Journal of
INDUSTRIAL HEMP™

Volume 7
Number 1
2002

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statistical treatment of the molecular data. In addition, markers tightly linked to the male sex and their applications are discussed, and a short protocol for direct amplification of such markers from hemp tissue is provided. A molecular map of hemp, including a number of RAPD markers obtained from a progeny of a cross between a female Carmagnola plant and a monoecious accession is also presented. Finally, the state of the art of sex genetics in hemp and the possibilities of developing molecular markers linked to different hemp chemotypes are discussed.

KEYWORDS. DNA markers, sex-linked markers, monoeciousness, molecular map, chemotypes



The hemp borer, Grapholita delineana, is newly described from feral hemp in Vermont, USA. It may pose a serious pest should hemp cultivation resume in the USA. A similar situation occurred in the 1960s, when G. delineana suddenly became a serious pest in southeastern Europe. Evidence suggests the pest was imported from its native range via infested hemp seed. Larvae of G. delineana bore into stalks and destroy fiber, or they infest flowering tops and destroy seed. The larvae and adults are described, along with their life history, geographic range, and host range. Careful phytosanitary measures can prevent the spread of G. delineana into quarantine areas, such as western Europe, Canada, and the entire southern hemisphere. Breeding hemp plants for resistance to G. delineana may prevent future epidemics. Vermont feral hemp appears to be more resistant to G. delineana than feral hemp growing in the Midwestern USA; the Vermont germplasm may have descended from plants imported in the 1830s, called "Smyrna" hemp, a western European landrace devoid of Chinese ancestry. Biological and chemical controls of G. delineana are described.

KEYWORDS. Control methods, differential diagnosis, geographic distribution, hemp borer, life history, taxonomy



Hemp (Cannabis sativa L.) and nettle (Urtica dioica L.) are both attractive candidates for high fibre yields with little or no biocide requirement. Separation of fibre fine enough for quality yarns to make hemp fabric or blends has been achieved in Western Europe in the last decades only on a laboratory scale because process costs are high. In Hungary, Romania, the Ukraine and Poland a hemp processing industry has continued retting mainly by water processes. Search for a commercially and environmentally viable method led us also to explore enzymatic separation, which was initiated by various researchers in the late 1960s and 1970s. This involves the use of various enzymes that dissolve pectin and hemicellulose between the cell walls thus freeing the fibre bundles and fibres. We tested various commercial and non commercial products (Röhm Enzyme GmbH and Novozymes AS/Bayer AG) and methods and then measured our results against samples of fibre separated by other methods using a Stelometer to determine tensile strength of fibre bundle collectives and OFDA (Optical Fiber Diameter Analyzer) to analyse fibre bundle width. Our results showed enzymatic separation capable of producing comparably fine and strong fibre suitable for quality textiles. These studies open the way for sustainable and local production of high value fibre with low impact on the environment.

KEYWORDS. Hemp, nettle, fibre separation, enzymatic degumming, hemp yarn and fabric, nettle yarn and fabric, Cannabis, Urtica



Defibrated flax and hemp fibres form new compatible composites substituting cabinets, car-inner panels, etc. The aim is to produce composites from Danish flax and hemp that are competitive to composites reinforced with fibres such as polypropylene, glass fibre, and metals. The plant fibre composites can be used in many applications with different purposes. The composites are based on a new process, where flax or hemp are defibrated into shortened fibres and subsequently formed into mats through a unique air-forming technique mixing the plant fibre and polymers in a strong web. The mats are then moulded in a hot-press for products in all kinds of shapes. The process is based on shortened fibres from flax and hemp. The stiffness of the composites and tensile strength properties are equal in all directions, and their values are higher than those of pure plastic composites. In the new Danish composites the tensile strength seems lower than in typical composites from flax and hemp, where mats are derived from carding followed by needle punching. The main advantage is the price as the composites based on mats from the shortened flax or hemp fibres can be produced much cheaper than carded mats from long fibres.

KEYWORDS. Plant fibre, flax, hemp, mat forming, press consolidation, thermoplastic composite, reinforced composite



Cannabis is grown and processed for a wide variety of uses. Many plant parts are used as medicine for humans and livestock; whole seeds and seed oil are eaten by humans; seeds and leaves are fed to animals, seed oil and stalks are burned for fuel. Whole plants, leaves and wood have environmental uses and bark, fiber and seeds are also of ritual importance. This paper introduces an interview schedule and questionnaire for investigating Cannabis use.

KEYWORDS. Cannabis use, data collection, ethnobotony, field research, plant use, survey

OTHER CONTRIBUTIONS

The largely technocratic debate over the way humanity should respond to the now very real problem of global climate change has reached a critical point. Almost every legislative and technological option has been explored–at least theoretically–without any real progress being made in terms of actually addressing the situation and we are now at a stage where we do not have time to discuss the merits of "wind power over nuclear power" or how we can "develop ways of freezing and storing" the anthropocentrically generated excess of greenhouse gases in the atmosphere–as president Bush recently suggested. This was the "debate" during the 1980's–and unfortunately also the 90's–when the representatives of world science on this issue, otherwise known as the Intergovernmental Panel on Climate Change (IPCC), were explaining to World leaders the (then) urgent requirement to take action. This article discusses the very real possibility that Cannabis could play a part in stabilizing a global environment.

KEYWORDS. Cannabis, climate change, environment



Traditionally, hemp is a plant very well suited to Italian pedo-climatic conditions. The textile fibre produced in the past was of the finest quality due to integrated selected local varieties, good agrotechnology and experienced retting techniques. Attempts to reintroduce this crop in Italy rely upon updating the chain of fibre production and its processing that lead to the textile and its derivatives. This article discusses a new research project designed to study production and utilization of the plant.

KEYWORDS. Hemp, fibre, genetics, processing



Fin-UK, Ltd. conducted agricultural trials with the Finola variety (previously known by the breeder's code FIN 314) at several locations in Europe during the year 2000. Finola is currently completing its last year of Value for Cultivation and Use (VCU) testing in Finland, and will soon be put forward for inclusion on the EU list of approved hemp cultivars.

KEYWORDS. Finola, agricultural trials, hemp

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# Welcome to the Journal of Industrial Hemp

It has been two years since the publication of the twelfth and final issue of the Journal of the International Hemp Association (JIHA), Volume 6, #2. We are extremely proud to present the first issue of its long awaited successor, Volume 7, #1 of the Journal of Industrial Hemp: Production, Processing and Products. We will remain true to the founding principles of the International Hemp Association, including our dedication to "the advancement of Cannabis through the dissemination of information." Like its predecessor, the Journal of Industrial Hemp (JIH) will be published biannually.

The JIH is the central source for both peer-reviewed scientific and reliable practical information on successfully growing, processing and introducing hemp products. Because the journal focuses strictly on hemp, it can offer practical information for farmers and business people as well as the latest research results for scientists interested in the industrial uses of Cannabis. Contributions concern fibre, seed, and resin, and can come from a wide range of disciplines such as agronomy, chemistry, ecology, economy and markets, ethnography, history, genetic resources and breeding, plant and crop physiology, phytopathology, products and applications, regulatory issues, and technology. The JIH is an essential resource for agronomists, environmental scientists, natural fibre and food advocates, hemp businesses, hemp enthusiasts, and libraries.

The wide range of subjects treated in the JIH is reflected in the contents of this charter issue. The section containing peer-reviewed papers opens with a contribution by Giuseppe Mandolino and Paolo Ranalli reviewing the most recent advances in hemp molecular biology and marker-assisted breeding. Special emphasis is given to the use of molecular markers for the evaluation and exploitation of hemp variability, for the study and the identification of sex, and for the application to the breeding of plants with specific cannabinoid compositions.

The contribution by John McPartland presents a wide ranging review of the epidemiology of the hemp borer, one of the major hemp pests.

Amongst other topics, the insects life cycle, its geographic range and taxonomy, and a range of control methods are discussed.

Jens Dreyer and his colleagues present their experimental results with respect to a comparison of enzymatic separation, chemical separation and separation by means of steam explosion applied to hemp and nettle fibre. Their results will contribute to opening the way for sustainable and local production of fibre for quality textiles with low impact on the environment.

A new process for the production of reinforced biocomposites from hemp and flax is presented by Bodil Engberg Pallesen and Tom Løgstrup Andersen. Their results suggest that the products resulting from this process are cheaper and present a more attractive environmental profile than comparable products resulting from processes currently in use.

A short methodological paper by Robert Clarke presents a new tool for researchers: an interview schedule and questionnaire for studying Cannabis use in any field situation. The schedule accommodates a wide range of possible uses including use as a raw material for industry, medicine, food, feed, and for both ritual and recreational usage. Ultimately, this interview schedule could be used as a basic structure for gathering and integrating information into a single world-wide Cannabis use database.

The first two papers in the section for popular (non-peer-reviewed) papers explore the northernmost limits of hemp growing. Jan Bojer Vindheim presents a very interesting and nicely illustrated overview of the history of hemp in Norway. We learn that, in the nineteenth century, Norwegians used to lift their hats in greeting when approaching a field of hemp. Hemp use in Norway goes back 2000 years and hemp cultivation was supported by kings and parish priests. The paper by Jace Callaway from Finland shows that hemp not only has a long history in the Nordic countries, but also current research activities and a promising future. The article reviews the potential of hempseed adapted to northern latitudes as both food and feed. Given the unique nutritional package in terms of dietary oil, protein, vitamins and minerals supplied by hempseed, the author sees its future as a viable replacement for imported soya in Northern Europe, both as human food and as a feedstock for animals.

Michael Karus and Markus Kaup look at the market potential of hemp in the European automobile industry. Their paper presents data showing the increased demand for non-wood natural fibres in car production over the past four years. In particular, the use of hemp and kenaf shows above-average rates of increased usage. The use of natural fibres in the German automotive industry increased by 19% from 1999 to 2000, while the use of hemp increased by 90%, making native hemp fibre the second most important natural fibre in the German automotive industry. Michael

Karus also supplied us with a short news-release presenting the European Industrial Hemp Association, which can be found at the and of this issue.

Marc Deeley presents a summary of his M.Sc. thesis, where he paints an optimistic picture of hemp's potential as a biomass crop for energy production, which may contribute to climate change mitigation.

Another contribution by Paolo Ranalli presents a short overview of a research project in progress, which is funded by the Italian Ministry of Agriculture. The project's name is: "Fibre hemp: From production to utilization," and focuses on key themes of the crop; e.g., genetic resources, breeding, biotechnology and molecular biology, agrotechnology, processing, spinning and final products. A contribution by Henry Gage rounds out this first issue by presenting the results obtained with the Finola cultivar in 2001.

To succeed, the Journal of Industrial Hemp not only needs the commitment of past and current IHA members, but also must reach out to new subscribers and contributors worldwide. As always, we are soliciting manuscripts for publication. We are interested in articles reporting on experimental works and review papers, as well as popular papers presenting scientific or technical results to the general public. In addition, we seek articles about new hemp developments, scientific conferences, and hemp museums. As always, letters to the Editor are welcome. We encourage any potential contributors to contact Hayo van der Werf, Editor at: derwerf@roazhon.inra.fr. The reawakening of the long dormant hemp industry promises an exciting future for all of those interested in hemp.

The JIH is the official journal of the IHA and a subscription to the JIH is included with IHA membership (US$ 45 / EURO 50 per year). Members will also receive an IHA members' directory, and access to advice from other IHA members, as well as access (by appointment) to the IHA library and our modest hemp collection with over 1000 hemp items from around the world. The IHA is a member-supported organization and always responds to the requests of its members over non-members. All IHA staff are unpaid volunteers. All twelve issues of the JIHA, printed on pure hemp paper, are still available through the IHA. The IHA can be contacted at: iha@euronet.nl. We hope you will join us in supporting this reintroduction of hemp!

Robert Clarke
Projects Manager, IHA

David Pate
Secretary, IHA

David Watson
Chairman, IHA

Hayo M. G. van der Werf
Editor

# LETTER TO THE EDITOR

I would like to comment on an article by Sytnik and Stelmah published in the June 1999 issue of this journal [JIHA], entitled "The character of inheritance of differences in cannabinoid content in hemp (Cannabis sativa L.)." The authors do not adequately distinguish between qualitative and quantitative modes of inheritance, both of which are involved in cannabinoid production. Qualitative traits are controlled by one or two "major" genes, while quantitative traits are controlled by several "minor" genes, each exhibiting a small effect. The ratio of THC/CBD is a qualitative trait, and the yield of THC + CBD is quantitative. These are two distinctly different traits, with different modes of inheritance. The investigators state that they crossed hemp strains "practically devoid of THC and CBD" to a strain containing "large amounts of cannabinoids." However, the segregation ratios that Sytnik and Stelmah observed in F2 and BC1 generations are qualitative, not quantitative. What they should have stated is that they crossed hemp lines having a low THC/CBD ratio to a line with a high THC/CBD ratio. Their "semi-quantitative" methodology of thin-layer chromatography is not suitable for comparing cannabinoid yields between plants. It merely provides a visual indication of the relative amounts of THC and CBD in a given sample. The investigators would have obtained similar segregation patterns, regardless of the quantitative levels of THC and CBD produced by their parental strains. The confusion between the two modes of inheritance carries over into their results. The authors state that, "in the F2 generation, a segregation of about a quarter of the plants with low CBD and THC content can be seen." What they should have

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stated is that about a quarter of the plants had a low THC/CBD ratio, not a low CBD and THC content. They concluded that the genetic control of THC and CBD biosynthesis is monogenic in character, but that it is more logical to assume that it is controlled by closely linked, independent genes. Their rationale is that the segregation ratios were not "exactly the same" in all combinations of crossing. This argument is faulty (although their conclusion may be correct), because it does not account for sampling error. In their introduction, Sytnik and Stelmah conclude that inheritance of cannabinoid production may be "blending, polygenic, and sex-linked," but they do not elaborate.

The distinction between qualitative and quantitative inheritance of THC and CBD is of practical importance to hemp breeders. Sytnik and Stelmah are correct in pointing out that it is a relatively simple procedure to produce certifiable hemp strains by selecting parents with a low THC/CBD ratio. It is much more difficult for a breeder to select for low THC + CBD yield, since there are undoubtedly a large number of genetic and environmental factors affecting this trait. Important factors likely to have a genetic component are the density of the glandular trichomes on the plant surface, and the size of the resin heads. Environmental factors affecting cannabinoid production include day length, and nutrient levels (to name a few). The confounding of environmental and genetic effects on cannabinoid yield makes it a difficult trait to select upon. Hemp breeders need to focus their attention on traits of economic importance, such as fiber strength and yield. The complexity of their task is compounded if they must simultaneously select for low cannabinoid yield. There is no economic advantage in growing a fiber crop of hemp that yields 0.01 % THC over one that yields 0.3 %, or even 1.0 % THC, unless perhaps the sticky resin interferes with processing. It is imperative that regulatory agencies establish maximum allowable limits of THC with regard to natural levels of variation. In practical terms, the acceptable limit should be set at a level somewhat higher than that which naturally occurs in plants having a low THC/CBD ratio. In my studies, several plants grown from certified hemp seed exceeded 0.3 % THC (dry weight of pistillate inflorescences), although THC levels rarely exceeded 1.0 %. I therefore recommend that the maximum allowable amount of THC in certified hemp be set at 1.0 %, rather than the value of 0.3 % originally suggested by Dr. Ernest Small.

Karl Hillig
khillig@bio.indiana.edu
17 November 2001

PEER-REVIEWED PAPERS

# The Applications of Molecular Markers in Genetics and Breeding of Hemp

Giuseppe Mandolino
Paolo Ranalli

ABSTRACT. Molecular markers were employed to the characterization and analysis of hemp genetic structure by using RAPD technique. The results are presented about the statistical treatment of the molecular data. In addition, markers tightly linked to the male sex and their applications are discussed, and a short protocol for direct amplification of such markers from hemp tissue is provided. A molecular map of hemp, including a number of RAPD markers obtained from a progeny of a cross between a female Carmagnola plant and a monoecious accession is also presented. Finally, the state of the art of sex genetics in hemp and the possibilities of developing molecular markers linked to different hemp chemotypes are discussed. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address:

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KEYWORDS. DNA markers, sex-linked markers, monoeciousness, molecular map, chemotypes

## INTRODUCTION

In the last fifteen years, the use of a number of techniques involving DNA manipulation and fingerprinting has greatly enhanced the possibility of studying the plant genomes; the advent of PCR-based techniques made these type of applications faster and more reliable. The unit cost of most of the molecular techniques useful in plant breeding dropped enough to allow widespread use of this approach, at the level of small-sized seed companies. As a consequence, in the last decade the information on genetic polymorphisms, molecular maps and trait-linked markers exponentially grew and was extended to plant species barely studied until a few years ago.

Hemp is no exception to this scheme. Until 1995, no papers dealing with molecular markers in the species Cannabis sativa had been published. The reintroduction of hemp cultivation in most European countries since 1996 led in the last few years to the application of many molecular biology techniques to this species, with a particular aim to the specific problems and the traits of value for this crop.

Since the beginning, the study at the molecular level of this plant species mainly focused on three aspects of biology and physiology: fibre, sex, and secondary metabolism. These traits are of paramount importance in hemp breeding, and many of the efforts devoted to the application of molecular tools to this ancient and traditional plant are aimed to understand, exploit and, in the future, modify these characters.

In this article, the most recent advances in hemp molecular biology and marker-assisted breeding are reviewed, with special emphasis on use of molecular markers for the evaluation and exploitation of hemp variability, for the study and the identification of sex, and for the application to the breeding of plants with specific cannabinoid compositions. The other important field of application of molecular biology to hemp genetics is represented by the study and the identification of the genes involved in fibre biosynthesis. The work on these topics is still at a very early stage, though the wide syntheny of plant genomes will probably soon allow the exploitation of molecular tools derived from studies on flax (genes, expressed partial sequences, markers; Ebskamp et al., 2000).

## GERMPLASM AND VARIETY CHARACTERIZATION

Molecular markers, and particularly DNA-based markers, have been widely employed to highlight the genetic structure of several crop species, to identify a molecular profile characteristic of a given variety (fingerprint), or to track specific traits of interest. Isozymes, RFLP, RAPD, AFLP, and microsatellite have been used for these purposes; each has advantages and disadvantages. The possibility to carry out the varietal characterization, and the strategy to use, strictly depends upon the genetic structure of a variety, that is extremely different in the different crop species. Hemp is a dioecious and obligate outbred species, with a high degree of variation. Early papers dealing with DNA markers highlighted the existence of a high level of molecular polymorphism (Faeti et al., 1996). This high degree of polymorphism makes the molecular fingerprinting of hemp varieties and germplasm difficult; it is necessary to estimate, from statistical parameters derived from the molecular pattern, the degree of variation within each variety and accession and to compare it with the extent of variation between the different materials that are to be characterized. This means that hemp characterization requires that the molecular pattern of a variety must be considered as an "average" for that variety, and a number of different plants must be considered for analysis. This is a situation similar to other outbred species like alfalfa, where many varieties are considered ecotypes; at the opposite side of the spectrum there is potato where, being each variety a clone, no within-varieties variation is found.

The question arises whether or not molecular markers are able to describe the genetic structure of a variety or accession, and which tools are required. In our institute during a work on RAPD markers in hemp (Random Amplified Polymorphic DNA, Williams et al., 1990, a technique relying upon the amplification of DNA segments mediated by single decamer oligonucleotides of random sequence acting as primers), we examined in detail six varieties: a dioecious landrace (Carmagnola), a dioecious selection derived from it (C.S.), a cross-bred cv. (Fibranova), a monoecious cv. (Fibrimon), a drug strain (Northern Light) and an inbred female line belonging to HortaPharm B.V., The Netherlands. The origin of these materials and the strategy used to breed them was known, and we evaluated the correlation between the information and the results obtained by examining 102 RAPD loci generated by 5 different decamer primers (Forapani et al., in press). Ten individual plants of each variety were examined, from the same seed lot. The results are shown in Table 1.

This table shows the total number of scorable band positions (loci), the number of fixed loci (i.e., bands present in all 10 plants examined per cultivar), the number and percentage of polymorphic loci, and the presence and quantity of variety-specific alleles (i.e., bands present in all plants of one variety and absent in all plants of the others). The average allele frequency and the heterozygosity averaged over all loci are also reported, as calculated by the TFPGA software (Tools For Population Genetic Analysis; Miller, 1999). This software, on the basis of a matrix derived from the presence/absence data for each plant, calculates all the parameters shown in Table 1, and their degree of significance, using a built-in bootstrapping procedure.

The data (Carboni et al., 2000) indicated the presence of different degrees of polymorphism within each variety. The number of scorable loci was highest for Fibranova and lowest for the inbred line. The number of fixed loci was very similar for the three Italian varieties (Carmagnola, C.S., and Fibranova), and significantly higher for the French fibre cultivar Fibrimon, for the drug variety Northern Lights, and for the female line 92.73.2.13. On the other hand, the number and percentage of polymorphic loci showed an opposite trend. Very similar

TABLE 1. Statistical parameters relative to the loci identified by RAPD analysis, and characterizing the six hemp varieties studied. The data relative to all the varieties cumulatively are shown in the last line.

| Cv. | Loci | | | | | Alleles | |
|---|---|---|---|---|---|---|---|
| | Total no. | Fixed | Polymorphic | Percent of polymor-phism | Cv. specific loci | Av. allele frequency | Av. heterozygosity |
| Carmagnola | 68 | 14 | 54 | 79.4 | - | 0.46 | 0.20 |
| CS | 71 | 15 | 56 | 78.9 | - | 0.47 | 0.20 |
| Fibranova | 83 | 12 | 71 | 85.5 | - | 0.42 | 0.26 |
| Fibrimon | 62 | 24 | 38 | 61.3 | - | 0.64 | 0.15 |
| Northern Lights | 61 | 26 | 35 | 57.4 | 3 | 0.67 | 0.15 |
| b92.73.2.13 | 45 | 31 | 14 | 31.1 | 5 | 0.79 | 0.05 |
| ALL | 102 | 3 | 99 | 97.1 | | 0.58 | 0.29 |

values were found for Carmagnola landrace and its derived selection C.S. (79.4% and 78.9%, respectively). A sharp decline in the number of scorable and polymorphic loci was observed for the monoecious fiber cultivar Fibrimon and for the dioecious drug variety Northern Lights. Finally, the lowest levels of all the parameters characterized the line 92.73.2.13, where the sex specific polymorphism was absent. As expected, the highest average allele frequency was found for this same line, while hybrid cultivar Fibranova showed the lowest value.

In the same table, estimates of unbiased heterozygosity are reported. 'Carmagnola' and 'CS' are indistinguishable (0.20), while 'Fibranova' showed the highest heterozygosity (0.26), and 'Fibrimon' and 'Northern Lights' had the same lower value (0.15). Finally, the average polymorphism (97.1%) and heterozygosity (0.29) are also reported, estimated for the hemp species as a whole.

The number of markers scored, of polymorphism and of heterozygosity is highest for the most heterogeneous materials, like the cross-bred 'Fibranova' or the ecotype 'Carmagnola'. The breeding work done to narrow the genetic basis of the cv. Fibrimon (necessary to maintain the monoecious trait) and of the cv. Northern Lights (necessary to keep high and uniform the THC content) is clearly visible as a decline in the number of bands and in a lower heterozygosity and a more pronounced tendency to fix a number of alleles. The inbred line used in this work can be viewed as an extreme situation, with the lowest number of bands, polymorphism and heterozygosity. The analysis also suggests that, if originally the cv. CS (a selection from Carmagnola) had a narrower genetic basis, this feature got lost, as no parameter is able to distinguish it from the variety it was derived from, at least on the basis of the seed lot considered. It can be concluded that the use of RAPD markers coupled with the use of specific population analysis softwares can describe the materials examined with good accuracy and agreement with the known characteristics of the varieties.

The huge variability present in most hemp cvs. has also been estimated by using a software, AMOVA (Analysis of Molecular Variance, Excoffier et al., 1992; Miller, 1998), that gives, on the basis of the RAPD patterns, the partition of the variability as within-cvs. or between-cvs. Considering all the primers used, the results averaged over all the cvs. examined indicate that 51.2% of the observed variation was explained by among-individual differences within cultivars, and only 48.8% was explained by among-cultivar variation. In other words, any given difference in the observed phenotype (i.e., in the DNA pattern observed) has quite the same chance to be due to differences between individual plants of the same variety or to differences between different varieties. This can be considered a good indication of the already suggested existence of a single gene pool with limited genetic separations among groups (de Meijer, 1999).

The results obtained by using RAPD markers on the materials described suggest that the heterozygosity level of hemp is such that F1 generations could be used for constructing a molecular map, because many of the scored markers appear to be present in an heterozygous state, as estimated by the statistical analysis performed, and confirmed by the examination of some specific crosses (see below).

## THE GENETICS AND MARKER-ASSISTED SELECTION OF SEX

### Molecular Markers for Sex

Hemp is a dioecious species in nature. Sex has long been recognized as a main trait to keep track of, for reasons concerned with chemotype breeding and selection (the maximum expression of cannabinoid content is obtained upon flowering of female plants) and with fibre quality selection (the Bredemann principle; Bócsa and Karus, 1998). In the breeding practice, therefore, it may be useful to have the possibility of a discrimination of the sexes, at a stage as early as possible. This would allow for example the identification of male plants well before flowering when their fibre content and quality need to be scored according to the Bredemann method before pollination of female plants. Besides, an early and safe identification of male individuals could be of value in the breeding of monoecious hemp, where the percent of genetically male plants must be kept at low levels in order not to lose the monoecious trait in the multiplication cycles. In monoecious hemp breeding, the use of a fast and reliable system to identify male plants could be considered a true quality check of elite seed stock.

In the past, several attempts have been made to early identify the sex of dioecious hemp by means of biometrical properties of the plants, using strictly vegetative traits (Lacombe, 1980). This type of analysis, however, only allows for a statistical differentiation of the two sexes. A molecular marker tightly linked to one or both sexes would provide an ideal tool for the purpose of early discrimination, as already done in other dioecious species of agronomical interest like hops (Polley et al., 1997), asparagus (Jiang and Sink, 1997; Reamon-Büttner and Jung, 2000), Actinidia (Gill et al., 1998).

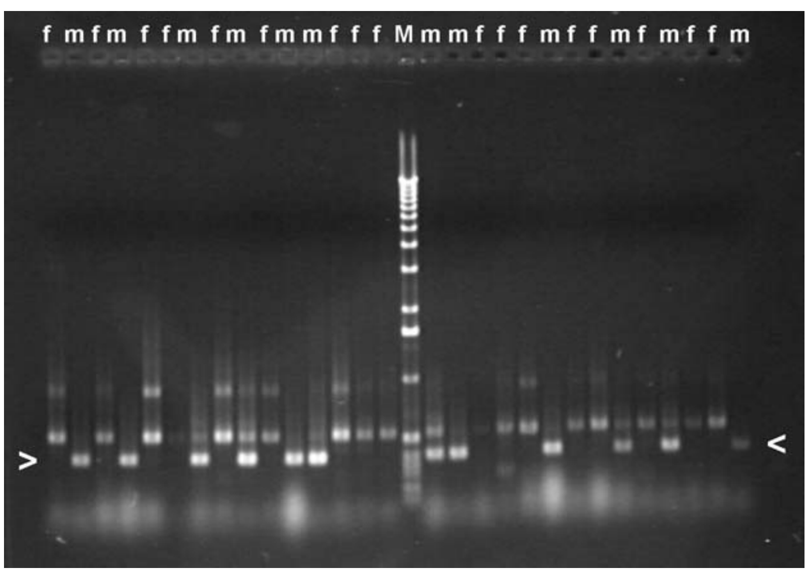
Besides, hemp has heteromorphic sex chromosomes, like Rumex and Silene, with a Y chromosome reported to be bigger than the X chromosome. The extra DNA content in the male plants has been evaluated to be about 3% of the whole genome (Sakamoto et al., 1998). These data suggest that there should be a number of male-specific DNA segments detectable on the hemp genome and potentially useful to discriminate male from female plants.

The first report of a male-associated DNA marker was from Sakamoto et al. (1995); these authors identified a DNA band present in five male plants tested and absent in five female plants. The strategy used to identify this marker was the RAPD technique already described above. The DNA band identified by these authors was 730 bp long, and a 164 bp subcloned fragment was found that, when used as a probe in Southern blot experiments on male and female digested DNA, yielded a number of sex specific polymorphisms. The male specific DNA fragment was sequenced and the sequence named MADC1 (Male Associated DNA from Cannabis 1). Despite these results the authors did not try to extend the validity of their markers to germplasm different from the single CBD strain used; besides, no attempts were made to apply their markers for a routine analysis based on markers such as STS (Sequence Tagged Sites) or SCAR (Sequence Characterized Amplified Region), more reliable than RAPD markers, for they are based on amplification of genomic DNA with specific, 15-25 bp long oligonucleotides as primers.

Mandolino et al. (1997) identified a 400 bp RAPD male-specific marker that was isolated, cloned and sequenced (MADC2; Mandolino et al., 1999). The presence of the marker, and the tight association with the male phenotype was validated examining a wide range of germplasm for the occurrence of the RAPD marker (Mandolino et al., 1998). Therefore, this marker was considered a good starting point for the development of more specific and reliable PCR based markers. A pair of primers was designed (5′-GTGACGTAGGTAGAGTTGAA-3′, and 5′-GTGACGTAGGCTATGAGAG-3′) able to amplify from male genomic DNA a 391 bp fragment as a single entity. The sequence constituting the marker is also present in female and monoecious plants, as demonstrated by the fact that this marker fails to discriminate sexes in Southern blots analysis when used as a RFLP probe; however, the result of PCR amplification using the SCAR marker (named $SCAR_{400}$) shows a clear polymorphism between male plants and female/monoecious plants in simple agarose-gel analyses (Figure 1). This marker has been shown to have a general validity, useful on very different materials and successfully tested also in a different laboratory (Dr. Andreas Peil, personal communication), it is public and immediately employable for all laboratories with a minimum equipment requirement. Its efficiency is close to 100%.

In the breeding practice, it would be useful a protocol as fast and simple as possible for the use of the described marker. In fact, a routine analysis should in principle avoid the isolation of genomic DNA from plant tissues, a procedure that still has labor intensive and time consuming steps (e.g., necessity to grind under liquid nitrogen the leaf or stem tissue from a high number of plants). However, simplified protocols do exist, particularly useful when specific primers are used in PCR assays, requiring higher annealing temperatures compared with the RAPD

FIGURE 1. Result of the screening with the $SCAR_{400}$ marker of 29 hemp plants at the 4-leaves stage. The method followed is described in the text. Lanes f, female plants; lanes m, male plants; lane M, molecular weight marker (1 kb ladder, Life Technologoies, U.K.). Arrows indicate the 400 bd male-specific band.



primers. In our laboratory, a protocol modified from Klymiuk et al. (1993; Table 2) was employed to speed up the analysis times and to cut down costs and labor as much as possible.

In this protocol, a crucial point is the quality of the tissue used for analysis. For successful analyses, particular care is recommended in respecting the times and the temperatures of incubation, as these appear to be the critical steps, along with the tissue quality. Using this method on a routine basis, we have normally a 98% success in amplification, and, therefore, it is possible to attribute exactly the sex to almost all the plants analyzed, as shown in Figure 1. The equipment required for this method is not sophisticated and expensive (water bath, table centrifuge, thermal cycler, horizontal electrophoresis equipment, UV transilluminator) and can easily be accommodated in a single room; therefore, we believe that the early and precise identification of the male plants in dioecious and monoecious hemp breeding practice is feasible at limited costs, and can, therefore, be profitably exploited for some specific application.

We now know that several are the male-specific RAPD markers; in a survey of 180 primers, corresponding to approximately 1500 DNA bands produced, 10 bands were found constantly present in all the male plants of all varieties and accessions found, and absent in the female or monoecious plants. This is a quite high percent, considering that in other dioecious species not endowed with heteromorphic chromosomes a much wider screening had to be done in order to find sex-associated markers (Hormaza et al., 1994). A progeny analysis, showing a complete lack of recombinational events between all the male-specific markers identified and the phenotype, suggests that these markers map

TABLE 2 . The main steps of the rapid determination of sex in hemp plantlets at the 4-leaves stage (modified after Klymiuk et al., 1993).

- Use the distal portion (about 4-5 mm long and 3-4 wide) of a leaflet from the first or second node;
- Place the tissue in an Eppendorf tube, add 40 µl NaOH 0.25 N;
- Place tube in a boiling water bath for 35 sec.;
- Neutralize adding 40 µl HCl 0.25 N and 20 µl Tris-Cl 0.5 M (pH 8.0)/ Triton X-100 0.25%;
- Spin briefly in a microcentrifuge;
- Place tube in a boiling water bath for 2 min.;
- Pick up the tissue fragment using a sterile micropipette tip, taking care not to damage the tissue;
- Transfer the tissue on the bottom of a PCR tube;
- Add 25 µl of amplification mixture: Taq polymerase 1 unit, $MgCl_2$ 1.5 mM, dNTPs 0.125 mM each, primers 4 ng/µl each (final concentrations in the mixture);
- Denature the mixture in a thermal cycler at 93°C for 2 min;
- Make 35 cycles of 1 min. at 94°C, 2 min. at 60°C and 2 min. at 72°C, with a final extension step of 10 min. at 72°C; and
- Run 10 µl of the amplification products at 5 V/cm. in a 1.5 % agarose gel.

on the part of Y chromosome excluded from pairing with the X chromosome during meiosis (Mandolino et al., Euphytica, submitted). Direct evidence for the position of male-associated markers also came from in situ hybridization experiments with the MADC1 sequence identified by Sakamoto et al. (2000) showing the presence of doublet signals only on the part of Y chromosome not pairing with the X at meiosis. The two sequences (MADC1 and MADC2) are not related, but share features like the G + C content and some degree of homology with plant retrotransposon sequences, and therefore it is likely that they derive from the same region of Y chromosome. Finally, it has been shown that a number of sex-related markers are also identifiable when the AFLP technique (Amplified Fragment Length Polymorphisms; Vos et al., 1995) is applied to the study of hemp genome (Peil et al., 2000), but no information on the involved sequence and behavior of the markers in progeny tests is available yet.

### Gene Expression of Sex

The flexibility of the sex expression in hemp can make the work on molecular markers more difficult, but does offer an unique chance to investigate the factors influencing the sex phenotype and the transduction pathways involved in its determination. Hemp can in fact be fully dioecious, but also monoecious plants occur, in which the same genetic background is differently regulated in the meristems giving origin to the two types of flower. Besides, it has been reported that even a full dioecious female plant can undergo partial or total reversion of the sex (Mohan Ram and Sett, 1982), which is likely to require a massive re-programmation of the genes involved in sex determination. Finally, the understanding of the expression of the genes involved in sex expression and of the factors regulating them might be of practical importance, as the mating system is one of the main objectives of breeding in hemp, as discussed above.

The possibility to obtain fully fertile male flowers by chemical treatment of a genetically female plant and the existence of several degrees of monoeciousness implies that the Y chromosome is not the only factor involved in the determination of male structures and functions. However, in an early stage of investigation, where the extent of male-female differences need to be evaluated at the level of gene expression, typical dioecious material is probably the best choice. We started recently a research program in which the cDNA-AFLP technique is being employed with the aim to identify the genes involved in the sex determination process. This approach uses the AFLP strategy not on the whole genomic DNA, but on the cDNA, i.e., the subset of DNA that is expressed in a particular tissue at a particular time. We used messenger RNA from hemp buds at different stages of development and determination; the tissue was picked up from plants of known sex, screened at a very early stage with the $SCAR_{400}$ marker described above. The mRNA is then copied into DNA, and this DNA undergoes AFLP analysis. The preliminary work led to the isolation of 919 bands corresponding to sequences expressed in the early stages after determination and absent in the vegetative stage. About 50% of these sequences were expressed only in male buds and 50% in the female buds. The following steps will be the identification of the subset of expressed sequences directly and effectively involved in the events leading to the determination of the meristems, and of the group of genes that are switched on and off during the reversion of sex identity.

## MOLECULAR MAPS

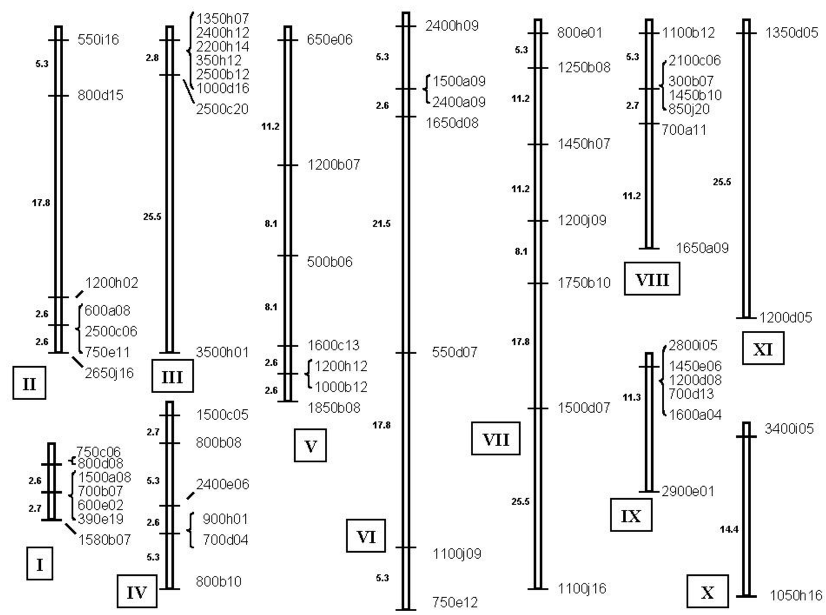
In our Institute, we are currently applying molecular markers to map a particular monoecious source, found in some plants of an accession from southern Italy. Monoeciousness occasionally occurs in different forms in hemp, and some of these forms have been introgressed in cultivated material to constitute monoecious varieties; the types of monoeciousness and the origin of the materials used has been reviewed extensively elsewhere (Neuer and von Sengbush, 1943; Bócsa, 1999; de Meijer, 1995). This trait is difficult to maintain, as even a small percent of male plants can bring, in the next multiplication generation, to the reversion of the stand to the dioecious state. Therefore, it would be particularly useful to couple the use of male-specific markers to some molecular marker able to follow in the breeding steps the monoecious trait. Unfortunately, the monoeciousness is strongly influenced in its phenotypic expression by environmental conditions and probably by a number of modifying genes still poorly studied, despite a number of hypotheses have been made (Migail, 1986). Apparently, there are different sources of monoeciousness, and a complete study on the differences and identities between them could take advantage from the use of molecular markers, as already done for different genotypes and accessions (Faeti et al., 1996).

The monoecious trait identified in our Institute (Dr. Giampaolo Grassi, personal communication) was relatively easy to maintain, as upon selfing of monoecious plants all the progeny showed some degree of monoeciousness; on the other hand, when crossed to a female dioecious plant (cv. Carmagnola), a 1:1 segregation ratio of female to monoecious individuals was observed on a 40 plants progeny. This behavior suggests a simple inheritance of the trait, though also for this material a strong influence of the environment was observed in the expression of the character in field conditions. This population, and their parental plants, was examined by RAPD markers. It was found that, also in hemp, DNA bands generated by decamer random primers can effectively and reproducibly be considered as genetic loci and their segregation followed in the progeny.

Up to now, 674 RAPD loci were scored. Among these, 269 (39.9%) were polymorphic between the two parentals. In an F1 mapping experiment, particular interest have those loci showing in the progeny a 1:1 segregation, i.e., belonging to the type Aa x aa. One hundred eighty-one out of the 269 polymorphic loci belonged to this category. Among the 405-non polymorphic loci, 46 segregated 3:1 in the progeny; however, these are not being used for F1 mapping. In general, the result of the $\chi^2$ test was not acceptable for only 16 loci out of 181 segregating 1:1 and for 14 out of 46 segregating 3:1. A number of markers segregating 1:1 were placed, by using the MapMaker software (version 3.0), on two maps, one for the 'Carmagnola' parent, and one for the monoecious accession. The 'Carmagnola' map is presently the most advanced, consisting of 66 markers distributed on 11 linkage groups (Figure 2).

The monoecious accession map consists presently of 9 linkage groups including 43 markers. The monoecious trait and several other markers were not yet included in any of the linkage groups under the conditions used; this is probably due to a still limited number of markers used, and/or to uneven or incomplete coverage of the hemp genome by the markers found. It should be noted that the map presented here does not include the Y chromosome, being the result of a cross between a female and a monoecious plant; because all the male-specific markers described in the previous sections do not show any recombination between them and with the male phenotype, the map of Y chromosome could only be a one-point, zero-dimensional map, as indeed has been shown by a preliminary map presented by Peil et al. (2000) where 19 AFLP markers and the sex locus all map in the same point with a 0 distance between them. This is not the case of our map, where a higher number of markers will probably lead to the identification of the 10 chromosomes.

FIGURE 2. Molecular map of hemp (Carmagnola variety). This map was obtained at LOD = 3.0, maximum distance 30.0




The improvement and saturation of the map derived from crosses involving monoecious plants could, in the near future, lead to identify DNA markers tightly linked to this important trait, with an important impact on the breeding of monoecious varieties.

## THE IDENTIFICATION OF MARKERS LINKED TO THE CANNABIS TYPE

In the last few years, molecular markers found an application to forensic problems concerning the species Cannabis sativa. In the recent past, a remarkable number of studies dealt with the identification of hemp from other plant species using a number of sophisticated techniques. The aim of many of these works was to allow the identification of Cannabis present on different type of residues for legal reasons, i.e., to develop tools useful in the drug repression activities.

Because of the importance of the problem of drug abuse and its repression, the hope was that the molecular technology could have provided a new and effective set of tools for this purpose. Therefore, several studies, during the last decade, investigated drug and fibre hemp types at the molecular level. The first report of a DNA-based analysis, aiming at the differentiation of hemp types was from Gillan et al. (1995), that compared RAPD patterns with the HPLC profile of a number of samples; a full differentiation between the different individual samples used was obtainable, but not correlated to the chemical profile, and hence not useful for legal purposes. However, another study described a method to use DNA markers to detect the presence of Cannabis material on skin (Wilkinson and Linacre, 2000) and the test was also patented. The limitation of these tests is that they are able to identify the presence of Cannabis sativa material and to distinguish it from other plant species, but fail to discriminate drug from fibre hemp. It is extremely likely that any fibre hemp farmer would result positive to this kind of tests, and get arrested even after having sown a zero-THC hemp variety. Other authors used particular sequences shown to be specific for Cannabis sativa; among these, the sequence of the spacer between two chloroplast genes, trnL and trnF (Kohjyouma et al., 2000; Linacre and Thorpe, 1998), or the ITS2 (nuclear ribosomal DNA Internal Transcribed Spacer II) sequence (Siniscalco Gigliano et al., 1997). In these experiments, specific primers for the target regions were used, leading in all cases to an effective identification of Cannabis DNA from other plant sources' DNA; however, once again these methods failed to correlate positively with the cannabinoid type or content of the plants, and cannot be used to discriminate fibre from drug hemp.

It is important, in our opinion, to realize that what is to be achieved is the distinction between chemotypes, that can be all present within a single population. This is particularly true in a scenario where hemp became a widespread culture for industrial purposes. Actually, the richness of hemp chemotypes is a potential resource to be investigated. In fact, in the latest years an increasing knowledge of the potential applications of several different cannabinoids present in Cannabis strains has raised the interest of pharmaceutical companies in several countries for the exploitation of these substances at an industrial level (Dr. Etienne de Meijer, personal communication; Mechoulam et al., 1998). In this changed context, where Cannabis is no longer considered to be an illicit plant and where (in an adequate normative frame) drug genotypes might have an useful application, also the main objective of the research should in our opinion be the identification of specific molecular markers associated with the different cannabinoid types and/or content. This is likely to be a difficult task, as it is well known that there are environmental influences (Bócsa et al., 1997; Lydon et al., 1987) and dependance on the developmental stage (Fournier, 1981). However, it is possible to realize crosses between pure drug strains and pure fibre plants, where the traits of interest, in this case THC and CBD, segregate. From these type of crosses–of little use from the breeding point of view–populations suitable for the mapping of the cannabinoids by standard genetical techniques could be derived. The availability of markers sufficiently linked to the cannabinoid type would make the selection work for cannabinoid content in hemp easier and safer. Alternative strategies could be the direct isolation of the genes involved in the metabolic pathway of cannabinoids (Shoyama, 2000). The long-term target of the mapping of the cannabinoid genes are their isolation and the possibility of their manipulation, for example, in hemp cell cultures to create bioreactors in which the different molecules with useful biomedical applications could be produced with high efficiency and low costs, or for the constitution of Cannabis varieties with different pure-cannabinoid profiles, for both fibre and pharmaceutical end-use.

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RECEIVED: 5 April 2001
ACCEPTED IN REVISED FORM: 27 June 2001

# Epidemiology of the Hemp Borer, Grapholita delineana Walker (Lepidoptera: Oleuthreutidae), a Pest of Cannabis sativa L.

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ABSTRACT. The hemp borer, Grapholita delineana, is newly described from feral hemp in Vermont, USA. It may pose a serious pest should hemp cultivation resume in the USA. A similar situation occurred in the 1960s, when G. delineana suddenly became a serious pest in southeastern Europe. Evidence suggests the pest was imported from its native range via infested hemp seed. Larvae of G. delineana bore into stalks and destroy fiber, or they infest flowering tops and destroy seed. The larvae and adults are described, along with their life history, geographic range, and host range. Careful phytosanitary measures can prevent the spread of G. delineana into quarantine areas, such as western Europe, Canada, and the entire southern hemisphere. Breeding hemp plants for resistance to G. delineana may prevent future epidemics. Vermont feral hemp appears to be more resistant to G. delineana than feral hemp growing in the Midwestern USA; the Vermont germplasm may have descended from plants imported in the 1830s, called "Smyrna" hemp, a western European landrace devoid of Chinese ancestry. Biological and chemical controls of G. delineana are described. 

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Journal of Industrial Hemp, Vol. 7(1) 2002
http://www.haworthpressinc.com/store/product.asp?sku=J237

KEYWORDS. Control methods, differential diagnosis, geographic distribution, hemp borer, life history, taxonomy

## INTRODUCTION

In September of 2000, inspection of a field of feral hemp (Cannabis sativa L.) revealed symptoms suggestive of the hemp borer, Grapholita delineana. The feral hemp was located along the banks of the Winooski River in Chittenden County, Vermont, USA. Plant symptoms included stalk galls (Figure 1) and damaged seeds (Figure 2). Breaking open stalk galls revealed feeding galleries but no larvae. Some stalk galls had exit holes, and these contained the remains of pupal casings. Hand threshing of flowering tops disclosed larvae pupatating within seed clusters. The larvae were identical to the description of G. delineana, given below. Voucher specimens were deposited at The Natural History Museum, London, UK. This is the first report of G. delineana in the northeastern USA.

## MORPHOLOGICAL DESCRIPTION

Larvae are pinkish-white to pale brown (Figures 2 and 3), eventually growing up to 9-10 mm long. Several pale bristles (setae) are barely visible per segment. MacKay (1959) provided detailed line drawings of segmental setae and pinacula, thoracic legs with claws, and abdominal prolegs with crochets (as "Grapholitha tristrigana"). The head is dark yellow-brown, hypognathous, with black ocelli, averaging 0.9 mm wide when fully grown. Larvae pupate in silken cocoons covered with bits of hemp leaf. Pupae are cylindrical with tapered ends, brown in color, up to 7 mm long (Figure 3). Adults are tiny moths, with greyish- to rusty-brown bodies and brown, fringed wings (Figures 3 and 4). Body length and wingspan average 5 mm and 9-13 mm, respectively, in males, and 6-7 and 10-15 mm, respectively, in females. Forewings exhibit white stripes along the anterior edge with four chevron-like stripes near the centre. Eggs are white to pale yellow, oval, 0.4 mm wide, and laid singly on stalks and undersides of leaves (Figure 3).

## LIFE HISTORY

Immediately after hatching, young larvae feed on leaves. In heavy infestations, leaves may become completely skeletonized (where larvae

FIGURE 1. Fusiform-shaped stalk gall caused by the hemp borer, G. delineana.

FIGURE 2. Damaged hemp seeds and young larva of the hemp borer, G. delineana.

selectively feed on the delicate leaf tissue between veins, leaving behind a skeleton of leaf veins). Rarely, according to Mushtaque et al. (1973), young larvae may bore into the narrow spaces insides leaves, causing leafmining symptoms. After several days, larvae stop feeding on leaves and bore into branches and stalks. Feeding galleries within stalks cause plants to form fusiform-shaped galls. Stalks may split or even snap at gall sites. The length of tunnels within galls averages only 1 cm (Miller 1982), or at most 2 cm (Nagy 1967). Boring near the terminal shoot may kill the shoot and cause the stalk to bifurcate at that point (Manolache et al. 1966).

Two or more generations arise per year, and late-season larvae feed on flowers and seeds, hence the common names “hemp leaf roller” and “hemp seed eater.” The larvae spin loose webs around terminal buds, especially the seed clusters of female plants (Kryachko et al. 1965;

FIGURE 3. Life cycle of the hemp borer, G. delineana. A. eggs, B. larva, C. pupa, D. female moth (McPartland redrawn from Tsao 1963).

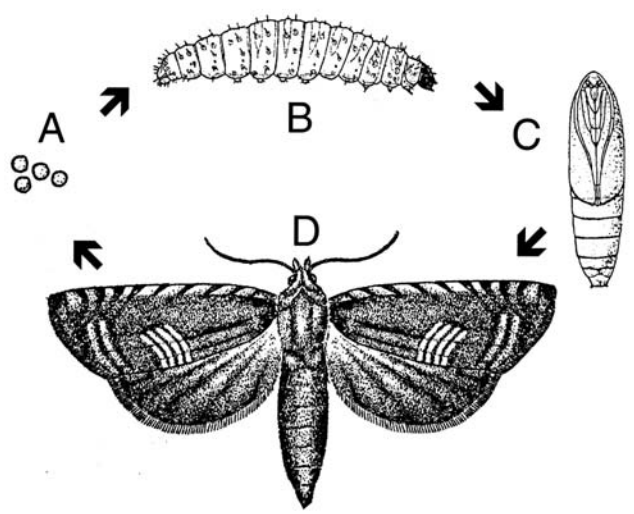



FIGURE 4. Adult moth of the hemp borer, Grapholita delineana.

Smith & Haney 1973). By September and October, the larvae go into hibernation. Day length under 14 hours induces diapause (Sáringer & Nagy 1971). Temperature also influences diapause–warm weather slows photoperiodic effects. Larvae overwinter in cocoons, in curled leaves or buds, bound together by strands of silk. Hemp borers also overwinter as pupae in stalks, roots, and stored seed (Shutova & Strygina 1969).

Overwintering larvae pupate in April, usually in soil under plant debris (Kryachko et al. 1965). Adults emerge in May or June, and migrate at night to new hemp fields. The moths are not strong fliers; Nagy (1979) calculated flight speeds of 3.2-4.7 km $hour^{-1}$ in a wind chamber. Upon finding a hemp field, females land quickly, usually within 3 m of the field's edge (Nagy 1979). After mating, females lay between 350-500 eggs, singly on stalks and undersides of leaves (Kryachko et al. 1965). Adults live less than two weeks (Nagy 1980). They are primarily nocturnal (Sandru 1972; Nagy 1979). Eggs hatch in five to six days at 22-25°C, or three to four days at 26-28°C. Out of 350-500 eggs, Smith and Haney (1973) estimated only 17 larvae survived to first instar.

Two generations of G. delineana occur in Hungary (Nagy 1979) and the Ukraine (Kryachko et al. 1965). Further south, three generations arise in Romania (Manolache et al. 1966), Illinois, USA (Smith & Haney 1973), and Armenia (Shutova & Strygina 1969). In Armenia, the three generations produced hatching peaks in early June, July, and September, and the September hatch was the largest (Shutova & Strygina 1969). In Romania, the three generations overlap, which resulted in the presence of adults throughout the season (Sandru 1967). The egg, larval, and pupal stages lasted 7-10, 22-29, and 10-21 days, respectively (Sandru 1972). In Pakistan, four generations overlap per year (Mushtaque et al. 1973).

## GEOGRAPHIC RANGE AND TAXONOMY

During the early 1960s, the hemp borer suddenly emerged as a pest across southeastern Europe, rather simultaneously. It was first detected in 1960, in the Ukraine (Kryachko et al. 1965) and in Russia (Danilevski & Kuznetsov 1968). The hemp borer subsequently appeared in Romania by 1963 (Manolache et al. 1966), Hungary by 1964 (Nagy 1967), and Bosnia-Herzegovina by 1967 (Bes 1967). After that it was reported in Armenia (Shutova & Strygina 1969), Moldavia (Shutova & Strygina

1969), Serbia and Montenegro (Lekic & Mihajlovic 1971), Bulgaria (Gerginov 1974), Greece (Vassilaina-Alexopoulou & Mourikis 1976), Slovakia (Bako & Nitri 1977), and Slovenia (Camprag et al. 1996).

To confuse researchers studying the emerging epidemic, the hemp borer was called different names in different places. It was called G. delineana in the Ukraine (Kryachko et al. 1965), Romania (Manolache et al. 1966), and Bosnia (Bes 1967), whereas it was called Grapholita sinana in Hungary (Nagy 1967) and Serbia (Lekic & Mihajlovic 1971). The name G. delineana was originally coined by Francis Walker, a British entomologist, in 1863. The name G. sinana was authored by Rudolf Felder, an Austrian entomologist, in 1874. In a careful comparison of specimens, Danilevski and Kuznetsov (1968) decided that G. delineana and G. sinana were identical, and therefore the name G. delineana had taxonomic priority over the later name G. sinana. Danilevski and Kuznetsov (1968) also placed G. mundana, G. terstrigana, and L. quadristriana in synonymy under G. delineana. This nomenclature was supported by Miller (1982):

Grapholita delineana Walker 1863 (Lepidoptera: Oleuthreutidae)
≡ Cydia delineana (Walker)
≡ Laspeyresia delineana (Walker)
= Grapholita sinana Felder & Rogenhofer 1874
= Cydia sinana (Felder) [Rogenhofer often omitted]
= Grapholita tristrigana Clemens 1865
= Grapholita mundana Christoph 1881
= Grapholita terstrigana Ragonot 1894
= Laspeyresia quadristriana Walsignham 1900

The genus name is commonly misspelled as Grapholitha (note the erroneous second “h”). Some workers refer the genus to the Tortricidae instead of the Oleuthreutidae. Common names include the Hemp borer, Hemp leaf roller, Hemp seed eater (in English), Kis kendermoly (in Hungary), Moliei cinepii (in Romania), and Konopljin savijac (in Yugoslavia).

Prior to the sudden outbreak of G. delineana in European hemp crops, the species was known in that region as a minor pest of hops (Humulus lupulus L.) (Nagy 1967). Researchers assumed G. delineana suddenly switched hosts, like the European corn borer, Ostrinia nubilalis Hübner, had switched from hemp to maize (Nagy 1986).

However, my inspection of the original descriptions by Francis Walker and Rudolf Felder revealed that the original specimens were collected in China, not in Europe (Walker 1863; Felder & Rogenhofer 1874). In fact, G. delineana is common in hemp-growing areas of Anhui and Jiangxi Provinces (Tsao 1963; Wang & Rong 1992). G. delineana also appears in Korea (Byun et. al. 1998), Japan (Walsignham 1900; Issiki 1957), Pakistan (Mushtaque et al. 1973; Baloch et al. 1974, 1975), the Uttar Pradesh of India (Sankaran & Ramachandran Nair 1973), and Nepal (McPartland et al. 2000).

Given this information, the strain of G. delineana that became a pest in southeastern Europe was probably imported from contaminated Chinese hemp seed. It was probably imported in the 1950s, and emerged as a problem by 1960. Indeed, the Hungarian government purchased several thousand metric tons of hemp seed from China in the early 1950s (Ivan Bócsa, personal communication); the Rudolph Fleischmann Agricultural Research Institute utilized this germplasm for heterosis breeding of hemp (Bócsa 1954). Similarly, the Vavilov Institute in Russia imported hemp seed from China in 1953 (Sergey Grigoryev, personal communication). But according to Russian regulations, the Chinese seed was sown in special quarantine areas to check for foreign pests and diseases. No Chinese seed was imported in the 1950s or 1960s by the Institute of Bast Crops, Ukraine (Paul Virovets, personal communication), nor by the Institute of Field and Vegetable Crops, Yugoslavia (Janos Berenji, personal communication). Dr. Berenji, however, noted that bird seed manufacturers imported tons of inexpensive Chinese hemp seed into Yugoslavia and Hungary.

The source of the new Vermont infestation is unknown. The situation in North America, like that in Europe, has been confused by researchers using different names. The borer was misidentified as Grapholita tristrigana (Clemens) by Hartowicz et al. (1971) in Kansas, and by Smith and Haney (1973) in Illinois. Dempsey (1975) called the pest Grapholita interstictana. The confusion was cleared by Miller (1982) who closely examined other researchers' specimens, and established their correct identity as G. delineana. Miller found museum specimens dating back to 1943 (in Kentucky and Wisconsin), and specimens collected later in Iowa, Illinois, Minnesota, Missouri, and New York (Miller 1982). Thus, G. delineana was introduced into the USA by 1943, possibly from contaminated bird seed or from seedstock imported for hemp cultivation during World War II. It may have been introduced into Kentucky as far back as the late 1800s. At that time, American missionaries stationed in China regularly sent hemp seed to Kentucky (Dewey 1902).

In Eurasia, the northern limit of G. delineana is reportedly 49°N, in Cherkassy, Ukraine (Kryachko et al. 1965), and the southern limit is 26°N in Pokhara, Nepal (McPartland et al. 2000). North American specimens have been collected as far north as 45°N, from Stearns Co., Minnesota (Miller 1982), and Chittenden Co., Vermont, and the southern limit is 38°N in Lexington, Kentucky (Miller 1982).

## HOST RANGE

There appear to be two strains of G. delineana, exhibiting different host-plant preferences. One is an indigenous hop-feeding strain that may have been present in Europe prior to 1960. Scattered populations of this strain continue to be found on hops in Hungary (Nagy 1979). The second strain of G. delineana prefers feeding on hemp. It apparently originated in Asia and moved into southeast Europe by 1960. In Pakistan, Baloch et al. (1975) described the Pakistani strain of G. delineana as "Cannabis-host-specific." Baloch and colleagues conducted a single-choice feeding experiment, and G. delineana larvae fed voraciously on Cannabis species, whereas they only "slightly nibbled hops leaves," and no or very little oviposition was observed on hops plants. Baloch and colleagues did not describe the Cannabis species they tested, but they probably used a drug strain of Cannabis. The drug strain may be a different host species than hemp; drug plants from Pakistan have been called Cannabis indica and Cannabis afghanica (McPartland et al. 2000).

## DIFFERENTIAL DIAGNOSIS

The stalk galls caused by G. delineana can be confused with galls caused by O. nubilalis, another common hemp pest. Nagy (1967) claimed G. delineana damage often arises in the top 1/3 of plants, whereas O. nubilalis usually forms galls in the lower 3/4ths of plants (Nagy 1959). But in Canada, over half of O. nubilalis-damaged plants were attacked above mid-height (Ernest Small, personal communication). Generally, O. nubilalis larvae drill longer tunnels than G. delineana larvae, but this trait may be variable. Symptoms are not sufficient to differentiate these pests, the insects themselves must be identified.

Other boring insects that cause similar stalk damage include larvae of the hemp weevil (Rhinoncus pericarpius L.), cabbage curculio (Ceutorhynchus rapae Gyllenhal), tumbling flower beetles (Mordellistena micans Germar, Mordellistena parvula Gyllenhal), and even the nettle midge (Melanogromyza urticivora Spencer). Late-season hemp borers that infest buds and eat seeds may be confused with budworms, such as the cotton bollworm (Helicoverpa armigera Hübner), the bollworm (Helicoverpa zea Boddie), and the flax noctuid (Heliothis viriplaca Hufanagel).

## ECONOMIC IMPACT

Larvae boring into stalks cause the rupture of fibers, damaging up to 100% of stalks in Romania (Manolache et al. 1966). Larvae feeding on terminal shoots cause excessive branching, resulting in short and inferior fibers (Manolache et al. 1966). Boring damage causes the plant to produce more "oakum" (Nagy 1967), interpreted as twisted fibers impregnated with extra lignin. Kryachko et al. (1965) described G. delineana destroying 80% of flowering tops in the Ukraine. Unprotected hemp in Yugoslavia suffered 41% seed losses (Bes 1978); 17-30% seed losses in Romania (Manolache et al. 1966), and up to 25% seed losses in Illinois, USA (Smith & Haney 1973). Each larva consumes an average of 16 Cannabis seeds (Smith & Haney 1973). Anti-marijuana biocontrol researchers considered the hemp borer "an excellent weapon" (Hartowicz et al. 1971; Mushtaque et al. 1973; Baloch et al. 1974; Scheibelreiter 1976). Forty larvae can kill a seedling that is 15-25 cm tall in ten days, and ten larvae per plant cripple growth and seed production (Baloch et al. 1974).

## QUARANTINE ZONES

The present geographic distribution of G. delineana is nearly palaeartic, including eastern Europe, temperate Asia, and the eastern and central USA. G. delineana has not been reported from hemp-growing areas in western Europe, such as Italy, Germany, France, Spain, the Netherlands, and the United Kingdom. Nor has it been reported from Canada. The pest has not been reported from hemp-growing regions in the southern Hemisphere (South America, Africa, Australia, or New Zealand).

The primary means of long-distance movement is via contaminated seed. Other hemp commodities (processed hemp fiber or retted hemp stalks) are not a major phytosanitary risk. Unretted stalks, however, are a hazard, because full-grown larvae survive in harvested but unprocessed stalks. Within China, a few regions are free of G. delineana, and these regions placed quarantine restrictions on hemp seed originating from infested regions (Tsao 1963).

Local dispersal may occur by trucking contaminated hemp stalks off-site, to retting ponds. Nagy (1979), however, suggested that moth flight was the primary source of local dispersal ("more important than translocation of the infestation in the course of transporting harvested material"). Nagy noted the moths are not strong fliers, and he estimated they rarely disperse more than 20 km.

## CONTROL METHODS

### Mechanical and Cultural Control

Hemp-growing regions free of G. delineana should exercise strict quarantine precautions, especially regarding seed imported from endemic areas (see "seed treatment" in the section on chemical control). Nagy (1979) described an "edge effect" in fields infested by G. delineana. Weakly-flying female moths land quickly after encountering a hemp field. Therefore, most egg-laying occurs in the first 3 m around the edge of a hemp field. In severe infestations, the edge zone should be cut down and buried or burned.

Early harvest of hemp may decrease the population of overwintering pests, because this would destroy a high percentage of the larval population (Nagy 1979). Manolache et al. (1966) reported good post-harvest control by destroying all hemp crop debris, and deep plowing of hemp fields in the autumn. Sandru (1972) emphasized that plowing buries the overwintering larvae and pupae too deep for the pests to emerge from the soil. Camprag et al. (1996) noted that that monocultured Cannabis attracts G. delineana, so the crop should be rotated. Consider destroying infested stands of feral hemp and hops.

Plants should be selected for resistance to G. delineana, just as Grigoryev (1998) selected hemp plants for resistance to O. nubilalis. Vermont hemp suffers less G. delineana damage than hemp I have examined in Illinois, Wisconsin, and Missouri. This variation may be due to genetic differences in the pest, or genetic differences in the plants. The ancestors of feral hemp in the Midwestern USA were varieties bred by Lyster Dewey, such as "Kymington," "Chington," and "Ferramington," and they all shared Chinese parentage (Dewey 1928). Chinese landraces are susceptible to G. delineana (Tsao 1963, Wang and Rong 1992), and G. delineana-susceptible hemp in southeastern Europe contained Chinese germplasm (Bócsa 1954). Vermont feral hemp, in contrast, may predate Dewey and his breeding efforts. Morphological evidence, combined with historical records, suggests the Vermont germplasm was introduced in the 1830s, has no Chinese heritage, and may represent a western European landrace called "Smyrna" hemp (Dewey 1902). Interestingly, feral hemp in Vermont is shorter in stature than Midwestern hemp; in the Midwest, Smith and Haney (1973) rarely found larvae attacking plants less than 30 cm tall.

Nocturnal light traps can be used to catch adults, and this is useful for scouting. Nagy (1980) used female sex hormones to attract and trap male moths, preventing reproduction. Nagy noted that female sex hormones of G. delineana also attracted males of Grapholita compositella F.

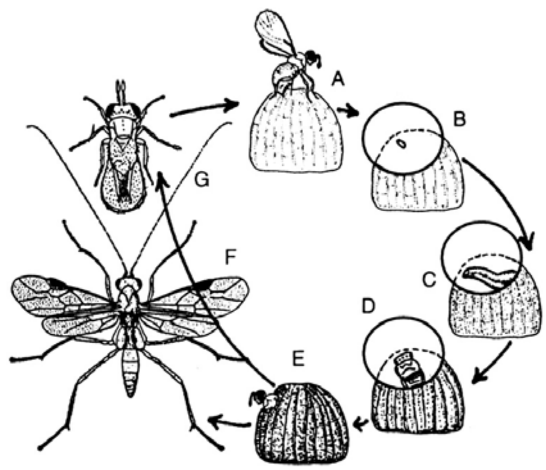
## Biological Control

G. delineana has many insect enemies. These parasites provide natural population control of G. delineana, and they may have brought a natural end to the epidemic in southeastern Europe. Scambus species parasitized 30% of hemp borers in Hungary (Scheibelreiter 1976). In Romania, larvae were heavily parasitized by Eubadizon extensor (L.) and Ephedrus plagiator (Nees) (Sandru 1967). Goniozus species parasitized G. delineana larvae in Pakistan (Mushtaque et al. 1973). In Illinois, Smith and Haney (1973) found 75% of larvae were parasitized by either Lixophaga variablis (Coquillett), a tachinid fly, or Macrocentrus delicatus Cresson, a braconid wasp. None of these native species has been commercially developed for biological control. Trichogramma and Macrocentrus species are commercially available.

### Trichogramma Species

At least 20 Trichogramma species have been mass-reared for commercial biological control. They efficiently parasitize and kill G. delineana eggs, before the pests can damage crops (Figure 5). Camprag et al. (1996) used a Trichogramma species to control the first generation of G. delineana with "51-68% efficiency." They released 75,000-100,000 wasps per ha, and repeated the release one week later. They did not report which Trichogramma species they used. Peteanu (1980) used Trichogramma evanescens Westwood against G. delineana, released at rates of 80,000, 100,000, or 120,000 wasps per ha, four times per season

FIGURE 5. Life cycle of Trichogramma species. A. Female wasp laying egg within egg of G. delineana. B. View of wasp egg within borer egg. C. Wasp larva feeding within borer egg. D. Pupal stage of wasp. E. Adult wasp emerging from dead egg. F. Adult male with open wings. G. Adult female with closed wings (McPartland redrawn from Davidson and Peairs 1966).




(presumably two releases against the first generation and two against the second generation). T. evanescens worked best at the highest release rate, and worked better against second generation larvae than first generation larvae. Peteanu combined T. evanescens with Bt and pesticides, with good results. Smith (1996) controlled a related Grapholita pest, G. molesta (Busck), with Trichogramma dendrolimi Matsumura, released at a rate of 600,000 wasps per ha, repeated every five days while moths were laying eggs.

Trichogramma adults are tiny wasps, 0.3-1.0 mm long, with a black thorax, yellow abdomen, red eyes, and short antennae. Females lay eggs in up to 200 pest eggs, which turn black when parasitized. Larvae pupate within pest eggs and emerge as adults in eight days, adults live another ten days. Trichogramma species are supplied as pupae within parasitized eggs, attached to cards made of cardboard, paper, bamboo, or within gelatine capsules. Trichogramma pupae can be manually distributed by hanging cards from plants in warm, humid places out of direct sunlight. This approach takes about 30 minutes per ha (Smith 1996). For a large-scale approach, attach Trichogramma pupae to carriers such as bran, and broadcast them from tractors or airplanes. Pupae and carriers can be coated with a sticky gel so they adhere to plant surfaces. Trichogramma species are compatible with Bt and NPV. Avoid insecticides while utilizing the wasps.

### Macrocentrus Species

Macrocentrus delicatus Cresson heavily parasitized G. delineana in Illinois, USA (Smith & Haney 1973). A related braconid, Macrocentrus ancylivorus Rohwer, has been mass-reared for field use against fruitworms, leafrollers, and stalk borers. The parasitoid is native to New Jersey, and has become established in an area ranging from Massachusetts to Georgia, west to the Mississippi River. In orchards, Grapholita molesta is controlled by releasing three to six M. ancylivorous females per tree (Mahr 1998).

Adults are slender wasps, 3-5 mm long, amber-yellow to reddish brown in colour, with antennae and ovipositors longer than their bodies. Female wasps are nocturnal, most active at 18-27°C and > 40% RH, and lay up to 50 eggs, one egg per borer (Mahr 1998). The wasps go after borers already within branches (second and third instars preferred). M. ancylivorus larvae initially feed within caterpillars, then emerge to feed externally, and pupate in silken cocoons next to the body of their hosts. One generation arises per generation of the host. They overwinter as larvae in hibernating hosts.

### Chemical Control

Nagy (1979) described an "edge effect" in fields infested by G. delineana. Spray this 3 m edge zone with pesticides as moths arrive. Spraying pesticides only works before hemp borers burrow into stalks. Once inside stalks, no surface sprays will affect borers.

Bako and Nitre (1977) successfully controlled young hemp borer larvae with aerial applications of Bacillus thuringiensi δ-endotoxin (Bt). Bt is a natural product; some workers consider it a form of biological control. The Nuclear polyhedrosis virus (NPV) is another hybrid of biological and chemical control. Most strains of NPV kill larvae of nocturids (e.g., budworms and cutworms), but there is a NPV strain that kills the codling moth, Cydia pomonella (L.), a pest related to G. delineana; it might also work on hemp borers. Many botanical-based chemicals work against hemp borers–neem, nicotine, rotenone, and ryania. Peteanu (1980) killed borers with sumithrin, a synthetic pyrethroid. The synthetic juvenoid fenoxycarb may work, it kills eggs of the related pest Grapholita funebrana (Godfrey 1995).

Sandru (1973a) tested the effectiveness of 12 insecticide sprays against G. delineana, and reported best results with malathion, chlorthiophos, and two formulations of fenitrothion. Manolache et al. (1966) previously reported success with malathion, as well as methyl-parathion, and DDT. Bes (1976) reported best control of first-instar larvae with fenthion, diazinon, phosalone, and dichlorvos; carbaryl and bromophos were less satisfactory, and dimethoate and thiometon were ineffective. Shutova and Strygina (1969) controlled larvae with sprays of sevin and methyl-parathion. Sandru (1973b) controlled overwintering larvae by mixing diazinon granules (5% or 10%) with seeds at the time of sowing. Effectiveness of this treatment depended on a good rainfall to activate the granules.

Seed infested by G. delineana larvae has been fumigated with methyl bromide (Tsao 1963), using 55 oz. per 1000 cu. ft, for 18 hours, at 9-10°C. Kryachko et al. (1965) used the same dose and same temperature, but required 24 hours to kill all larvae; they reported the moisture content of the seed was 11-12% (fumigation of seed with higher moisture content resulted in reduced germination). Kryachko et al. (1965) also report complete kill of larvae in cocoons with methallyl chloride at 100-150 oz. per 1000 cu. ft for 24 hours at 6-15°C. Tsao (1963) suggested storing seed for one year before dispatch would reduce seed infestation.

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RECEIVED: 19 March 2001
ACCEPTED IN REVISED FORM: 25 May 2001

# Comparison of Enzymatically Separated Hemp and Nettle Fibre to Chemically Separated and Steam Exploded Hemp Fibre

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ABSTRACT. Hemp (Cannabis sativa L.) and nettle (Urtica dioica L.) are both attractive candidates for high fibre yields with little or no biocide requirement. Separation of fibre fine enough for quality yarns to make hemp fabric or blends has been achieved in Western Europe in the last decades only on a laboratory scale because process costs are high. In Hungary, Romania, the Ukraine and Poland a hemp processing industry has continued retting mainly by water processes. Search for a commercially and environmentally viable method led us also to explore enzymatic separation, which was initiated by various researchers in the late 1960s and 1970s. This involves the use of various enzymes that dissolve pectin and hemicellulose between the cell walls thus freeing the fibre bundles and fibres. We tested various commercial and non commercial products (Röhm Enzyme GmbH and Novozymes AS/Bayer AG) and methods and then measured

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Journal of Industrial Hemp, Vol. 7(1) 2002
http://www.haworthpressinc.com/store/product.asp?sku=J237

our results against samples of fibre separated by other methods using a Stelometer to determine tensile strength of fibre bundle collectives and OFDA (Optical Fiber Diameter Analyzer) to analyze fibre bundle width. Our results showed enzymatic separation capable of producing comparably fine and strong fibre suitable for quality textiles. These studies open the way for sustainable and local production of high value fibre with low impact on the environment. 

KEYWORDS. Hemp, nettle, fibre separation, enzymatic degumming, hemp yarn and fabric, nettle yarn and fabric, Cannabis, Urtica

## INTRODUCTION

Growing well in central Europe with low input of biocides, hemp (Cannabis sativa L.) and stinging nettle (Urtica dioica L. convar. fibra) are promising candidates for non-food market production. Although both plants do produce fine and strong fibre, commercial extraction of fibre fine enough for high quality yarns has only been achieved commercially with hemp by chemical separation in People's Republic of China, India and some other countries. Other possibilities for refining coarse fibre bundles are the steam explosion process (Kessler et al., 1994) and the ultrasonic technique (von Drach et al., 1999) both unfortunately not yet industrially viable. For mechanical fibre extraction adequate facilities exist in Europe, many of them new. But the retted hemp and nettle fibre has, so far, not been fine enough to be spun into quality yarns. We began to explore enzymatic separation in 1998 as an alternative to physical or chemical separation for producing fine fibre.

The qualities of hemp fibre as related to cultivation, harvest and post-harvest treatment and fibre processing have been intensively discussed over the last years (Van der Werf et al., 1995; LWK-WE IPP, 1997; Martens et al., 1997; Gusovius, 2000). Good quality hemp has high commercial value for paper (Brunet, 2000) and for some fabrics. The fibre quality of nettle has just begun to be investigated (Kohler and Wedler, 1996; Lützkendorf et al., 2000; Dreyer et al., 2001).

Briefly field-retted, mechanically separated bast fibre from hemp and nettle is often sufficient for technical applications (Hanselka, 1998;

Gassan et al., 1998; Lützkendorf et al., 2000; Müssig, 2001). To get really fine, clean and strong fibre for textile use, there must be further treatment through physical or chemical fibre processing and finishing. Another means of extracting textile fibre is enzymatic retting of the stem or the enzymatic separation of coarse bast fibre bundles after mechanical processing. Basic work on this alternative method has been done on flax in the last 30 years by Hurdequint et al. (1994) (Demande de Brevet D'Invention No 8121646), Sharma (1986; 1987); Sharma and Van Sumere (1992); Petrova et al. (2001) (DE 4012351 A1), Brühlmann et al. (1994), Henriksson et al. (1997), Akin et al. (1997; 1999) and others. There is much activity in Eastern Europe today in the areas of retting and biotechnology, i.e., the Bast Fibrous Plants Today and Tomorrow, Breeding, Molecular Biology and Biotechnology Beyond 21st Century Conference held on 28-30 September, 1998 in St. Petersburg. The Institute of Natural Fibres in Poland is working on enzymatic treatment of short and waste bast fibres for their use in the production of high quality textile products.

Enzymatic separation of bast fibre bundles has been researched but up to now no commercial process has been developed (Dreyer et al., 2000). In Switzerland, activities have begun for developing a new biologically based separation technique for bast fibre bundles (Leupin, 1999). Today enzymes play a prominent role in the finishing of yarn, fabric, and garments of cotton, silk and wool (Cavaco-Paulo, 1998).

## EXPERIMENTAL PROCEDURE

### Hemp and Nettle Cultivation

The botany and cultivation of hemp has been described in various papers (Van der Werf et al, 1995; NOVA-Institut, 1997; FNR e.V., 1997; Bócsa and Karus, 1997; Bassetti et al., 1998; Höppner and Menge-Hartmann, 1999) and need not be discussed here. The botany of stinging nettle and nettle culture is a new topic presented in only a few papers (Dreyer et al., 1996; Schmidtke et al., 1998; Scheer-Triebel and Franken-Welz, 2000) and one book (Dreyer, 1999b) and is described here briefly with reference to fibre production. Stinging nettle (Urtica dioica L.) is a perennial, nitrophilous herb widely distributed throughout the temperate regions of the world containing sclerenchymatic fibres in the bark (Tobler-Wolff, 1951). In the 1940s, 100 tons of pure fibre were extracted mechanically and chemically from stinging nettle grown on 150-200 ha of cultivated land. The nettle yarn was woven into quality textiles (Bredemann, 1959) as an alternative fibre to ramie (Boehmeria nivea [L]. Gaud.). The fibre of stinging nettle is remarkable for its high tensile strength, fineness, excellent spinning quality, and the fact that its cell walls are unlignified (Herzog, 1955; Bobeth, 1993). Latest investigations on the technical characteristics of nettle fibre show that it also has potential as reinforcement fibre in some application areas for polymer matrix composites (Lützkendorf et al., 2000). Advantages of nettle fibres are low specific weight, high strength and good stress rating (elasticity module).

Studies in the biology and agronomy of some cultivars of Urtica dioica L. began again in 1992 at the Institute of Applied Botany, University of Hamburg. Cultivation and research on Urtica dioica L. started originally in 1927, initiated by Bredemann, and were a very important activity at the Institute until 1950. After cross-breeding of the phenotypically best wild plants with high fibre content, new varieties called fibre nettle with a high fibre yield were cultivated. The most suitable nettle clones were cultivated in field experiments to gather facts on productivity. The economic and ecological reasons for cultivation of stinging nettle are: (1) perennial culture with low resource input (fertilizer and pesticides), (2) production of new, high-quality agricultural raw material, (3) high potential for cultivation on greenland areas, (4) potential for improving soils over-fertilized with nitrates and phosphates, (5) extensive cultivation and utilization of a single planting is possible for 10-15 years, and (6) promotion of population diversity in local flora and fauna.

Different cultivars of fibre nettle (Urtica dioica L. convar. fibra ) have been cultivated in field experiments northeast of Hamburg since 1993. The loamy sand soil of our fields can be characterized as Parabraunerde (Ah-Al-Bt-C) and the value of the soil is 35 Bodenpunkte. Fibre nettles are cultivated in rows approximately 125 cm apart with a distance in the row of approximately 60 cm. Between the rows, spontaneous vegetation is allowed to grow and has to be mowed when higher than 20 cm. Vegetative propagation is done with cuttings. Plants are now available on a large scale thanks to commercial greenhouse propagation (Greenhouse Dr. J. Dreyer, E-MAIL: Jens_Dreyer@gmx.de). After four to eight weeks preculture in the greenhouse, the seedlings are transplanted in the field by machine. Fertilizer levels are low: 60-80 kg N/ha, 150-180 kg $K_2O$/ha and 40-50 kg $P_2O_5$/ha. Fertilizing with nitrogen, although normally done in spring, can be optimized by two half dosages, one in April and another in June.

For maximum production of fibre there is a need to use only plants with high dry stem weight and a high to very high fibre content. The low input agro-system for fibre nettles with clover rows between the nettles leads to very high dry stem weight per plant. One important statistic on using fibre nettle as a fibre crop for textile and technical purposes is the fibre production per ha. The highest pure fibre yield (dew retted, then chemically separated fibre with no impurities) has been 1,430 kg/ha. The average fibre yield of the clones ranged from 812 kg/ha to 858 kg/ha. Amounts could be doubled by using mechanical fibre separation because of inclusion of parenchymatic cells with the fibre.

## MATERIAL

### Hemp and Nettle Fibre

Hemp cultivar "Felina 34" was grown at the trial station of the Weser-Ems chamber of agriculture in Wehnen, near Oldenburg, in northern Germany in 1996. The experimental plants were the subject of 4 random repetitions. The test plots were 3 x 6 m wide, the seeding time was the last week in April. The seeds were planted 200 /m$^2$ and fertilized with 100 kg/ha of nitrogen. After experimentation with different harvesting methods, the material for this study was harvested by rotary disk mower (designated as "c") on 17-18 Sept., 1996. The material was field-retted for four different time periods. Two out of the four batches were used in this study–very briefly retted (designated as "2") and retted for three weeks until 50% of stem had darkened (designated as "4"). Samples described here were taken September 23 (sample 2 unretted hemp) and October 14 (sample 4 retted hemp). In parallel with the use of the scoring system (1-5), near-infrared spectroscopy (NIRS) was used to analytically measure the degree of retting. This technique has already delivered identifiable and reproducible results in the determination of the degree of retting of flax stalks (Quint 1996). Here a so-called "retting sensor" was established that primarily registers the blackening of the flax stalk by the spread of mycelium fungus (Müssig et al., 1998). The stems were dried in swath, baled and stored. The fibre bundles were separated mechanically with a Bahmer-Flaksy laboratory unit. After six passages through this unit the hurdless fibre bundles were refined with a coarse separator (designated as "CS"). We developed and used our own laboratory coarse separator. Our results with separation in the lab are comparable to industrial separation techniques (Müssig, 2001).

Fibre nettle cultivar 'Clone 13' was grown in the University of Hamburg experimental fields (Dreyer et al., 1996). Harvesting was done on 5 Sept. 1996 using a cutter bar (Dreyer, 1999a). The nettle was field-retted in the same fashion as described above for hemp (designated as "2 HH" and "4 HH"). The stems were then dried in swath, baled and stored. The fibre was separated mechanically with a Bahmer-Flaksy laboratory unit. After six passages through this unit, a laboratory coarse separator was used.

## Enzyme Procedure

The mechanically processed hemp and nettle bast fibre just described was then upgraded to textile quality in the laboratory by degumming with enzymes. Various formulations and procedures were tested resulting in the choice of MPG (macerising polygalacturonase, 1,220,000 PGU/mg [PGU is measured by viscosimeter degradation of a pectin solution at 30 C and pH 3.9] ) and PE (pectinmethylesterase, 135,780 PE/g [1 PE/g is the active turnover of 1µval acid per minute under condition 30 C, pH 4.5 and 0.55% substrat concentration]) formulations from Röhm Enzyme GmbH and BioPrep3000L® (Etters et al., 1999, Krebs Lange et al., 2000) from Novozymes AS (Bayer: BAYLASE EVO).

The preparations tested consist primarily of pectinolytic and hemicellulolytic enzymes that work by dissolving pectin and hemicellulose from the cell walls thus freeing the fibre. Enzymes are produced through the submerged fermentation of genetically engineered Bacillus microorganisms and the culture of Aspergillus niger. Different classes of enzymes with varied capacity for pectin degradation were tested including: polygalacturonase (EC 3.2.1.15), pectinmethylesterase (EC 3.1.1.11), and pectinlyase (EC 4.2.2.10).

The culture filtrates with stabilized and concentrated enzymes were dissolved in an unbuffered water solution of pH value 4.0 for pectinases PE and PGU and pH 8.0 for BioPrep3000L®. Ten gram of fibre was treated in a beaker containing 300 ml of enzyme solution (30 to 1 liquor to fibre ratio for optimum test conditions). The samples were mechanically agitated for 0.5 to 2 hours at about 40 C for pectinases PE and PGU and 60 C for BioPrep3000L®. They were then neutralized and rinsed for 2 minutes in cold water.

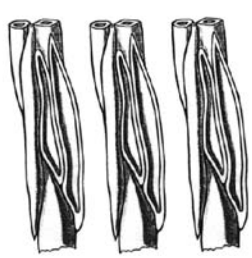
## Test Procedures

The resulting hemp and nettle fibres were tested for strength by Stelometer and for fineness by OFDA, an Optical Fiber Fineness Analyzer. For comparison the hemp and nettle raw fibre was chemically separated by using a 0.4% sodium carbonate solution for 1 hour at 100 C and tested. We also separated hemp by steam explosion at the IAF in Reutlingen for comparison and tested this fibre. We used the Stelometer and OFDA to test several other commercially produced fibres, also for comparison. A brief description of the two testing methods follows. A Stelometer or Strength Elongation Meter is used to test fibre bundle collectives as in Figure 1. Samples were clamped in a Pressley clamp with plexiglas jaws with a free clamping length of 3.2 mm. The device was adjusted to ASTM D 1445. It could be argued that greater accuracy might be obtained by measuring individual fibre bundles. In these tests, however, the large number of measurements taken (> 200) insured a level of accuracy gained in a fraction of the time that would be required to achieve similar results by measuring individual strands. OFDA or the Optical Fiber Fineness Analyzer was developed for measuring the diameter of wool fibre. This apparatus efficiently measures width distribution of bast fibre bundles. Results have been found to correlate well with those of other methods. Because of the large number of measurements taken the results can be well reproduced (Drieling et al., 1999).

## Materials Tested

As well as the hemp and nettle samples that were treated with enzymes in the laboratory, we also tested the following materials by Stelometer and OFDA:

FIGURE 1. Hemp fibre bundle collective [adapted from the Herzog, 1926]



Hemp fibre bundle collective;

aligned collective consisting of a small number of hemp fibre bundles that are gathered together and clamped in the measuring device

Soda 2c/4c or soda 2HH/4HH = alkaline separated hemp (2c/4c) and nettle (2HH/4HH). For purpose of comparison, the same fibres as separated by enzymes were also treated chemically in a 0.4% sodium carbonate solution (1 h at 100 C).

SE = steam explosion hemp 2c/SE and 4c/SE. The same fibres as above were separated by the steam explosion process under development at the Institute for Applied Research (IAF) in Reutlingen, Germany for use on flax and hemp. The material is coarsely separated by machine, impregnated with a solvent, and then exposed to saturated steam in an autoclave (Müssig et al., 1998).

HNF A Hemp: chemically separated short hemp fibres from the HNF Company, China

HTEX Hemp: chemically separated short hemp fibres from the Hemptex Company, China

NTEX Hemp: chemically separated short hemp fibres from the Naturetex Company, China

FLASIN flax: chemically separated flax fibres from the Flasin Company, Germany.

## RESULTS AND DISCUSSION

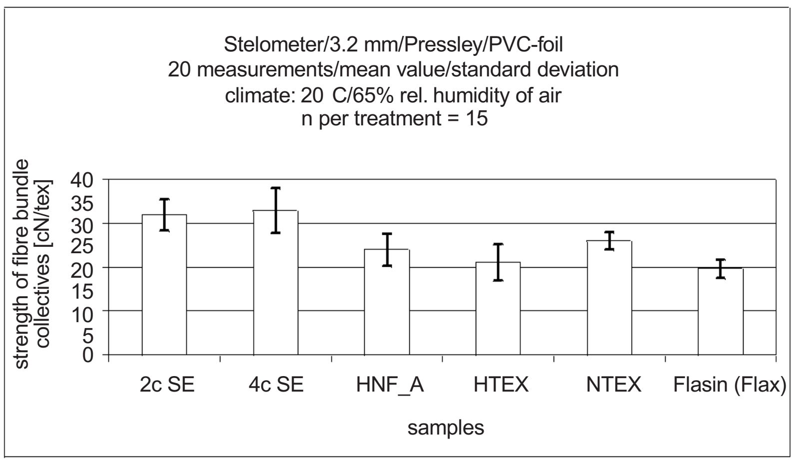
The first table (Table 1) and figure (Figure 2) show the test results on comparison samples (steam explosion and Chinese hemp and Flasin fibre).

As shown in column five of Table 1 a very large number of fibre bundles were measured ranging from 59,516 up to 89,031 bundles. Thus a very good confidence level of < 0.3% was attained. The mean fibre diameter value ranged from 19.1 μm to 15.3 μm. This shows a high fineness variation in the different samples. Fineness of the three Chinese varieties was shown to be much the same, ranging from 16.3 to 17.4. Fineness of fibre bundles was greatly improved by steam explosion and the rating of 18.1 and 19.1 μm is much the same for both differently retted types. Retting of hemp by using steam explosion afterwards had no significant advantage with respect to fineness. The Flasin flax fibre was definitely the finest variant amounting to about 15 μm.

TABLE 1. OFDA results for comparison samples

| | Mean value in µm | Median in µm | Part < 30 µm in % | Part > 100 µm in % | Number of measured fibre bundles |
|---|---|---|---|---|---|
| 2c/SE | 18.1 | 15.2 | 91.9 | 0.2 | 59,516 |
| 4c/SE | 19.1 | 15.2 | 89.8 | 0.5 | 63,471 |
| HNF_A | 17.4 | 14.5 | 92.2 | 0.1 | 72,279 |
| HTEX | 16.3 | 13.9 | 94.4 | 0.0 | 75,900 |
| NTEX | 17.0 | 14.4 | 93.1 | 0.1 | 77,648 |
| FLASIN | 15.3 | 13.2 | 96.9 | 0.2 | 89,031 |

FIGURE 2. Stelometer results for the comparison samples




The strength of tested fibre bundle collectives from hemp and Flasin shown in Figure 2 ranged from an average of 33 cN/tex to 20 cN/tex. A large strength variation in the different samples was apparent. The fibre bundles treated by steam explosion were the strongest fibre. Strength of both samples was about 32 cN/tex with no significant difference between them. Although slightly coarser than the Chinese hemp samples, they were much stronger than this slight difference would make. Apparently the steam explosion process is responsible for a marked retention of fibre strength. The comparison also showed the finest fibre, Flasin, to be the weakest.

FIGURE 3. OFDA ratings on 4c bundles before and after enzyme treatment

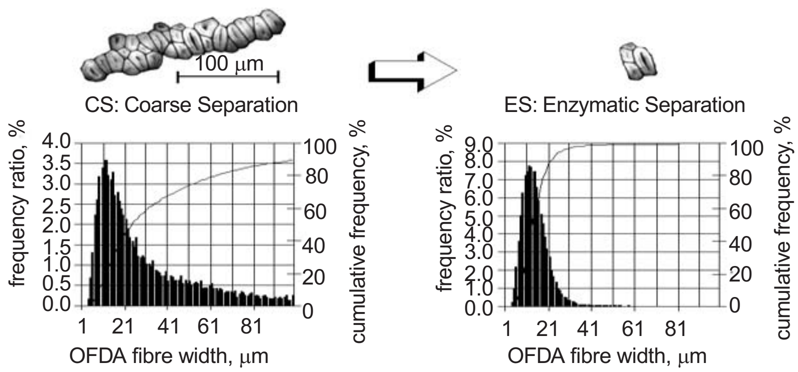
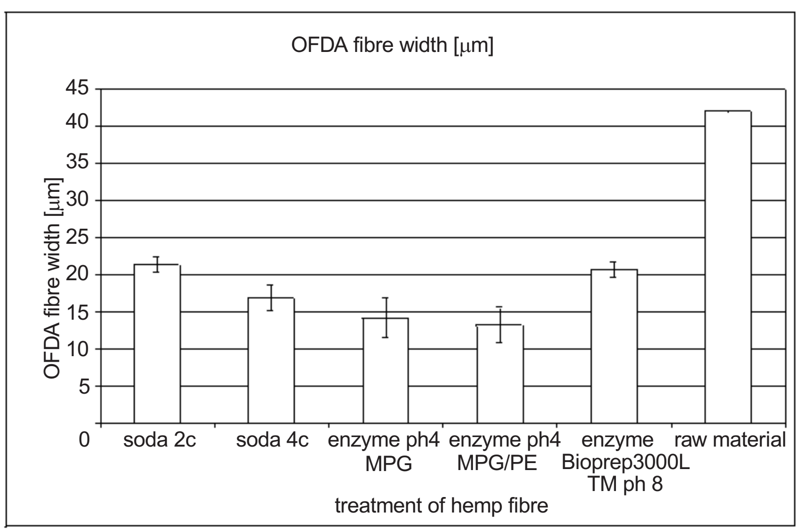


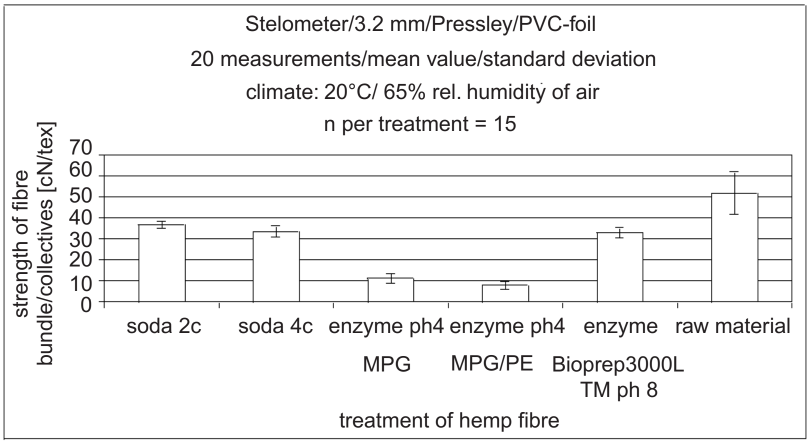

FIGURE 4. OFDA results on chemically and enzymatically separated hemp bundles




The next figures (Figure 3-Figure 7) are concerned with fineness distribution and strength data on the target hemp samples that were treated enzymatically.

Figure 4 shows that 4c soda hemp was finer than 2c soda hemp with an average width of 16.8 μm, similar to the Chinese samples in fineness (16.3–17.4 μm, Table 1). This seems to indicate that well retted fibre

FIGURE 5. Stelometer results on chemically and enzymatically separated hemp bundles




like 4c hemp is upgraded by the alkaline process. The strengths of the 2c and 4c soda samples were about 33-37 cN/tex (Figure 5) with no significant difference to 2c and 4c steam explosion process (Figure 2, 2c/SE 4c/SE). Figure 4 also shows that enzyme MPG and MPG + PE at pH 4 produced quite fine fibre (13.1-14.1 μm), comparable to Flasin flax fibre (Table 1) but much weaker amounting only to 8–11 cN/tex (Figure 5). Enzyme treatment with BioPrep 3000L® maintained fibre strength of 33 cN/tex (Figure 5) compared with strength of raw material (51.6 cN/tex and 42 μm fibre width) and produced fine fibre amounting to 20.6 μm (Figure 4). These fibres were comparable in strength to steam explosion hemp (Figure 2, 2c/SE and 4c/SE hemp) and soda 2c/4c hemp (Figure 5). These fibres were also comparable in fineness to steam explosion hemp 4c (Table 1, 4c/SE 19.1 μm), the Chinese HTEX hemp sample and Flasin flax fibre. Enzyme MPG and MPG + PE at pH 4 weakened the fibre of both 2c and 4c compared with the alkaline chemical and enzymatic treatment up to one-fourth (Figure 5).

Enzyme BioPrep3000L® treatment resulted in quite fine fibre, comparable to the 2c soda, and was also a strong fibre in all of the above Stelometer tests. Correct enzyme mixture formulation with low cellolytical activity seems to be very beneficial in maintaining fibre

FIGURE 6. OFDA results on chemically and enzymatically separated nettle fibre collectives

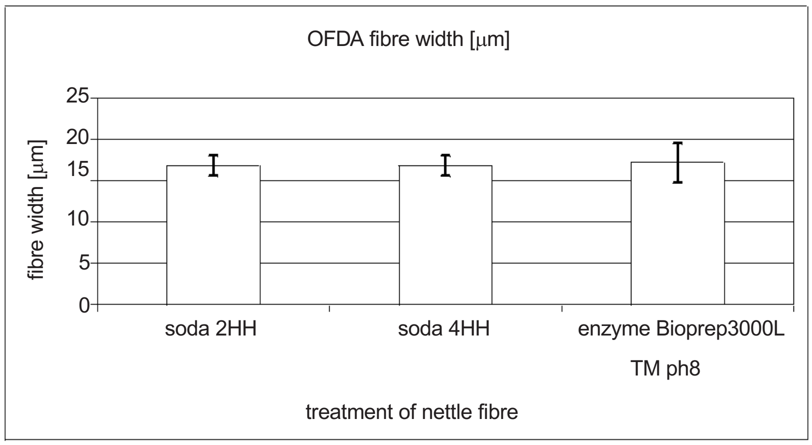
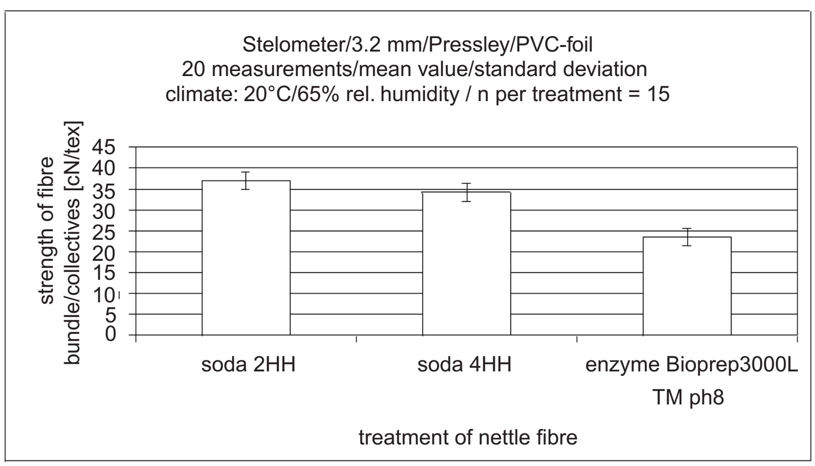



FIGURE 7. Stelometer results on chemically and enzymatically separated nettle fibre collectives

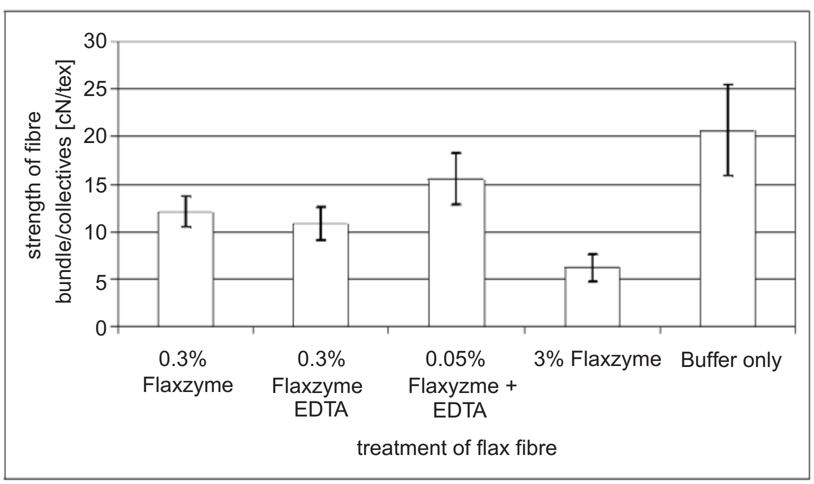



strength. Further investigation should be done on getting better fineness by the same or lower concentration level with varied duration, temperature, and additives.

Figures 6 and 7 show OFDA and stelometer results on enzymatically and chemically separated nettle fibre.

For the three tested treatments, nettle fibre fineness was nearly the same amounting to 17 μm (Figure 6). Fibre fineness between 2 HH and 4 HH soda process was identical probably due to sufficient retting of 2 HH in the field. This indicates that a shorter retting period is needed for nettle than for hemp. Fineness of all three treated nettle fibres was the same as Chinese hemp samples HNF_A, HTEX and NTEX and finer than steam explosion 2c/SE or 4c/SE (Table 1). Alkaline and enzyme treatment of nettle fibre resulted in the same values for fineness, but there were some differences in strength (Figure 7). Briefly retted nettle amounted to 37 cN/tex (soda 2 HH) and was a little stronger (4 cN/tex) than more extensively retted nettle. The strength of the enzymatically separated nettle fibre treated with BioPrep3000L® was about 23.5 cN/tex. Although there was a difference of about 10 cN/tex in strength between soda and BioPrep3000L® treated nettle fibre, its fineness indicates that nettle treated with enzymes potentially has values comparable to the alkaline treated variety. The strength of enzymatically separated nettle fibre was little higher than the Chinese HTEX hemp sample and Flasin flax fibre. Further investigation must be done on getting better strength by enzyme treatment of nettle.

FIGURE 8. Stelometer results in enzymatically separated flax fibre bundles [adapted from Akin et al., 1999]



Comparing the strength of enzymatically separated flax fibre bundles with Flaxzyme (Fig. 8, Akin et al., 1999) and enzymatically separated hemp and nettle fibre with BioPrep3000L®, we found much higher strength ratings in our trials for both plants. The micronaire for flax fibre of between 4.3 and 8.0 did not explain the loss in strength (Akin et al., 1999). Some reasons for these differences could be variations in raw material, enzyme formulation, and conditions.

## CONCLUSION

The investigation of enzymatic separation of coarse hemp and nettle fibre has shown that this process has good potential for producing fine strong hemp and nettle textile fibre. Hemp variant BioPrep3000L® /BAYLASE EVO is the best enzymatically treated variety. It is comparable to alkaline separated fibre. These results have now been verified by commercial trials.

There are clear environmental advantages for the enzymatic technique. No special apparatus, other than normal galvanized steel tubs, are required. The relatively low water temperature that appears from our studies to be best could be generated with low environmental impact. Unlike the alkaline treatments, the waste water from enzymatic retting is biodegradable and poses no particular disposal problems. Because of organic matter in waste water there must be efficient waste water treatment. Further investigations in this area are ongoing.

And because the process is relatively low-tech as far as equipment it could be done locally, close to the fields where the fibre plants are grown, thus reducing transportation and fossil fuel use. Future developments along these lines might include research into retting with specific microorganisms and optimizing enzyme formulations with auxillaries.

## ACKNOWLEDGMENTS

This study is being supported by the "Deutsche Bundesstiftung Umwelt," Osnabrück and AiF "Otto von Guericke" e.V. Koln. The authors gratefully acknowledge Novozyme AS and Röhm Enzyme GmbH for supplying enzymes, Editor of this presentation: Gregory Warne, American English Editor & Translator, g.warne@wanadoo.fr.

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RECEIVED: 28 March 2001
ACCEPTED IN REVISED FORM: 19 September 2001

# Reinforced Biocomposites from Flax and Hemp

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ABSTRACT. Defibrated flax and hemp fibres form new compatible composites substituting cabinets, car-inner panels etc. The aim is to produce composites from Danish flax and hemp that are competitive to composites reinforced with fibres such as polypropylene, glass fibre, and metals. The plant fibre composites can be used in many applications with different purposes. The composites are based on a new process, where flax or hemp are defibrated into shortened fibres and subsequently formed into mats through a unique air-forming technique mixing the plant fibre and polymers in a strong web. The mats are then moulded in a hot-press for products in all kinds of shapes. The process is based on shortened fibres from flax and hemp. The stiffness of the composites and tensile strength properties are equal in all directions, and their values are higher than those of pure plastic composites. In the new Danish composites the tensile strength seems lower than in typical composites from flax and hemp, where mats are derived from carding followed by needle punching. The main advantage is the price as the composites based on

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The authors wish to thank the Danish Ministry of Food, Agriculture and Fishery, the Directorate for Food, Fishery and Agro Business, The Ministry of Environment and Energy and The Danish Environmental Protection Agency who have supported the project.

Journal of Industrial Hemp, Vol. 7(1) 2002
http://www.haworthpressinc.com/store/product.asp?sku=J237

mats from the shortened flax or hemp fibres can be produced much cheaper than carded mats from long fibres. 

KEYWORDS. Plant fibre, flax, hemp, mat forming, press consolidation, thermoplastic composite, reinforced composite

## INTRODUCTION

The National Department of Plant Production at the Danish Agricultural Advisory Centre, has been working on development of new products from plant fibres, especially flax and hemp fibres. One of the results is the development of new fibre mats from flax and hemp. The concept is based on a completely new technology, i.e., forming mats in all sizes and thicknesses from shortened fibres derived from flax and hemp through a new air-forming technique.

The mats are suitable for a whole range of applications, first of all in insulation products and composites. The thickness of the mats varies from few millimetres to, e.g., 200 mm and various densities. Mats for insulation purposes normally contain 95 to 98 percent plant fibre and only 2 to 5 percent synthetic fibres, mainly polymers. The polymer is used as a matrix. In thermoplastic composites the percent of fibres and polymers varies according to the application-in some composites for example up to 70 percent flax fibre is used. One of the main advantages of the new fibre mats is the price, which is competitive to, e.g., mineral wool and glass wool (Pallesen, 2000b).

The new composites can replace composites reinforced with plastic, glass fibres, etc., Reinforced flax and hemp composites from carded mats have already turned out to be well suited for the motor industry. Many parts of car interiors and exteriors have already been replaced with natural fibre composites such as flax and hemp. In Daimler-Chrysler more than 20 prototype parts from flax and hemp are used in cars (Schlosser, 2000).

The National Department of Plant Production has patented the "Method for manufacturing a fibre mat, the fibre mat itself and the use of such a fibre mat." The air-forming technique used represents a new generation of air-forming techniques, and is patented by Marianne E.

Eriksen (www.MEconsulting.dk). The new air-forming system forms natural fibres with a very high capacity compared to current air-forming systems of the same size.

The National Department of Plant Production and Risoe National Laboratory have in co-operation carried out trials with composites made from press-consolidated mats of flax and hemp. The main target was to find the potential in the fibre composites for different applications, depending on the fibre quality, the polymer, etc. The raw material was Danish grown flax or hemp at different degrees of retting, harvested and collected in alternative ways.

The Materials Research Department, Risoe National Laboratory, has worked with polymer composites for many years and has a simplified model of the press consolidation technique. The quality of the moulded composites has subsequently been tested at Risoe National Laboratory.

The new fibre mats are commercialised through the company Danish Natural Insulation Inc. (Figure 1). Situated in the southern part of Denmark in Sakskobing the production plant initiated production of mats for insulation in April 2001. The plant capacity will as a start be

FIGURE 1. The new production line of Danish Natural Insulation Inc.

600 kg per hour, increasing to 1200 kg per hour depending on market demands. The new approach making composites, for example, for the motor industry will hopefully soon become a reality.

The Danish Agricultural Advisory Centre acts as advisor to the new company offering know how. The production is based on a license production.

The objective of this investigation is to find out how we can make composites, without adding chemicals, which are cheap to manufacture, and where the strength is of secondary importance. We have been interested in investigating which plastic types–polymers–give the best properties, still being suitable for the new mat forming process. Furthermore we have studied the influence of the degree of retting on the material properties of the composite.

## METHODS

### Raw Material

The quality of the raw material plays a major role in the final application. With the new technique plant fibre composites from un-retted flax and hemp fibres will result in a badly defibrated fibre material containing a large amount of shives-wood core-in the final product. This will, in general, cause a poor quality with reduced strength properties.

Well-retted flax and hemp fibres will, on the other hand, result in a fine quality and a lower content of shives. With the applied technology it is difficult to achieve composites which are almost 100 percent free of shives.

### Hemp

Hemp can be grown in Denmark, but in general the climate is too cool compared to, e.g., Germany and France. Hemp with low content of THC was first legalized in 1998 in Denmark. At Koldkaergaard, Research Farm under the National Department of Plant Production, we have carried out demonstrations of different harvest techniques. The best results have been achieved with hemp cut and laid on swath at the end of August and cut twice in two layers with a MacDon. After a retting period of 2-3 weeks the straw will have changed color from green to dark grey and the fibres will have turned light grey. The straw is pressed into bales when the moisture content of the straw is 15-16 percent. The yield of the stem varies. At Koldkaergaard Research Farm, 8-10 tons per ha of well retted stem were harvested. The fibre percentage was 25-30 (Pallesen, 2000b).

### Flax

Harvesting flax for fibre production is also fairly complicated in Denmark, also due to the unstable climate. The idea has been to grow fibre flax for both seed and fibre production. One way is to cut the crop, lay in swaths and combine-harvest after 1-3 weeks. In Denmark, harvesting time for fibre flax is August, approximately 1 month earlier than combining linseed. The straw is cut app. 8-10 cm above ground level. The swathing process is carried out when the leaves on the lower two thirds of the stems have fallen off and the stems have turned yellow. At Koldkaergaard Research Farm, The National Department of Plant Production, we have achieved an average of 1600 kg seed per hectare over 3 years (9% moisture) (Pallesen, 1998).

After combining the seeds, the flax straw is dew-retted on the field until the colour has changed from yellow to light grey–normally after two weeks of retting. After combining, the straw is spread in all directions, and will not be suitable for a traditional scutching process. The retted straw is pressed into bales and is ready for processing.

Wet summers can cause many problems with respect to cutting the straw and harvesting the stem with a combine harvester, due to the very strong flax fibres. One alternative method used with success has been to use a “stripping-combine harvester,” in order to avoid trouble with the stem passing through the traditional combine harvester (Pallesen, 2000b).

### Tested Raw Material

In order to evaluate the potential of the raw material for thermoplastic composites, four different raw materials have been tested:

- Semi-retted flax 1997. This quality is very suitable for mat forming resulting in very soft and fluffy mats. The color of the fibres is light grey. Flax S.
- Almost unretted flax 1999 with a yellowish color. Flax U.
- Retted hemp 1998 with a grey color. Hemp S.
- Unretted hemp flax 1999 with a yellowish color. Hemp U.

## Defibration of Fibres

The National Department of Plant Production has developed an alternative method to process hemp and flax into short fibres at a size of approximately 15-25 mm, fibres mainly occurring as single fibre bundles. In a short fibre process line at Koldkaergaard Research Farm the stem is decorticated into single fibre bundles through a completely mechanical process. The result is a production of shortened and cleaned flax and hemp fibres suitable for a number of industrial purposes such as mat forming (Figure 2).

The principle of the process is a bale opener feeding a hammer mill, where sharp-edged hammers crush the raw material which subsequently is drawn off by suction through an adjustable revolving screen, and the shives and fibres are easily separated. This method enables the removal of 95% to 99% of the flax or hemp core, called the shives (Pallesen, 1996).

## Mat Forming

The fibres produced can be formed into mats using a special air forming technique. In order to achieve the highest possible capacity for handling very long fibres, a new former head has been developed which is able to handle 20-40 mm long fibres. The new technique has been invented and patented by M. Eriksen (Eriksen, 2000).

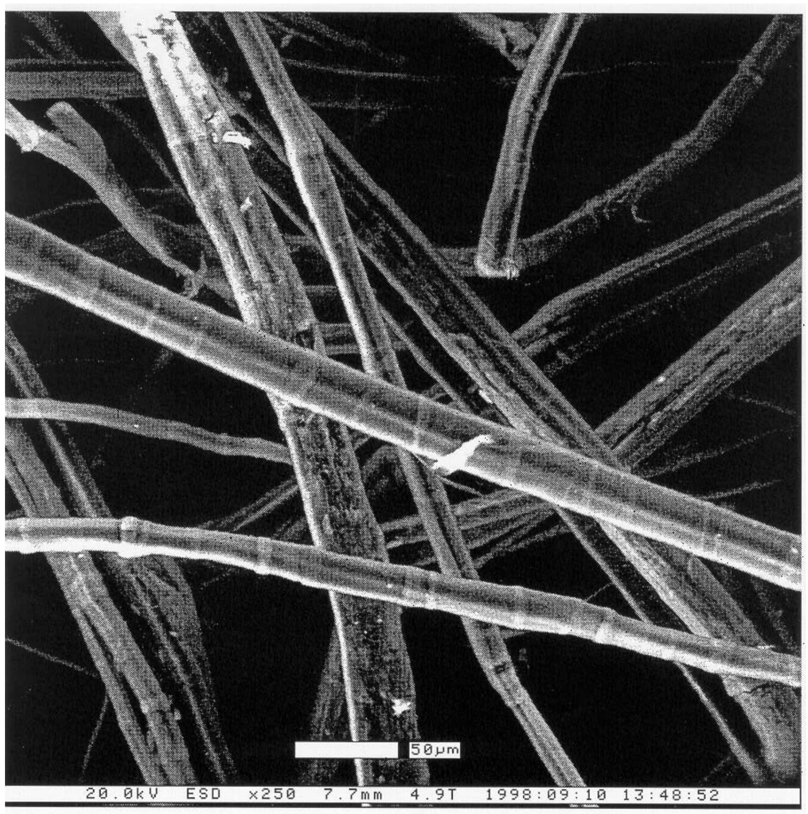
The mats are formed in a separate line with a forming wire and a vacuum box. The dry fibres are led into a forming box and are mixed in an air stream passing several rotating rollers, which are provided with radically extending spikes. The fibres enter the forming wire and are formed into the requested thickness or weight per square meter. The mats are then transferred to an open air oven for fixation (Pallesen, 2000a).

In comparison, the use of traditional air forming techniques has met insuperable difficulties, and it is not possible to form the described fibres from flax and hemp using the traditional technique. This means that a realistic productivity cannot be obtained.

The fixation of the fibre mats takes place in a true air oven, where synthetic binders such as polymers are bonded with the natural fibres through melting. The true air oven can fixate a mat of, e.g., 100 mm in a few seconds. The principle of the oven is based on recycling of air, allowing the airflow to pass through the material.

The mats can be used for many purposes depending on the size of the mat, the quality of the fibre and binder, modification of the fibres, etc. When moulded, the mats can be fluffy or hard as structural composites.

FIGURE 2. Flax fibre, SEM-photo × 250




Mats, where the strength is of secondary importance, contain only 2-5% of polymers in order to manufacture a stable mat, e.g., for insulation purposes. For manufacturing composites the amount of polymers is increased compared to the proportion of polymer for insulation purposes.

Polymer Matrix

Plastic-fibre polymers work as a binder in the mat, and are thoroughly mixed with the plant fibre during the mat forming process. In the press consolidation the polymer has the matrix function.

The tested binders represent polymers that are already adapted for use in the air-forming industry as binders. The binder has an influence on the strength of the composite. The different binders are chosen in order to achieve a high tensile strength in combination with the plant fibre.

The bi-component PP/PE has a matrix core with polypropylene 65% with a melting point at 160°C which is surrounded by polyethylene 35% with a melting point at 127°C. In the oven, the surface of the polymer melts and binds the plastic and the plant fibre together to form a relatively elastic mat. The internal core of the polymer is unaffected, and contributes to the strength and elasticity of the mat. The bi-component fibres are used in many combinations in non-woven products, and offer product strength and an environmentally friendly binder function.

The bi-component Polyester T-255 (50:50) consists of a polyester core surrounded by polyethylene. The T-255 has the advantage of a broader melting point area, ranging from 127-180°C. The principle is similar as explained for PP/PE fibre.

Polypropylene PP has a higher melting point than the other two polymers. PP is often used in composites, e.g., the automobile industry, geo-textile, etc.

## Press Consolidation

The natural fibres have high tensile strength and stiffness, whereas the function of the matrix is to tie the fibres together through warm fixation.

At Risoe National Laboratory, a simplified model of the press consolidation technique is used for the trials. For the press consolidation technique, the following sequential steps can be identified: lay up, pre-heating under vacuum, consolidation and cooling. Five basic pieces of equipment are required to fulfil the process operation: press, pre-heat unit, mould, conveyor system and a control unit (Andersen, 1997).

## Press

The press is a single active hydraulic press with a press force of 30-200 kN and a maximum press area of $1000 \times 800$ mm$^2$. The piston with the crosshead is integrated in the bottom of the press and has a maximum press stroke of 400 mm. The speed of the crosshead can be adjusted from 0-100 mm/s.

TABLE 1. Different polymers tested in the fibre composites.

| Bi-component polymer fibre | Pct. of plant fibre/binder | | Processing temperature in the mat forming process/ fixation (°C) |
|---|---|---|---|
| | (Wt-%) | (Vol-%) | |
| T-255 (50/50) Polyester/Co polyethylene | 50/50 | 48/52 | 145 |
| PP/PE (65/35) Polypropylene/Polyethylene | 50/50 | 37/63 | 145 |
| PP Polypropylene | 50/50 | 37/63 | 160 |

## Pre-Heat Unit

In the pre-heat unit the material is conduction heated under vacuum in order to prevent/minimise the degradation of the thermoplastic matrix material and to remove the air (porosity) from the puffy semi-raw material. The pre-heat units consist of two 1300-Watt heaters. The design of the vacuum tool has been specially developed to handle postpreg semi-raw materials, where it is necessary to melt the matrix material in order to ensure a high level of fibre wet out in the consolidated laminate-composite.

## Conveyor System

In the forming phase, the material has to stay hot for as long as possible to facilitate the rearrangement of the fibre reinforcement and matrix flow in the material. Fast and accurate handling of the material from the pre-heat section to the press is, therefore, necessary. The speed of the conveyor can be adjusted from 0-3 m/sec with a position accuracy of 1/10 mm. The flexible silicone vacuum sealing is placed on top and bottom of the conveyor frame.

## Mould

The press and heating unit was set up for pressing flat (2D) test laminates of maximum 300 $\times$ 300 $mm^2$. The two mould parts consist of 1300-Watt heated press plates attached to both the stationary top part and the crosshead.

## Control Unit

The PC/PLC control unit takes care of both the process parameters according to the fix values and automatic execution of the experiment.

## Experimental Plan

Two serials have been carried out. Serial I, a pretrial, was carried through in order to determine the optimum temperature for press consolidation. Temperatures of, respectively, 140 and 190° C were used on flax mats during press consolidation (Krex, 1986). The best results were achieved at 190° C.

In serial II the trials were carried out at 190° C, as the preferred temperature for the warm pressed composites. Process time was 10 minutes.

## Characterisation

The density, content of fibre, polymer matrix and porosity was determined by gravimetric measurements using a modified version of ASTM D 3171-76 (Standard test method for fibre content of reinforced resin composites) (ASTM, 1978). In this procedure, composite samples were weighed, the PP or PP-PE matrix dissolved in hot xylene and the residual flax or hemp fibres were isolated, dried and then weighed. With the known density of the fibre material and of the polymer matrix, it was possible to calculate the weight and volume fractions and the porosity of the composite. Calculations have been performed with flax and hemp fibre density of 1.54 g/cm$^3$ and a matrix density of 0.91 g/cm$^3$ in PP/PE and PP. The porosity expresses the amount of air in the composite after pressing, the lower percent means in general a higher strength.

It was not possible to dissolve and remove the T255 matrix material totally from the consolidated laminates–composites. Therefore, there are no results of fibre content and porosity for these material combinations. The matrix density for polyester is 1.4 g/cm$^3$.

Four tensile test specimens were cut and tested in both the main directions of each laminate allowing a comparison of tensile strength and stiffness properties between samples cut in the same direction as the mat (zero degrees or warp) and samples cut across the mat direction (90 degrees or weft). Tensile testing of "dog bone"-shaped specimens, measuring 180 mm in length, 25 mm in maximum width and 15 mm in the narrowest section, was performed on a mechanical Instron testing ma-

TABLE 2. Optimisation of process temperature in flax composites.

| Serial I Trial no. | Material | Temperature-[°C] | Process time [min] | Consolidation power [kN] |
|---|---|---|---|---|
| 1 | Flax/PP-PE | 140 | 5 | 200 |
| 2 | Flax/PP-PE | 190 | 5 | 200 |

TABLE 3. Composites with flax and hemp, and varying polymers as matrix.

| Serial II Trial no. | Raw material* | Matrix | Temperature [°C] | Process time [min] | Consolidation power[kN] |
|---|---|---|---|---|---|
| 1 | Flax S | PP | 190 | 10 | 200 |
| 2 | Flax U | PP | 190 | 10 | 200 |
| 3 | Hemp S | PP | 190 | 10 | 200 |
| 4 | Hemp U | PP | 190 | 10 | 200 |
| 5 | Flax S | PP/PE | 190 | 10 | 200 |
| 6 | Flax U | PP/PE | 190 | 10 | 200 |
| 7 | Hemp S | PP/PE | 190 | 10 | 200 |
| 8 | Flax S | T255 | 190 | 10 | 200 |
| 9 | Flax U | T255 | 190 | 10 | 200 |
| 10 | Hemp S | T255 | 190 | 10 | 200 |
| | * S = Semi-retted, U = Un-retted | | | | |

chine with a 5 kN load cell. The machine was operated in displacement control at a speed of 2 mm/min. Longitudinal strain of each specimen was recorded with two back-to-back extensometers. Readings of load and strain were sampled at 4 Hz with a PC-based data acquisition system (Labtech Notebook). The average strain was used and the stress was calculated as load divided by initial cross-section. From the measurements, the average stiffness (E-modulus) was calculated in the range from 0%-0.2% strain and the average ultimate tensile strength was recorded.

## RESULTS

The tensile strength and stiffness (E-module) has been measured in the preliminary serial I, where two levels of temperature, 140° and 190°

C during the press consolidation were tested. The reason for choosing 140° C was that we wanted to test the properties in the composite at a lenient treatment. We also wanted to investigate whether the polymer was damaged at high temperature. Since the core in the PP/PE matrix, the polyethylene, melts at 140°C, we would have attachment between the plant fibre and polymer-matrix. The PP fibre is semi-crystalline, with a broad melting interval. At 140°C, the polymer is softened up, and makes an attachment through the PE, that has melted and fastens to the plant fibres. The PP fibre works as reinforcement of the composite.

Treatment 190°C during press consolidation gives a higher tensile strength and a higher stiffness than at 140°C, but only at direction 0° of the mat length, whereas the direction 90° of the mat length has the same tensile strength (Figure 3). The result is presumably due to a better attachment to the fibre. The level of strength in the pre-trial is not very high, but shows that direction of the mat is of some importance. Furthermore, the high temperature, 190°C, gave the lowest porosity which is a great advantage.

A reference would be a 100% plastic composite. The tensile strength in polypropylene (PP) is 28.5 MPa and stiffness is 1.7 GPa. The tensile strength in bi-component PP/PE is 20.0 MPa and stiffness is 1.4 GPa. The polyester is not measured.

The high temperature gave the best results, and showed that the polymer was not damaged. But the contribution to the tensile strength in flax and hemp is negative in the preliminary trials. This is due to a lack of contact between the plastic and the plant fibre.

The light microscope image in Figure 7 shows the fibrillation and the attachment between the fibre and matrix. There are only few air holes in the composite, trial 8. The fibres occur as single fibres and in fibre bundles. In trial no. 3 there are parts of the composite with no attachment to the matrix.

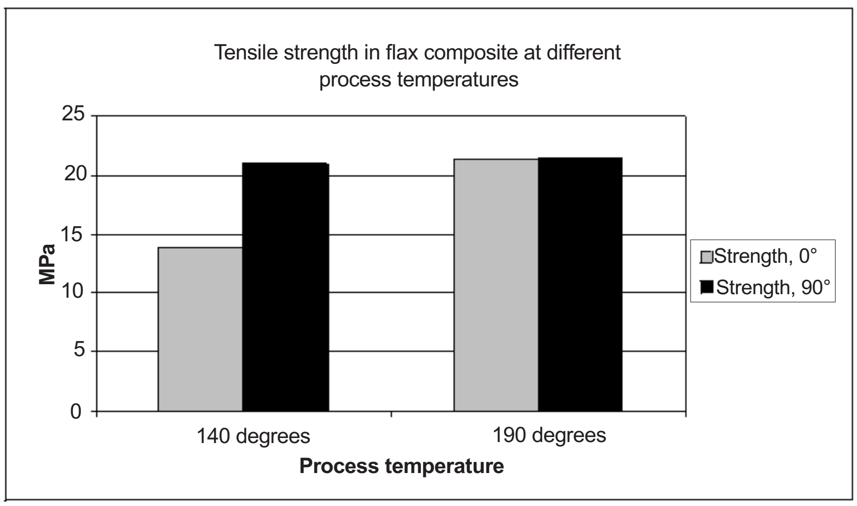
The variation of the matrix and the raw material in serial II shows a wide variance in strength. In general the un-retted flax gives a higher strength than semi-retted flax, whereas the un-retted hemp has a lower tensile strength. The best results, 43 MPa (0°, length direction), are achieved in hemp with a polyester matrix T-255 (Figure 8).

The fibre volume fraction is normally in the range 20-35 vol. %, corresponding to a fibre weight fraction of 35-55 weight %, and the porosity is normally in the range 2-10% (Figure 9). It is possible to determine the porosity in composites with very high porosity contents, up to 50 vol. % (Andersen et al., 1999).

TABLE 4. Process temperature–flax fibres in warm pressed composites, serial I.

| Process temperature | Fibre content, pct. of weight | Fibre content, pct. of volume | Porosity, volume pct. |
|---|---|---|---|
| 140 | 44.6 | 27.0 | 18.2 |
| 190 | 45.1 | 33.0 | 1.5 |

FIGURE 3. Tensile strength at two process temperatures. Tensile strength at 0°, length direction of the mat and 90° cross direction in flax composites

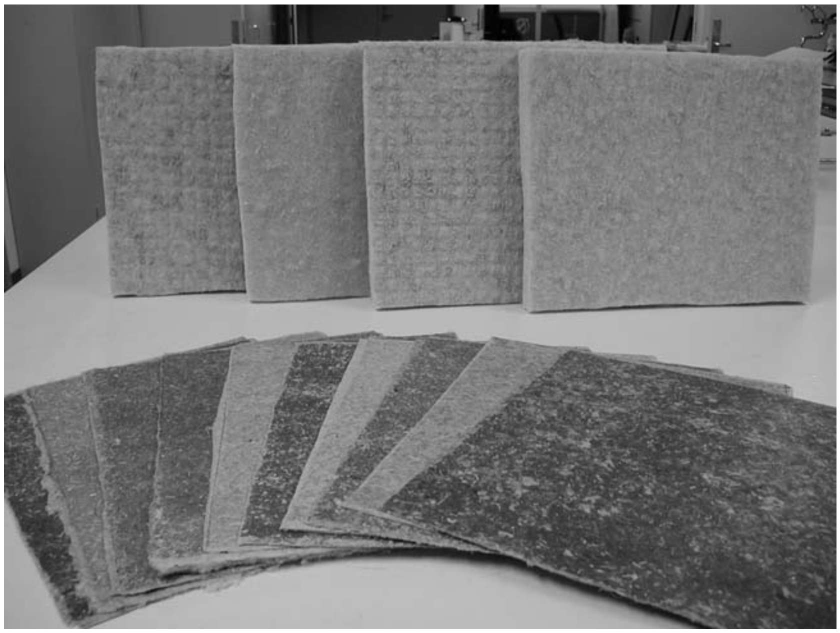



The chosen 50% weight content of plant fibre (from 27%-48% vol.) is relatively high in order to achieve a good adhesion and wetting between fibre and matrix. A higher content diminishes the possibilities to achieve a good fibre wetting due to an insufficient amount of polymer.

There are no results of fibre content and porosity for the polyester-composites since it was not possible to totally dissolve and remove the T255 matrix material from the consolidated laminates-composites.

The highest stiffness is achieved in polypropylene, whereas the T-255 has the lowest stiffness (Figure 10).

FIGURE 4. Mats and press consolidated fibre composites



## DISCUSSION AND CONCLUSION

### Comparison with Other Composites

Compared to other composites derived from flax and hemp fibres the tensile strength in the trials is rather low, the highest being 43 MPa in un-retted hemp and polyester composite. Flax/PP composite has 20-25 MPa, which is lower than PP-composite at approximately 30 MPa.

Generally, 50-70 MPa in tensile strength (0°, length direction) and 3-6 GPa (0°) in stiffness are easily achieved in flax composites from carded and needle-punched mats based on short flax fibres, a typical by-product from the textile industry (Snijder, 2000). This is measured in hemp-fibre/PP composites, using 30–50% of fibre. The highest content of fibre resulted in the highest tensile strength and the highest E-module.

The tensile strength can be improved with a modification of the fibres. At Risoe National Laboratory, an improvement of the tensile strength, is

FIGURE 5. Test composites for tensile strength and E-module–"dog bone" shaped composites

found typically 25%, in jute composite modified with MAPP (Maleic anhydride grafted polypropylene). The improvement is due to a better chemical bonding between the natural fibres and the matrix.

ATO-DLO also works on the improvement of the fibre composites. Flax-polypropylene composites modified with, e.g., MAPP have resulted in a tensile strength increased by 25% or even more (Snijder et al., 1997), reaching 95 MPa for flexural strength.

The tensile strength varies according to the orientation of the fibres: Direction 0° of the mat length or 90° cross direction. The strength is normally higher for the cross direction, but not in the new composites. Random needle-punched mats-in this case Danflax mats-produced from flax tow with 50% PP, have obtained a tensile strength at 28 MPa in length direction versus 57 MPa at cross direction, and a stiffness of 3.3 GPa in length direction versus 5.5 GPa in cross direction (Nielsen, 1999), which is not very different from the new mats.

FIGURE 6. Stiffness (E-module) of flax composites at 0°, length direction of the mat and 90°, cross direction, at two process temperatures

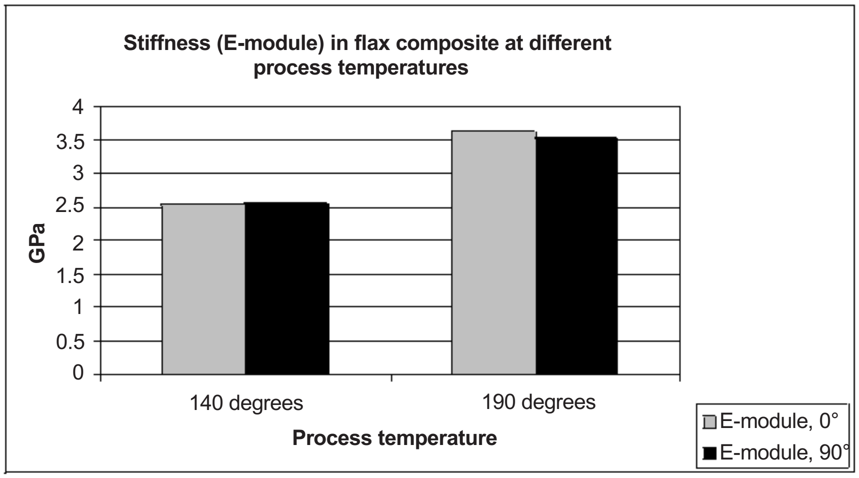



FIGURE 7A. Light microscope image of composite with semi-retted flax and polyester (T-255), X126; trial no. 8

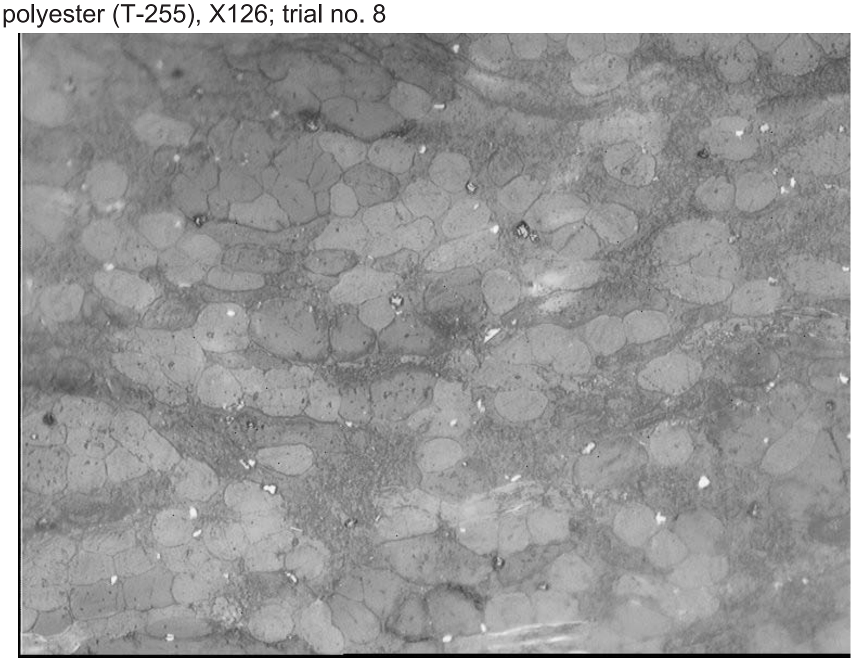


FIGURE 7B. Light microscope image of composite with semi-retted hemp and polypropylene, X31; trial no. 3

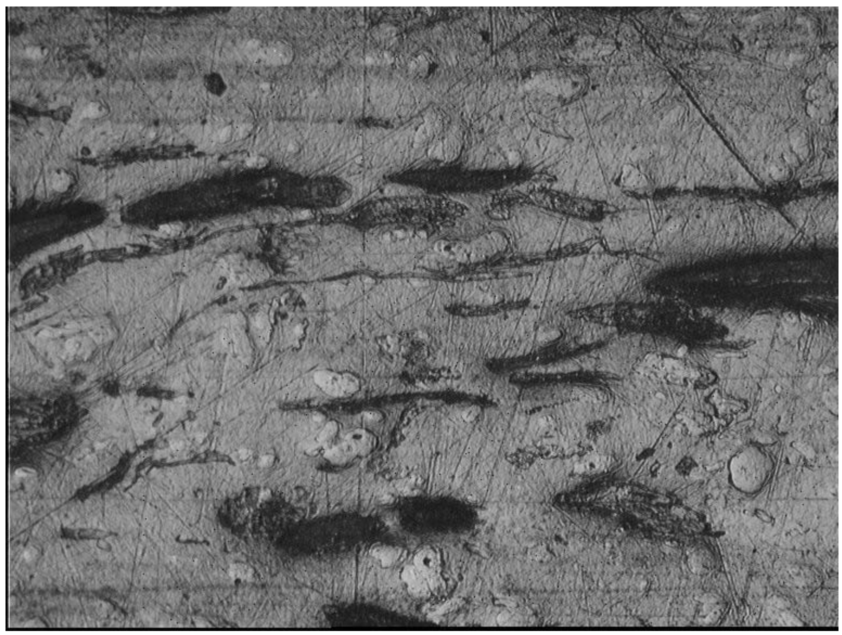


FIGURE 8. Tensile strength in composites with flax and hemp, at varying degrees of retting and with different polymers

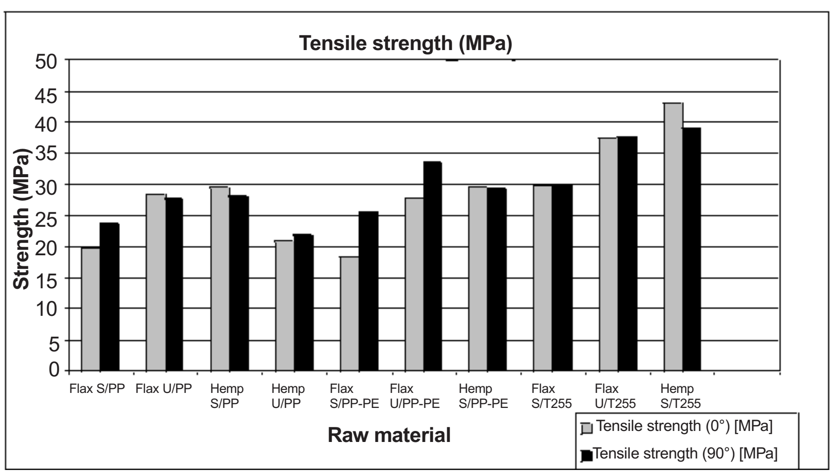


FIGURE 9. Porosity and fibre volume, composites from flax and hemp, serial II

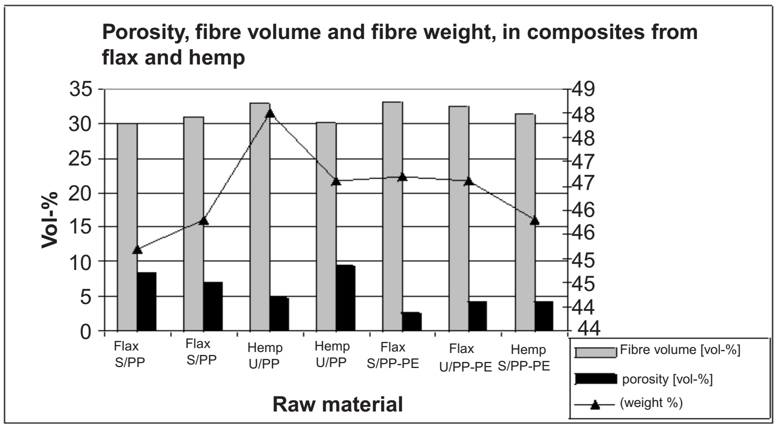
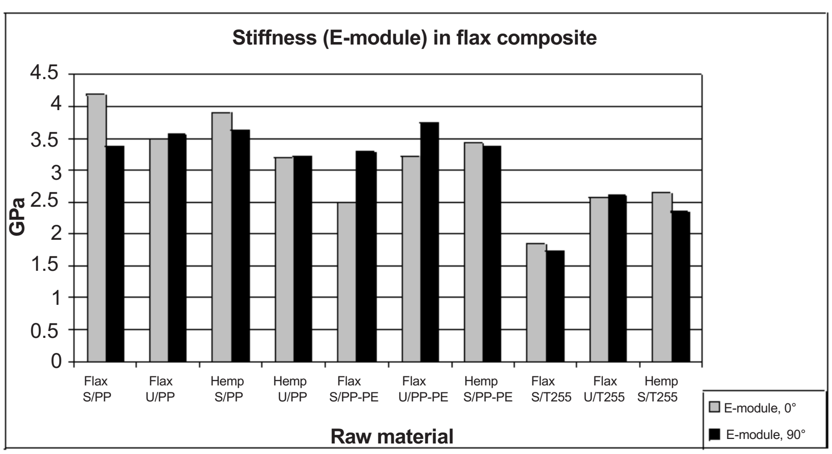



FIGURE 10. Stiffness in composites of flax and hemp, at varying degrees of retting and with different polymers



In comparison to the stiffness and strength of the fibres themselves, flax fibres obtain strength in the elementary fibre at 290 MPa in strength and 44 GPa in stiffness (Lilholt and Toftegård, 1999).

In general, flax and hemp have a higher strength than many other natural fibres. Jute typically varies from 30-40 MPa in tensile strength in length direction, and between 40-60 MPa at cross direction. The E-module varies from 4 GPa at length direction to 6-7 GPa at cross direction (Andersen; 1999).

The Danish flax and hemp mats processed with the new technique have a potential that has not yet reached the maximum. The mats contain a large surface area due to the defibration of the flax or hemp containing a large amount of fibre-to-fibre bindings. But the fibres are easily damaged through the mechanical defibration resulting in a relatively low tensile strength.

In some cases, the porosity is very high, this is due to an insufficient mix of the plant fibre and the plastic fibre before press consolidation. In the light microscope one can also see whether the composite is fully consolidated or not. The fibres without matrix are seen as dark parts of the composite. In such cases, the plant fibre and the synthetic fibre need to be mixed sufficiently and, in some cases, the amounts of plastic fibre need to be increased.

In the trials, we have utilised the proportion 50% plant fibre to 50% synthetic fibre, on a weight basis. Since the specific gravity of the PP/PE is 0.9 g/cm$^3$ and 1.4 g/cm$^3$ for polyester (T-255), the utilised volume percentage is not enough to cover all the plant fibres. This means that the potential is not fully explored for this matrix. We estimate that we can increase the strength by using a higher content of synthetic fibre for this type of composite.

The adhesion will also be improved having a high fibre bundle degree of fibrillation as seen in the light microscope photos showing the composite with good adhesion and one with a low adhesion in Figure 8A and 8B. The smaller the fibre bundles are, the easier it will be to achieve a good bond between fibre and matrix.

The composites can be optimised with respect to the quality of the fibres, the quantity of fibre bundles, the type of matrix, the right combination of fibre and matrix, an efficient mix of plant fibre and matrix, through a modification of the fibres, e.g., with MAPP, etc. A balance between mechanical damages and higher expenses should be found. Major possibilities to improve the characteristics of the composite exist.

At the new production line in Denmark, the problem with efficient mixing of plant fibre and matrix is solved with the development of a new mixing system in the mat-forming process (Eriksen, 2001), which is under patenting.

## CONCLUSION

Our objective was to manufacture environmentally correct products from natural fibres and to avoid using chemistry to modify the properties.

In general, the tensile strength of the new composites made from the mats processed with the new technique is lower than that of traditional composites from carded fibre mats. On the other hand, the cost of processing these mats is only half of that of processing carded mats.

The potential of this new technique is not fully exploited. By using a compatibiliser such as MAPP, it will be possible to increase especially the strength in the composites but the price will increase. Furthermore, finding the right combination of fibre quality and matrix will result in an improvement of the strength.

Another advantage is that the new mats are homogeneous and have the same tensile strength in the length direction as in the cross direction. The new production concept has made it possible to use almost green-retted flax and hemp and not only retted qualities.

The great advantage of the new type of mats is their price. The mats can be produced at a lower price than the typical needle-punched mats from flax or hemp. Approximately, half the price at 1.3–1.4 EURO per kg formed mat, compared to 2.8–3.0 EURO per kg needle-punched mats from flax. Furthermore the plant fibres are cheaper than the plastic fibres, they are recyclable, and the energy balance is improved.

So far, the new company–Danish Natural Insulation Ltd.–has concentrated production on mats for the insulation market. However, we expect that the future composite market will be interested in the new mats for many purposes.

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RECEIVED: 7 May 2001
ACCEPTED IN REVISED FORM: 29 November 2001

# Field Interview Schedule and Questionnaire for Investigating Cannabis Use

Robert C. Clarke

ABSTRACT. Cannabis is grown and processed for a wide variety of uses. Many plant parts are used as medicine for humans and livestock; whole seeds and seed oil are eaten by humans; seeds and leaves are fed to animals, seed oil and stalks are burned for fuel. Whole plants, leaves and wood have environmental uses and bark, fiber and seeds are also of ritual importance. This paper introduces an interview schedule and questionnaire for investigating Cannabis use. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com> © 2002 by The Haworth Press, Inc. All rights reserved.]

KEYWORDS. Cannabis use, data collection, ethnobotony, field research, plant use, survey

## INTRODUCTION

It is most important that an interview schedule be easy for field researchers and their respondents to use. Cannabis field research requires an interview strategy that can be used to thoroughly investigate the many possible uses of this single plant genus. Such a survey requires a broad question set relying on fixed and uniquely numbered answers when possible, in order to allow the inclusion of additional data as it is accumulated. Structured data sets are acceptable for answering many

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Journal of Industrial Hemp, Vol. 7(1) 2002
http://www.haworthpressinc.com/store/product.asp?sku=J237

questions but offer limited possibilities for responses and they should also be accompanied by an interview strategy allowing free text options. However, an interview strategy must also invite and accommodate new knowledge, such as a previously unrecognized use for the plant.

Presented here, is an interview schedule for studying Cannabis use in any field situation. The initial incentive to compile a field questionnaire results from the author's ongoing survey work investigating the traditional uses of Cannabis hemp. The inspiration for assembling a detailed question and data set came from reading Popular Tools of Hemp-Work in Hungary by Ébner Gönyey (1936), a Hungarian ethnologist. The logical arrangement of the interview schedule and the phrasing of many of the questions were adapted directly from Selected Guidelines for Ethnobotanical Research: A Field Guide by Miguel Alexiades (1996). Additional general background questions (Sections A., B. and C.), as well as the term "interview schedule," were gleaned from Ethnobotany–A Methods Manual by Gary Martin (1995). The format for the data set and the majority of the descriptors are adapted directly from Economic Botany Data Collection Standard (EBDCS) compiled by Frances Cook (1995) and her colleagues at the Royal Botanical Gardens, Kew. Their exhaustive work provided a model for compiling answer sets for the interview schedule. Alexiades' text provided a logical question structure, including the majority of the questions, but few of the responses. Cook's data collection standard provided hierarchically arranged question sets with fixed descriptor states (responses). The intended use of the EBDCS is to collect flexibly structured, yet discrete data concerning the economic uses of a wide range of plants to supplement the taxonomic data accompanying herbarium sheets. The questions are narrowly focused, and are accompanied by many possible answers to allow use by a wide variety of researchers with differing academic interests. Cook provided lists of the plant parts utilized and their myriad possible uses, and the author of this paper was able to supplement her work with additional possible responses. Cook also provided several questions of a more botanical nature which were added to this interview schedule. Although the EBDCS is structured in a question/response format, the author felt it would be difficult to employ in this ethnographic research.

Although this interview schedule and question set was primarily designed for interviewing respondents from rural indigenous ethnic groups, it can also be applied to investigating Cannabis use in clinical and industrial situations. Cannabis is used for such a wide variety of purposes that with only slight modification this interview schedule could also be used to study other economic plants. All answers are assigned a unique number corresponding to the number of the question. It is not necessary for a respondent to answer every question for the partial data to be valid, as responses can be integrated into the collective data set. The logical organization of the interview strategy and closed data sets adapt the survey for data entry on a hand-held computer, easing data collection, storage and evaluation. Researchers interested in utilizing this interview schedule, or developing it for computerized use, are invited to contact the author.

## EXPLANATION OF INTERVIEW SCHEDULE AND STRATEGY

In actual practice, interviews usually begin with the general question, "Is Cannabis used in this area?" This is the simplest way to determine early-on the extent of knowledge of the potential respondent and identify individual use categories for deeper investigation. Intensive focused questioning is reserved for those respondents selected as knowledgeable. During the course of the interview, broad questioning gradually becomes more focused as the depth of the interview increases, finishing with detailed questioning on specific topics. Interviews should begin with general questions of a less structured format to set a casual tone to the questioning, build the trust and confidence of the respondent(s), establish a rapport and allow them to expound freely on their knowledge of the local region, its inhabitants, and their use(s) of Cannabis.

The interview schedule is divided into two major sections; General Survey Information and Questions for Individual Respondents. Answers to general Geographical (Section A), Population (Section B) and Conservation (Section C) questions found in the General Survey Information section will be much the same for neighboring geographical and cultural areas and may not require answers from each respondent. Much of this general basic information may be found in books and articles about the region. The Questions for Individual Respondents begins with a Respondent Profile (Section 1) which is based on information provided by each respondent about themselves before beginning any direct questioning about Cannabis. The Current Status of Cannabis Use (Section 2), Management Status of Cannabis Resources (Section 3) and Distribution of Cannabis Resources (Section 4) of Cannabis resources should be determined from several respondents, although conditions may be similar throughout each local area.

Questions for individual respondents begin in Plant Resource Use Determination (Section 5) and are structured to determine the specific topics of Cannabis use knowledge possessed by each respondent. To begin the more focused phase of the interview, the interviewer should ask a short series of direct closed questions such as: "Do you use Cannabis for (whatever use the respondent did not already mention)?" This will allow the interviewer to close any use categories for which there is no use in the area (or the respondent has no knowledge) and to continue along more potentially rewarding lines of questioning. Once the respondents breadth of knowledge concerning Cannabis use is determined the remainder of the interview schedule concentrates on the details of individual uses. Plant Resource Use Statistics (Section 6) should be collected for each use established in Section 5. These questions are mostly economic in scope and answers may vary widely for each use in each area. The first questions in each subset call for Yes/No answers, or finite descriptors and lead the interviewer through the early investigative parts of the question sets while probing the depth of knowledge of the respondent.

The remaining questions concerning Individual Uses (Section 7) are semi-structured and more focused, corresponding to uses known to the respondent established earlier in Section 5, each with a particular subset of questions presented in fixed order (see Outline of Interview Schedule). The respondent is asked a more highly structured subset of direct questions pertaining to each individual use category, with closed questions providing discrete answers to indicate the plant part and its use. Both discrete and open responses describing preparation and use allow the elucidation of fine detail. Respondents commonly revert to the more comfortable unstructured conversational setting, moving freely between several topics, and the order of questioning then becomes impossible to maintain. In this case, the structured format of the questionnaire allows the interviewer to "fill in the blanks" as the respondent touches on various aspects of Cannabis' use. In an effort to avoid confusion, interviews should be conducted with individual respondents when possible. However, interviews were frequently carried out with a group of respondents who discuss the question together, before the dominant member of the group (often the senior weaver, head of family or village leader) answers. Open questions were applied to complex and culturally sensitive situations such as funerary and other ritual use to allow elaboration on each subject. The questions and responses in Sections 5 and 6 make up the bulk of this survey summary. More detailed questions calling for textual explanatory answers follow, and these are also presented here.

In Cannabis-rich regions, it can be very time consuming to complete every aspect of this interview schedule. However, in practice, only a few of the individual use types will usually apply in each geographical and cultural region, and it proves expedient to focus on the individual sections most appropriate to the particular study and setting.

## DATA ANALYSIS

The majority of the information collected in this interview schedule consists of closed data sets that can be analyzed statistically. Textual data resulting from open questioning can be summarized using respondent consensus approaches and added to the closed data sets along with novel responses. This provides the flexibility needed to incorporate both closed and open responses, while allowing statistical comparisons of use frequency, geographical ranges, ethnic groups, subsistence vs. commercial uses, etc. The most appropriate strategies for statistical analysis will be determined by the intended use for the data.

## OUTLINE OF DATA SUMMARY

General survey information
- A. Geographical Survey
- B. Population Survey
- C. Conservation Status

Questions for individual Respondents
1. Respondent Profile
2. Current Status of Cannabis Use
3. Management Status of Cannabis Resources
4. Distribution of Cannabis Resources
5. Plant Resource Use Determination
6. Use Statistics
7. Individual Uses
    - 7.1. Material uses
        - 7.1.1. Cordage
        - 7.1.2. Paper
        - 7.1.3. Textiles
        - 7.1.4. Building materials
    - 7.2. Medicine
    - 7.3. Human Food

7.4. Animal feed
7.5. Bee plants
7.6. Fuels
7.7. Poisons
7.8. Environmental uses
7.9. Ceremonial / Social / Recreational / Ritual use
7.10. Literary and historical references
7.11. Other uses
8. Local Cannabis Terminology

## CONCLUSION

The interview schedule and questionnaire prove quite useful for gathering detailed information concerning traditional Cannabis use. It is hoped that researchers will use it and make improvements, possibly using it to investigate other economic plants. Ultimately, such an interview schedule could be used as a basic structure for gathering and integrating information into a single worldwide Cannabis use database.

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RECEIVED: 3 May 2001
ACCEPTED IN REVISED FORM: 9 November 2001

## OTHER CONTRIBUTIONS

# The History of Hemp in Norway

Jan Bojer Vindheim

ABSTRACT. Around the year 1000 we may assume that hemp was grown in several places in Norway, but at all times the importation has been greater than local production. This article discusses the history of hemp in Norway and the many ways the plant has been used, including both ritual and common purposes. 

KEYWORDS. Hemp, Norway, fibre, archaelogy

In the Norwegian valley of Gausdal, people in the nineteenth century would lift their hats in greeting as they approached a field of hemp. The plant was known to house a vette, a nature spirit best treated with respect.

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In Norwegian folklore, hemp cloth symbolised the beginning and end, and it was the first as well as the last in which people were swathed in this life (Reichborn-Kjennerud, 1922). These traditions may be relics from a time when hemp had a function in the pre-Christian religion, but the central use of hemp in Norway for the last thousand years has been as a source of fibre.

Hemp may have been grown in Norway in pre-historic times. Pollen samples suggest hemp growing in the vicinity of the Oslo fjord in the Roman Iron Age, around the beginning of the Christian era (Hafsten, 1956).

All this is, however, uncertain. The first certain proof of hemp in Norway is from the Viking age. Woven textiles of hemp were placed in graves in Southwestern Norway around the year 1000. They were probably fragments of sail. Otherwise, the usual material for Viking ship sails was wool or nettle fibre (Jørgensen, 1986).

Hemp fibre was most important to the Vikings as material for cordage. Until hemp came into use, ropes and lines were mainly produced from the bast of lime trees. The long, supple hemp fibres made it possible to produce better cordage, which in turn was an important precondition for the long sea journeys of the Vikings. Hemp was, therefore, an important item of trade as well as an important bounty from their regular armed raids.

## THE OSEBERG QUEEN

The richest archaeological material from Viking times in Norway is the Oseberg find. Two women were buried in a mound in the county of Vestfold around the year 850 in a splendid ship with ample equipment. The find includes a small piece of hempen material, the use of which has not been determined, but even more interesting is the fact that four seeds of Cannabis sativa were also found. One of these seeds was discovered in a small leather pouch.

The well respected archaeologist, Anne Stine Ingstad, who was responsible for excavating the medieval Norse settlements in Newfoundland, is prominent among many historians who believe the younger of the two buried women–usually called the Oseberg Queen–was a priestess of the great Norse goddess Freya, and not only a secular queen as the first excavators thought. Ingstad sees the presence of the Cannabis seed in the (talismanic) pouch as an indication of possible ritual use of Cannabis as an intoxicant in pre-Christian Scandinavia (Ingstad, 1992).

The find of hemp seeds in the Oseberg ship may be interpreted in various ways. Without a doubt the presence of these seeds proves that the hemp plant had reached Norway by the early Viking days, but we do not know whether the seeds were grown in Norway, or how they arrived in the country. We also do not know how the hemp, once cultivated, was utilised.

In the ninth century, there were active trade routes both eastwards through Russia and westwards along the European coasts and waterways. It is also reasonable to suppose a cultural connection to Cannabis seeds that were placed in tombs in central Europe more than a thousand years ago.

Worth noting in connection with the Oseberg find is the lack of ropes and textiles made from hemp. This is one reason for suggesting a ritual use for the Cannabis seeds. The women in the Oseberg ship had clothes made from flax, wool, silk and nettle, but not from hemp. The ropes were made from lime fibres in spite of the better quality of hemp rope.

Ingstad's suggestion that Asiatic ritual use of hemp may have reached Scandinavia corresponds well with the origin of hemp in central Asia and with etymological theories tracing the word Cannabis to finnish-ugric roots. The existence of female noaides (saami shamans) has been established by May-Lisbeth Myrhaug (1997), but ritual use of hemp seems to be unknown in saami noaide tradition.

In addition to the theory of ritual use of hemp in Viking times or earlier, it is prudent to consider another, more mundane, theory: the hemp seeds were placed in the pouch of the Oseberg Queen for their rarity. These were highly prized seeds of an exceedingly useful plant, as yet rare in the north. The hemp seeds were valuable for their promise of better cordage and more durable textiles. Such a theory does not, of course, exclude the possibility that hemp may also have had ritual uses.

## ONE THOUSAND YEARS OF HEMP

Around the year 1000 we may assume that hemp was grown in several places in Norway, but at all times the importation has been greater than local production. "Hamp" and "harp" are common elements of place names in several parts of the country and also occur in Norwegian forms of speech like "inn i hampen" ("in the hemp field," meaning "beyond credibility"). From the thirteenth century, the king had taxed hemp growers, and from the sixteenth century, we have records of state income from hemp growing in Vestfold (Holmboe, 1927).

In Norway, hemp fibre has mainly been the raw material for ropes and rough textiles. If hemp might have supplied textiles as fine as flax, it has, at least with fibres grown in Norway, required more work. Flax has usually been finer than hemp, but Italian hemp varieties could provide a quality comparable to linen textiles. As recently as in 1977, Olav Skarsvaag, a fisherman from the island of Frøya, recalled his family tradition:

> Until the end of the last century lines and nets were made from hemp. Cotton came into use around the turn of the century. I guess the cotton was not as strong as the hemp, but it was a little cheaper.
>
> The hemp mostly came from Russia and Italy, and I believe the Italian was considered finer and smoother than the Russian. Until around 1880 it was usual to spin hemp-yarn in the household. (Heien et al., 1977)

In "The King's Mirror," an instruction book from the thirteenth century, written for an unknown noble youth, the young man is advised to cover his shirt with a (woollen) cloak before approaching the king, since "no man can make himself attractive in linen or hemp" (Hellevik, 1965). It would seem that hemp was regularly used closest to the body, as underwear. However, hemp has also been used for finer textiles, and occasionally even for decorative weavings. Examples of these may be seen at the Nordenfjeldske Kunstindustrimuseum in Trondheim.

In more recent times, most Norwegian farms of any size have had their own hemp field, often in the vicinity of the dung cellar. There are reports of hemp cultivation as far north as Velfjord in the county of Nordland. In the central parts of southern Norway, hemp was used as a textile fibre, but along the coast, the hempen fibres were utilised for all manner of lines, ropes, nets, etc., in the boats and ships. It seems the more beneficent climate in the inland valleys was better suited for hemp cultivation than the stormy coastal districts, and therefore more hemp was grown inland than along the coast where the biggest demand was.

There are also a few reports that hemp has been utilised in popular medicine, against snakebite, mostly, but also for "heatedness of heart" and for eye problems (Reichborn-Kjennerud, 1922).

In a Norwegian dictionary from the late nineteenth century, we learn that "hampebraak" or "hampedengje" signifies a "noisy or slothful woman." Of talkative people it might be said that "the mouth went like a hampeklove" (Schjøtt, 1914). These words refer to well known tools from the various stages in the preparation of hemp fibres. Much has been the same as for the preparation of flax fibres, but the hemp tools were rougher than the corresponding tools for flax (Hoffman, 1991).

Traditionally, the male plants have been harvested first, the females a little later. The plants were pulled up with the roots and dried in bundles. The seeds could, then, be shaken off before the plants were soaked in water for retting. When the soft parts of the plant have been dissolved, the stalks are dried once more and pulled bit by bit through the hampebraak. This has a "mouth" that breaks the stalks, and makes it possible to remove the fibres from the wooden parts. This is heavy work that was usually done by the menfolk.

The next step is pulling the fibres through the hackle, a kind of comb that separates the fibres. This was considered lighter work and was done by the women. The hackled fibres could be spun and then woven, or used for making cordage.

Those who bought imported fibres in "dolls," bundles of fibres weighing 6-9 kg, avoided much of this preparatory work. The hempdolls were used for rope making and other purposes on the farms in wintertime, as reported by Mr. Skarsvaag.

## THE KING CALLS FOR HEMP

In the northernmost parts of the country, it is unlikely that hemp has been cultivated, but hempen fibres have been items of trade, as is evident from a letter sent in 1591 by King Christian IV of Denmark-Norway to the Swedish King Sigismund. Christian complains that Swedish officials are selling nails, hemp, liquor and butter to the saami fisher people, and thereby replacing the Danish and Norwegian traders (Holmboe, 1927). This hemp was probably in the form of dolls which the Swedes brought up from the Baltic sea.

On the Danish island of Fyn, tradition holds that the art of growing hemp was brought back by sailors from the Baltic lands (Brøndegaard, 1979). In the Danish parts of the realm, the conditions for growing hemp were much better than in Norway, and King Christian IV, in 1629, demanded of the Danish farmers that they grow hemp to supply his navy. He supplied seeds through his sheriffs. His successor, Christian V, even included the duty of growing hemp in his Danish Law of 1683, which states:

> Every farmer who holds a full farm, and does not sow a bushel of hemp seed, and he, who holds half a farm, half a bushel, should by his lord be charged and punished as an obstinate and reluctant servant, unless he proves that he has no suitable soil therefore.

In the corresponding Norwegian law, the passage on hemp cultivation was not included, but we must suppose that the king's officials tried to stimulate hemp-production in those parts of Norway where this was possible. The growing import of hemp fibres must have been cause for worry. To the port of Bergen alone, the amount imported during the period 1650-1654 was 3064 ship pounds of hemp (approximately 500 tons), mainly from the Baltic countries. The regularity of the Baltic trade in hemp and linen is also testified in two lines from a well known poem written by the priest and poet Petter Dass in 1702:

> To Revel and Riga your Voyage did go
> Your Hemp and your Flax to acquire. (Holmboe, 1927)

It was quite common for hemp and flax to be mentioned in the same context, and it is not easy to distinguish between materials made from these two types of fibre.

The Dane Gerhard Schöning travelled on behalf of the Danish king through parts of Norway in 1773-75. His report to the king is an important source of information on this era. Describing the state of industry and agriculture in the towns and in the countryside, he complains that too little hemp is grown in most villages, although some hemp is grown even in mountain villages like Oppdal. All the more pleased did he become when approaching the village of Sparbu:

> As soon as I entered Sparboen, I immediately encountered a large field of hemp. There was little or nothing to see of hemp in the previous villages in Indherredet, in Sparboen however a great deal thereof. (Schöning, 1979)

Some places, like in "Surendalen" much hemp was grown. "The crops of hemp and flax are many places buxom" B.H. Loevenskiold reports from Bratsberg, near Trondheim, in 1784. But local production has evidently not been sufficient to cover demand, since farmers from Jämtland, across the Swedish border, every year made the trip into Norwegian markets with hemp as well as flax (Holmboe, 1927).

The authorities constantly sought to stimulate further production of hemp. In 1775 the Royal College of Sciences in Trondheim offered a prize for the best treatise on hemp cultivation, and the winner was a parish priest, Claus Finde, from the west coast district of Sogn. His winning essay was never published, but the manuscript is kept at the library of the Royal College of Sciences in Trondheim. Father Finde describes the various kinds of soil that are suitable for hemp and flax, the procedures for sowing and reaping, including the method of separating the male plants (Giaedder) from the females (Finde, 1775).

The newspapers also did their bit for the cause of hemp. Adresseavisen in Trondheim, in 1802 published an article on the cultivation of hemp, including the following passages:

> A sensible farmer cultivates for himself as much hemp as is needed for the running of his farm.
>
> At the beginning of May, after the conditions of the year, the hemp should be sown, when the soil is dry, harrowed down with a wooden harrow and rolled. Thereafter small creatures such as chickens should be kept well away from it.
>
> Once the hemp is sown and treated as shown, it will grow so that it in the month of August has progressed far enough that some of it is ripe, and can be pulled.
>
> The Male hemp ripens first and it is this that first must be pulled, or rather separated from the female. One starts therewith from one edge of the field, takes out or pulls up the ripe hemp in one’s front, in this way one obtains space to enter the hemp without damaging the plants that remain standing. The female hemp, the one that bears the seed, should yet remain standing 3 to 4 weeks to ripen. (Adresseavisen, 1802)

During the Napeolonic wars, the ports of Denmark and southern Norway were blockaded by the English navy. No cargo could enter the country from the Baltic. Merchants from Trondheim and Bergen, therefore, sent ships northwards to the Russian port of Arkhangelsk to fetch cereals, hemp and other goods, but this was not sufficient for the needs of the country. When Norwegian privateers, in the year 1809, captured a British ship loaded with hemp, and brought it into the port of Trondheim, the government wanted to secure this valuable cargo for state needs. The privateer captains refused to bow to this demand, since "the country is in the greatest need of hemp for the fisheries and merchant navy purposes." They managed to sell 1500 voger of hemp, (around 27 tons) before the government could secure the remaining cargo (Mykland, 1997).

Against this background, it is only natural that the Royal Society for the Welfare of Norway, which was founded in 1809, agitated strongly for increased cultivation of hemp in Norway. Sheriff Sivert Aaarflot from Volda, in 1805, published a small book in Copenhagen on "The Cultivation and Preparation of Hemp." He also disseminated information about hemp cultivation through his own magazine "Norsk Landboeblad" (Hoffman, 1991).

## ROPEWALKS

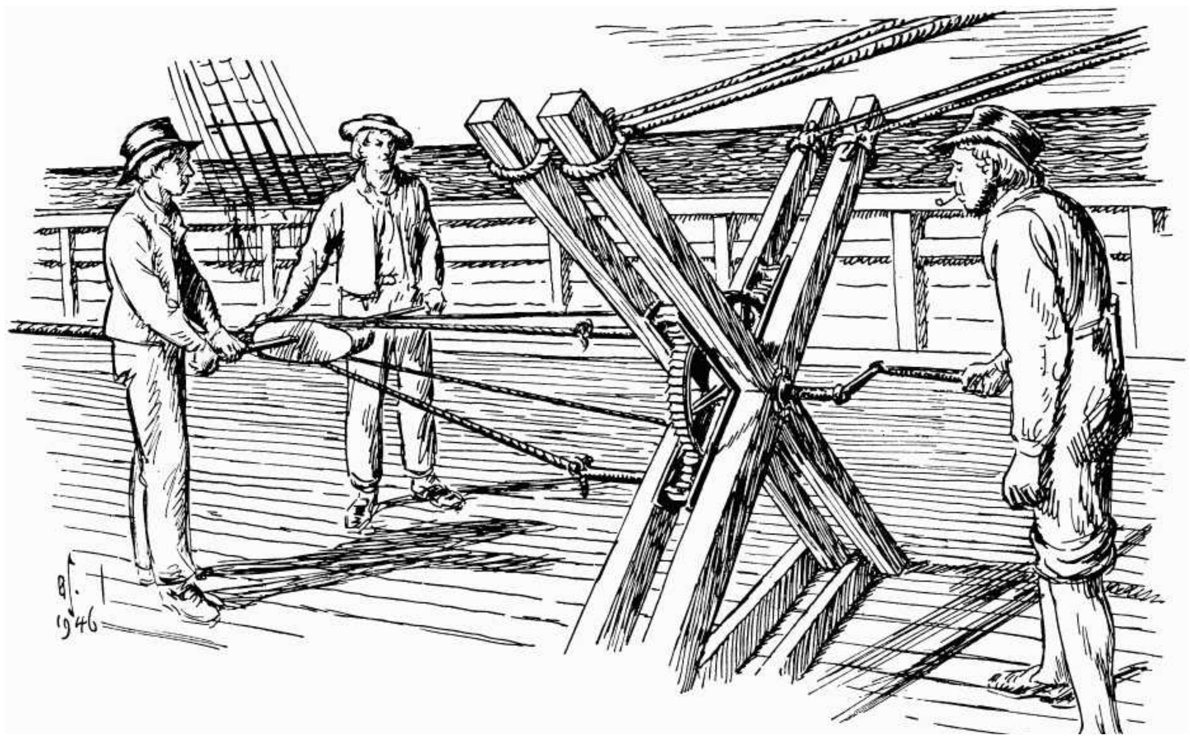
The main use of hemp in recent centuries was for cordage to supply the Norwegian merchant navy as well as the fishing fleet (Figure 1). The rope walk is one of the oldest forms of industry, and rope walks were established at several locations along the Norwegian coast, becoming characteristic features of many Norwegian towns. In many localities, roads and squares still have names recalling the presence of ropewalks in former days. Making ropes was a necessary practice wherever ships and boats were built or repaired.

The Danish-Norwegian kings took steps to organize and tax the rope-making industry from the seventeenth century. In 1607, a "new rope walk" was established in Bergen, at that time the main Norwegian harbour, implying that one or more such institutions had been in existence previously. In Trondheim, two local merchants were given the royal privilege of producing rope in the year 1637 (Vindheim, 2000).

The production of rope was a handicraft requiring a building or an open space the same length as the rope to be made, since it was only in the twentieth century that machinery was developed allowing cordage to be coiled during production. If the rope walk had no roof, ship's cordage could only be made in dry weather. It was illegal to sell cordage made from wet hemp as naval equipment (Stavseth, 1948).

To make the hempen ropes strong and supple they had to be impregnated with tar. The tar was heated in large receptacles through which the rope could be pulled or dipped. A rope walk was, therefore, a fire hazard, and was for this reason often placed outside the town centre.

FIGURE 1. Rope making on the deck of a sailing ship



The necessary amount of hemp fibre was huge. One single sailing ship needed several miles of various types of cordage. The pinnacle of Norwegian naval rope making was the anchor cable for the frigate Kong Sverre, made from 4 tons of Russian hemp. It was carried through the streets of Tønsberg, in the year 1864, by 120 sailors led by a marching band (Figure 2) (Stavseth, 1948).

In the years 1870-1874, Norway imported more than 18,000 tons of hemp and 1,200 tons of cordage. In times of war and crisis, trade was often interrupted, and the rope walks therefore needed large stocks of hemp fibre. One large ropewalk, Tønsberg Reperbane, had storage capacity for 2,000 tons of hemp. During World War I, Norwegian merchants were forced to reopen the old Northern sea route to Arkhangelsk, and in 1918, a cargo of 900 tons of hemp arrived in the Northeastern town of Vardø, where the local authorities did not have enough storeroom. Parts of the cargo, therefore, had to be stored at Hammerfest, further west (Stavseth, 1948).

After World War I, the demand for hempen cordage dwindled swiftly. The amount imported in the period 1920-24 was only half of the amount a decade earlier. However, in the late 1930s an increase was caused by the upsurge in Antarctic whaling. The whalers were fussy about their equipment, and the harpooners were especially careful about the "forerunner," a slender rope connected directly to the harpoon (Figure 3). Only hand spun Italian hemp had the desired quality (Stavseth, 1948).

In the period 1930-35, the importation reached a new high level of almost 20,000 tons of hemp (Figure 4), while the export of cordage reached more than 3,000 tons.

## A NEW ERA OF HEMP CULTIVATION?

With the demise of the large fleets of sailing ships, demand for cordage sank, and what production remained was shifted to fibres other than hemp. In spite of a small increase during the Second World War, by the end of the 1950s, there was no registered cultivation of hemp in Norway. With new drug legislation introduced in 1964, hemp cultivation even became illegal, although seeds were not banned until the year 1999.

During the 1990s, hemp cultivation was reintroduced in several northern European countries, but a side-effect of the hard-line drug policies in Norway and Sweden was that all applications to grow hemp,

FIGURE 2. The anchor cable for “Kong Sverre” was carried by 200 sailors led by a marching band

FIGURE 3. The harpooner on a whaling ship had to depend on the quality of the forerunner rope

from scientific institutions as well as individual farmers, were turned down.

In the summer of 2001, however, a public controversy arose when a small number of farmers in the county of Vestfold turned over 3000 illegally cultivated low-THC hemp plants to the police, and demanded to be prosecuted. The Minister of Agriculture immediately promised to look into the restrictions on industrial hemp, and a few weeks later his Ministry delivered a report on the problems of separating intoxicating from industrial hemp strains. The ministry did not foresee any large problems in this regard, and recommended a system of licensed growing. However, at this writing (October 2001), it is uncertain whether or not these recommendations will be put into practice by the new centre-right government.

FIGURE 4. Unloading hemp fibre in Tønsberg

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# Hemp as Food at High Latitudes

J. C. Callaway

ABSTRACT. Hempseed offers a unique nutritional package, in terms of dietary oil, protein, vitamins and minerals, which can be produced at high latitudes ( > 50° latitude). Hempseed oil is highly unsaturated and contains both essential fatty acids (linoleic acid and alpha-linolenic acid) in a nutritionally balanced ratio, in addition to considerable amounts of biochemically important gamma-linolenic acid (GLA) and stearidonic acid (SDA). The protein in hempseed is complete, in that it contains all of the essential amino acids in nutritionally significant amounts, and lacks the nutritional inhibiting factors found in soya. Hempseed could become a viable replacement for imported soya in Northern Europe, particularly as feed stock for animals. 

KEYWORDS. Cannabis, hemp, essential fatty acids, linoleic acid, alpha-linolenic acid, GLA, SDA, vegetable protein, edestin, albumin

## INTRODUCTION

Most varieties of Cannabis are adapted to temperate (typically less than 50° latitude) or equatorial climes. At these latitudes, the length of

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Journal of Industrial Hemp, Vol. 7(1) 2002
http://www.haworthpressinc.com/store/product.asp?sku=J237

daylight during the growing season is significantly shorter than that of boreal regions ( > 50° latitude). As day length usually determines the time of flowering for Cannabis, and because seed production follows flowering, the ideal variety for high latitudes would be one that was capable of flowering during long photoperiods. Hemp varieties that are bred in Central Europe, for example, seldom flower or produce significant amounts of seed at latitudes greater than 60° from the equator. In contrast, an early blooming variety would not only survive the short sub-arctic summer, but actually take advantage of the long days (20+ hours in June and July) to produce abundant amounts of seed. Furthermore, seeds produced at high latitude show a higher degree of unsaturation in the oil profile, particularly higher amounts of gamma-linolenic acid (GLA) and stearidonic acid (SDA), which are biologically important secondary metabolites of the two essential fatty acids.

For fiber production, the effect of light sensitivity for some hemp varieties can also be exploited to geographical advantage at high latitudes. A hemp variety from Central Europe, for example, grown under sub-arctic conditions would be tall and asexual; i.e., a higher yield of biomass and a more consistent fiber quality (Callaway and Hemmilä, 1996). Before the emergence of "cannabiphobia" in North America during the 1930s, a concerted effort was made in the United States to take advantage of this phenomenon; that is, seed stock was produced in Kentucky (at lower latitude) to be grown for fiber in Minnesota (at higher latitude) (Roulac, 1997). A similar strategy can be applied to optimize hempseed production, however the rule of latitude is reversed; i.e., varieties from the North are used to produce maximal seed yields at southern latitudes by exploiting the early-blooming characteristic.

It is not entirely obvious that a strategy for the production of seed is almost diametric to that of fiber. This realization can only come from direct experience with the production of seed, because historically the cultivation of hemp has been primarily for the production of fiber. For fiber production, flowering is not desirable, provided that a reliable source of planting seed can be secured. For the production of seed, of course, flowering is paramount, and a northern-acclimated seed stock is ideal for increasing yields at lower latitudes by hastening maturity.

Unfortunately, logic does not always prevail in modern agriculture, and particularly so for regulations concerning the cultivation of hemp. In Europe, for example, a hemp crop that does not produce seed is not eligible for the fiber subsidy, according to EU regulations. And unlike flax, there are presently no approved varieties of hemp that are subsidized for the production of seed in Europe. It is difficult, then, to comprehend why subsidies for the production of hemp fiber must depend on the development of mature seed, especially when there are no subsidies for the production of hempseed, or even varieties that are certified for grain production. It is actually surprising that hemp does not enjoy a dual use subsidy like flax, as both a fiber and food crop, especially in light of hempseed's superior position to flax in terms of yield, production ecology, taste and nutrition.

## A NUTRITIOUS FOOD FROM HEMPSEED

A certain amount of enthusiasm has recently developed in Western cultures around food products derived from hempseed. The fresh, raw seed has a pleasant nutty flavor that can also be easily modified to taste. Asiatic cultures have used Cannabis seed as a food and medicine for thousands of years. Toasted seeds are still sold in the markets of China, although most seed is exported (untoasted) as birdseed. In the Eastern European countries, Cannabis seed oil has been used as a butter substitute, typically by those who could not afford dairy products. As a consequence, hempseed "butters" have developed as delicacies in those regions.

## A "FOOD" VARIETY OF HEMP

Finola (previously known by its breeder code "FIN-314") is an early-blooming variety of hemp (i.e., non-drug Cannabis) that was developed in Finland (Callaway and Laakkonen, 1996; Laakkonen and Callaway, 1998). It is the only variety of Cannabis that is registered as an early-blooming variety within the UPOV/OECD. Finola is currently under commercial cultivation in Finland, France, England, Canada, and Australia. This variety of hemp has produced over 2000 kg of seed per hectare in Canada (Przytyk, 1999). At latitudes greater than 60° N, a recent agricultural trial at a government station in Finland measured the average yield to be 1700 kg/ha. The parents of Finola were selected from the Vavilov collection in St. Petersburg in cooperation with the International Hemp Association.

Although no early-blooming varieties currently appear on the EU list of approved cultivars, Finola would satisfy a curious regulation that requires the production of mature seed in order to receive the fiber subsidy for hemp. EU-approved hemp cultivars, for example, often fail to produce any seed at latitudes greater that 50° N. Finola is frost tolerant at all stages of growth, does not require herbicides or pesticides at high latitudes, and matures in less than 115 days. Under bio-dynamic cultivation, a successful crop can follow clover from the previous year, without additional nitrogen in the Spring. The content of THC for this variety has typically varied between 0.04-0.16% in dry, mature female flowers, according to analytical tests conducted by forensic laboratories in Finland, England and Canada, which is well below the arbitrary EU and Canadian cutoff level of 0.3% THC. Moreover, the CBD/THC ratio is greater that 10:1, which is well above the more recent EU requirement of 2:1. Such a cannabinoid profile betters that of any other currently approved EU variety of hemp, and essentially means that Finola is useless as a recreational drug.

Finola is also short in stature (less than 2 meters at latitude 62° N) and readily harvested by conventional methods. It is substantially shorter when grown at lower latitudes, and cannot compete with other varieties of hemp in terms of overall biomass production, but can compete with flax in terms of yield and quality of fiber. While the bast fiber content is less than 15% for Finola, this fiber is almost entirely primary fiber and thus softer than that of fiber hemp varieties. Rather than the production of fiber or hurd, however, the entire stalk from Finola may be more useful when left in the field, under bio-dynamic cultivation, to decompose and increase soil humus levels.

Cannabis is an open pollinated genus, which does not require pollinating insects to help in the production of viable seed, although apiarists should consider the benefits of placing bee hives in close proximity to fields of Finola, due to the abundant amounts of pollen produced by this crop. At high latitudes, Finola produces pollen somewhat later than most plants. This additional pollen allow bees to work well into late summer, thus increasing their yields of honey.

## CHARACTERISTICS OF FINOLA

Dioecious; typically at a 1:1 ratio of male:female
Early blooming; crop maturity in less than 110 days

Short in stature; ca. 1.5 meters tall at latitudes above 60° N
Little or no branching, even as isolated individuals
Frost tolerant to −5°C at all stages of growth
Drought resistant, high seed yields
Seed is high in oil (ca. 35%) and protein (ca. 30%)
Seed oil is high in polyunsaturated fatty acids (ca. 80%)
Seed protein contains all essential amino acids
Bast fiber is high in primary (i.e., long) bast fiber
Low in THC, with high ( > 10) CBD/THC ratio

## HEMPSEED OIL AND OTHER FOOD OILS

Consumer awareness of nutrition, especially concerning dietary fats and oils, is extremely low. This is due in part to the fact that the importance of certain oils in the diet was not fully recognized until about 60 years ago, and partly because deficiencies in either of the two "essential fatty acids" are not as readily apparent as with other dietary deficiencies (Horrobin, 1997). In addition, the food oil industry has successfully marketed vegetable oil products that have long shelf lives, such as margarines and other non-dairy spreads, over potentially more healthy products obtained from fresh cold-pressed oils. Unfortunately, most consumers are reluctant to give up the habit of spreading a thick fatty substance onto their crackers and breads.

From a physical perspective, fats and oils have clearly defined differences. Simply stated, a fat is a solid at room temperature while an oil is a liquid. Aside from very few exceptions (e.g., palm and coconut fats), most vegetable "fats" are, in fact, oils. Although there are very small amounts of "free" fatty acids in vegetable oils, it is of some importance for taste and freshness to keep this value as low as possible. More typically, native fatty "acids" in vegetable oils exist in the form of triglyceride esters, where the three fatty acid molecules are attached to one molecule of glycerol. However, for consistency and simplicity's sake, the term "fatty acid" will be used here in discussing the oils found in hempseed.

Hempseed oil contains very high amounts of the two fatty acids that are essential to human health (Deferne and Pate, 1996); linoleic acid (LA, 18:2n-6)) and alpha-linolenic acid (LNA, sometimes "ALA," 18:3n-3), in addition to oleic acid (OA, 18:n-9), gamma-linolenic acid (GLA, 18:4n-6) and stearidonic acid (SDA, 18:4n-3). In the numerical nomenclature for each of these fatty acids, the number 18 refers to the number of carbons in each individual molecule. It is extremely rare in vegetable oils to have significant amounts of individual fatty acids with more than 18 carbons. The number appearing after the colon indicates the number of unsaturated points on the molecule, and the number following the "n" is the position of the first point of unsaturation on the molecule, counting from the end, or "omega" portion of the molecule. These five molecular entities, all of which are unsaturated to some degree, typically account for almost 90% of hempseed's oil (Callaway et al., 1997). Hempseed oil, in general, only has about 10% of its composition as saturated fatty acids. When fresh, this oil is green because of the chlorophyll that is naturally found within the mature seed, which is technically an achene, or nut. LA and LNA are required by the body to form membranes for nerve cells, in addition to serving as biochemical substrates for short-lived chemical messengers, such as prostaglandins, leukotrienes and eicosanoids (Horrobin, 1994). Because they cannot be manufactured by the body, LA and LNA are considered essential to human nutrition, and must be consumed on a daily basis for optimal health. Current estimates of adequate intake for adults is approximately 4.5-6.7 grams/day of LA and 2.2-3.3 grams/day for LNA (Simopoulus et al., 1999).

LA, LNA, GLA, and SDA are all polyunsaturated fatty acids that typically account for approximately 80% of hempseed oil. They are great for health, but have rather short shelf lives, due to oxidation. In order to lengthen shelf life and avoid rancidity, unsaturated vegetable oils are often bleached, "deodorized," and "partially hydrogenated" by industrial processes, meaning that these oils are sometimes chemically transformed from unsaturated to more saturated oils, with many novel fatty acids formed as unavoidable side-products. These side-products include geometric and positional changes in the molecular structures to form chemicals that are not found in nature. Positional changes mean that sites of unsaturation, or "double bonds," have been shifted from one place to another within the molecule. Geometric changes mean that the orientation of the double bond has changed from cis (pronounced "sis") to trans. It is surprising how little work has been done by medical researchers to identify these novel products, much less evaluate their potential health risk. Trans-fatty acids in the diet have been estimated to cause 25,000-30,000 premature deaths a year in North America through coronary artery disease (Koletzko and Decsi, 1997). Trans-fatty acids typically enter the diet through deep-fried foods, non-diary margarines, butter, cheeses, sausages and chocolate-nougat spreads (Callaway, 1998).

As a further example of consumer ignorance being manipulated by mass marketing campaigns, "CLAs" (i.e., conjugated linolenic acids) are being hyped on the packages of some food products, in an attempt to capitalize on the recent fame of GLA. Some claims of anti-cancer properties for some CLAs have surfaced, along with evidence of their potential toxicity. They are mostly found in dairy products, and should not be confused with the demonstrated benefits of GLA from certain vegetable oils.

Few other food oils even approach the exceptional fatty acid profile found in hempseed oil. The following table compares information on the typical unsaturated fatty acid profiles from Finola seed, common hempseed, rapeseed ("Canola"), soya and flax seed oils. Many other food oils are available and could be listed, but these are the most common food oils that have the highest amounts of both LA and LNA:

| | OA | LA* | LNA* | GLA** | SDA** | LA/LNA ratio |
|---|---|---|---|---|---|---|
| Finola seed | 9% | 54% | 22% | 4% | 2% | 2.5 |
| Hempseed | 10 | 55 | 20 | 2 | 1 | 2.8 |
| Rapeseed | 60 | 22 | 12 | 0 | 0 | 1.8 |
| Soy bean | 23 | 51 | 7 | 0 | 0 | 7.3 |
| Flaxseed | 15 | 15 | 61 | 0 | 0 | 0.3 |

*essential to human health

**biologically important metabolites of LA and LNA

Most informed consumers now recognize terms such as "triglycerides," "unsaturation" and "omega" in the context of food oils. To understand the meaning of these words, it is important to imagine the fatty acid molecule as having a "head," where it is individually attached to glycerol, along with two other fatty acid molecules to form a triglyceride. The other end of the fatty acid molecule is the "tail," or end, or "omega" portion of the molecule. Thus, omega-3 means that the first point of unsaturation on a particular fatty acid is three carbons from the end of the molecule's tail, and omega-6 means that the first site of unsaturation is six carbon atoms from the end.

For nutritional purposes, it is important to know that there must be a balance in the daily intake of omega-3 and omega-6 fatty acids, and that the only fatty acids that are essential for human health are linoleic acid (LN), an omega-6 fatty acid, and alpha-linolenic acid (LNA), an omega-3 fatty acid. Recent advertizing by the fish oil industry suggests that increasing omega-3 fatty acids in the typical Western diet, usually in the form of gelatin capsules, can offset some of the putative health problems caused by too much LA, which is found in many common vegetable oils that are often lacking in sufficient amounts of LNA (e.g., olive, soy, corn, sunflower and safflower seed oil). This suggestion rides on the tacit assumption that cold-water ocean fish liver oil products have been carefully processed, packaged and stored in a way to avoid oxidation, the bane of any highly unsaturated food oil. Otherwise, the trusting consumer might as well be eating expensive capsules of varnish! This is particularly true for fish liver oils that contain high amounts of eicosapentanoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are long-chain, super-unsaturated omega-3 fatty acids that react even faster with oxygen than the shorter chain omega-3s (i.e., LNA and SDA) found in some vegetable oils.

The optimal ratio of LA to LNA is presently considered to be near 2:1, according to modern nutritional studies (Simopoulus et al., 1999), and an estimated ratio for the pre-agricultural human diet has been suggested to be near 1:1 (Crawford et al., 2001 and Simopoulus, 2001). To date, there are no medical studies suggesting that the overall daily intake should be less than 1:1. For many years, however, when nutritional studies were made with soy bean oil as the standard, a ratio of about 7:1 was considered to be optimal (see the table for soy and flaxseed oils vs. hemp). It is truly amazing that the nutritional community has independently arrived at a hypothetical ratio of 2:1, which is so similar to that of hempseed oil, without even considering it as a source of the two essential fatty acids. Clearly, this oversight has been due to the lack of nutritional research on hempseed over the last 65 years.

## LA:LNA RATIOS AND DELTA-6-DESATURASE ACTIVITY

Most healthy humans do not need a dietary supplement of GLA or SDA, because we already have an enzyme (delta-6-desaturase) that produces both GLA and SDA from LA and LNA, respectively. This enzymatic action, however, is either lacking or absent in infants, the elderly, and in some disease states, such as alcoholism. The enzyme kinetics of delta-6-desaturase also favor a lower amount of LA, relative to LNA, because the production of SDA from LNA by this enzyme is relatively slow, while the production of GLA from LA by the same enzyme is relatively fast. Although both GLA and SDA are important in the body as substrates for other useful products, such as prostaglandins (Series 1 from GLA, and Series 2 and 3 from SDA), leukotrienes and eicosanoids, the over production of SDA from excess LNA can alter prostaglandin levels unfavorably over time. Also, excessive amounts of LNA, as in flaxseed oil, can effectively compete with LA for access to delta-6-desaturase, and subsequently decrease the production of GLA (Schwab et al., 2001).

## HEMPSEED PROTEIN

A tasty and nutritional "milk" can be easily produced from hempseed by grinding with water. Other non-dairy products, such as baked goods and frozen desserts, are also readily made from whole hempseed. Such products could easily replace those made from soya, which must be imported by Nordic countries. The protein in hempseed is considered "complete" in the sense that all of the essential amino acids are present in nutritionally significant amounts (Wirtschafter, 1997). This protein is approximately one third albumin, an important protein that is also found in egg whites and human blood, and two thirds edestin, another important globular protein of similar character. Unlike soya, which contains trypsin inhibiting factors, hempseed proteins are easy for the body to digest. Vitamins and minerals of biological importance are also found in hemp seed. Combined with its potential for medicine and fiber, it is no wonder that humans have cultivated Cannabis since prehistoric times.

## CONSIDERATIONS FOR FOODS DERIVED FROM HEMPSEED

Frying with any unsaturated oil is not a good idea, and this is especially true for hempseed oil. Frying with butter, or some other highly saturated fat would actually be much healthier, if used sparingly and balanced by exercise. This runs contrary to what we are often told about fats and oils, and serves as an indication of how confused the entire issue has become. When unsaturated fats are heated above 160°C, as in deep frying, the molecules twist from their natural cis positions to trans conformations. The problem with excessive amounts of trans forms of both LA and LNA (e.g., foods deep fried in rapeseed oil) is that they inhibit the enzyme delta-6-desaturase, thus inhibiting the body's production of GLA, SDA and a subsequent cascade of metabolically important components. The health effects from this practice are insidious, and take a long time to manifest as age-onset diabetes, psoriasis, coronary heart disease or even mental disorders. Aside from the production of trans-fatty acids, the heating of polyunsaturated oils rapidly increases the amount of potentially carcinogenic peroxides present.

The best way to obtain high quality food oils would be to support the local economy by purchasing freshly cold-pressed hemp or rapeseed oils, if they are available. For optimal benefits, these oils should be pressed under an inert atmosphere (such as nitrogen) from fresh, viable seeds, and the oil must be stored in a cool, dark place. If the oil were pressed and used within the same day, one might opt for flaxseed oil as an occasional nutritional supplement, but this oil alone is not suitable as a staple food because of its disproportionately high amount of LNA. Moreover, the delicate taste of flax seed oil is often lost after only a few days. Taste is also a problem with fish liver oils, no matter how fresh it is, although such oils are also a valuable source of biologically important omega-3 fatty acids.

So far, the number of scientific publications on Cannabis seed's nutritional potential is relatively small, and these results have yet to appear in widely circulated scientific journals. This may be due to the political bias against Cannabis over the last 65 years (Kassirer, 1997; Lancet, 1995; Zimmer and Morgan, 1997), which has inhibited scientific inquiry into this seed's dietary potential until only recently. In short, news on the food potential of hemp seed has not yet reached most clinical nutritionists, much less the global market, and it may be a few more years before in-depth studies on hemp seed's nutritional benefits are published and recognized. Until that time, informed consumers in Europe and North America, who are concerned about the quality of their diet and health, have already created a significant demand for hempseed oil.

Another real potential health hazard with highly unsaturated vegetable oils, such as the oils from flax and hempseed, is in their vulnerability to light and oxygen. The sites of unsaturation on the oil molecules are vulnerable to attack by oxygen, and this process is hastened by light. Peroxides are the product, and these can become toxic when taken over long periods of time. Industrial oil-based coatings, such as paints and varnishes, take full advantage of this property because these peroxides initiate a polymerization process that "dries" to a protective finish. In fact, rather than a source of nutrition, most Cannabis seed oil was used as a paint base until the late 1930s. Flax seed oil and synthetics are primarily used for this purpose today.

The psychoactive cannabinoid "THC" is not found within the seed. It is, however, found in high concentrations on the bract that surrounds the seed, especially with drug varieties of Cannabis. This bract must not be confused with the seed's shell, or "hull." Thus, it is possible to have small amounts of THC in food products that have been prepared from non-drug varieties of Cannabis seed, such as hempseed oil (Callaway et al., 1997), but such low amounts are not a threat to public health, and more recent research has shown that the hemp industry can respond to political pressures imposed by urine testing for cannabinoids (Leson et al., 2001).

## CONCLUSIONS

It is likely that important components of fresh and organically grown produce have yet to be fully recognized by medical science. For this reason, the overall impact of nutritional deficiencies on human health remains an open, and poorly understood, question. Judging by what is already known about diet and health, hempseed can be considered as one of the most complete single sources of vegetable nutrition. Hempseed has a complete protein and a balanced oil that even tastes good, both of which are advantages over soy and flaxseed products.

Extreme care is required to produce high quality foods from hempseed, and producers of these products must ultimately accept this responsibility. Hempseed oils should be pressed under nitrogen, with added antioxidants and stored in a dark, cold place until use. Two important points must be kept in mind, which apply to all vegetable oils: (1) polyunsaturated oils are easily oxidized over time by light and air to form toxic peroxides, (2) frying with any polyunsaturated oil will permanently twist a portion of the molecular configurations from (healthy) cis- to (unhealthy) trans-fatty acids. As too little is presently known about the chronic toxicity of these by-products, caution is suggested and their ingestion should be minimized, if not avoided entirely.

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# Natural Fibres in the European Automotive Industry

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ABSTRACT. In the eighties, studies carried out in Germany and the EU forecasted very large market potentials for composites from flax and other natural fibres. Although considerable research and development was carried out,[1] the development of these markets proved far more difficult and long-term than previously expected. The ambitious German flax program, backed by substantial funding, did not survive these hard times. Only in recent years, did an actual industrial demand for natural fibres develop. Nowadays, the use of natural fibres in certain applications has already become a matter of course, something which no one had dared to expect only five years ago. The most important customer is the automotive industry. 

KEYWORDS. Fibres, hemp, automotive industry, markets, composites, technology

## GERMANY AND AUSTRIA

Table 1 shows the results of different surveys by nova Institute[2] from the years 1996, 1999 and 2000 (1, 2, 3). Almost all relevant suppliers to

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Journal of Industrial Hemp, Vol. 7(1) 2002
http://www.haworthpressinc.com/store/product.asp?sku=J237
 

TABLE 1. Use of natural fibres in the German and Austrian automotive industry in tonnes/year (composites, excluding seat upholstery) (1, 2, 3)

| Fibre | 1996 | 1999 | 2000 | Tendency 1999 -> 2000 |
|---|---|---|---|---|
| Flax | 2,000 | 11,000 | 12,700 | +15 % |
| Hemp | 0 | 1,100 | 2,100 | +90 % |
| Jute | 1,000 | 700 | 240 | −65 % |
| Sisal | 1,000 | 500 | 100 | −80 % |
| Kenaf | 0 | 1,100 | 1,400 | +25 % |
| Coconut fibre | 0 | 0 | 600 | - |
| Total | 4,000 | 14,400 | 17,140 | +19 % |

the automotive industry in both Germany and Austria have been included. All three surveys were carried out in writing and verbally. Most important were the suppliers to the automotive industry who contributed their data almost without exception. By means of additional surveys of fibre producers, traders and finally the automotive manufacturers, the validity of the data could be verified.

The table clearly illustrates the increased demand over the past four years and especially the above-average rates of increase for hemp and kenaf, whereas the use of jute and sisal decreases. The use of natural fibres in the German (and Austrian) automotive industry increased by 19% from 1999 to 2000; the use of hemp even increased by 90%, which made native hemp fibre the second most important natural fibre in the automotive industry.

The demand in Germany alone is expected to increase to about 20,000 tonnes/year in the near future, medium-term forecasts expect 25,000-45,000 tonnes/year. The introduction of every new car model increases the demand–depending on the model–by 500 to 3,000 tonnes/year. Due to its sizeable sales and innovative power, the German automotive industry represents–with a 2/3 share of the total natural fibre use–by far the most important customer in Europe.[3]

## EUROPE

In Europe the use of natural fibres in the automotive industry in 1999 was about 21,300 tonnes and in 2000 about 28,300 tonnes (Table 2).

TABLE 2. Use of natural fibres in the European Union 1996–2010 in tonnes/year (composites, excluding seat upholstery) (2,3,4)

| Fibre | 1996 | 1999 | 2000 | 2005 (forecast) | 2010 (forecast) |
|---|---|---|---|---|---|
| Flax | 2,100 | 15,900 | 20,000 | - | - |
| Hemp | 0 | 1,700 | 3,500 | - | - |
| Jute | 1,100 | 2,100 | 1,700 | - | - |
| Sisal | 1,100 | 500 | 100 | - | - |
| Kenaf | 0 | 1,100 | 2,000 | - | - |
| Coconut fibre | 0 | 0 | 1,000 | - | - |
| Total | 4,300 | 21,300 | 28,300 | 50,000-70,000 | >100,000 |

Due to the fact that the automotive industries in France, Italy, Spain and Sweden discovered the potentials of natural fibres with a delay of about 2 years compared to Germany, in these countries especially high rates of increase are expected in the medium term. Accordingly, in 2005 the use of natural fibres could increase to 50,000 to 70,000 tonnes and in 2010 to more than 100,000 tonnes, provided that the EU end-of-life vehicle directive does not unfavourably influence this development (see below). At a price of about 0.50-0.60 Euro/kg fibre, this translates into an annual market of more than 50-60 million Euro (2).

## NORTH AMERICA

According to an up-to-date study by Kline & Company, the North American market for composites from natural fibres will increase from US$ 155 million in 2000 to US$ 1,380 million in 2005 (5).

## $CO_2$-SAVING POTENTIALS OF NATURAL FIBRE COMPOSITES

According to a study (1) that was conducted by IFEU and nova Institute in 1996, each kg hemp fibre that substitutes glass fibres in composites, saves 1.4 kg $CO_2$ during its whole life cycle (from cultivation to recycling/disposal). On the assumption that (1) similar savings will arise from use of other natural fibres and (2) natural fibres will primarily substitute glass fibres (which is, with the present state of the art, not yet the case, see below), then 140,000 tonnes of $CO_2$ per year would accordingly be saved by the use of 100,000 tonnes/year natural fibres in the EU (Table 2).

## WHY DOES THE AUTOMOTIVE INDUSTRY EMPLOY NATURAL FIBRES?

There are numerous weighty arguments in favour of the use of natural fibres composites instead of wood fibres, textile waste fibres, glass fibres composites or for example ABS parts. While in the eighties and at the beginning of the nineties mainly ecological reasons were much of a concern, nowadays the favourable mechanical properties and the production costs play a major role. The following overview lists the main advantages of natural fibre composites:

Natural fibre composites have:

- Low density: weight reduction of 10 to 30%.
- Favourable mechanical and acoustic properties.
- Favourable processing properties; e.g., low wear on tools.
- Potential for one-step manufacturing, even of complex construction elements.
- Favourable accident performance (high stability, no splintering).
- Favourable eco-balance for part production and due to weight savings during vehicle operation (several studies on this subject have been conducted).
- Occupational health benefits compared to glass fibres.
- No off-gassing of toxic compounds (in contrast to phenol-resin bonded wood and recycled cotton fibre parts).
- Price advantages compared to previously used technologies and to synthetic fibres, which became increasingly costly due to the increase in oil prices, and
- Positive effects on agriculture.

## ORIGINS OF NATURAL FIBRES

The flax short fibres used in the automotive industry come from the EU and Eastern Europe (especially Lithuania). Due to the lack of data, it is hard to ascertain the quantities coming from EU cultivation and production, estimates reach from 20% to over 50%. Flax short fibres are regularly produced as by-product of flax long fibres, that are used in the apparel industry.

In contrast, the hemp fibres used in the automotive industry come practically without exception from German (about 50%), Dutch, English and French total fibre production. The new hemp processors in the EU are highly focused on the automotive industry (followed by the insulation materials market).

As of yet, no significant quantities of hemp fibres are imported, in the future imports from Eastern Europe could come about. Especially in Romania, a modern hemp industry is arising from the ruins of the old one, being supported by German capital.

The other natural fibres used mainly come from Asia (jute, kenaf, coconut fibre/coir), as well as Africa and South Africa (Sisal). It is planned to grow and process kenaf in Spain in the future.

## EXISTING USE IN AUTOMOBILES

The following overview, which covers only recent car models (Table 3), illustrates how established the use of natural fibres for serial parts in the automotive industry already is.

## FURTHER MARKET DEVELOPMENT

The sales forecast in Table 2 solely refers to production technologies that are already used in serial production nowadays. The currently used natural fibre composites are mainly press-moulded parts. They consist of nonwovens from natural fibres and a binder (duroplast/thermoset resp. thermoplast), which are pressed into the desired form. Typical applications are door inserts, hat racks, pillar cover panels and boot linings (Table 3). At the current state of technology, 5-10 kg of natural fibres per automobile can be used (excluding seat upholstery). For the approximately 16 million (5.7 million alone in Germany) vehicles (automobiles and lorries) annually produced in Western Europe, this would correspond to a current technical potential of 80,000-160,000 tonnes of natural fibres per year in Western Europe in the sector of nonwovens for press-moulded parts alone (also see the digression "technology").

TABLE 3. Use of natural fibres for serial parts in the automotive industry (1997-2001)

| Manufacturers/Customers | Model / Application (dependent on model) |
|---|---|
| Audi | TT, A2, A3, A4, A4 Avant (1997), A4 Variant (1997), A6, A8 (1997), Roadster, Coupe<br>Seat back, side and back door<br>panels, parcel tray, boot lining, rear flap lining, rear storage panel, spare tire lining |
| BMW | 3, 5 and 7 Series and others<br>Door inserts/door panels, headliner panel, boot lining, seat back |
| Citroen | C4 (2001)<br>Door inserts |
| DaimlerChrysler | A-Klasse, C-Klasse, E-Klasse, S-Klasse<br>Door inserts,<br>windshield/dashboard, business table, column cover |
| Fiat | Punto, Brava, Marea, Alfa Romeo 146, 156, Sportwagon |
| Ford | Mondeo CD 162 (1997), Cougar (1998), Mondeo (2000), Focus<br>Door inserts, B-column cover, parcel tray, in the future also motor protection (cover undershield) |
| MAN | Bus (1997)<br>Headliner panel |
| Mitsubishi | Miscellaneous models (since 1997) |
| Nissan | Miscellaneous models |
| Opel | Astra, Vectra, Zafira<br>Headliner panel, door inserts, column cover, instrument panel, rear shelf panel |
| Peugeot | New model 406 |
| Renault | Clio, Twingo |
| Rover | Rover 2000 and others<br>insulation, rear storage panel |
| Saab | Coupe (1998)<br>Door inserts |
| SEAT | Door inserts, seat backs |
| Toyota | Miscellaneous models |
| Volkswagen | Golf A4, Golf 4 Variant (1998), Passat Variant, BoraDoor inserts, seat backs , rear flap lining, parcel tray |
| Volvo | C70, V70, Coupe (1998)<br>Door inserts, parcel tray |

(2,3,4,6,7)

If new production technologies, like natural fibre-reinforced plastics used with injection moulding technology could be successfully established, an additional market share in the approx. 1 million tonnes sized EU-market for glass fibre-reinforced plastics could be captured. This amounts to 60,000 tonnes glass fibre-reinforced plastics per year alone in the vehicle construction sector. Furthermore, in case of environmental laws being tightened up, the use of bio-composites (natural fibres + bio-plastics) could become a matter of interest in the future.

Whether and how fast these new markets might be conquered, is mainly dependent on the time that it takes to fully develop the injection moulding technology for natural fibres for serial production. Numerous institutes and companies in the EU are working very intensively on this new technology; a first serial production is expected for the year 2002.

Running parallel to this, developments of the modification of natural fibres with the means of vapour pressure (steam explosion), ultrasonic and enzymatic fibre separation are in progress. After many years of research and development work, the first serial production is in prospect for the year 2002. The modified fibres are very suitable for the injection moulding technology, also in the exteriors of the vehicles.

The present applications of natural fibre-reinforced composites are nearly exclusively confined to the automobile sector. Markets in the field of lorry, bus, train and aeroplane construction could supervene in the future, because the above-mentioned advantages of fibre products apply to these, too.

## DIGRESSION: TECHNOLOGY

### Natural Fibre Press-Moulded Parts

Technically speaking, natural fibres in composites are almost exclusively used in press-moulded parts up to now. Typical applications are door panels, rear shelf panel, column cover and parcel tray.

There are two state-of-the-art production techniques, which are most often used in serial production. According to a survey by the nova Institute from 2000, both techniques have a market share of about 50%.

In the one process, natural fibres are blended regularly with polypropylene (PP) fibres and formed to a fibre mat, or natural fibre nonwovens and polypropylene mats are applied in multiple layers, and then pressed under heat into the desired form (thermoplast). In the other process, nonwovens from natural fibres are soaked/sprayed with synthetic binders, e.g., epoxy resin or polyurethane, and then moulded into the desired form. The composite matrix is formed during the moulding process through polymerization and hardening (thermoset/duroplast).

On the fibre side, blends of natural fibres, e.g., flax and jute or flax and hemp, are interesting for technical reasons. The finer flax fibre imparts high stability to the part but prevents the complete permeation with the thermoset binder. This may result in fractures during use. Only a blend with the coarser jute or hemp fibres achieves an optimum balance between stability and saturation with binder. When using thermoplasts, it is sufficient to use only one natural fibre and to blend it, for example, with PP fibres.

The press-moulded parts are used especially in door panels, rear shelf panel, column cover and parcel tray (Table 3). For a door panel, typically 1.2-1.8 kg (front) resp. 0.8-1.5 kg (rear) of natural fibres are used, a parcel tray typically require between 1.5 and 2.5 kg of natural fibres. Other parts may requires similar amounts, within a range of 0.8 to over 2 kg.

In 1998, Daimler/Chrysler already used about 5-6 kg of plant fibres per vehicle (this corresponds to about 20,000-24,000 tonnes/year for the entire company). At the current state of technology, 5-10 kg of natural fibres per automobile can be used (excluding seat upholstery).

In 2000, press-moulded parts from natural fibres for the exteriors, specifically the undershields of the vehicles, were introduced for the first time. This could help to develop further substantial market shares. So far, however, a series production does not take place, although practical tests succeeded.

## Natural Fibre Injection Moulding

In an automobile, the described natural fibre press-moulded parts substitute especially wood and recycled cotton fibres as well as ABS parts. A relevant substitution of glass fibres will not come to pass until natural fibre-reinforced plastics can be used in series production with injection moulding.

In past years, various research and development projects demonstrated the technical feasibility of a replacement of glass fibres in fibre-reinforced plastics with flax and hemp fibres, even for exterior applications of vehicles. The diverse natural fibre injection moulding technologies differ considerably from each other: The wide range of variation reaches from long fibre injection moulding over elementary fibre injection moulding to the use of different adhering agents. It still remains unclear, which way is the silver bullet.

Costs, however, are still an obstacle. Even though prices for natural fibres per kg are lower than for glass fibres, the production process is entirely geared towards glass fibres and retooling is what makes the use of natural fibres still more costly. As soon as these production problems are resolved, a market for natural fibres could be developed within this field, the size of which, according to expert opinions, resembles the current market for press-moulded parts from natural fibres.

The efforts to translate the natural fibre injection moulding technology from research to series production have also been strongly intensified by the EU end-of-life vehicle directive. Compared to other natural fibre composites, the re-use seems to be more simple (Table 4). Experts forecast that the natural fibre injection moulding technology will be used in series production possibly as soon as in 2002.

## PRICES AND QUALITY MANAGEMENT

Prices for flax and hemp fibres have become predictable and range from Euro 0.50 to 0.60/kg for fibres used in technical nonwovens especially composites. Over the past 5 years, the quality of these fibres has essentially improved in terms of low hurd content and fibre fineness, with the price level being constant.

Important to the future development of an EU natural fibre economy is the implementation of a quality management system from cultivation through harvesting, fibre processing, nonwovens production to end

TABLE 4. Re-usability of different composites in the automotive industry

| Composite | Re-usability | Market share 2000 |
|---|---|---|
| Natural fibre press-moulded part with thermoset binder | hardly possible (at the most as filler) | about 50% |
| Natural fibre press-moulded part with thermoplast binder | possible (even though in inferior components or as supplement) | ca. 50% |
| Natural fibre injection moulding | possible (even again in injection moulding) | < 1% |
| Natural fibre composites | - | 100% |
| Glass fibre-PP-injection moulding | probably possible (even again in injection moulding) | - |

product. To the same degree as the use of natural fibres is becoming accepted, the demand for higher and consistent fibre qualities is growing-independent of climate factors during cultivation, harvesting, and retting. This may provide opportunities for the EU fibre production. The "European Industrial Hemp Association (EIHA)" (www.eiha.org ) aims at developing uniform quality standards that specifically refer to technical fields of application as well as to certain composites.

Should the young fibre industry be able to consistently meet the manufacturers' price and quality requirements, flax and hemp will become an important natural and renewable raw material resource for the industry, next to oil and starch plants.

## DIGRESSION: EU END-OF-LIFE VEHICLE DIRECTIVE

### The EU End-of-Life Vehicle Directive and Its Consequences on the Future Use of Natural Fibres

The market analyses presented on the previous pages are based on mostly unaltered basic conditions. Since the EU end-of-life vehicle directive has a major influence on the future use of materials in automobiles, it depends on the concrete implementation of the EU end-of-life vehicle directive, whether the described positive tendencies will persist, come to an end or whether new natural fibre product lines will have to be established.

### Basic Facts About the End-of-Life Vehicle Directive

After the EU Council of Ministers had approved of the EU end-of-life vehicle directive in July 2000, the European Parliament also approved of the proposal by the Conciliation Committee on September 7th. The member states have to translate the new directive into national law within 18 months.

One of the main points of the EU end-of-life vehicle directive are the scheduled re-use quotas. From 2015 on, re-use and recovery shall be increased to a minimum of 95% by an average weight per vehicle and year. Within the same time limit, the re-use and recycling shall be increased to a minimum of 85% by an average weight per vehicle and year, i.e., in general only 10% can be used for energy recovery and a maximum of 5% are allowed for disposal in landfills. Due to the fact that composites, regardless, whether on a synthetic or a renewable basis, can only be recycled under high technical and financial expenses, the question arises, whether the “thermal quota” for these materials is sufficient or whether its use in automobiles will decrease.

## Lightweight Construction, Renewable Resources and Ecology

The re-use quota for vehicles using lightweight materials will become particularly critical, as composites are heavily used and at the same time the quota, that is based on weight percentages, results in very small amounts of utilisable remaining quantities. The automotive industry might feel impelled to increasingly use other materials than hitherto for lightweight construction. Amongst other things, the use of foamed aluminium or composites made of only one petrochemical plastic (matrix and fibres consisting of the same plastic, e.g., PP), is a matter of concern (8).

Regarded solely from a point of view that focuses on disposal, one could appreciate this development, but looking at the whole life cycle of an automobile it reveals possible ecologically negative effects. Different studies show that particularly the phase of use and consequently the weight of the vehicle are ecologically relevant. Exactly this is the field where composites and predominantly natural fibre-reinforced composites have great advantages.

In addition, thermal recycling of natural fibre composites is to be regarded as an ecologically reasonable re-use alternative, because of the far-reaching carbon dioxide neutrality, similar to the burning of biomass.

On the whole, there are various hints that natural fibre-reinforced composites have ecological advantages in many respects–in the phases of production, utilisation and disposal–even though (or just because?) they are finally thermally utilised so that their substitution contradicts the ecological and agro-structural aims of the EU policy. What is missing, however, is a reliable scientific eco-balance which actually proves these advantages. Such a study could possibly prompt the EU-Commission in Brussels to incorporate special provisions for natural fibre components into a revised version of the EU end-of-life vehicle directive, which is planned for 2005/2006. If special provisions for natural fibre components should come about, regardless of the details, this would mean a decisive advantage in the competition with synthetic products and bring forth the improvement of the market chances for natural fibre products.

It is hard to estimate at the moment, which consequences the EU end-of-life vehicle directive (in the current or in a possibly modified version) will finally have on the use of renewable resources. We do know that the situation at the time of writing (autumn of 2001) is that:

- The demand by the automotive industry for natural fibres is persistent and the use of natural fibres still increasing.
- Miscellaneous suppliers have managed to realise re-use and material recycling methods for their natural fibre composites and guarantee this to the automotive manufacturers.
- The automotive manufacturers keep on fighting any quota-based regulation within the total re-use quota of 95% weight and wish to make a free choice between material and energy recovery. Ecological arguments, as brought forward by the natural fibre and lightweight construction lobby, are highly welcome, since they point out the inanity of the current quota-based regulation.

## NOTES

1. The European Union (EU) and its member states have comprehensively sponsored the development of new application fields for flax and hemp fibres. According to an analysis by nova Institute, from 1982-2002 more than 50 million Euro in EU subsidies (DG VI, DG XII, DG XIV) have been directed towards to the development of new flax and hemp applications as well as harvest and fibre separation technologies, respective national projects in addition. In Germany alone, between 1989 and 2000 more than 90 million Euro (of which more than 45 million Euro were raised from federal funding and funding by the German states) were invested in research and development, as well as in new harvest, fibre separation and processing techniques (2).

2. The nova Institute is the one and only institute in Europe that has specialised in market research and marketing in the field of industrial crops. Numerous research projects and industry consultings have been carried out since 1994 (detailed references at: www.nova-institut.de).

3. To complete the picture, it is worth mentioning, that the German automotive industry uses about 50,000-60,000 tonnes/year of recovered cotton and 50,000-70,000 tonnes/year of wood fibres in 1999, in addition to the already mentioned natural fibres. However, these figures clearly show a downward trend. Reasons are found in the inferior mechanical properties of these fibres and the fact that composites from these fibres release odours (e.g., formaldehyde from phenol resin-bonded wood and cotton fibres). This downward trend in the use of cotton and wood fibres will benefit both bast fibres such as flax and hemp fibres and ABS components (2).

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# Could Cannabis Provide an Answer to Climate Change?

Marc R. Deeley

ABSTRACT. The largely technocratic debate over the way humanity should respond to the now very real problem of global climate change has reached a critical point. Almost every legislative and technological option has been explored–at least theoretically–without any real progress being made in terms of actually addressing the situation and we are now at a stage where we do not have time to discuss the merits of "wind power over nuclear power" or how we can "develop ways of freezing and storing" the anthropocentrically generated excess of greenhouse gases in the atmosphere–as president Bush recently suggested. This was the "debate" during the 1980's–and unfortunately also the 90's–when the representatives of world science on this issue, otherwise known as the Intergovernmental Panel on Climate Change (IPCC), were explaining to World leaders the (then) urgent requirement to take action. This article discusses the very real possibility that Cannabis could play a part in stabilizing a global environment. 

KEYWORDS. Cannabis, climate change, environment

As we can see there are very few–if any–places in the world where the effects of global climate change are not being felt to varying de-

This article is a summary of the author's thesis titled "Cannabis: An environmentally and economically viable method for climate change mitigation" University of Strathclyde, Glasgow, Scotland, 2000 (revised 2001).

grees. The scientific consensus is that these "symptoms" (floods, droughts, storms, etc.) will persist, increase in their severity and actually perpetuate the problem of climate change by further contributing to it's causes such as desertification. Like a cancer patient, the World will not recover without immediate and effective long term treatment which targets the cause(s) of ailment–fossil fuel consumption and unsustainable land use conversions being most responsible. IPCC projections of climate change within the context of an industrial and therefore fuel dependent World consider the best possible strategies to remedy the situation; these strategies explicitly link the areas of agriculture, land use and society's demand for resources with the industrial utilisation of biomass. "If the development of biomass energy can be carried out in ways that effectively address concerns about other environmental issues and competition with other land-uses, biomass could make major contributions in both the electricity and fuel markets, as well as offering prospects of increasing rural employment and income" (IPCC, 1996b, p. 15).

Utilisation of biomass in both the energy and transport sectors holds several benefits not least because these can be used to offset or substitute directly for fossil fuels thereby reducing emissions of Greenhouse Gases (GHGs), particularly carbon dioxide ($CO_2$), while simultaneously sequestrating atmospheric $CO_2$ via photosynthesis by creating and enhancing terrestrial "carbon sinks" (IPCC, 1996b). Following the United States' refusal to consider serious reductions in their emissions, "carbon sinks" are now a universally agreed method to achieve atmospheric carbon reductions as set out in the Kyoto Protocol. The IPCC (1996b) considers fast-growing hardwoods to be the best possible option. Cannabis is, therefore, perfectly placed to be utilised in this area given its chemical composition, which is comparable to that of a hardwood (van der Werf et al., 1999) and rapid growth cycle compared to other high cellulose content organisms.

Moreover, there exists at present much of the technology to translate this into a pragmatic climate change mitigation option with higher energy efficiency and lower unit capital costs than conventional methods of energy production (IPCC, 1996b). This is especially significant given that "analysis of future global trends in greenhouse gas emissions has shown that reducing emissions from fossil fuels will have the greatest effect on atmospheric carbon concentrations between 1990 and 2100" (Adger and Brown 1994, p. 229).

According to a paper published in Biomass and Bioenergy, "Assessing the Ecological and Economic Sustainability of Energy Crops" which considers the viability of nine possible biomass contenders[1] via comprehensive life cycle assessments, Hanegraaf et al. (1998, p. 351) conclude that, "hemp comes out as one of the best options for energy cropping." I would be inclined to go further. An ideal approach to climate change mitigation would include the following objectives:

- Sequestration of atmospheric carbon dioxide and/or reduced fossil fuel consumption.
- Prevent the destruction of natural ecosystems (biodiversity).
- It would not burden developing countries with costly socio-economic regulations.
- It would not require significant changes to current land use (i.e., displacing people or activities).
- It would have a minimal environmental impact and/or address other environmental/pollution problems.
- It would also provide (socially equitable) economic incentives for global implementation.

(Adapted from UNFCCC, 1992 and IPCC 1990, 1996a, 1996b)

Cannabis cultivation has the potential to satisfy all of the above criteria. While farmers would find the cultivation of another annual crop easier than trying to integrate perennials, the adoption of Cannabis as a key rotation crop (irrespective of farm size) would also yield several direct benefits including the reduction of pesticides while increasing the yield of crops following from it in rotation (Roulac, 1997) thereby assisting the goal of achieving sustainable agricultural systems. It is also the case that years of mono-culture (and, relative to Cannabis, protein deficient)[2] cereal production will require alternative and rotational crops rather than for instance genetically modified crops to, "allow control of those weeds, pests and diseases that still cannot be controlled in the cereal crops themselves, and perhaps more importantly [would] help restore organic matter to the soil following years of depletion by cereal crops" (Forbes and Watson, 1992, p. 257).

Cannabis cultivation could, therefore, be used to promote environmentally beneficial methods of agriculture (especially via rotation cultivation) which could actually help secure a long-term strategy of land management, ensuring that food shortages do not occur. This would be greatly enhanced by taking advantage of the multiplicity of possible uses Cannabis presents us with according to local economic, social and environmental needs. For example, depending on these local variables Cannabis could be used for either food (see footnote 2 above), fibre or as a bioremediation crop to restore unproductive land (especially that degraded by the overuse of chemicals high in heavy metals) back to agricultural productivity while at the same time providing industrial quantities of cellulose for fuel and/or energy production. According to Ranalli (1999, p. 69) Cannabis is "able to extract heavy metals from the soil in amounts higher than many other agricultural crops" and it is the case that agricultural land shortages are arguably far more likely to occur in areas where there is a deficit of suitable land due to intensive agricultural practices combined with inadequate land management (IPCC, 1996b).

However, the greatest advantage for Cannabis cultivation as a method of climate change mitigation is in terms of logistics and the comparative ease with which this particular form of biomass could be integrated into the existing fossil fuel economy. With the ability to be grown at all but the very coldest latitudes, Cannabis could form the basis of an internationally distributed (yet locally determined) fuel industry. The chemical composition (high cellulose) and physiology of Cannabis make it an ideal feedstock for ethanol production in comparison to the starch based crops currently used in the US and South America (Lorenz and Morris, 1995). Ethanol is not only a complementary product to the oil economy (combining ethanol with gasoline increases quality of gasoline and produces significant environmental benefits) but can also be used as a direct replacement requiring only modest alterations to industrial operations.

The key determining variable is global land use and contrary to popular belief there is more than enough available cropland to satisfy the World's rapidly growing population. Taking into account the unsuitability of some soils and terrain, the FAO considers there to be 3000 Mha of potential cropland of which only about 50 percent is at present cultivated (around 1450 Mha)(IPCC, 1996b, p. 809). In light of this, many of the analyses (Hall et al., 1994 and IPCC, 1996b) that consider between 10 and 15 percent of total global cropland to be available for biomass production specifically for energy (and transport) applications represent conservative assumptions. When taken along with the potential use of Cannabis as a bioremediation crop for land suffering "light" to "moderate" degradation, (750 Mha and 910 Mha, respectively) much of which is caused by the over cropping of erodible soils, unsustainable land use conversions (i.e., forest to livestock) and over use of chemical inputs (IPCC, 1996b) the possibilities have even more practical relevance for future development, especially in the agricultural sectors of developing countries.

The World urgently needs a replacement for fossil fuels and while there are many overtly technological options the only realistic possibility rests in finding a comparatively similar substitution feedstock. Cellulose derived ethanol would appear to be an ideal industrial successor to fossil fuels with Cannabis appearing to be the most environmentally sound and economically viable feedstock for ethanol production. In addition we should consider all the products ranging from plastics to building composites currently dependent on fossil fuels which the utilisation of highly versatile cellulose such as Cannabis could replace. In effect we would be replacing an unsustainable industrial feedstock for one which is not only sustainable but addresses some very serious environmental and socio-economic issues. There is certainly enough supportive evidence to get such projects underway–the rest is politics.

## NOTES

1. These include Rape seed, Sugar beet, Winter wheat, Silage maize, Hemp, Miscanthus, Poplar, Willow and Grass fallow.
2. Cannabis seed (often referred to as a nut or fruit) is a unique and highly nutritious food source which according to Pate (1999) contains 20-25 percent protein, 20-30 percent carbohydrates, 10-15 percent insoluble fibre and 25-30 percent oil in addition to a variety of minerals including phosphorus, magnesium, sulphur, calcium with modest amounts of zinc.

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# Hemp in Italy: A New Research Project

Paolo Ranalli

ABSTRACT. Traditionally, hemp is a plant very well suited to Italian pedo-climatic conditions. The textile fibre produced in the past was of the finest quality due to integrated selected local varieties, good agrotechnology and experienced retting techniques. Attempts to reintroduce this crop in Italy rely upon updating the chain of fibre production and its processing that lead to the textile and its derivatives. This article discusses a new research project designed to study production and utilization of the plant. 

KEYWORDS. Hemp, fibre, genetics, processing

The logistic chain of agricultural production to end users of plant fibres has to be structured: breeders must develop cultivars for specific uses and regions of growth, new fibre extraction methods must be adaptable to different raw fibre properties and different uses, innovative procedures of traditional water retting have to be found and new knowledge underlying the spinning and end-use markets is needed.

A research program named "Fibre hemp: From production to utilisation" funded by the Italian Ministry of Agriculture is in progress. It is focussed on key themes of the crop, i.e., genetic resources, breeding,

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biotechnology and molecular biology, agrotechnology, processing, spinning and final products. More in detail, it deals with the following tasks: (1) the maintenance of hemp germplasm accessions in a viable condition and their reproduction in sufficient amounts to distribute seeds to researchers world-wide. Besides, new methods of hemp genetic resource evaluation based on DNA analysis, as well as new ways of increasing the genetic diversity using biotechnological methods and their incorporation into breeding processes are in progress; (2) the identification of molecular markers for specific traits and their allocation in a saturated linkage map. The perspectives seem to be good, given the high level of polymorphism at the DNA level already observed by our group; the close association found in our lab between male phenotype and specific molecular markers, even in the absence of a molecular map, can already become a rapid diagnostic tool in breeding. However, saturated molecular maps will be needed in order to find markers associated with other important characters, probably quantitative in nature, such as THC and fibre content; mapping these QTL is certainly the most important task for the future; (3) integration of conventional and molecular techniques in the breeding schemes in order to develop new dioecious and monoecious varieties and to gain insight into the genetic factors underlying the expression of the most important cannabinoid categories stored in the plant; (4) experimental investigations in the cultivation phase, particularly needed in the sectors of fertilizers, of plant density (in relation to the variety planted, the cultivation environment and fertilizers used), of the best time to harvest the crop (which has a bearing on the productivity and the quality of the fibre), of full mechanisation of harvesting and operations involved in hemp processing; (5) retting procedures. Experiments involving microbiological retting are in progress; they are aimed at the following objectives: (i) to reduce the length of the process, (ii) to obtain a better control of the retting process, in order to have reproducible results, (iii) to improve the fibre quality. In the past, in Italy and other European regions, traditional water retting was performed by pulling the hemp by hand or machine, tying it into sheaves and soaking it in retting tanks filled with water for a period of up to 14 days. Since this procedure is time expensive and labour intensive, facilities have to be developed in order to make retting more predictable, reproducible and cheaper; (6) THC detection. The labs currently involved in THC assessment use traditional methods based on gas-chromatography and sometimes GC in combination with mass-spectrometry. Gas chromatography, like high performance liquid chromatography methods, requires quite expensive instruments and highly experienced technicians. For large scale THC analysis (such as that required in breeding programs) these traditional methods are not convenient and immunological procedures are most suitable. Our group has raised antibodies against THC and successfully used them for serial tests in breeding programs; these results have placed us in a pioneering position and the current program gives us the opportunity to gain further insight in the competitive-ELISA and in assessing tests performed directly in the field (with a very simple strip test or similar format). The optimisation of the extraction procedure for field tests is still in progress; (7) fibre quality assessment. Research work on assessing non-retted straw, retted straw and fibre for their quality by a number of traditional, chemical and instrumental methods is in progress. The program also includes: comparing data and measurements, organoleptic and instrumental quality methods, defining a unified vocabulary on quality and establishing standards and testing methodology.

Conclusively, the fibres go, sequentially, from farmers to scutchers, traders, spinners, weavers, tailors, the retail trade and finally, to the customer: the logistic chain of agricultural production to end users of plant fibres relies on an interdisciplinary approach in which different disciplines must be integrated simultaneously.

# Finola Progress 2000-2001

Henry Gage

ABSTRACT. Fin-UK, Ltd. conducted agricultural trials with the Finola variety (previously known by the breeder's code FIN 314) at several locations in Europe during the year 2000. Finola is currently completing its last year of Value for Cultivation and Use (VCU) testing in Finland, and will soon be put forward for inclusion on the EU list of approved hemp cultivars. 

KEYWORDS. Finola, agricultural trials, hemp

Trial plots of 1-2 hectares were planted in northern France and Norfolk, England on 4th and 5th May 2000, and one plot was planted in Switzerland on 15th June. Seed beds were well prepared and raked before drilling and no fertilizer was used. Due to low germination, the seeding rate was 50 kg/hectare, twice the amount usually required, in order to ensure a dense growth. The seed was planted to depth of about 1 cm. Flowering occurred remarkably early with this variety: five weeks after planting in both France and the U.K. The crop showed a good resistance to weeds as relatively fast growth in the first few weeks smothered competing vegetation.

Harvest occurred later than usual, on the 3rd and 5th of September in the UK and France, respectively, and late September in Switzerland. This was due mainly to wet weather, and the late planting of the Swiss cultivation plot. Desiccation was carried out in England but did not seem to have much of an effect. Harvesting was carried out successfully

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with conventional combine harvesters. Small deposits of hemp residue collected on the stripper-header, which may require cleaning if larger areas are harvested. Because the height of the plants was unevenly distributed in the case of the English plot, the cutting angle needed adjustment several times during harvesting. Uneven growth was put down to the excessively high seeding rate, which caused the plants to compete fiercely for sunlight. This was compounded by the uneven distribution of quality soil in the cultivation plot. Despite this, the harvest results were better than the trials taking place in France and Switzerland, with 1.65 tonnes/hectare compared with 1.5 tonnes in France and 1.25 tonnes in Switzerland. The average plant height was also higher at 130 cm in England compared to 100 cm on the continent. The fact that the hemp was planted into high quality sandy loam soil in the English trial plot explains why the plant height and seed yield was better than in northern France. The late seed drilling date in Switzerland may help explain the poorer results there.

The seed was dried from a moisture of about 30% to 10% which is near optimal for storage as moulding can occur if the seed has a higher moisture content.

In 2001, agricultural trials continued in Central Europe on different soil types. This year, the germination rate was higher, which allowed a substantial reduction from the previous year's seeding rate, resulting in more even growth. It is hoped that Finola will be included on the EU list of approved hemp cultivars next year, and thus be eligible for the subsidy that is currently offered to other varieties. Although the overall yield of fiber for Finola is relatively low, compared to other hemp varieties, its fiber is almost exclusively primary bast fiber (i.e., long fiber) and more comparable to flax in both yield and fineness. More importantly, however, is the production of grain from this variety, which has exceeded all others in agricultural trials in Europe and Canada. In addition to its fine fiber quality, Finola offers a unique source of high quality oil and vegetable protein.

Fin-UK Ltd manages Finola hempseed cultivation within Europe with the exception of Norway, Sweden and Finland. Any questions should be directed to henry@motherhemp.com

# European Industrial Hemp Association (EIHA)

On 14th September 2000, the European Industrial Hemp Association (EIHA) was founded in Wolfsburg, Germany, at the occasion of the BIORESOURCE HEMP 2000 conference. The European Industrial Hemp Association regards itself as an informal conjunction of hemp primary processing companies within the European Union (EU). Almost all hemp primary processing companies have joined the association.

Aims of the EIHA are:

- to establish a regular exchange of information between the member companies,
- to encourage the exchange of information between the national ministries,
- to promote EIHA as a contact partner for EU institutions and national ministries,
- to formulate a statement on the "end-of-life vehicle directive" and to subsequently practice national and EU-lobbying, and
- construction of the internet site "www.EIHA.org" featuring the foundation press release and a list of the member companies including links to the respective homepages.

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Michael Karus (Germany) and Francois Desanlis (France) keep their status as co-ordinators of the EIHA until further notice.

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