.0
883041	48	F	F	18.0	4.5	1	1	0.25	1.02	0.24	4.30	204	258	265	570	34	17.0	60.7	4.0	19.8	23.8	172.4	99.9
883042	52	F	F	17.4	4.7	1	1	0.08	0.99	0.08	4.70	163	237	252	475	33	14.2	57.3	2.6	21.8	24.4	143.2	67.8
883043	47	F	F	15.8	4.6	5	5	0.62	0.86	0.70	4.35	182	264	264	554	33	16.6	54.8	7.2	20.9	28.1	156.3	112.3
883044	46	F	F	17.2	4.3	1	3	0.25	1.36	0.18	4.25	226	267	333	504	34	15.6	53.0	6.0	20.2	26.3	130.3	71.3
883045	47	F	F	17.8	4.5	3	3	0.35	0.92	0.38	4.30	213	263	285	538	32	17.7	52.5	8.6	22.1	30.8	160.0	89.2
883046	47	F	F	17.6	4.4	1	3	1.33	0.43	3.04	4.15	233	296	263	532	35	15.4	49.6	7.8	23.9	31.7	142.1	61.8
883047	47	F	F	21.4	4.9	3	3	0.63	1.01	0.67	4.10	223	273	278	548	31	17.5	56.6	6.6	19.3	25.9	131.3	64.2
883048	47	F	F	19.8	4.4	1	3	0.10	1.51	0.07	4.70	234	275	330	538	37	14.6	50.3	6.7	22.4	29.1	130.0	63.8
883049	47	F	F	19.6	4.8	1	3	0.25	1.08	0.23	4.00	198	275	257	531	34	16.6	52.2	12.6	19.6	32.2	79.1	27.1
883050	47	f	F	16.0	4.5	5	3	0.37	0.67	0.55	4.35	206	262	308	500	33	15.3	62.8	4.3	17.7	22.1	148.4	84.2
883051	47	f	f	23.6	4.4	3	3	0.57	0.87	0.68	3.75	170	257	269	526	32	16.1	72.9	1.3	11.5	12.8	132.2	90.9
883052	47	f	f	19.4	4.4	5	5	0.12	1.69	0.07	3.95	169	250	232	513	35	15.5	74.4	1.8	10.2	12.0	104.5	37.5
883053	47	f	f	13.8	3.8	5	5	0.25	1.34	0.18	3.90	164	249	254	563	32	17.5	66.7	2.9	16.1	19.0	133.7	71.9
883054	47	f	f	17.2	4.3	5	7	**0.30**	1.16	**0.41**	**4.06**	**178**	**244**	**215**	**557**	**35**	**16.3**	**69.1**	**1.8**	**10.1**	**12.4**	156.0	83.0
883055	47	F	F	21.8	4.6	1	3	0.79	0.83	0.91	4.30	233	279	256	594	34	17.3	55.8	4.1	20.0	24.1	81.9	36.9
883056	47	f	f	17.2	4.5	1	3	**0.24**	**1.53**	**0.33**	**4.22**	**203**	**264**	**249**	**561**	**29**	**20.7**	**62.7**	**2.2**	**12.1**	**14.5**	*	*
883057	47	f	f	23.1	5.0	3	3	0.50	1.38	0.36	3.70	171	253	248	528	30	18.3	75.0	0.8	11.4	12.2	105.6	44.7
883058	47	f	f	15.0	4.5	3	3	0.32	1.09	0.29	3.95	179	253	279	550	32	16.9	66.5	1.8	15.8	17.6	165.9	88.7
883063	*	*	d	14.0	3.9	1	3	0.59	0.21	2.68	3.25	234	289	254	575	30	19.3	58.5	1.8	15.0	16.8	158.4	100.6

Appendix 2. continued

a	b	c	c'	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
883065	48	F	F	17.8	5.1	1	3	0.26	1.40	0.20	4.25	168	250	221	538	32	17.6	63.7	3.3	16.2	19.5	159.9	94.3
883066	48	F	F	15.2	3.9	1	3	0.32	1.76	0.17	4.05	215	262	292	536	33	16.3	62.3	6.7	17.0	23.7	158.9	65.2
883067	48	F	F	16.0	4.3	1	3	0.54	1.69	0.30	4.30	198	250	238	551	30	19.0	67.5	3.3	14.1	17.5	169.7	95.8
883080	50	*	f	20.2	4.6	3	3	0.02	0.75	0.25	5.85	137	217	207	516	34	15.9	70.6	2.5	11.5	14.3	106.5	60.2
883081	44	*	f	19.8	4.8	3	3	0.23	1.51	0.33	4.29	194	259	241	555	31	20.4	66.3	2.8	12.9	15.8	*	*
883082	47	*	f	14.2	4.3	7	3	0.13	1.26	0.30	4.07	148	205	228	518	37	14.9	64.3	1.9	13.3	14.9	*	*
883083	44	*	F	17.2	4.4	5	9	0.37	1.65	0.38	4.11	207	270	286	560	26	23.0	64.5	2.1	17.9	18.7	*	*
883110	47	w	w	13.8	3.7	5	7	0.04	0.88	0.26	4.58	161	244	202	532	29	20.3	69.4	1.9	8.4	11.3	*	*
883113	47	w	w	11.6	3.9	7	5	0.03	1.32	0.24	4.80	148	201	218	541	22	23.8	65.2	1.1	12.8	13.6	285.0	217.5
883114	47	w	f	15.4	4.2	5	7	0.13	1.00	0.32	4.64	158	233	217	551	26	21.2	67.8	2.2	9.6	12.6	123.8	65.5
883140	53	w	w	16.0	4.1	1	3	0.09	1.52	0.06	4.75	187	283	148	520	33	15.8	66.8	0.6	10.0	10.6	253.7	116.5
883141	53	w	w	6.8	3.2	7	9	0.06	0.74	0.09	5.00	165	233	177	497	30	16.8	75.2	0.3	9.2	9.5	301.7	155.3
883154	44	w	w	10.4	3.8	5	7	0.06	1.50	0.25	4.57	154	203	243	588	28	22.8	66.1	2.2	16.5	17.8	278.6	200.0
883161	*	*	f	17.8	4.2	3	3	0.64	2.26	0.43	4.20	175	240	238	577	34	19.5	68.2	1.3	9.0	11.0	106.8	53.0
883172	44	F	F	15.4	4.3	5	3	0.75	1.13	0.68	4.80	206	253	308	544	31	18.0	59.4	6.0	20.5	26.5	153.8	88.5
883173	44	F	F	19.8	4.8	3	5	0.66	1.29	0.54	4.35	184	263	282	493	35	14.4	60.7	5.7	16.1	21.8	148.6	70.3
883174	44	F	F	17.6	4.7	1	3	0.24	1.53	0.16	4.10	192	262	282	554	27	20.5	70.4	2.2	14.1	16.3	145.1	48.5
883213	35	f	f	29.4	4.8	1	3	0.08	0.68	0.12	4.15	260	323	328	534	28	18.8	65.9	1.8	10.3	12.7	*	*
883247	57	f	f	19.8	4.5	1	1	0.13	1.21	0.12	4.70	148	206	207	509	30	18.5	72.4	1.9	10.2	12.1	200.7	126.6
883248	54	f	f	20.8	4.5	3	3	0.13	1.03	0.13	4.85	162	213	202	538	30	18.1	72.3	1.3	11.4	12.7	125.4	62.8
883249	*	F	f	18.0	4.3	1	5	0.91	1.28	0.74	2.95	234	289	215	568	29	19.9	73.1	1.0	11.4	12.4	116.4	68.6
883250	52	F	f	17.4	4.3	1	3	0.17	0.77	0.22	4.45	156	210	222	529	33	16.3	74.1	2.2	10.0	12.2	160.3	106.6
883251	*	F	f	17.0	4.3	3	3	0.19	0.72	0.25	4.30	157	214	229	504	33	15.3	68.9	2.1	12.2	14.3	173.6	103.6
883260	*	d	d	14.0	4.1	3	3	1.77	0.23	7.46	3.90	178	268	191	549	28	19.5	70.9	1.1	10.0	11.1	220.4	154.5
883262	*	*	F	15.8	4.2	1	5	0.09	1.47	0.27	4.68	174	244	266	533	31	18.5	58.8	5.2	15.9	20.9	127.7	70.8
883270	*	d	d	13.8	3.9	1	7	1.46	0.27	5.03	4.40	226	279	194	602	28	22.3	66.7	0.0	14.1	14.1	159.8	70.1
883271	35	d	d	16.0	4.2	1	3	1.15	1.59	0.72	3.25	260	307	177	575	29	20.0	*	*	*	11.4	143.1	75.0
883272	39	f	f	21.4	4.7	1	3	0.18	1.31	0.14	3.85	230	296	294	547	27	20.3	66.1	1.5	14.1	15.6	105.3	46.1
883289	59	f	f	12.4	3.6	3	3	0.07	0.54	0.13	4.85	140	192	72	506	29	17.0	*	*	*	14.7	192.2	120.4
883290	59	f	f	14.4	4.1	1	3	0.11	0.89	0.12	5.20	143	190	63	503	27	19.0	68.3	0.1	9.8	10.2	139.7	80.0
883291	*	F	f	19.2	4.6	1	3	0.26	0.98	0.26	4.70	148	213	187	511	31	16.4	72.2	2.3	10.7	13.0	162.9	93.3
883292	*	f	f	18.5	4.5	1	3	0.09	1.59	0.06	4.40	148	210	187	546	30	19.0	69.7	1.7	13.2	14.9	149.9	79.3
883293	*	F	f	21.0	4.5	1	3	0.56	1.06	0.47	4.45	158	244	195	524	28	18.6	73.5	2.7	9.6	12.3	116.2	66.3

Appendix 2. continued

a	b	c	c′	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
883294	45	F	f	19.2	4.5	1	3	0.32	1.08	0.29	4.10	164	253	244	538	30	18.0	72.4	1.8	10.1	12.0	174.8	74.3
891001	*	*	f	18.0	4.5	3	1	0.35	1.33	0.30	4.65	161	255	218	505	32	15.5	67.3	2.5	13.5	15.9	125.6	95.7
891046	47	f	f	19.2	4.1	1	5	0.26	1.11	0.23	3.60	175	246	252	540	33	17.4	76.9	0.6	11.9	12.5	96.7	44.4
891047	47	f	f	21.6	4.3	5	7	1.03	0.98	1.03	3.45	185	259	222	542	31	17.0	70.1	1.2	10.8	12.1	122.3	56.4
891048	47	f	f	21.8	4.5	1	3	0.37	1.09	0.36	3.90	183	259	271	578	27	22.3	72.5	1.2	10.7	11.9	113.3	60.7
891049	47	f	f	19.4	4.4	1	3	0.72	0.90	0.81	3.65	186	240	253	524	32	16.5	72.2	0.6	11.2	11.9	114.1	58.0
891051	47	f	f	15.4	4.2	3	5	0.61	0.88	0.68	3.95	197	259	256	557	34	16.9	73.1	1.3	11.0	12.3	157.8	95.6
891054	47	f	f	19.4	4.3	1	5	0.67	1.78	0.38	3.80	168	249	255	505	34	15.6	70.8	0.8	11.0	11.8	123.8	82.6
891055	47	f	f	21.2	4.6	3	3	0.59	1.17	0.50	3.55	177	251	264	553	28	20.3	73.3	1.9	10.3	12.2	167.5	119.2
891056	47	f	f	18.0	4.4	1	3	0.64	1.19	0.54	3.95	182	259	259	508	29	18.9	76.9	1.2	10.5	11.7	167.3	95.8
891057	47	*	f	16.8	4.1	3	3	0.11	1.41	0.08	3.85	160	249	167	479	32	15.5	72.4	1.8	8.4	10.2	178.8	85.4
891058	47	f	f	18.0	4.2	1	5	0.59	0.83	0.71	3.85	177	265	281	531	31	18.3	70.2	1.3	12.6	13.9	165.0	107.8
891059	47	f	f	15.4	4.2	3	3	0.42	1.08	0.38	4.35	172	248	259	554	27	20.9	66.4	3.0	13.0	16.0	160.1	95.9
891060	47	f	f	16.6	4.3	1	3	0.26	1.09	0.24	3.95	186	254	283	515	37	14.8	59.9	1.9	17.3	19.3	136.5	60.9
891061	47	f	f	16.6	4.1	5	5	0.17	0.94	0.17	3.90	167	218	213	520	29	18.4	66.9	1.7	13.4	15.1	172.9	90.4
891062	47	f	f	14.8	4.2	3	7	0.14	0.87	0.18	4.40	174	223	228	504	29	18.1	69.3	1.7	11.8	13.6	161.0	87.9
891063	47	f	f	19.8	4.4	3	3	0.26	1.11	0.23	3.75	183	263	273	547	34	15.6	72.3	1.2	11.7	12.9	142.8	77.2
891064	47	f	f	20.0	4.2	1	1	0.76	0.79	1.15	4.15	182	253	259	516	31	16.5	71.7	0.8	12.1	12.9	176.8	100.1
891065	46	F	F	18.2	4.2	1	5	0.18	1.19	0.16	4.10	230	266	285	524	34	15.6	59.4	7.2	18.4	25.6	128.8	63.3
891066	47	f	f	13.0	3.8	7	7	0.36	0.81	0.45	4.10	164	213	206	497	30	17.4	73.2	0.8	10.1	10.9	141.2	80.4
891067	47	f	f	22.0	4.3	1	3	0.25	1.34	0.21	4.10	186	250	272	539	32	16.6	74.2	1.2	11.6	12.7	121.8	68.3
891068	50	*	f	18.8	4.1	1	5	**0.52**	**0.53**	**0.99**	**3.67**	**164**	**248**	**249**	**551**	**32**	**18.7**	**71.3**	**2.6**	**8.8**	**12.4**	171.6	84.5
891069	47	F	F	18.8	4.2	1	7	0.15	1.38	0.11	4.30	233	274	247	549	31	17.8	53.5	9.9	18.1	28.0	148.3	78.5
891070	47	F	F	17.0	4.2	1	3	0.22	1.21	0.18	4.35	219	264	258	537	34	16.5	54.3	10.3	18.4	28.8	142.8	80.6
891071	47	F	F	21.8	4.6	1	1	0.69	0.92	0.85	4.20	213	271	272	556	37	15.3	57.1	7.4	18.5	25.9	113.4	63.3
891088	39	f	d	18.4	3.8	1	3	1.32	1.41	0.95	3.80	195	298	175	533	26	20.4	62.1	0.2	14.5	14.6	*	*
891090	39	*	f	18.6	4.5	1	1	0.11	1.62	0.07	4.10	198	272	261	544	32	17.9	71.3	1.2	13.3	14.5	120.0	57.4
891092	39	*	f	16.8	4.1	1	1	0.51	1.05	0.49	4.60	219	279	240	570	26	22.3	71.1	0.4	12.8	13.2	116.8	44.4
891093	39	*	f	20.4	4.2	1	1	**0.03**	**1.13**	**0.24**	**4.13**	**227**	**286**	**294**	**582**	**30**	**19.7**	**67.8**	**1.8**	**10.4**	**12.6**	100.7	40.1
891094	39	*	f	18.8	4.5	1	1	0.10	1.17	0.09	4.45	235	294	285	550	25	22.5	71.9	0.2	12.0	12.2	130.6	56.3
891131	*	*	f	17.0	4.2	3	3	**0.10**	**1.17**	**0.29**	**4.18**	**187**	**244**	**239**	**509**	**31**	**16.9**	**67.7**	**2.2**	**11.6**	**14.0**	166.2	94.1
891137	*	*	f	16.8	4.5	1	3	0.59	1.37	0.42	4.35	163	253	219	502	28	17.8	70.5	1.7	11.4	13.2	114.1	44.8
891158	48	F	F	16.4	4.4	3	3	0.21	1.07	0.20	4.40	213	260	273	526	27	21.0	62.7	3.6	18.9	22.5	143.3	83.6

Appendix 2. continued

a	b	c	c'	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
891186	*	F	F	19.0	4.8	3	3	0.12	0.96	0.12	4.15	193	246	247	541	31	17.6	57.4	5.6	19.1	24.7	161.5	102.1
891187	*	F	F	24.4	4.4	7	3	0.05	1.12	0.26	4.57	189	248	240	538	24	22.2	59.0	6.9	19.2	25.5	176.3	110.6
891191	28	w	w	6.6	3.4	5	7	0.06	0.82	0.29	5.22	243	317	232	610	23	26.6	62.2	1.9	13.0	14.7	126.3	43.2
891192	28	w	w	8.2	3.5	7	7	0.44	0.55	0.98	4.49	216	296	228	572	32	18.7	63.5	2.6	12.2	15.1	*	*
891193	28	w	f	19.0	4.4	1	1	1.17	0.62	1.95	5.05	246	338	315	598	27	22.8	68.5	0.5	14.4	14.9	67.7	21.9
891194	34	d	f	23.8	4.5	1	1	0.93	0.40	2.30	3.75	225	280	235	570	32	18.5	75.8	0.5	9.5	10.0	117.1	81.0
891195	*	d	d	18.4	4.3	3	5	0.89	0.29	2.87	3.82	161	236	169	494	34	14.9	63.2	0.4	13.7	13.4	201.4	105.5
891196	*	d	f	12.6	3.9	3	9	0.64	0.89	0.69	4.35	170	240	246	535	24	22.6	68.3	1.4	11.6	13.1	103.2	20.2
891197	*	d	d	11.8	3.7	1	3	1.17	0.93	1.20	3.37	227	300	233	539	31	16.9	61.9	0.9	13.5	13.9	121.7	73.1
891198	*	d	d	12.0	3.6	3	3	1.71	0.96	2.81	3.59	197	259	253	549	32	19.3	68.4	1.9	11.3	13.4	138.1	48.1
891199	*	d	d	16.8	4.0	3	5	1.57	0.93	1.58	4.06	201	271	233	565	29	19.7	66.1	0.9	12.4	13.1	170.5	70.6
891200	*	d	d	13.2	3.8	1	3	1.00	0.55	1.52	3.37	223	292	265	554	32	18.3	65.3	2.9	13.6	16.4	*	*
891201	*	d	d	16.4	4.0	3	3	2.00	1.18	1.40	3.19	227	306	253	594	28	24.6	60.8	0.7	11.9	12.4	126.1	64.2
891203	*	d	d	12.6	3.9	1	3	1.29	0.36	2.66	4.08	233	320	244	554	26	21.8	63.6	3.0	10.8	14.4	132.1	78.6
891204	*	d	d	15.3	4.1	5	5	2.29	0.89	1.92	3.70	220	282	248	582	21	29.1	65.9	2.4	11.9	14.6	111.1	57.9
891223	53	F	F	14.0	3.9	5	3	0.26	0.58	0.43	5.20	176	238	292	489	31	15.4	52.9	6.6	22.9	29.5	143.7	85.8
891228	*	F	F	15.4	4.0	3	7	0.03	0.92	0.25	4.70	140	234	261	533	34	16.3	56.6	4.1	22.6	24.7	141.4	66.0
891229	*	F	F	18.2	4.3	3	5	0.05	0.66	0.28	4.27	154	205	232	528	24	21.0	57.3	3.4	22.3	23.6	157.4	79.0
891240	*	*	f	14.0	4.2	3	3	0.33	1.29	0.39	4.41	184	248	256	548	26	21.6	69.5	1.8	9.3	11.9	89.8	41.0
891285	53	F	F	13.6	4.0	3	5	0.27	1.12	0.24	4.20	167	248	252	539	31	16.9	57.6	4.0	24.2	28.2	136.8	86.4
891286	53	F	F	13.6	4.1	3	5	0.23	1.11	0.20	4.75	166	253	262	536	33	16.0	59.7	7.5	18.7	26.2	127.0	68.3
891287	53	F	F	14.6	3.9	5	7	0.31	1.11	0.28	4.25	167	240	254	551	29	19.4	54.7	3.9	27.2	31.1	128.5	80.8
891288	53	F	F	14.6	4.2	5	5	0.21	0.89	0.24	4.25	171	232	255	486	33	15.0	56.1	4.6	24.2	28.8	152.6	94.6
891326	*	F	F	14.0	4.4	1	7	0.06	0.64	0.29	3.75	186	244	263	522	35	15.7	59.8	6.0	16.3	22.2	154.6	78.0
891327	*	f	w	13.6	3.9	1	5	0.03	1.07	0.25	5.67	130	185	164	525	20	24.8	62.6	0.6	14.7	14.5	169.4	105.7
891328	*	f	f	21.6	4.4	1	5	0.31	0.74	0.50	4.41	164	244	243	534	38	15.5	69.2	2.7	13.2	15.9	116.4	48.5
891329	45	F	F	16.2	3.9	1	7	0.05	1.09	0.25	3.96	148	236	262	535	39	14.3	59.7	6.0	19.9	25.0	171.9	93.1
891330	*	F	f	18.8	4.5	3	5	0.58	0.86	0.67	4.03	184	247	267	530	32	16.7	61.5	3.3	13.9	17.2	141.1	89.1
891331	*	F	f	17.6	4.4	3	3	0.20	1.08	0.36	4.22	154	220	237	544	27	21.8	60.8	3.3	15.2	18.0	128.7	70.9
891332	45	F	f	22.0	4.4	5	7	0.73	2.11	0.46	3.87	200	264	286	543	35	15.9	69.4	3.6	11.2	15.5	*	*
891333	45	F	F	15.4	4.4	3	5	0.24	1.19	0.36	4.63	142	224	238	531	33	15.3	59.6	5.1	18.8	23.0	131.4	62.2
891342	47	F	F	17.4	4.2	1	1	0.96	0.36	2.55	4.25	221	264	253	559	31	19.0	61.1	6.3	20.3	26.6	130.1	58.5
891343	47	F	F	22.2	4.4	3	1	0.55	0.78	0.76	4.20	229	266	263	556	34	16.4	55.2	8.2	18.8	26.9	109.5	54.1

Appendix 2. continued

a	b	c	c'	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
891348	*	*	f	22.6	4.3	1	1	1.25	0.27	4.54	3.80	230	272	302	577	26	21.6	*	*	*	8.8	126.9	67.4
891383	35	d	d	14.8	4.3	3	3	1.69	0.25	5.12	3.05	213	288	221	604	23	27.3	69.4	0.1	11.5	11.6	110.5	62.2
891384	28	d	d	10.8	3.6	3	5	2.25	0.37	4.19	5.03	249	342	243	514	35	13.9	66.2	1.1	9.5	11.1	127.9	51.8
891385	30	d	d	16.4	4.1	1	7	3.96	0.19	14.13	3.94	230	286	269	567	27	22.4	66.8	0.3	11.5	11.7	124.6	67.0
891386	19	d	d	9.2	3.8	5	3	1.24	0.54	1.96	4.64	252	308	248	573	33	19.1	67.4	0.9	16.6	16.2	123.1	66.0
901047	44	w	w	7.2	3.6	5	7	0.03	1.66	0.24	4.50	148	194	174	579	26	24.6	65.7	0.1	10.9	11.0	237.2	246.7
901048	44	w	w	8.6	3.7	3	3	0.03	1.15	0.24	4.62	151	220	196	612	25	27.0	67.2	0.4	12.1	12.3	280.8	184.9
901072	44	*	w	10.4	4.0	5	5	0.61	2.07	0.43	3.81	213	274	225	577	24	25.4	68.5	1.2	10.9	12.3	194.3	147.5
901078	*	*	w	18.0	4.2	3	7	0.04	0.75	0.26	3.62	252	337	216	591	34	18.9	64.5	0.2	7.0	8.2	130.8	84.0
901107	42	F	f	21.8	4.5	5	9	0.24	1.41	0.34	3.99	207	282	298	556	33	18.5	70.9	1.5	10.2	12.2	*	*
901161	36	f	f	24.4	4.5	3	5	1.52	0.43	2.57	3.35	246	308	269	557	29	20.3	69.5	0.7	9.9	10.9	*	*
901162	36	f	d	15.6	4.0	3	5	1.80	0.38	3.34	3.81	240	300	262	567	30	19.9	71.5	1.0	7.2	9.3	164.3	103.6
901163	36	f	f	18.4	4.1	3	5	1.29	0.33	2.77	3.88	246	298	234	593	27	23.6	70.2	1.2	7.5	9.8	89.0	61.7
910914	47	*	d	9.0	3.8	5	3	0.86	1.20	0.63	4.00	220	295	192	539	30	19.2	57.2	1.1	12.9	13.7	197.1	102.4
910915	47	F	F	19.0	4.4	3	5	0.08	1.44	0.26	4.12	223	282	275	561	42	14.3	54.7	11.4	18.2	30.2	155.4	78.0
910972	*	d	d	10.6	3.7	5	5	2.22	0.16	9.07	5.06	236	317	161	564	32	20.6	61.4	0.3	15.5	14.7	*	*
921017	39	*	f	18.2	4.4	1	3	0.23	3.24	0.27	4.51	203	274	276	524	30	17.5	67.4	1.6	11.7	13.5	*	*
921018	39	*	F	22.2	4.7	3	3	0.19	4.57	0.26	4.29	223	309	267	528	26	19.1	68.1	1.6	12.6	14.1	*	*
921019	52	F	F	15.2	4.2	7	5	0.13	1.33	0.30	4.13	181	240	263	536	32	17.7	53.7	6.1	23.0	27.3	*	*
921020	52	F	F	20.2	4.4	1	3	0.06	0.89	0.27	4.62	145	221	216	521	28	19.7	63.0	4.5	15.2	19.6	*	*
921040	52	F	F	13.0	4.2	3	3	0.34	1.15	0.43	4.07	178	240	259	526	32	16.1	53.9	7.8	24.9	30.9	*	*
921049	45	*	f	17.4	4.3	5	5	0.24	0.93	0.41	4.21	148	212	231	557	35	18.3	70.2	2.6	12.0	14.8	*	*
921050	44	*	f	17.2	4.3	3	7	0.12	0.80	0.33	4.34	154	224	245	526	31	18.1	68.2	2.4	11.6	14.4	*	*
921051	*	*	d	20.8	4.5	3	5	2.32	0.85	2.37	3.82	181	264	269	542	26	20.7	67.9	1.3	11.6	13.0	*	*
921052	47	*	f	16.2	4.3	5	5	0.18	1.16	0.33	4.53	187	247	251	546	28	20.1	67.6	3.3	11.5	15.3	*	*
921053	*	F	f	17.4	4.6	5	5	0.42	0.93	0.54	4.25	165	240	245	563	32	18.1	68.8	2.9	11.0	14.4	*	*
921054	*	F	F	14.8	4.4	3	3	0.20	1.27	0.33	3.75	168	244	284	550	40	14.5	65.0	4.5	16.2	20.4	*	*
921198	20	*	f	19.2	4.5	1	3	0.77	0.17	3.03	3.78	252	323	269	545	27	21.0	70.0	1.0	10.9	12.1	*	*

Appendix 2. legend

Passport data	a : accession no.		
	b : adaptation latitude (degrees)		
	c : pre-defined plant-use group (see Chapter 8)		
Experimental data *) **)	c': plant-use group as assigned on the basis of discriminant function scores (see Chapter 8)		
	1 : ACHtsw	11 : PLAhei	
	2 : ACHlen	12 : FIBlen	
	3 : ACHabc	13 : FIBwid	
	4 : ACHmar	14 : FIBrat	
	5 : THCcon	15 : WOOcor	
	6 : CBDcon	16 : SECfib	
	7 : CANrat	17 : PRIfib	
	8 : LEAind	18 : TOTfib	
	9 : ANTday	19 : HAPgal	
	10 : MATday	20 : HAPegg	

*) Data in bold are adjusted scores from the 1993 field evaluation (procedure Chapter 8).
**) Abbreviations are explained in Chapter 8, Table 1.