Composition and desiccation-induced alterations of the cell wall in the resurrection plant *Craterostigma wilmsii*

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Resurrection plants have the unique capacity to revive from an air-dried state. In order to tolerate desiccation they have to overcome a number of stresses, mechanical stress being one. In leaves of the *Craterostigma* species, an extensive shrinkage occurs during drying as well as a considerable cell wall folding. Our previous microscopically analysis using immunocytochemistry on the resurrection plant *Craterostigma wilmsii*, has shown an increase in labelling of xyloglucan and unesterified pectins in the cell wall during drying. In this study, we have undertaken a biochemical approach to separate, quantify and characterize major cell wall polysaccharides in fully hydrated and dry leaves of *C. wilmsii*. Our results show that the overall cell wall composition of *C. wilmsii* leaves was similar to that of other dicotyledonous plants with respect to the pectin content. However, the structure of the hemicellulosic polysaccharide xyloglucan was characterized to be XXGG-type. The data also demonstrate marked changes in the hemicellulosic wall fraction from dry plants compared to hydrated ones. The most conspicuous change was a decrease in glucose content in the hemicellulosic fraction of dry plants. In addition, xyloglucan from the cell wall of dry leaves was relatively more substituted with galactose than in hydrated walls. Together these findings show that dehydration induces significant alteration of polysaccharide content and structure in the cell wall of *C. wilmsii*, which in turn might be involved in the modulation of the mechanical properties of the wall during dehydration.

**Introduction**

Over one third of the earth’s surface is considered to be arid or semi-arid because it is subjected to permanent drought (Bewley and Krochko 1982). Plants found in dry areas have developed astonishing ways to survive water deficit stress. Water-deficit tolerance is a strategy in which plants are able to experience protoplasmic dehydration without permanent injury (Bewley and Krochko 1982). Many lower order species (mosses and ferns) can tolerate tissue desiccation (Bewley and Krochko 1982, Oliver 1996, Oliver et al. 1998). In higher plants, while seeds and pollen are frequently desiccation tolerant, the vegetative tissues rarely are. However, there are a few angiosperms, termed resurrection plants (Gaff 1971) in which the vegetative tissues have the remarkable capacity to withstand severe water loss. During dehydration, desiccation-tolerant plants have to ameliorate against numerous stresses as the water is gradually lost from the cells (Bartels et al. 1990, Tuba et al. 1994, Dace et al. 1998, Farrant 2000, Scott 2000, Kranner et al. 2002). To do so, resurrection plants have evolved a number of molecular mechanisms, including the biosynthesis of LEA (late-embryogenesis abundant) proteins, or modification of lipid composition and carbohydrates (reviewed in Vertucci and Farrant 1995, Ingram and Bartels 1996, Walters et al. 2002).

One of the first and major stress plants have to overcome in order to survive desiccation is mechanical stress (Iljin 1957). For some of the resurrection plants such as...
Selaginella lepidophylla and the Craterostigma species, upon drying, the cell walls of vegetative tissues fold, becoming highly convoluted (Sherwin 1995, Thomson and Platt 1997). When the plant is rehydrated, cells return to their original volume without apparent injury. In the resurrection plant _C. wilmsii_, a diminution of approximately 78% of the initial cell volume occurs when the plant is dried (Farrant 2000). This reduction in cell size is due almost entirely to an extensive folding of the cell wall. It has been proposed that the folding of the cell wall is a strategy developed by the cell to avoid the tearing of the plasmalemma from the cell wall during dehydration, thus allowing the cell integrity to be maintained (Farrant and Sherwin 1997). Such folded cell walls are also a common feature in dry seeds and the manner of the cell wall collapse is characteristic of a given species (Webb and Arnott 1982). These authors have suggested: (1) that wall folding in seed cells is essential for preserving the structural integrity of the tissue and retaining the viability of the seeds upon rehydration; and (2) that the extent and the manner of cell wall folding observed in various seed species depend upon cell wall chemical composition and structure.

The cell walls of plants are dynamic structures which play critical roles in plant morphology, growth and development (Albersheim et al. 1994, Penell 1998). Cell walls are also fundamentally involved in mediating plant responses to pathogenic and environmental stress (Zwiazek 1991, Boudart et al. 1998). Despite this ascribed importance, our understanding of the function of plant cell walls is still not complete. The recent characterization of many _Arabidopsis_ mutants with altered cell walls is promising in providing a better understanding of the biosynthesis and function of plant cell walls (Reiter et al. 1991, Peng et al. 2000, His et al. 2001, Andéme-Onzighi et al. 2002). Nevertheless, resurrection plants such as _Craterostigma_ species also provide an excellent system to explore the function of the cell walls upon environmental changes. Many reports have illustrated the involvement of the cell wall in the response to varying environmental factors such as wounding (Cardemil and Riquelme 1991), pathogen attacks (Boudart et al. 1998), cold acclimation (Weiser et al. 1990, Fujikawa et al. 1999, Kubacka-Zebalska and Kaeperska 1999, Stefanowska et al. 1999), osmotic stress (Hohl and Schopfer 1995, Wakabayashi et al. 1997, Marshall et al. 1999) saline stress (Iraki et al. 1989a, 1989b, 1989c) and dehydration (Zwiazek 1991, Ha et al. 1997).

To date there have been no studies on the characterization of folded cell walls of the resurrection plant _C. wilmsii_ upon drying, except our previous microscopic analysis (Vicré et al. 1999). In that report, using immunocytochemistry, we studied the distribution of xyloglucan (XG) and various pectic epitopes in the cell walls of _C. wilmsii_ leaves during drying and rehydration (Vicré et al. 1999). We showed that the cell wall folding induced by dehydration was accompanied by a marked increase in XG and polygalacturonic acid/rhamnogalacturan I (PGA/RG-I) epitopes, with levels declining again during rehydration.

On the basis of these observations, we wanted to further analyse the chemical composition of _C. wilmsii_ walls using biochemical methods. In the present study, we describe the fractionation and structural analysis of polysaccharides isolated from the cell walls of fully hydrated and dehydrated leaves. This represents the first report on the cell wall chemical composition of desiccation-tolerant higher plants.

### Materials and methods

#### Plant material

Plants of _C. wilmsii_ (Scrophulariaceae) were collected from Buffelskloof Nature Reserve, Mpumulanga, South Africa and were maintained in a glasshouse at the University of Cape Town as previously described (Sherwin and Farrant 1996). Whole plants were dried by withholding water and allowing the plant to dry naturally under ambient conditions for 15 days.

#### Sample preparations for scanning electron microscopy (SEM)

Leaves from both hydrated and dry plants were cryofixed in liquid nitrogen slush and sublimed at −70°C (frozen hydrated samples). They were coated with gold palladium and viewed using the cooled stage in a Hitachi s-570 scanning electron microscope.

#### Sample preparations for transmission electron microscopy (TEM)

Leaf fragments (1–2 mm) were excised from the midblade of the plant rosette. These were fixed overnight in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) containing 0.5% caffeine (Sherwin and Farrant 1996). Post fixation was in 1% osmium tetroxide in phosphate buffer. Following ethanol dehydration, the material was embedded in epoxy resin (Spurr 1969). Thin sections (90 nm) were cut using a Reichert Ultracut-S ultramicrotome and collected onto 200 μ mesh copper grids. Sections were stained with uranyl acetate (10 min) and lead citrate (10 min) as previously described in Vicré et al. (1999) and were observed at 80 kV on a Zeiss transmission electron microscope (EM 109).

#### Isolation of cell wall material (CWM)

Leaves from hydrated and dry plants were frozen in liquid N₂. The freeze dried leaves (2 g) were ground to a powder in a mortar and suspended in boiling ethanol (1 g: 100 ml) for 15 min. After filtration, the residue was suspended in 100 ml of methanol/acetone (1:1) under agitation for 24 h then washed thoroughly with 80% acetone. The isolated cell walls were filtered and allowed to dry in an oven at 80°C overnight. The weights and the yields were then determined.
Fractionation of CWM

CWM (dry powder) was extracted as follows: three times with water (500 mg/150 ml) at 100°C for 2 h each with constant stirring, three times with 0.1% EDTA (pH 7.5) at 100°C for 2 h each and once with 1.5 M boiling NaOH (Girault et al. 2000). The NaOH solution contained 1% (w/v) NaBH₄ and the extraction carried out under N₂ with continuous stirring. The NaOH extract was neutralized with amberlite IRH resin. All fractions were filtered, dialysed against deionized water for 24 h, lyophilized and weighed. The yield was determined for each fraction. The remaining cell wall residue (CWR) was dried and weighed, and sometimes hydrolysed with trifluoroacetic acid (TFA) in order to analyse the monosaccharide content of non-cellulosic polysaccharides. All results expressed were usually obtained from three experiments or more.

TFA hydrolysis of the remaining cell wall residue (CWR)

For further analysis of sugar composition determination, 5 mg of CWR was dissolved in 3 ml of 2 M TFA for 30–60 min at 100°C in sealed tubes. Then the supernatant was dried under air stream and used for sugar analysis by GC as described below.

Sugar quantification

Total sugar content of each fraction was determined by the phenol sulphuric method using galacturonic acid (Gal A) as standard (Dubois et al. 1956). Uronic acid content was determined by the m-hydroxyphenyl method (MHPD) according to Blumenkrantz and Absoe-Hansen (1973) using Gal A as a standard sugar. All experiments were performed 10 times.

Monosaccharide composition analysed by Gas Chromatography (GC)

The sugar composition of cell wall fraction was determined by gas chromatography analysis of their trimethyl-silyl glycosides according to York et al. (1985). Samples (0.5 mg of polysaccharide) were mixed with methanol/HCl 1 M (500 µl) for methanolysis 24 h at 80°C. Inositol was used as internal standard. The solution was then evaporated under an airstream and the samples were washed with methanol and dried. This step was repeated three times. Samples were methysialylated with 100 µl of Tri-Sil for 2 h at 80°C. The solution was evaporated under an air stream and the residue was suspended in 20 µl of pyridine and 1 ml of cyclohexane. One µl was loaded on the injector and injected directly on a DB-1 column (DB-1 Supelco). Chromatographic data were integrated with GC Star Workstation software (Varian), each surface being corrected according to its response factor. Sugars detected by GC were arabinose (Ara), fucose (Fuc), galactose (Gal),galacturonic acid (Gal A), glucose (Glc), glucuronic acid (Glc A), mannose (Man), rhamnose (Rha) and xylose (Xyl).

Detection of arabinogalactan-proteins (AGPs) with the Yariv reagent

β-D-glucosyl Yariv reagent (Biosupplies, Australia), which binds and precipitates AGPs (Fincher et al. 1983) was used to detect AGPs in different cell wall fractions. 1% agarose gel containing 0.15 M NaCl and 30 µg ml⁻¹ Yariv was placed in a Petri dish. Five µl of each fraction as well as AGP standards was loaded into a small well made in the gel. Diffusion was carried out at 37°C in a humid chamber for 24 h. Standards were made of a red wine purified AGP (Pellerin et al. 1995) and used in a range of concentration between 0.2 mg ml⁻¹ to 3 mg ml⁻¹.

Mass spectrometry analysis of XG fragments

XG fragments were generated by treating 1 mg of the NaOH fraction with 5U of endo-β-(1,4) glucanase (Mega-zyme International, Ireland, EC 3.2.1.4) in 500 µl of 10 mM AcNa for 18 h. Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectra (MALDI-TOF MS) of the resulting xyloglucan solubilized fragments were recorded on a Micromass (Manchester, UK) Tof spec E MALDI-TOF mass spectrometer. Mass spectra were performed in the reflector mode. This instrument was operated at an accelerating voltage of 20 kV with a reflector potential of 26 kV, and a pressure of approximately 10⁻⁷ mbar in the source and 10⁻⁶ mbar in the analyser. Samples were desorbed and ionized from the probe tip with a nitrogen laser (λ = 337 nm) with a pulsewidth of 4 ns. More than 12 laser shots were sums for each mass spectrum. Two µl of the endoglucanase-generated-xyloglucan oligosaccharides solution was dissolved in the same volume of matrix prepared by dissolution of 2 mg of 2, 5-dihydroxybenzoic acid in 200 µl of 70% (v/v) acetonitrile in 0.1% (v/v) TFA. The sample-matrix mixture obtained was homogenized and 2 µl of this solution was deposited onto probe tips and allow to dry for a few minutes under vacuum. The target was then applied in the MALDI-TOF mass spectrometer.

Monosaccharides content of XG fragments

XG fragments after enzymatic digestion were purified by using a carboigraph (Altech) column (Packer et al. 1998). A carboigraph column was prewashed before use with use with 5 ml acetonitrile 90% with 0.1% TFA and by 5 ml deionized water. The samples were placed on the graphite column and washed with 5 ml water. Neutral oligosaccharides, like XG fragments, were selectively eluted using 5 ml of acetonitrile 25%. This fraction was then collected, freeze-dried, treated with MeOH/HCl and silylation reagent for GC analysis (York et al. 1985).

Results

Morphology of dry plants

The morphological changes undergone by the resurrection plant C. wilmsii during drying are presented in Physiol. Plant. 120, 2004 231
In fully hydrated plants, the leaves were green and expanded (Fig. 1A). As the plant dried, leaves curled inward and a purple colour characteristic of anthocyanin pigments was visible on the abaxial surface. In the dry state the leaves were tightly folded with abaxial surfaces of the outermost leaves exposed to sunlight (Fig. 1B).

**Microscopical study**

Figure 2A-D show cryo-scanning electron micrographs of hydrated and dry leaves. In transverse sections from hydrated leaves, cells were characterized by a rounded shape in both spongy and palisade parenchyma cells (Fig. 2A). In contrast, cells in dried leaves were folded and highly shrunken (Fig. 2B). It is interesting to note that cell wall undulations were apparent in the three dimensions without any regular pattern or orientation (Fig. 2B). Only xylem, being more rigid, maintained its original shape. The boundary between palisade and spongy parenchyma was no longer discernible and the surface of lower epidermis (abaxial surface) exhibited numerous hairs. This is the face exposed to the light when the plant is dried and we assume that their role is essentially a protection against light stress.

Figure 2C,D show surface views of epidermis from hydrated and dry tissues, respectively. In Fig. 2C stomata and glands are visible. Epidermis of dry leaves appeared convoluted and highly folded (Fig. 2D). It is worth noting that stomata of dried leaves were always open. Schwab et al. (1989) reported that in *Craterostigma plantagineum*, drying results first in a closure of the stomata, but when the water potential drops further they could be passively reopened. This may also be the case with *C. wilmsii*, and tensions created as the epidermis folds could lead to a passive reopening of stomata.

The ultrastructure of chemically fixed hydrated and dry leaf cells is shown in Fig. 3A,B. In hydrated leaf cells, the cytoplasm, including organelles such as chloroplasts were peripherally located along the cell wall, with normal organization (Fig. 3A). The cell wall was also normal in appearance and a large vacuole, which completely fills the cell, was present. In the cells of dried leaves the cytoplasm occupied the cell volume as the central vacuole disappeared and considerable cell wall folding was observed (Fig. 3B). Most of the chloroplasts were rounded in shape and were localized in the middle of the cell. Such protoplasmic dehydration-induced alterations of the cellular anatomy have been previously reported in *C. plantagineum* by Schneider et al. (1993). Note that the thylakoid membranes were well defined in dry cells and the plasmalemma was not always immediately adjacent to the folded cell walls, with some withdrawal evident in some cells.

We have previously demonstrated that folded cell walls in dehydrated leaves of *C. wilmsii* presented significant alterations of XG and some pectin epitopes (Vicré et al. 1999). Therefore, we wanted to gain better information on the precise chemical composition of cell walls of hydrated and dry *C. wilmsii* plants.

**Yield and total sugar content of cell wall fractions**

Cell walls of hydrated and dried cells were isolated and polysaccharides sequentially extracted with hot water (HW), the calcium chelator EDTA and 1.5 M NaOH (see Materials and methods). The total amount of the cell wall isolated from hydrated leaf cells was quite similar to that of the walls from dried plants (317 versus 350 mg g⁻¹ of lyophilized leaves). As shown in Table 1, the yield of the material extracted from the cell wall of hydrated plants...
represented 15, 28 and 37%, in HW, EDTA and NaOH fractions, respectively, and was not significantly different from that of dried plants.

In contrast, dehydration resulted in a marked increase in the neutral sugars in the EDTA fraction, whereas only a slight decrease was detected in HW and NaOH fractions. Similarly, uronic acid content exhibited a 2-fold increase in the EDTA fraction of dehydrated plants. Nevertheless, it is worth noting that polysaccharide fractions extracted from dry plant cell walls were much more easily solubilized in water than those of hydrated ones.

Fig. 2. Cryoscanning electron micrographs of hydrated and dried *C. wilmsii* leaves. (A-B) Transverse sections of hydrated (A) and dried *C. wilmsii* leaves (B). (C-D) Surface view of the epidermis of hydrated (C) and dried (D) leaves. Glands are present in the epidermis. Note that in the dry leaves, the stomata are largely opened. g, gland; h, hair; le, lower epidermis; st, stomata; sp, spongy parenchyma; pp, palissadic parenchyma; ue, upper epidermis; vs, vessels; →, cell wall folding. Bars: a, b = 200 µm; c,d = 40 µm.

**Chemical composition of cell walls**

The composition of cell walls isolated from hydrated and dried plants is presented in Table 2. The amount of each monosaccharide in the three fractions as well as in the remaining cell wall residue (CWR) was determined using GC.

**Hot water extract**  The major sugars found in HW soluble material are characteristic of pectins. In both hydrated and dry plants, the HW cell wall fraction was mainly composed of Gal A, Ara and Gal although Glc, Glc A
and small amounts of Rha, Xyl and Man were also present. Gal A was the predominant sugar in both cell walls, accounting for 32.1 ± 5.9% and 38.9 ± 3.7% of total sugars from hydrated and dry plants, respectively. No major difference in the content of various sugars was detected. Although the sugar composition of this fraction was indicative of pectins, some AGPs were also present, as revealed by gel diffusion and Yariv staining (Fig. 4).

**EDTA extract** The sugar composition of chelator-soluble fractions comprised mainly Gal A, in both hydrated (61.3 ± 5.9%) and dry (60.2 ± 6.1%) plants (Table 2). Small amounts of Gal and Ara were also detected, but all other sugars were present in very minor proportions. The content of sugars was not significantly different between dry and hydrated plants. In addition, as for HW fractions, gel diffusion and Yariv staining revealed the presence of AGPs in the EDTA fractions for both hydrated and dry plants (Fig. 4).

**1.5 M NaOH extract** In both hydrated and dry plant cell walls, the alkali-soluble fraction represented the main fraction accounting for about 37% (Table 1). The major sugars detected were Glc, Gal and Xyl in both hydrated and dry plants demonstrating the hemicellulosic nature of this fraction and suggesting the presence of XG. In hydrated plants, Glc was the predominant neutral sugar in the NaOH fraction representing 30.5 ± 3.1%, whereas Gal and Xyl represented 17.4 ± 3% and 16.2 ± 3%, respectively. Interestingly, the content of the predominant sugar, Glc, was reduced by approximately 2-fold in dry plants (17.7 ± 1.9%). Another notable finding of this analysis is that, unlike Glc, Gal was lower in hydrated than in dry cell walls. The presence of high levels of Gal A indicates that some pectins were also solubilized by the alkali treatment.

**CWR after TFA hydrolysis** Although the CWR left after sequential extraction by HW, EDTA and NaOH was expected to contain mainly cellulose, the analysis of its monosaccharide contents has shown that non-cellulosic polysaccharides were still present in this fraction. The CWR fraction was treated with 2 N TFA to hydrolyse all polysaccharides except cellulose, and sugar composition was determined using GC (Table 2). The presence of relatively high levels of Glc, Gal and Xyl suggests that XG was still present in the CWR. This was probably tightly bound to cellulose. Again, the Glc content was reduced in dry plants as compared to hydrated ones, confirming the result obtained with the NaOH fraction. Interestingly, the contents of both Xyl and Gal were higher than Glc content in the cell walls of dry plants. As Glc is usually the predominant sugar in XG, this indicates that Xyl and Gal may originate from other polymers than XG or that XG from dry plants evolved a different structure and sugar composition.

**Determination of XG structure** To analyse further the change in the Glc content of the NaOH fraction, we focused on XG, the major hemicellulosic polysaccharide in primary walls of dicot plants, which is the main source of Glc in this fraction. To determine the XG structure, the NaOH soluble fraction was digested with endoglucanase to generate specific XG-fragments that were analysed by MALDI-TOF mass spectrometry. For hydrated and dry plants, three main ions were obtained which corresponded to XG-derived...
Table 1. Amount of the cell wall material and carbohydrates solubilized with hot water, EDTA and NaOH from hydrated and dry plants. Yields are expressed as weight percent of the total cell wall material. Assays for total sugars and uronic acid were repeated 10 and 6 times, respectively*. Values are expressed in µg mg⁻¹ cell wall. Protein content was estimated according to Bradford assay.

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<th>Hydrated plants</th>
<th>Dry plants</th>
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<tr>
<td>Cell wall (mg g⁻¹ lyophilized leaves)</td>
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<tr>
<td>Hot water</td>
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<tr>
<td>Yield (W/W)</td>
<td>15%</td>
<td>15%</td>
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<tr>
<td>Total sugars*</td>
<td>128±10</td>
<td>110±16</td>
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<tr>
<td>Uronic acids*</td>
<td>42±12</td>
<td>36±7</td>
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<tr>
<td>Proteins</td>
<td>4.3</td>
<td>5.6</td>
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<tr>
<td>EDTA</td>
<td></td>
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<tr>
<td>Yield (W/W)</td>
<td>28%</td>
<td>32%</td>
</tr>
<tr>
<td>Total sugars*</td>
<td>140±38</td>
<td>259±29</td>
</tr>
<tr>
<td>Uronic acids*</td>
<td>50±25</td>
<td>102±25</td>
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<tr>
<td>Proteins</td>
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<tr>
<td>NaOH</td>
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<td>Yield (W/W)</td>
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<td>36%</td>
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<tr>
<td>Total sugars*</td>
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<td>97±14</td>
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<tr>
<td>Proteins</td>
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<td>4.7</td>
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<tr>
<td>Residue (CWR)</td>
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<tr>
<td>Yield (W/W)</td>
<td>20%</td>
<td>17%</td>
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oligosaccharides and were composed only of three to five hexoses, interestingly with only two pentoses (Fig. 5). This MALDI-TOF analysis enabled us to conclude firstly that the XG of *C. wilmsii* is not fucosylated, as the mass spectra did not show any ion compatible with structure containing a deoxy-hexose. Secondly, the core structure of this XG appeared to be of a XXGG-type structure containing a deoxy-hexose. Thirdly, the content and structure of certain matrix polysaccharides such as XG was altered during dehydration.

**Discussion**

The result of this study demonstrates that: (1) leaf cell walls of *C. wilmsii* contain the same major polysaccharides found in the primary cell walls of many dicot plants; (2) that the hemicellulosic polysaccharide XG has a particular structure with no Fuc substitutions like XG from Solanaceous plants (Vincen et al. 1997) and the storage XG of tamarind seeds (York et al. 1993); and (3) that the content and structure of certain matrix polysaccharides such as XG was altered during dehydration.

**Overall composition of *C. wilmsii* cell wall**

The carbohydrate components of *C. wilmsii* cell walls were separated into four fractions, the water, EDTA, and NaOH-soluble fractions, as well as the remaining cell wall residue. Leaf cell walls of *C. wilmsii* were characterized by a large proportion of homogalacturonans as judged from the EDTA fraction which contained up to 60% Gal A. This fraction represents around 30% of leaf cell walls twice that of the water fraction. Similarly, sugar composition of water-soluble polysaccharides revealed Gal A as a major sugar (around 30%) indicative of the presence of homogalacturonan (HG). According to the classification of pectins by Schols and Voragen (1996), which is based in part on the ratio of Rha to Gal A, rhamnogalacturonans are differentiated from homogalacturonans by having a ratio Rha/Gal A varying between

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<td>Ara</td>
<td>16.5±4.9</td>
<td>25.7±7.7</td>
<td>12.4±1.8</td>
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<td>8.7±0.6</td>
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<td>6.4±1.5</td>
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<td>Xyl</td>
<td>3.1±1.6</td>
<td>2.8±1.1</td>
<td>4.8±0.7</td>
<td>3.5±2.0</td>
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<td>19.9±1.0</td>
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<tr>
<td>Man</td>
<td>1.7±1.1</td>
<td>2.5±0.3</td>
<td>0.9±0.1</td>
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<td>7.2±1.0</td>
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<td>Gal</td>
<td>18.1±11.2</td>
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<td>9.7±2.4</td>
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<td>17.4±2.9</td>
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<td>Glc</td>
<td>11.8±3.7</td>
<td>7.2±1.7</td>
<td>3.1±1.6</td>
<td>3.1±0.7</td>
<td>30.5±3.1</td>
<td>17.7±1.9</td>
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<td>GlcA</td>
<td>14.0±6.9</td>
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<td>1.6±0.4</td>
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<tr>
<td>Gal A</td>
<td>32.1±5</td>
<td>38.9±3.7</td>
<td>61.3±5.9</td>
<td>60.2±6.1</td>
<td>13.1±1.8</td>
<td>17.4±1.6</td>
<td>11.8±4.3</td>
<td>4.7±3.7</td>
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Table 2. Monosaccharide composition of the cell wall fractions from hydrated (H) and dry (D) *C. wilmsii* leaves. Values are mol percentage of the sugar recovered. Data are means of at least 3-values.* Sugars solubilized from residual material by TFA, does not include cellulose.
0.05 and 1. The Rha to Gal A ratio in the water fraction ranged between 0.13 and 0.16 for hydrated and dry plants, indicating that rhamnogalacturonan was also present. Calculation of this ratio for the EDTA fraction indicates the presence of the polysaccharide rhamnogalacturonan I (RG I), probably in lower amounts than in the water fraction. The presence of Ara and Gal in both fractions suggests the occurrence of these sugars on side chains of RG I. However, these residues may reflect the presence of arabinogalactan or arabinogalactan-proteins, the latter being detected by Yariv staining on agarose gel in both fractions (Fig. 4). Glycosyl-residue composition of the alkali-soluble fraction showed that it contains predominantly Glc, Xyl and Gal, which are the primary sugars of XG of primary walls (Bacic et al. 1988). Thus, as in all dicot plants, XG seems to be the major hemicellulosic polysaccharide in C. wilmsii leaf cell walls. However, other hemicelluloses such as xylans or glucomannans might also be present. In addition, xyloglucan-like polysaccharide was released upon TFA treatment of the cell wall residue, indicating that some XG in C. wilmsii walls was also tightly bound to cellulose. However, in this study we did not check for the presence of rhamnogalacturonan II (RG II). Nevertheless, using anti-RG II antibodies (Matoh et al. 1998) and immunocytochemistry we could detect this polysaccharide in the cell walls of C. wilmsii leaves (Vicré 2001).

Desiccation-induced alterations of cell wall polysaccharides

In a previous immunocytochemical study, we showed that dehydration of C. wilmsii was accompanied by cell wall folding and alteration of XG and pectin epitopes content. No such changes were observed in a control non-tolerant plant Pisum sativum (Vicré et al. 1999). In the present study, we found that the total sugars and uronic acids content in the EDTA soluble fraction was much higher in dry than in hydrated plants (Table 1), which is suggestive of a possible increase in pectins. This increase would explain the increase in the immunogold-labelling of acidic pectins we previously observed (Vicré et al. 1999). However, a complete solubilization of EDTA fraction prepared from hydrated leaves was always difficult to achieve. Attempts to further solubilize these fractions with TFA significantly reduced the difference in the content of total sugars between hydrated and dry plants. Thus the difference in uronic acid observed could be attributed to modifications of pectin solubilization properties rather than specific changes in the amount of these polysaccharides. This is interesting as it reflects changes in the reorganization and may be bonding of pectins to other wall polymers, which may occur upon desiccation. Such changes would affect the overall architecture of the cell wall, thereby contributing to the observed folding (i.e. extensibility) and alterations in the level of pectic
It is worth noting that Sherwin (1995) showed that the walls, increasing its elasticity in response to dehydration. Another striking difference in sugar composition is that Glc content of the alkali-soluble fraction was much lower in dry plants than in hydrated ones. This was also the case for the TFA-soluble fraction after hydrolysis of the cellulose-rich residue. In theory, any starch present could suggest a possible XG trimming during dehydration. Considering the large degree of shrinkage in cell volumes and the great capacity of the wall to fold, it seems reasonable to expect the cell walls of leaves of *C. wilmsii* to be remarkably elastic during drying in order to prevent any irreversible damage. Furthermore, changes in XG have previously been reported to play a role in many plant species in response to water stress. Zwiazek (1991) indicated that white spruce needles had increased hemicellulose content after preconditioning treatment or severe drought stress exposure and Kubacka-Zebalska and Kacperska (1999) found that the hemicellulose fraction of cold acclimated leaves of winter oilseed rape subjected to frost treatment had decreased Glc and Xyl contents.

Sugar metabolism plays a major role in desiccation tolerance of *C. plantagineum* (Bianchi et al. 1992, Ingram et al. 1997, Norwood et al. 1999, 2000). This resurrection plant accumulates a large amount of octulose (a rare sugar in higher plants) in fully hydrated leaves tissues, whereas upon dehydration octulose level decreases and sucrose concentration increases up to 90% of the total sugar content (Bianchi et al. 1992). It would appear that *Craterostigma* species have evolved a capacity to accumulate sucrose very rapidly from carbohydrate sources already present in the leaf. In the case of *C. wilmsii*, one of these carbohydrate sources could be the cell wall, and more particularly XG. Thus, an additional explanation for the loss of Glc in dry plants would be that Glc is used for sucrose synthesis. The Glc released either from the cell walls or starch might be an important energy source within the cytoplasm, for the induction of protection mechanisms involved in desiccation tolerance in this species.

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