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ANATOMICAL CHARACTERISATION AND VARIABILITY OF THE THISTLE *CYNARA CARDUNCULUS* IN VIEW OF PULPING POTENTIAL

Teresa Quilhó¹, Jorge Gominho² & Helena Pereira^{1,2}

SUMMARY

The thistle *Cynara cardunculus* L. is an herbaceous perennial with high productivity that is harvested annually and is a potential fibre crop for paper pulp production. The anatomical variation within stalks was studied (base, middle and top) and compared in *C. cardunculus* plants at different development phases. The stalk of *C. cardunculus* includes an epidermis, cortex and a central cylinder with fibro-vascular bundles with phloem, xylem and a fibrous sheath that is variable in arrangement and size within and between plants.

At harvest, the pith represents 37% of the stalk transectional area and 7% of the total weight. There was a slight variation in quantitative features of, respectively, the three development groups studied; mean fibre length was 1.04 mm, 0.95 mm and 1.05 mm; mean fibre width was 15 µm, 16 µm and 21 µm; mean fibre wall thickness was 3.2 µm, 3.4 µm and 4.9 µm. Fibre length and width decreased within the stem from base to top, while fibre wall thickness increased. Mean vessel diameter was 22 µm and mean vessel element length 220–483 µm. In mature plants, parenchyma represents 39% of the total transectional area and fibres 25%. The proportion of fibres increases during plant development and in mature plants is highest at the stalk base.

As regards anatomical features, *Cynara* stalks compare favourably to other annual plants and fibre biometry indicates good potential for paper sheet forming and strength properties.

Key words: *Cynara cardunculus* L., anatomy, density, tissues proportion.

INTRODUCTION

Non-wood fibre crops have long been an industrial raw material, namely for pulp and paper (Atchison & McGovern 1993). Bamboo, bagasse and cereal straws are important resources of fibres, and in some regions are the only or main source of pulp fibres (Kynklund 1994), while flax, hemp, cotton, miscanthus, kenaf and esparto (Van Dam et al. 1994) are seen as potential raw-materials.

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The research on non-woody plants has been focused on species with high biomass production that can be grown in set-aside and marginal areas as non-food industrial crops. The thistle *Cynara cardunculus* L. is a new fibre crop that recently has been investigated in research programmes of the European Union and has yielded promising results.

Cynara cardunculus grows naturally in many regions of the world, in Europe, North Africa, Madeira and Canary Islands, and South America, very often in harsh conditions with high temperatures and water stress in summer, and on thin, unproductive and stony soils (Bailey & Bailey 1976; Tutin 1976). Traditionally, the plant has been used in regional food dishes (e.g., soups, salads) and as a protease source for milk coagulation in cheese making (Pires et al. 1994).

Cynara cardunculus is an herbaceous perennial of the family Asteraceae with a high growth and biomass production. The vegetative cycle of the plant begins by seed germination with the first rains in autumn, and in winter it develops a rosette of large leaves. The stalk starts elongation in spring and the first capitula appear in June-July. During summer, the aboveground part of the plant dries off and the underground part (a tuberous root system) remains in dormancy until a new cycle begins with the burst of leaves formed from root tissue with the autumn rainfall (Quer 1962; Bailey & Bailey 1976).

The green leaves from winter growth may be used for food and fodder; the biomass harvested at the end of the cycle may be used for energy (biomass combustion) and the stalks for pulp production and the seeds are a source of protein and oil (Fernandez & Manzanares 1989; Fernandez & Curt 1994). The average biomass production is 20 tons/ha.yr, but 30–35 tons/ha.yr of dry material may be attained in optimal conditions, including about 40% stalks, 25% leaves and 35% capitula (Fernandez 1992; Dalianis et al. 1996).

The pulping quality of *Cynara cardunculus* stalks has been evaluated showing high pulp yields and good strength properties. Most process limitations referred to its anatomical characteristics, which were only roughly described (Gominho et al. 2001). Several aspects were not investigated, i.e., cell type distribution, as well as between and within plant variation. We did not find any anatomical descriptions of *Cynara cardunculus* in the literature. Carlquist (1965) described the wood of *Cynareae*.

In this paper we describe anatomy, cell biometry and cell type distribution of the stalk of *Cynara cardunculus* in different plants and growth stages as related to its pulping potential.

MATERIAL AND METHODS

Plant material

The material used in these studies was collected from experimental trial plants of *Cynara cardunculus* in the fields of the Instituto Superior de Agronomia, Lisbon, Portugal, established in October 1998.

In June 1999, after the elongation of the stalk, but before the development of the capitula, the plants were classified into three groups: group A, corresponding to the less developed plants with an height under 50 cm and not flowering; group B, 50–1.50

m high and on the onset of flowering; and group C, higher than 1.5 m and flowering. Three plants were randomly collected from each group for analysis. The leaves and flowers were removed and only the stalks kept. Samples for anatomy were cut from the stalk of each plant as transectional discs at the base, mid-height and near the top.

The trial was harvested in September 1999 at the end of the annual cycle, and four plants were randomly selected for physical and structural characterisation.

Physical characterisation

The plants harvested at the end of the cycle were characterised in relation to moisture content, dimensions and density. Using an image analysis system, the cross-sectional area and the mean diameter of stalks and pith were measured at three height levels: at the base, mid-height and at the top. The basic density of the material was calculated using the oven-dry weight and the green volume.

The bulk density of air-dried chips with the approximate dimensions of $18 \times 20 \times 5$ mm³ was also determined. Three measurements were made for each parameter.

Anatomical characterisation

Transverse and longitudinal sections approximately 17 µm thick were prepared with a Reichert sliding microtome after impregnation with DP 1500 polyethylene glycol. The sections were stained with acridine red and astra blue and mounted in Euparal.

In transverse section the number of vascular bundles per mm² and their size were determined using a projection microscope. The mean diameter of at least 10 metaxylem vessels was determined for each sample, as the average of two measurements taken at right angles to one another. In the tangential section, the length of a minimum of 10 vessel elements was recorded.

Observations were also made on macerated elements from samples treated with glacial acetic acid: 20% hydrogen peroxide 1 : 1, at 60 °C for 48 h, then stained with astra blue. The length, width and wall thickness of 40 fibres per sample were measured using a semi-automated system (Leitz - ASM 68K).

The proportion of tissue types was calculated in the transverse section on each of five areas from epidermis to pith using an image analysis system coupled to a microscope with a 54-point grid.

RESULTS AND DISCUSSION

General description

At the end of the first year cycle, the stalks of *Cynara cardunculus* had an average height of 129 cm. Branches occurred approximately halfway up the stalk, on average 5 branches per plant.

The stalk was approximately circular in cross section, with longitudinal ridges about 1–2 mm above the stem surface. A circular crown of light brown fibrous tissues surrounded an internal circular pith of white colour.

The stalk diameter at the base was on average 26 mm with a 16 mm pith (Table 1). The proportion of pith was rather constant within the plants, representing on average

Table 1. Diameter of stalk and pith, pith content (% area and % mass) and basic density of stalks at different height levels of *Cynara cardunculus* plants harvested at the end of the cycle. Mean of four plants and standard deviation.

	Base	Mid	Top
Stalk diameter (mm)	26 ± 5	21 ± 3	15 ± 3
Pith diameter (mm)	16 ± 3	13 ± 2	9 ± 2
Pith content (% or area)	37.8 ± 3.8	38.2 ± 4.4	36.4 ± 3.6
Pith content (% of mass)	8.0 ± 1.2	7.3 ± 2.1	6.0 ± 1.7
Stalk basic density (kg/m ³)	196 ± 21	195 ± 21	182 ± 23

Table 2. Quantitative anatomical features in the stalk of *Cynara cardunculus* in different plants' development phases (groups A, B and C); mean of four plants per group; standard deviation and in parentheses minimum and maximum.

		Plant A	Plant B	Plant C
Fibres	length (mm)	1.037 ± 0.247 (0.554–1.960)	0.947 ± 0.137 (0.454–1.920)	1.049 ± 0.063 (0.453–2.006)
	width (µm)	15 ± 2 (8–26)	16 ± 2 (8–25)	21 ± 2 (8–36)
	wall thickness (µm)	3.2 ± 0.8 (1.5–7.2)	3.4 ± 0.9 (1.5–6.1)	4.9 ± 0.4 (2.2–8.2)
Fibro-vascular bundles				
	no/mm ²	1–9	1–5	1–5
(type Δ)	height (µm) ¹	1065 ± 550 (325–2134)	1211 ± 254 (659–1755)	2901 ± 611 (1671–4773)
	width (µm) ²	138 ± 43 (55–292)	160 ± 20 (78–236)	316 ± 28 (151–545)
(type □)	height (µm) ¹	351 ± 36 (128–836)	554 ± 63 (178–880)	579 ± 173 (169–1356)
	width (µm) ²	238 ± 21 (96–700)	351 ± 57 (192–612)	382 ± 110 (122–951)
Vessel elements				
(type Δ)	length (µm)	353 ± 78 (122–864)	394 ± 45 (115–965)	483 ± 146 (180–1549)
	diameter (µm)	23 ± 6 (7–59)	23 ± 5 (8–53)	25 ± 4 (5–69)
(type □)	length (µm)	268 ± 25 (107–450)	220 ± 46 (59–601)	293 ± 21 (152–645)
	diameter (µm)	21 ± 5 (7–54)	21 ± 3 (8–47)	23 ± 4 (9–60)

¹) Maximum dimension of the fibro-vascular bundle in radial direction.

²) Maximum dimension of the fibro-vascular bundle in tangential direction.

Table 3. Cell type proportion (% or area) in the stalk of *Cynara cardunculus* (plants A, B and C) as the mean of measurements at three height levels, and standard deviation.

Tissues (%)	Plant A	Plant B	Plant C
Parenchyma	54 ± 5	46 ± 11	39 ± 23
Fibres	24 ± 7	24 ± 7	25 ± 12
Medullar rays	14 ± 10	19 ± 2	21 ± 8
Vessel elements	4 ± 1	9 ± 1	14 ± 3
Phloem	4 ± 3	4 ± 4	5 ± 4

37% of the total area and 61% of the total diameter. The mean area ratio between the fibrous tissues and the pith was 1.7 with a low between and within plant variation. Pith represented on average 7.1% of total weight.

Moisture and density

At harvest at the end of the cycle, the stalks of *Cynara cardunculus* had an average moisture content of 18%, and 14% after air-drying. The harvested biomass is thus practically dry, thereby eliminating one of the disadvantages of agricultural biomass production for industrial uses, where high moisture contents affect transport, storage and conservation.

The mean basic density of stalks was 191 kg/m³. The bulk density of air-dried chips was 138 kg/m³ for the whole stalk and 160 kg/m³ for the depithed stalk. Pith therefore impacts negatively on bulk density, which is lower when compared to hardwood chips, e.g., 333 kg/m³ for *Betula* sp. (Hakkila 1989) but similar to other annual plants, e.g., 184 kg/m³ for *Arundo donax* (Shatalov et al. 2001). The basic density of *Cynara* stalks is below the values for hardwoods, e.g., 367 kg/m³ for *Populus tremula*, 470 kg/m³ for *Betula* sp. (Hakkila 1989) and 543 kg/m³ for *Eucalyptus globulus* (Miranda et al. 2001a), as well as for *Arundo donax* (420 kg/m³) (Shatalov et al. 2001).

Anatomical description

The structure of the three plants in different development stages (groups A, B and C) were observed and compared. Their anatomical structure was very similar and major differences were found only in tissue proportion and biometry (Table 2 & 3). In general the structure followed the description made by Gominho et al. (2001) on mature *Cynara* stalks.

The stalks of *Cynara cardunculus* showed a thin epidermis (Ep) to the outside, a cortex (Cx) and a central cylinder (Cc) with outer, central and inner parts (Fig. 1–3). The epidermis covered the stem as a single compactly unignified cell layer with an average 15 µm thickness (Fig. 4). The cuticle was sometimes difficult to observe. In the transection the cells appeared rounded and with contents, sometimes elongated near hairs and stomata (Fig. 4, arrow).

The cortex represents the area between epidermis and the first fibro-vascular bundles of the central cylinder. The first cell rows (2–6 cells) under the epidermis were denser,

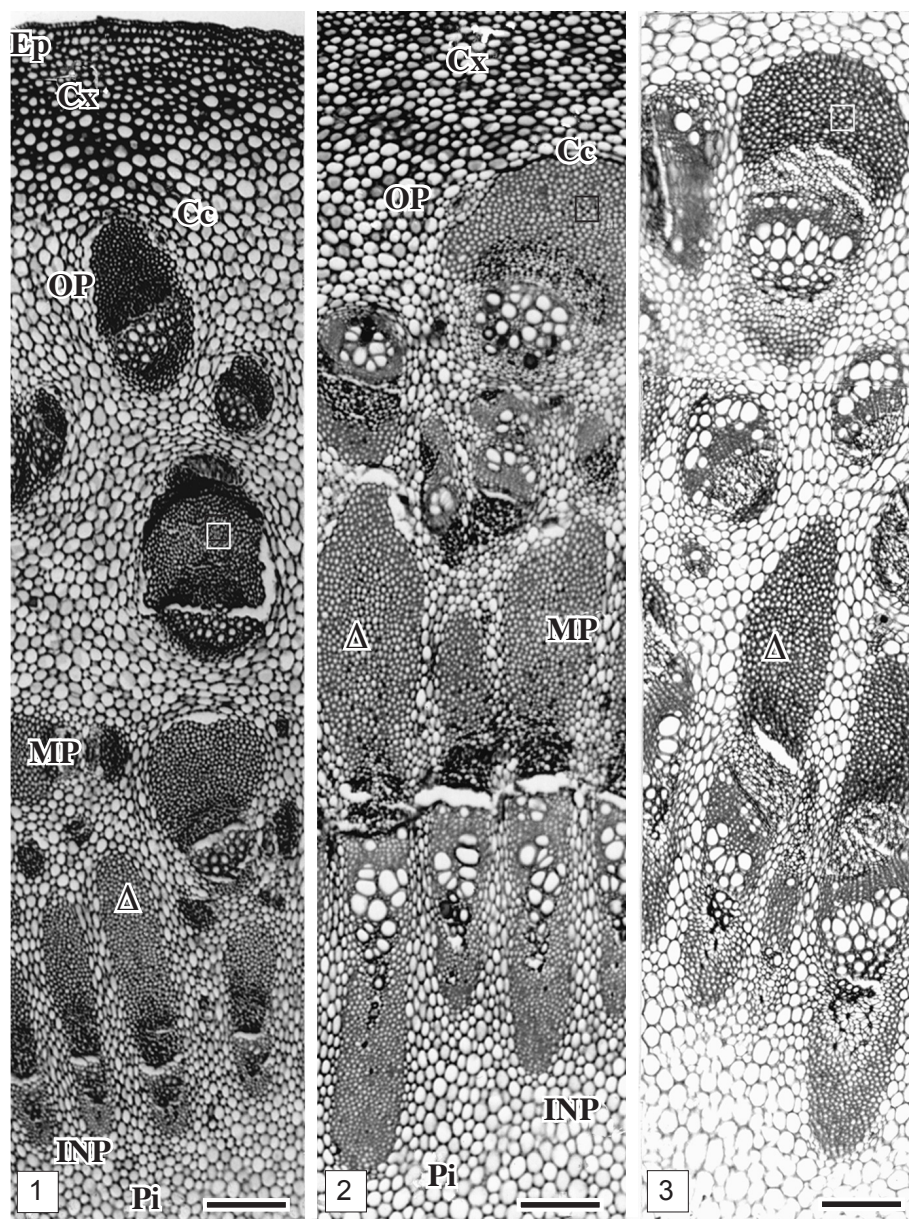
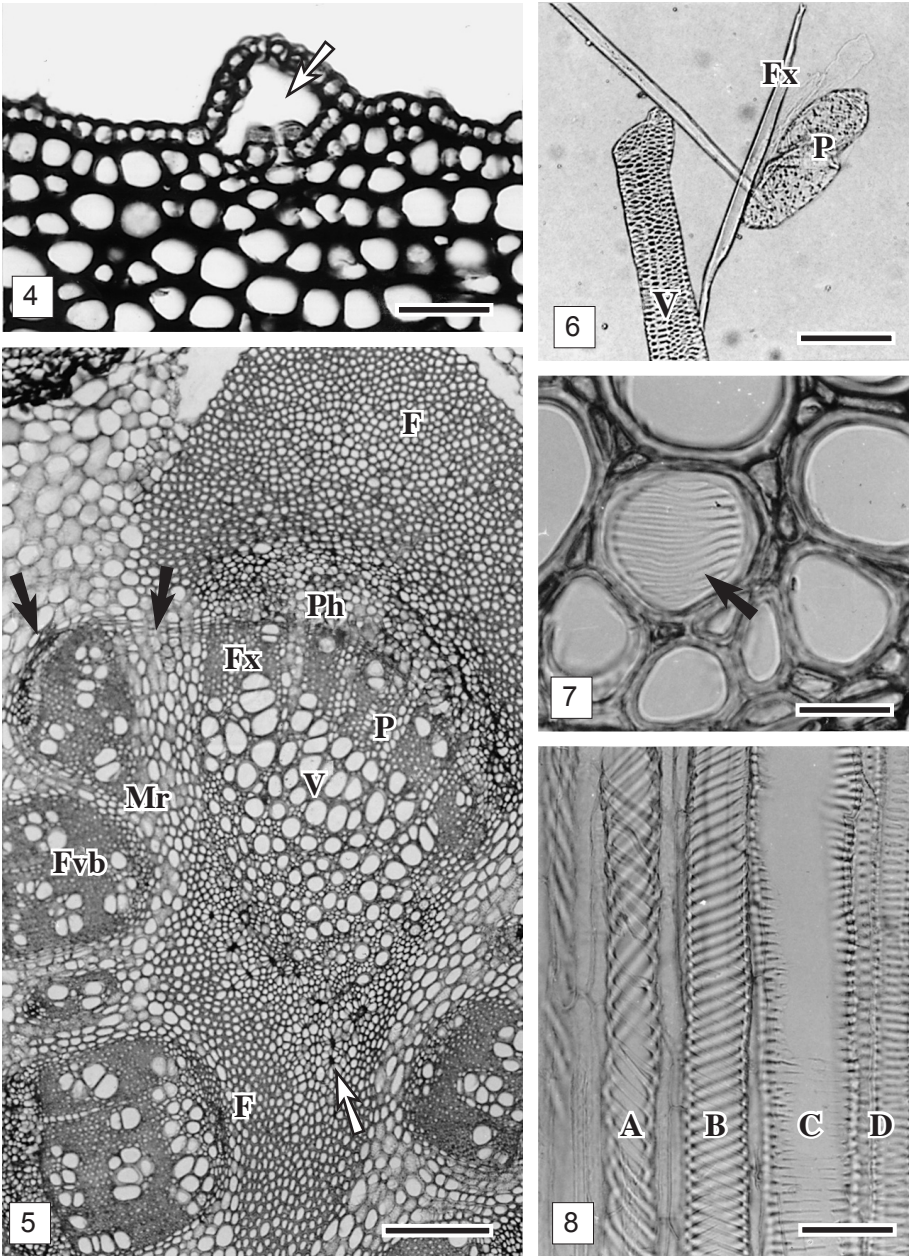


Fig. 1–3. Transverse sections of a *Cynara cardunculus* stalk: epidermis (Ep), cortex (Cx) and central cylinder (Cc) with outer part (OP), medium part (MP) and inner part (INP). Fibro-vascular bundles – type A (Δ), type B (□). Pith (Pi). — 1: plant A. — 2: plant B. — 3: plant C. — All scale bars = 20 μm.

→
 Fig. 4. Epidermis of *Cynara cardunculus* (transverse section): stomata (arrow). — Fig. 5. Fibro-vascular bundles (Fvb) of *C. cardunculus*; Ph = phloem; Xm = xylem; F = extraxylary fibres; Fx = libriform fibres; V = vessel elements; P = parenchyma; medullar ray, intrafascicular cam-



bium (black arrows); nonliving element of protoxylem stretched and destroyed (white arrow). — Fig. 6. Macerated material of *C. cardunculus*; V = pitted vessel element; P = parenchyma cells; Fx = libriform fibre. — Fig. 7. Vessels of fibro-vascular bundles of *C. cardunculus*; helical thickenings of the vessel lateral walls (arrow). — Fig. 8. Protoxylem vessels with thickenings: A: annular; B: helical; C: scalariform-reticulate; d: pitted. — Scale bar of Fig. 4 & 7 = 4 μ m, of Fig. 5, 6 & 8 = 8 μ m.

with small and rounded cells, sometimes more numerous and evident to the outside of the vascular bundles (arrow). To the interior, cells were larger, spherical, and sometimes tangentially elongated (Fig. 2).

In the periphery of the central cylinder (outer part, OP), fibro-vascular bundles were irregularly dispersed. Concentric rings of fibro-vascular bundles were not found and large bundles were interspersed with small ones. In the middle (medium part, MP) bundles were more frequent, denser and parallel to one another with interspersed large and small bundles (Fig. 1–3). This feature was more evident in the beginning of stalk elongation (A, Fig. 2). In the mature stalks (C, Fig. 3) the number of vascular bundles increased and had a more irregular distribution pattern.

The pith (Pi), in the inner part of the central cylinder (INP), consists of parenchyma (Fig. 1–3).

The fibro-vascular bundles (Fvb) consist of phloem (Ph), an intrafascicular cambium (arrow), xylem (Xm), and a fibrous sheath (F) of extraxylary fibres (Fig. 5). The general structure of fibro-vascular bundles in different plants was illustrated by Shaw et al. (1969). This structure was the same within the stem of the different plants, but arrangement, distribution and size varied (Fig. 1–3 & 5). Two types of fibro-vascular bundles were present (Table 2): the inner fibro-vascular bundles have a large radial diameter and the cambium showed an approximate tangential orientation (type Δ); the outer fibro-vascular bundles are smaller, with similar radial and tangential diameters (type \square) and the cambium showed various orientations (Fig. 1–3 & 5).

The phloem is located outside the metaxylem and is composed of sieve-tube elements and associated companion cells (Ph) (Fig. 5).

The xylem has a variable number of metaxylem vessels (V) with interspersed parenchyma cells (Fig. 5).

The vessels were grouped (two or three, sometimes more than four) obliquely and radially. The vessel elements had simple perforation plates (arrow, Fig. 6), as do other *Cynareae* (Carlquist 1965). An image showing what apparently was identified as a scalariform perforation (Fig. 7) was on subsequent observation of the tangential oriented perforation bars assigned to an artefact arising from the helical thickenings of the vessel lateral walls. A similar observation was made by Carlquist (1965) on different *Cynareae* species.

Vessel elements showed reticulate and pitted secondary thickenings (Fig. 6 & 9). The protoxylem vessels vary in number (often more than 6) and have annular and helical wall thickenings (Fig. 8). The nonliving elements of the protoxylem were unable to accompany the extension of adjacent cells, stretched (white arrow, Fig. 5) and were destroyed.

The fibres (Fig. 6 & 9) form a sheath surrounding phloem and xylem (F in Fig. 5) as extraxylary fibres and provide structural support. The shape and size of the fibrous sheath differs from the outside of the plant inwards: the fibrous strands are rounded in the outermost zone, and radially elongated near the pith (Fig. 1–3). The fibres also appeared interspersed with metaxylem vessels (Fx in Fig. 5). These libriform fibres or xylary fibres (Fx) were much less abundant than extraxylary fibres. In general, extraxylary fibres were described having a long spindle-like shape, frequently very thick with their ends blunt rather than tapering (Esau 1953), depending on the species. The

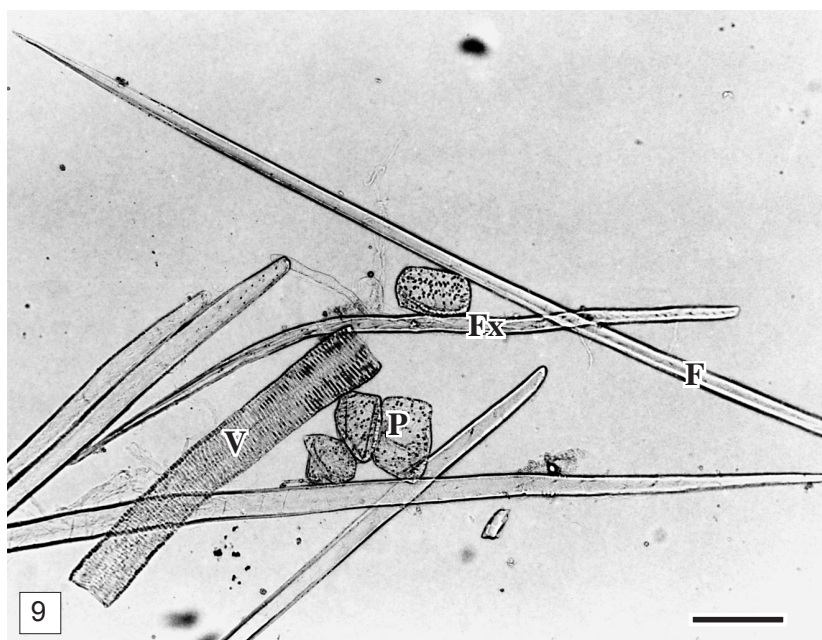


Fig. 9. Macerated material of *Cynara cardunculus*. — V = reticulate vessel; P = parenchyma cells; Fx = libriform fibre; F = extraxylary fibres. — Scale bar = 8 μ m.

observation of macerated material (Fig. 6 & 9) showed that there was not a significant difference in fibre wall thickness between extraxylary and libriform fibres of *Cynara cardunculus*.

Parenchyma is a homogeneous tissue of rounded cells (Fig. 5, 6 & 9), with thin walls and irregular and small intercellular spaces. Parenchyma surrounds the fibro-vascular bundles in the central cylinder as medullar rays (Mr, Fig. 5) and in the inner zone of the central cylinder (pith) where cells are larger and with more intercellular spaces.

Cell dimensions

Table 2 shows the mean values of cell dimensions in the three *Cynara cardunculus* plants.

Vessel elements in the inner fibro-vascular bundles (type Δ) were always longer than vessel elements in outer fibro-vascular bundles (type \square) (e.g., 483 μ m and 293 μ m, respectively, in the mature plants). Gominho et al. (2001) observed shorter vessel elements in mature stalks of *Cynara* and Carlquist (1965) also observed short vessel elements in species of *Cynareae*. Vessel element length increases from the base to the middle of the plant and then decreases towards the top (Fig. 10). The mean vessel diameter is similar in both types of fibro-vascular bundles (24 μ m), but narrower than those observed by Gominho et al. (2001). Mean vessel diameter decreases from the base towards the top (Fig. 10). Voulgaridis et al. (2000) found in *Hibiscus cannabinus* a similar tendency of length and diameter variation of vessel elements. The mean fibre

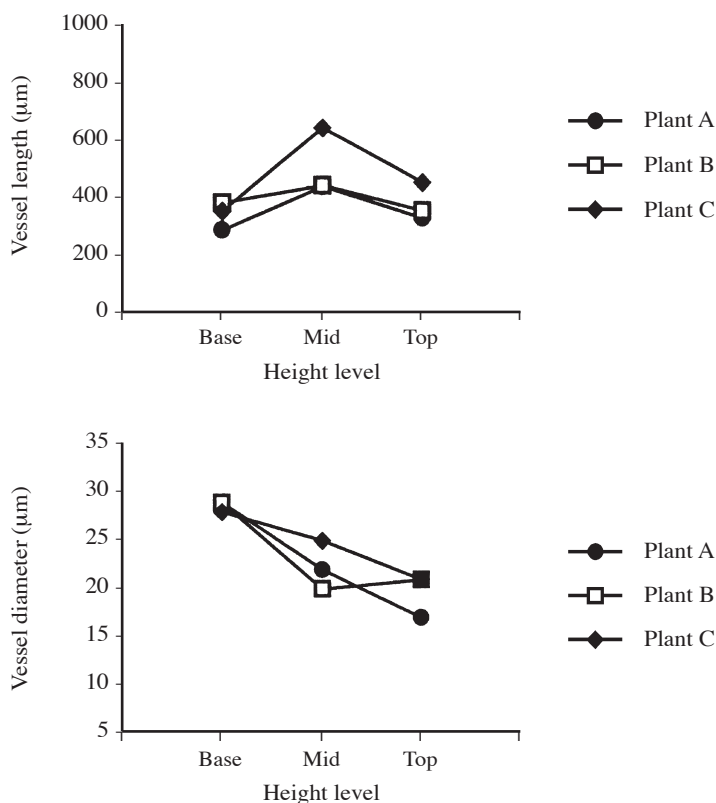


Fig. 10. Longitudinal variation of vessel element length and diameter at the base, mid and top of *Cynara cardunculus* stalks.

length, width and wall thickness ranged from 0.947–1.049 mm, 16–21 µm and 3.4–4.5 µm, respectively (Table 2). These values are in the same range of those determined by Gominho et al. (2001) for *Cynara cardunculus*. Fibre length and width decrease from base to top of the stalk (Fig. 11). Bhat et al. (1993) observed a similar longitudinal variation in *Calamus* sp. Fibre wall thickness shows an opposite variation, increasing from the base upwards (Fig. 11) with statistical significance ($P < 0.05$).

Proportion of tissues

The percentages of areas of the component tissues of *Cynara cardunculus* stalks are shown in Table 3 as plant means.

Axial parenchyma occupies the largest area with 39% to 54% of the total area, followed by the fibres with approximately 24%. Plants of group A, which were not flowering, had more parenchyma (54%) than the others; the mature C plants had the largest proportion of fibres (25%), rays (21%) and vascular tissue (14%). The content of fibres did not differ very much between the three groups (Table 3). It was lower than in *Arundo donax* (approx. 36%) (Shatalov et al. 2001).

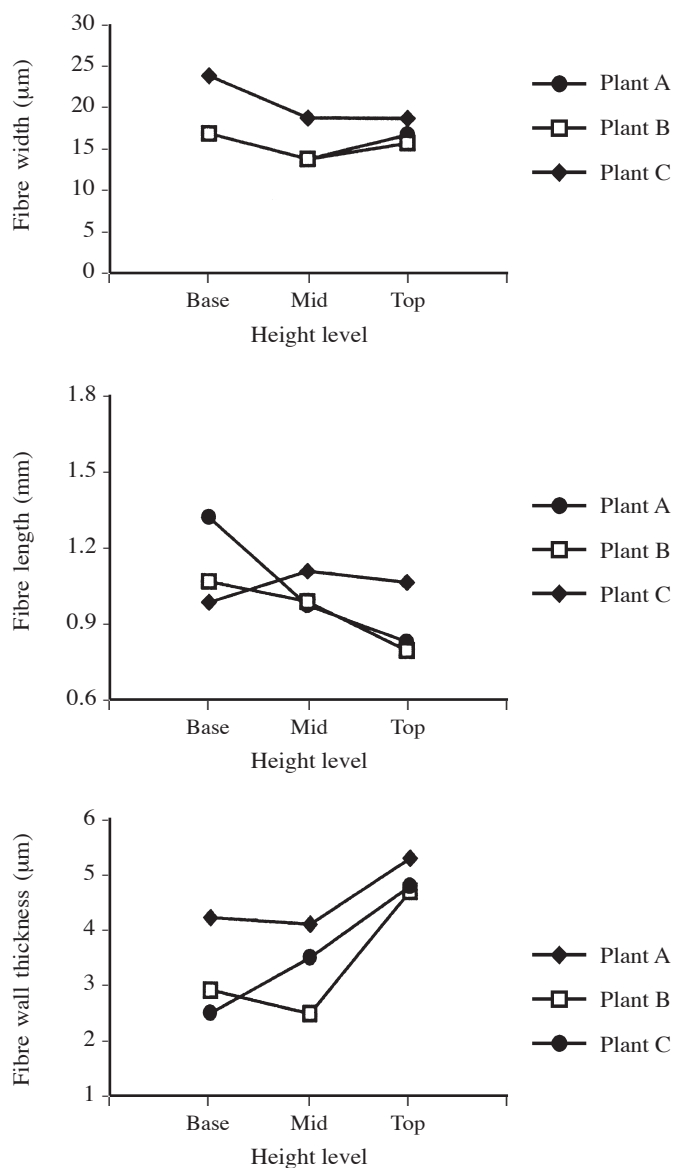


Fig. 11. Longitudinal variation of fibre width, length and wall thickness at the base, mid and top of *Cynara cardunculus* stalks.

The proportion of axial parenchyma is higher in the top, especially for the more mature plants. From base to top, the mean values are, respectively: 58–48–56% in A plants, 38–42–59% in B plants and 17–36–63% in C plants.

The proportion of the fibres is higher at the stalk base with a range from base to top, respectively of 33%, 30% and 39% in plant A, 30%, 25% and 16% in plant B and 39%,

18% and 17% in plant C. Bhat et al. (1993) and Abasolo et al. (1999) verified the same longitudinal variation in *Calamus* species with more fibres situated at the base than at the top of the plant.

The proportion of rays, vessels and phloem showed no trend of axial variation.

Suitability as a fibre source for paper

In papermaking, fibres are the cell elements that impart mechanical strength to the paper sheet. On the contrary, parenchyma with spherical and small cells are considered to decrease the raw material quality. Parenchyma has a low density and decreases the bulk density of the chip charge to the pulping digester, it consumes chemicals without participating in paper strength and it makes pulp water drainage more difficult.

In *Cynara cardunculus*, parenchyma is present in the pith and in the intermedullary rays between the fibre-vascular bundles, but in lower amounts than many other annual plants. The volume content of parenchyma in *C. cardunculus* was low when compared with *Arundo donax* (58%) (Shatalov et al. 2001), wheat straw (68%) and similar to cornstalks (50%) (Atchison 1993). While the volume of parenchyma ratio in *C. cardunculus* may seem significant (39%), in terms of mass ratio it probably will represent only about 10% of the material (7% as pith). However, the presence of parenchyma will be a characteristic of the *Cynara* raw material and even if the stalks are depithed by a mechanical pre-treatment, parenchyma will still be present. For some uses, namely when bulk of paper is favoured, this is acceptable, but for others, e.g. printing papers, screening techniques might have to be considered.

The important papermaking elements in *Cynara cardunculus* stalks are the fibres that surround phloem and xylem in the fibre vascular bundles (extraxylary fibres). Fibres represent approximately 25% of the volume. In mature *Cynara* stalks, fibres have average dimensions (1.1 mm length, 21 μm width, 3.6 μm wall thickness) that favour paper properties and are similar to such highly valued paper fibres as those of *Eucalyptus globulus* (Miranda et al. 2001b).

Average fibre length of *Cynara cardunculus* is in the range of other annual plants, such as *Arundo donax* (1.2 mm) (Shatalov et al. 2000), esparto (1.5 mm), wheat straw (1.5 mm) and bagasse (1–1.5 mm) (Atchison 1993). *Cynara* species fibres have a somewhat wider diameter than *Arundo donax* (16 μm) (Shatalov et al. 2001), wheat straw (15 μm), bamboo (15 μm) and esparto (12 μm) and are in the same range as cornstalks (20 μm) and bagasse (20 μm) (Atchison 1993). *Cynara* fibres have thinner walls than those of *Arundo donax* (5 μm) (Shatalov et al. 2001). Therefore, dimensional ratios often used to characterise paper fibres in terms of flexibility and sheet forming aptitude are favourable for *Cynara* fibres: coefficient of flexibility 0.67 and felting coefficient 50. For other non-wood fibre crops these ratios range as follows: felting coefficient of *Arundo donax* 71–79 (Shatalov et al. 2001), of wheat straw 67 (Leminen et al. 1996) and of bamboo 79 (Bhargava 1993); coefficient of flexibility of *Arundo donax* approximately 0.37 (Shatalov et al. 2001), of wheat straw 0.46 (Leminen et al. 1996) and of bamboo 0.27 (Bhargava 1993).

In summary, as regards anatomical features, *Cynara cardunculus* stalks compare advantageously to other annual plants, with fibre biometry comparable to *Eucalyptus*

globulus wood fibres, indicating favourable paper properties. This was confirmed by the first experiments of pulping of *Cynara* stalks and by the good strength properties of the corresponding paper sheets (Gominho et al. 2001). As regards plant development, harvest should be made at the end of the cycle, since the papermaking favourable anatomical characteristics increase with plant maturation, i.e., decrease of parenchyma content, due to the increase of the size of fibro-vascular bundles and of fibre dimensions.

CONCLUSIONS

The stalk of *Cynara cardunculus* includes an epidermis, a cortex and a central cylinder. The central cylinder contains the fibro-vascular bundles with phloem, xylem and a fibrous sheath variable in arrangement and size, within and between plants. The pith is abundant and parenchyma corresponds to about one third of the stalk volume at harvest.

Some trends of longitudinal variation are observed from the base to the top of the plant: fibre length and width decrease, and fibre wall thickness increases; vessel elements diameter shows a progressive decrease and length increases to the middle of the plant and then decreases towards the top. The proportion of fibres increases during plant development with a decrease of axial parenchyma.

As regards anatomical features, *Cynara* stalks compare favourably to other annual plants with approximately 25% fibres in volume. Fibre biometry indicates good potential for paper sheet forming and strength properties.

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