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Mucilages and polysaccharides in *Ziziphus* species (Rhamnaceae): localization, composition and physiological roles during drought-stress

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Abstract

The drought-tolerant tree species *Ziziphus mauritiana* Lamk. and *Z. rotundifolia* Lamk. were shown to have similar high mucilage concentrations (7–10% dry weight) in their leaves, with large numbers of mucilage-containing cells in the upper epidermis and extracellular mucilage-containing cavities in the leaf veins and stem cortex. The main sugar constituents of the water-soluble mucilage extract were rhamnose, glucose and galactose. During drought-stress in two independent studies, foliar mucilage content was unaffected in both species, but glucose and starch contents declined significantly in crude mucilage extracts from droughted leaves. Enzymatic hydrolysis of the mucilage extract using α-amylase and amyloglucosidase released glucose, indicating that a mucilage-associated water-soluble glucan, with α-1,4- and α-1,6-linkages, may exist which was extracted together with the mucilage. From the current data, it is not possible to localize the glucan to determine whether or not it is associated with mucilage-containing cells. Data from pressure–volume analyses of drought-stressed and control leaves showed that, in line with their similar mucilage contents, the relative leaf capacitance isotherm (change in relative water content per unit change in water potential) was similar in both species. During drought-stress, reduced relative capacitance resulted from osmotic adjustment and decreased wall elasticity. Data suggest that in *Ziziphus* leaves, intracellular mucilages play no part in buffering leaf water status during progressive drought. In *Ziziphus* species, growing in environments with erratic rainfall, the primary role of foliar mucilage and glucans, rather than as hydraulic capacitors, may be as sources for the remobilization of solutes for osmotic adjustment, thus enabling more effective water uptake and assimilate redistribution into roots and stems prior to defoliation as the drought-stress intensified.

Key words: Drought, mucilage, polysaccharides, water relations, *Ziziphus*.

Introduction

Polysaccharide hydrocolloids including mucilages, gums and glucans are abundant in nature and are commonly found in many higher plants. These polysaccharides constitute a structurally diverse class of biological macromolecules with a broad range of physicochemical properties which are widely used for applications in pharmacy and medicine (Franz, 1989).

Although mucilages can occur in high concentrations in different plant organs, their physiological function in most cases is unclear. Mucilages found in rhizomes, roots and seed endosperms may act primarily as energy reserves (Franz, 1979) whereas foliar mucilages appear not to serve as storage carbohydrates (Diestelbarth and Kull, 1985). Generally, it has been assumed that foliar mucilages are merely secondary plant metabolites (Naglschmid *et al.*, 1982), but there are reports that they may play a role in frost tolerance (Goldstein and Nobel, 1991; Lipp *et al.*,

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1994), water transport (Zimmermann *et al*., 1994), wound responses (Clarke *et al*., 1979), plant host–pathogen interactions (Davis *et al*., 1986), the ionic balance of plant cells (Pollak and Albert, 1990; Trachtenberg and Mayer, 1982), and as carbohydrate reserves (Pimienta-Barrios and Nobel, 1998).

Due to the high concentration of hydroxyl groups in the polysaccharide, mucilages generally have a high water-binding capacity and this has led to studies of their role in plant water relations. It has been suggested that the ability of mucilage to hydrate may offer a mechanism for plants to resist drought (Clarke *et al*., 1979). There is growing evidence that, due to the high water-holding capacity of the polysaccharide, extracellular mucilages in particular may play an important role in the drought resistance of certain plant species (Goldstein and Nobel, 1991; Morse, 1990; Nobel *et al*., 1992). In *Hemizonia luzifolia*, extracellular mucilages have been demonstrated to buffer leaf water status against environmental fluctuation during the middle of the day (Morse, 1990). By acting as an apoplastic capacitor (Nobel *et al*., 1992), mucilages can also enable leaves to maintain water potential when soil water deficits develop (Robichaux and Morse, 1990).

Mucilaginous substances have been reported as occurring widely in the Rhamnaceae, including the genus *Ziziphus* (Metcalf and Chalk, 1950). However, previous reports on mucilaginous cells in leaf and stem material of *Ziziphus* have not addressed the abundance, localization, chemical characteristics or potential role of mucilages in this genus (Metcalf and Chalk, 1950).

Trees and shrubs of *Ziziphus* are characteristically found in many semi-arid regions of the world and can thrive in water-limited environments which may be unsuitable for other forms of annual crop cultivation (Jawanda and Bal, 1978).

Recently, research into the mechanisms for drought tolerance and avoidance in *Ziziphus* species has shown that *Z. mauritiana* and *Z. rotundifolia* exhibit a range of drought avoidance adaptations to progressive drought-stress. Under well-irrigated conditions, *Ziziphus mauritiana* exhibited high rates of net photosynthesis and transpiration compared to other fruit tree species (Clifford *et al*., 1997), but during early stages of drought-stress, the stomata were very sensitive to water deficit and reduced stomatal conductance effectively increased intrinsic water use efficiency in the short term (Arndt *et al*., 2001). However, as water deficits increased, osmotic adjustment occurred concomitant with an increased root : shoot ratio to access deep soil moisture reserves, followed by leaf loss and ultimately drought-enforced dormancy (Arndt *et al*., 2001; Clifford *et al*., 1998).

This paper examines the location of mucilages in *Z. rotundifolia* and *Z. mauritiana* and studies the effects of drought-stress treatments imposed in two separate experiments on the changes in abundance and chemical composition of crude mucilage extracts. The ecophysiological implications of mucilages and associated polysaccharides in *Ziziphus* species are discussed.

Materials and methods

Localization of mucilages

Samples for anatomical studies were taken from the collection of *Z. mauritiana* (Gola) and *Z. rotundifolia* held in the glasshouse at HRI, Wellesbourne UK. Trees were grown in 65 l pots in a free-draining growing medium containing equal parts Levingtons C2, grit and John Innes No 2. Pre-sampling, the average day/night temperatures were 24/17 °C, with 2.14/1.45 kPa vapour pressure deficit and 370 μmol m^{-2} s^{-1} photosynthetically active radiation. Small (not > 5 mm square) pieces were cut from the central leaf lamina and secondary stem. Material was either mounted in Tissue Tek O.C.T™ medium (Sakura bv, The Netherlands) and sectioned fresh using a cryo-microtome (Bright Instruments Ltd, Huntingdon, UK), or vacuum infiltrated for 3 h in 3% glutaraldehyde (0.1 M phosphate buffer pH 7.2), dehydrated through a graded ethanol series and embedded in London Resin White resin. Fresh sections were stained with 1% alcian blue for polyanionic polysaccharides (O'Brian and McCully, 1981), whilst 1 μm resin sections were cut (Reichert-Jung Ultracut) and stained for light microscopy using azure/methylene blue (Richardson *et al*., 1960), periodic acid-Schiff (PAS) for starch and polysaccharides (Jensen, 1962), or toluidine blue-O, a general polyanionic stain (Feder and O'Brien, 1968).

Chemical analyses of mucilage

Changes in mucilage content during drought-stress: Foliar samples for mucilage analysis were derived from two separate studies investigating drought-stress: one using *Z. mauritiana* and the second using *Z. rotundifolia*. (1) Potted *Z. mauritiana* trees were exposed to a single 12 d drought-stress cycle as described previously (Clifford *et al*., 1998). (2) In the second experiment, 2-year-old potted *Z. rotundifolia* seedlings were exposed to either gradual or rapid drought-stress development imposed through regulated deficit irrigation over a 34 d cycle as described earlier (Arndt *et al*., 2001).

For chemical analyses, leaf material was collected from both treatments pre-stress and after 12 d in experiment (1), whilst in experiment (2), comparisons were made between control and both stress regimes after 34 d of treatment. In all treatments, foliar samples were collected and analysed from four trees per treatment.

Extraction and purification of water-soluble polysaccharides: Data on content and chemical composition of *Ziziphus* leaf mucilage were compared with that of commercially available (Flos Tiliae EAB, Vienna, Austria) lime tree flower mucilage (*Tilia cordata* L.) for which content and composition is well documented (Kram and Franz, 1983, 1985). Samples of *Ziziphus* and lime tree flower mucilage were analysed in parallel.

For *Ziziphus*, dried leaf samples were ground to a fine powder in a ball mill (Retsch MM2, Vienna, Austria). Water-soluble polysaccharides were extracted from 100 mg dried leaf material in 2 ml water at 20 °C. The extracts were shaken, put in an ultrasonic bath for 2 min, incubated for 15 min in a water bath at 35 °C and shaken for 2 h on a horizontal shaker. After

centrifugation, the superanatant was removed and stored. The pellet was re-extracted twice more using the same procedure, and the aqueous supernatants combined prior to dialysis (MWCO 12–14 kDa, ZelluTrans, Roth, Karlsruhe, Germany) for 16 h, to remove all low molecular weight compounds. The dialysed extracts were freeze-dried and the dry weight of the lyophilized crude mucilage determined.

Enzymatic hydrolysis of polysaccharides and starch: Water-soluble polysaccharides extracted with the leaf mucilage were enzymatically degraded to glucose monomers using a combined α-amylase and amyloglucosidase hydrolysis. Glucose was quantified by high performance anion exchange chromatography as described previously (Arndt *et al*., 2000).

Starch: After repeated extraction of low molecular weight carbohydrates of homogenized leaf samples with ethanol : water mixtures (Ho, 1976) and enzymatic degradation of starch with heat stable α-amylase and amyloglucosidase, glucose was quantified by high performance anion exchange chromatography (HPAEC-PAD; DX 500 and ED 40, Dionex, Vienna, Austria (Arndt *et al*., 2000)).

Acidic hydrolysis of crude mucilage: Acid hydrolysis of the crude mucilage was performed by adding 300 µl 2 M trifluoroacetic acid to 1 mg of crude mucilage and incubating at 120 °C for 90 min. After centrifugation (5 min, 15 000 rpm), the sample was dried under vacuum. Twice, the sample was redissolved in water and redried under vacuum to remove any remaining acid. Carbohydrates were determined using gas chromatography (Arndt *et al*., 2000).

Water relations: Pre-dawn leaf water status was determined for each treatment using a pressure chamber as described previously (Clifford *et al*., 1998), in eight trees per treatment in experiment 1 (3 outer canopy fully expanded leaves per tree), and in shoots from five trees per treatment in experiment 2.

From the second drought experiment described above, pressure–volume curves were derived for three trees in each of the control and gradual stress treatments (three replicate shoots per tree), both pre-stress, and after 34 d of treatment, with irrigated controls measured on each occasion. The repeat pressurization technique was used as described previously (Clifford *et al*., 1998). Water potential isotherms were constructed from the pressure–volume data.

Data analysis: Data for replicate trees were analysed by analysis of variance as a 2×2 factorial using Genstat 5 for Windows (version 4.1; Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

Results and discussion

Leaf anatomy and localization of mucilage

In *Z. mauritiana*, the abaxial leaf surface was characteristically densely pubescent, with numerous stomata arranged in the interveinal regions, whereas the adaxial surface was glabrous, with comparatively few, sunken stomata. Leaf hairiness, hypostomatous distribution and sunken stomata are all characteristic features of species that exist in drought-prone regions. Transverse sections from fresh leaf lamina material showed that both *Ziziphus* species have characteristic C_3 anatomy, with an abundance of mucilaginous material exclusively localized in the adaxial epidermal cells which stained intensely with the mucopolysaccharide stain, alcian blue (Fig. 1a). The mucilage-cell contents also stained positively and uniformly with the periodic acid-Schiff reagent (PAS), indicating a high polysaccharide content throughout the cell (rather than localized as would be seen in amyloplasts). When stained with the PAS/toluidine blue-O combination for light microscopy, there was no discernible nucleus, vacuole or cellular organelles in the epidermal mucilage cells, but with numerous starch grains and nuclei clearly visible in the mesophyll parenchyma (Fig. 1b) as previously reported for *Salix* (Mariani *et al*., 1988). Mucilage, produced in Golgi, accumulates initially between the plasmalemma and the cell wall, and after prolonged mucilage deposition, the remaining cytoplasm becomes compressed against the outer periclinal cell wall and degenerates (Bakker and Gerritsen, 1992). In contrast to the intracellular mucilage secretion observed in *Ziziphus*, in the high polysaccharide subspecies of *Hemizonia luzifolia*, leaves have been shown to have large volumes of extracellular mucilage within the spongy-mesophyll layer (Morse, 1990).

Although mucilages in the leaf lamina of *Ziziphus* were intracellular, the cortical and peripheral parenchyma of the major leaf veins and stems contained significant amounts of extracellular mucilage. The leaf veins had mucilage-filled spaces between the large-thin-walled outer collenchymatous cortical cells (Fig. 1c), whilst in the stem, intercellular spaces in the central cortex and in the peripheral cortex surrounding the phloem were filled with mucilage (Fig. 1d) which, when viewed in longitudinal section, formed intercellular channels (Fig. 1e). A similar pattern of schizogenous mucilage has also been reported for *Hibiscus schizopetalus*, in which the degeneration of mature mucilage cells resulted in canal like, mucilage-filled cavities running parallel to minor veins (Bakker and Gerritsen, 1992).

Impact of drought-stress on leaf mucilage content

Data from both drought experiments showed that the total amount of mucilage was similar in both species with, on average, leaves of *Z. mauritiana* and *Z. rotundifolia* typically containing 7.1% and 9.6% mucilage, respectively, on a leaf dry matter (DM) basis (Table 1). Compared to most other species, and particularly tree species, these values are high and most of the species listed in Table 1 contained not more than 3% mucilage in their leaves (expressed on DM basis). In contrast to this are species that accumulate high levels of extracellular mucilage, such as the annual composite *Hemizonia luzifolia* (4–30% DM depending on subspecies (Morse, 1990)), and cacti up to 37% DM, (Nobel *et al*., 1992).

In neither experiment was the total amount of mucilage in the leaves of *Ziziphus* affected by drought

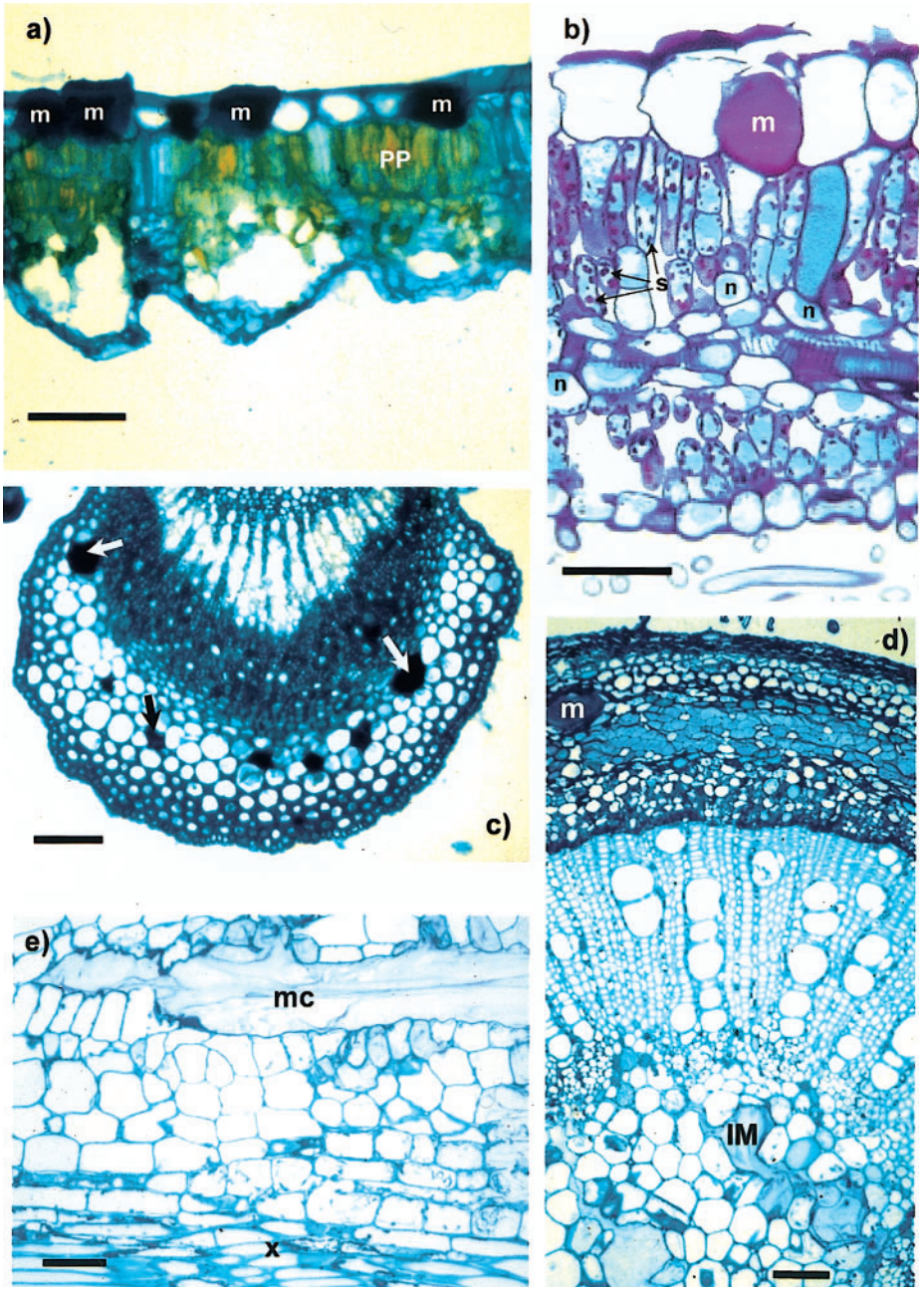

Fig. 1. (a) Fresh transverse section of leaf lamina of *Ziziphus mauritiana*; ×136 magnification, stained with alcian blue. Abundant mucilage (m) in adaxial epidermal cells stain an intense blue. Chlorophyll in the palisade parenchyma (PP) appears green. Scale bar represents 100 μm. (b) Transverse section of resin-embedded leaf lamina of *Ziziphus rotundifolia*; ×157 magnification, stained with a periodic acid-Schiff/toluidine blue O combination. Mucopolysaccharide material (m) is uniformly stained in the thick-walled adaxial epideramal cell. Abundant starch grains (s) are present in all palisade and spongy parenchyma, with nuclei (n) clearly stained. Scale bar represents 50 μm. (c) Fresh transverse section of a main leaf vein in *Zizpihus mauritiana*; ×97 magnification, stained with aniline blue. Intensely staining tracts of intercellular mucilage (arrows) are present in the peripheral cortex. Scale bar represents 100 μm. (d) Transverse section of resin-embedded stem of *Ziziphus mauritiana*; ×154 magnification, stained with azure/methylene blue. Large mucilage-filled intercellular spaces (IM) are present in the inner cortex, with additional extracellular mucilage (m) also present in the peripheral cortex. Scale bar represents 50 μm. (e) Longitudinal section of resin-embedded stem of *Ziziphus mauritiana*; ×175 magnification, stained with azure/methylene blue, showing an intercellular mucilage channel (mc) running through the inner cortex adjacent to the primary xylem (x). Scale bar represents 50 μm.

(Table 2). This is similar to the report that there were no differences in the water-soluble mucilage content extracted from Boraginaceae from wet and dry habitats (Pollak and Albert, 1990), but contrasts with early findings in *Prunella* (Volk, 1938), *Brunella grandifolia* (Jeremias, 1966) in which mucilaginous substances increased during drought-stress. Seasonal variation in leaf mucilage content was reported (Diestelbarth and

Table 1. *A comparison of crude foliar mucilage content and sugar composition of water-soluble polysaccharides of* Z. mauritiana *and* Z. rotundifolia *after acid hydrolysis, compared to leaf mucilage sugar compositions published for other plant species and flower mucilage from* Tilia cordata

Plant species	Content (% DW)	% of total mucilage sugar content							Reference
		Rha	Gal	Ara	Xyl	Man	Glc	Div	
Ziziphus mauritiana	7.1	26.4	25.6	17.4	8.6	–	22.0	–	
Ziziphus rotundifolia	9.6	31.6	18.0	12.6	4.1	2.5	31.3	–	
Cassia angustifolia	2.5	26.1	26.7	40.9	tr	tr	6.3	–	Müller *et al*., 1989
Corchorus olitorius	2.0	20.4	61.2	12.3	6.1	–	–	–	Hegnauer, 1973
Ginkgo biloba	5.9	35.3	20.2	26.7	0.5	8.0	9.3	–	Höllriegl *et al*., 1986
Dicerocaryum zanguebarium	–	–	39.6	22.6	35.8	1.9	–	–	Benhura and Marume, 1993
Malva sylvestris	–	64.4	35.6	–	–	–	–	–	Tomoda *et al*., 1989
Malva sylvestris	–	15.0	46.3	25.0	5.0	–	8.7	–	Franz, 1966
Opuntia ficus–indica	–	13.1	40.1	24.6	22.2	–	–	–	Trachtenberg and Mayer, 1981
Plantago lanceolata	0.8	6.9	44.4	32.4	1.9	4.0	9.2	1.1[a]	Bräutigam and Franz, 1985*b*
Taxus baccata	3.0	–	–	–	–	–	–	–	Diestelbarth and Kull, 1985
Thuja occidentalis	2.1	–	–	–	–	–	–	–	Diestelbarth and Kull, 1985
Tilia cordata, flowers	4.1	36.9	26.1	13.7	7.3	6.5	9.6	–	
Tilia cordata, flowers	3.0	30.3	32.9	22.1	5.9	3.0	5.8	–	Kram and Franz, 1983

Rha, Rhamnose; Gal, Galactose; Ara, Araboinose; Xyl, Xylose; Man, Mannose; Glc, Glucose; Div: a, Fucose; b, Ribose; tr, trace.
Cassia, Caesalpiniaceae; *Corochis*, Tiliaceae; *Ginkgo*, Ginkgoaceae; *Dicerocarium*, Pedaliaceae; *Malva*, Malvaceae; *Opuntia*, Cactaceae; *Plantago*, Plantaginaceae.

Table 2. *Amounts of crude mucilage in drought-stressed leaves of* Z. mauritiana *and* Z. rotundifolia *together with glucose content derived from enzymatic hydrolysis of the water-soluble crude mucilage extract*

Composition of *Tilia cordata* flower mucilage, analysed using the same techniques, are included for comparison (*/** significant at $P < 0.05/0.01$).

	Day	Pre-dawn Ψ_{leaf} (MPa)	Crude mucilage content (% DM, leaf)	Glucose from water-soluble crude mucilage extract, enzymatically hydrolysed		Starch content (% DM, leaf)
				(% DM, crude mucilage)	(% DM, leaf)	
Experiment 1: *Z. mauritiana*						
Control	day 0	−0.50	7.05	30.66	2.05	6.50
Stressed	day 0	−0.48	7.38	30.71	2.31	6.94
Control	day 12	−0.49	7.22	27.82	2.03	6.65
Stressed	day 12	−1.83**	6.90	4.32**	0.30**	1.69*
5% LSD (df)		0.304 (28)	1.699 (12)	11.38 (12)	0.963 (12)	3.674 (12)
Experiment 2: *Z. rotundifolia*						
Control	day 34	−0.10	7.95	11.60	0.91	3.29
Gradual stress	day 34	−1.52**	8.80	6.12*	0.55	0.91*
Rapid stress	day 34	−1.94**	9.76	3.48**	0.35*	0.45*
5% LSD (df)		1.08 (12)	3.291 (10)	4.938 (10)	0.483 (10)	2.022 (9)
Tilia cordata						
Flowers			4.10	2.00	0.19	–

Kull, 1985) and it is possible that, either the duration of stress in the current studies may have been too short (12 d and 34 d) to affect gross mucilage metabolism, or that any underlying decrease in mucilage may have been offset by the accumulation of another unidentified polymer.

Mucilage composition

Acid hydrolysis of crude leaf mucilage extract in both *Z. mauritiana* and *Z. rotundifolia* revealed that the main sugar constituents were rhamnose, glucose, galactose, and arabinose (Table 1). The most interesting observation was the extremely high glucose content in the crude mucilage extracts for both *Ziziphus* species, with at least 2-fold higher glucose content compared with other species for which data are available (Table 1). The hexuronic acids, galacturonic acid and glucuronic acid, were also present in the hydrolysate, but at lower concentrations than the main sugar components (data not shown), and the high levels of the neutral sugars rhamnose, galactose and arabinose are consistent with published leaf mucilage composition for other species (Table 1). Leaf mucilages usually have pectin like structures with large amounts of D-galactose and L-arabinose (Bräutigam and Franz, 1985*b*), with the disaccharide 4-α-galacturonosylgalacturonic acid as the major repeating unit (Bailey, 1965). However, mucilages generally comprise a mixture of polysaccharides (Bräutigam and Franz, 1985*a*; Müller *et al*.,

1989) and the high glucose levels resulting from the acid hydrolysis of the water-soluble mucilage extract in *Ziziphus* may suggest the presence of abundant glycosidic polysaccharides, or glucans. To test this idea, crude leaf mucilage extract from *Ziziphus* was enzymatically hydrolysed with α-amylase and amyloglucosidase, and compared with the well-characterized mucilage from lime tree (*Tilia*) flowers (Table 1). Alpha-amylase and amyloglucosidase are two specific enzymes that hydrolyse α-1,4 and α-1,6 glycosidic linkages and are commonly used in starch determination to degrade the two major starch constituents amylose and amylopectin. Compared to *Tilia* flower mucilage, relatively high glucose concentrations were measured following enzymatic degradation of *Ziziphus* mucilage extracts. It is extremely unlikely that the glucose was starch-derived, since foliar starch would be insoluble in water at 30 °C, the extraction temperature for mucilages in the current study, which was well below the gelatinization point (Morrison, 1992). Alpha-amylase and amyloglucosidase do not possess the mixed linked β-glucanase activity necessary to hydrolyse β-glycosidic bondages (Åman and Hesselman, 1984), and therefore it is also unlikely that glucose was released directly from a mucilaginous polysaccharide, since glycosides, which contain uronic acid, are commonly linked in β-glycosidic bonds (Bailey, 1965) as has been demonstrated for leaf mucilage from *Plantago lanceolata* (Bräutigam and Franz, 1985*b*). Consequently, the most likely source of the glucose would be a water-soluble glucan, present at high concentrations in *Ziziphus* leaves, that was co-extracted with the mucilage, as has been reported previously for *Tilia* flower mucilage, in which it was also suggested that the glucose was glucan- rather than starch-derived (Kram and Franz, 1983).

Data from enzymatic hydrolysis also revealed that in both *Ziziphus* species, the glucose content in crude mucilage declined significantly during drought-stress (Table 2), and that the extent of the decrease was associated with the degree of drought-stress, with significantly lower glucose contents in rapidly- than in gradually-stressed leaves at the end of the 34 d drought cycle. The large reductions in glucose content indicates that the foliar glucan may be relatively easily metabolized or remobilized. However, from the available data, it is not possible to localize the glucan within the leaf tissue. However, if one assumes that the majority of mucilage cells are metabolically inactive (see above), the observed decrease in glucose during drought-stress indicates that the glucan may be located in the metabolically active mesophyll or epidermal cells. In addition, it is conceivable that water-soluble glucans occur more frequently also in other plant species, but that they have not been detected due to the conventional procedures used for starch analysis. During starch determination in plant samples low molecular weight compounds are removed by repeated washing of ground plant material with ethanolic solutions. Therefore, any water-soluble glucans, which are not soluble in ethanol, are degraded with starch in the subsequent enzymatic hydrolysis and consequently, their presence may result in erroneous starch determination.

Previous studies showed that during drought-stress, starch levels declined, with large increases in the concentrations of hexose sugars in the crude bulk leaf extract. The current work indicates that although stored starch may contribute to increases in hexoses during drought-stress, a high proportion of solute accumulation in bulk leaf tissue may be accounted for by the high levels (1–2% DM, derived from Table 2) of water-soluble glucan extracted together with the mucilage. This would provide a source of easily degradable carbohydrate contributing to rapid remobilization of sugars and solutes for osmotic adjustment, cyclitol and proline accumulation (Clifford *et al*., 1998), and increased root : shoot ratio (Arndt *et al*., 2000) observed in *Ziziphus* during drought-stress.

Effects of drought-stress on relative leaf capacitance

Data in Fig. 2 superimpose the water potential isotherms (changes in Ψ_{leaf} with relative water content (θ)) from both experiments for bulk leaf tissue of control irrigated and drought-stressed *Z. rotundifolia* and *Z. mauritiana*. For each irrigation treatment, concomitant with the similar amounts of mucilage in both species, the water potential isotherms were very similar. In *Z. mauritiana*, θ changed more quickly per unit change in Ψ_{leaf} over the first 5% drop in θ than in *Z. rotundifolia*, but was similar thereafter. This shows that, under irrigated conditions, *Z. rotundifolia* may generate a slightly more negative Ψ in the plant, thereby improving water uptake under these conditions for favourable growth in this vigorous species.

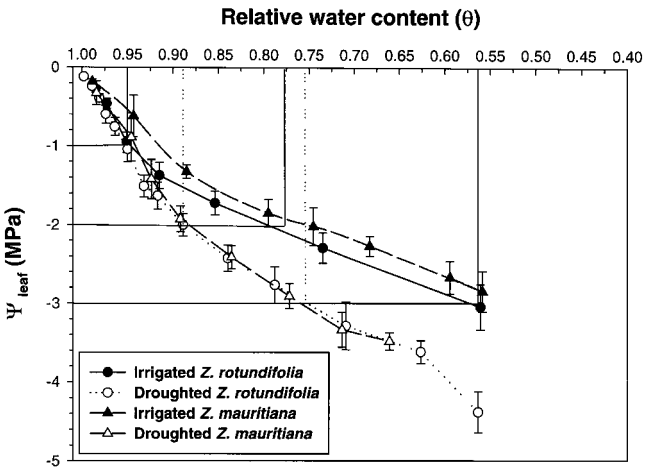

Fig. 2. Water potential isotherms for rehydrated control and drought-stressed leaves from *Ziziphus rotundifolia* and *Z. mauritiana*. Solid (control) and dotted (stressed) lines extending to the *x*-axis indicate the extent to which θ varied between irrigation treatments at Ψ_{leaf} −1, −2 and −3 MPa (±s.e.; $n = 3$).

Indeed, this may be one reason why *Z. rotundifolia* has commonly been used as a rootstock for selectively improved, large-fruited (less vigorous) cultivars of *Ziziphus* in India.

Comparing the isotherms from the different irrigation treatments, below θ of ~92%, Ψ_{leaf} in the drought-stressed treatments continued to decline more rapidly per unit change in θ than in the irrigated controls (Fig. 2). This is consistent with the accumulation of solutes in the bulk leaf tissue and osmotic adjustment as reported previously for both species (Clifford *et al*., 1998; Arndt *et al*., 2001), with a higher θ maintained for a given value of Ψ_{leaf}. From the pressure–volume curves, it was clear that although Ψ_{leaf} was depressed during drought-stress, the point of turgor loss was the same in droughted and irrigated controls (Clifford *et al*., 1998). This was due to a decrease in cell wall elasticity as drought developed and may be a short-term adaptation both to improve the ability of the plant to extract moisture from a progressively drying soil profile, and to maintain cell volume and metabolic activity at low soil water potential.

Due to the colloidal nature of mucopolysacchardes, their capacitance is generally high (Goldstein and Nobel, 1991), so they can lose significant amounts of water without large changes in water potential (Morse, 1990). In comparison with other species in which mucilages are reported to play an important role in plant water relations, the relative capacitance of leaves in *Ziziphus* is no greater than 0.21 MPa^{-1} (Fig. 2), which lies towards the lower end of the range reported for the high and low polysaccharide subspecies of *Hemizonia luzifolia*: 0.7 MPa^{-1} and 0.08 MPa^{-1}, respectively. In cacti, relative capacitance of the cladode parenchyma is 1.04 MPa^{-1}, with large amounts of mucilage-associated solutes which may aid their function as an apoplastic capacitor (Nobel *et al*., 1992). The most important difference between *Ziziphus* and these genera is that *Hemizonia* and cacti characteristically have large amounts of extracellular mucilage, whereas *Ziziphus* appears to have predominantly intracellular mucilage in the leaf lamina adjacent to the mesophyll cells. These observations confirm the idea that extracellular mucilages are effective in affecting cell water relations and increasing capacitance, with intracellular mucilages, as observed in *Ziziphus*, making no contribution to cellular capacitance (Morse, 1990).

As drought-stress intensified, leaf loss ensued, with an 80% reduction in leaf area by the end of the drying cycle in the rapid stress treatment (Arndt *et al*., 2001). Following the trial, the stressed trees were irrigated after 2 months without water. There was a rapid regrowth of shoots with recovery of the canopy (unpublished data).

In multi-site field trials of *Ziziphus* in Zimbabwe, trees grown in the Highveld areas subjected to sub-zero temperatures suffered high levels of foliar damage and loss, indicating that the foliar mucilage was ineffective in conferring low temperature tolerance. One potential role for foliar mucilages in *Ziziphus* may be as a store of secondary metabolites to deter herbivory or pathogens (Davis *et al*., 1986), but from the current study, there are no data available to test this. Therefore mucilages do not appear to benefit plant water relations in transpiring tissues, and following drought-enforced leaf loss, remobilization of solutes from water-soluble glucans and starch in the stems and roots (Arndt *et al*., 2001), act as significant carbohydrate and solute sources for survival during prolonged drought, facilitating rapid regeneration of shoot material once conditions become more favourable.

Conclusion

Large amounts of intracellular mucilage were present in the leaf lamina of *Ziziphus*, with extracellular mucilages in the main leaf veins and stem cortex. Data from the two drought experiments showed that there were no changes in total mucilage content during drought-stress, and that the predominantly intracellular foliar mucilages were ineffective in altering leaf capacitance during drought-stress, with water relations being dominated by changes in solute concentration and decreasing wall elasticity.

Biochemical analysis of the water-soluble polysaccharides, extracted together with the mucilage using enzymatic hydrolysis, indicated that a water-soluble glucan may be present in the mucilage extract. The main role of the glucan, together with foliar starch, may be as significant sources of monosaccharide sugars contributing to osmotic adjustment in the short-term, and to remobilization of solutes to other sink organs as drought intensified. By providing a form of energy and solute storage in non-transpiring tissue, glucans may together with starch, support survival during dry periods and promote rapid regrowth following rainfall.

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References

Åman P, Hesselman K. 1984. Analysis of starch and other main constituents of cereal grains. *Swedisch Journal of Agricultural Research* **14,** 135–139.

Arndt SK, Clifford SC, Wanek W, Jones HG, Popp M. 2001. Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought-stress. *Tree Physiology* **21,** 705–715.

Arndt SK, Wanek W, Clifford SC, Popp M. 2000. Contrasting adaptations to drought-stress in field-grown *Ziziphus mauritiana* and *Prunus persica* trees: water relations, osmotic

adjustment and carbon isotope composition. *Australian Journal of Plant Physiology* **27,** 985–996.

Bailey RW. 1965. *Oligosaccharides*. Oxford: Pergamon Press.

Bakker ME, Gerritsen AF. 1992. The development of mucilage cells in *Hibiscus schizopetalus*. *Acta Botanica Neerlandica* **41,** 31–42.

Benhura MAN, Marume M. 1993. The mucilaginous polysaccharide material isolated from ruredzo (*Dicerocaryum zanguebarium*). *Food Chemistry* **46,** 7–11.

Bräutigam M, Franz G. 1985*a*. Schleimpolysaccharide aus Spitzwegerichblättern. *Deutsche Apotheker Zeitung* **125,** 58–62.

Bräutigam M, Franz G. 1985*b*. Structural features of *Plantago lanceolata* mucilage. *Planta Medica* **4,** 293–297.

Clarke AE, Andreson RL, Stone BA. 1979. Form and function of arabinogalactans and arabinogalactan-proteins. *Phytochemistry* **18,** 521–540.

Clifford SC, Arndt SK, Corlett JE, Joshi S, Sankhla N, Popp M, Jones HG. 1998. The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* Lamk. *Journal of Experimental Botany* **49,** 967–977.

Clifford SC, Kadzere I, Jones HG, Jackson JE. 1997. Field comparisons of photosynthesis and leaf conductance in *Ziziphus mauritiana* and other fruit tree species in Zimbabwe. *Trees* **11,** 449–454.

Davis KR, Darvill AG, Albersheim P, Dell A. 1986. Host–pathogen interactions. XXIX. Oligogalacturonides released from sodium polypectate by endopolygalacturonic acid lyase are elicitors of phytoalexines in soybean. *Plant Physiology* **80,** 568–577.

Diestelbarth H, Kull U. 1985. Physiological investigations of leaf mucilages. II. The mucilages of *Taxus baccata* L. and of *Thuja occidentalis* L. *Israel Journal of Botany* **34,** 113–128.

Feder N, O'Brien MS. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* **55,** 123–142.

Franz G. 1966. Die Schleimpolysaccharide von *Althena officinalis* und *Malva sylvestris*. *Planta Medica* **14,** 89–110.

Franz G. 1979. Metabolism of reserve polysaccharides in tubers of *Orchis morio* L. *Planta Medica* **36,** 68–73.

Franz G. 1989. Polysaccharides in pharmacy: current applications and future concepts. *Planta Medica* **55,** 493–497.

Goldstein G, Nobel PS. 1991. Changes in osmotic pressure and mucilage during low-temperature acclimation of *Opuntia ficus-indica*. *Plant Physiology* **97,** 954–961.

Hegnauer R. 1973. *Chemotaxonomie der Pflanzen, Band 6: Dicotyledoneae: Rafflesiaceae–Zygophyllaceae*. Basel und Stuttgart: Birkhäuser Verlag.

Ho LC. 1976. The relationship between the rates of carbon transport and photosynthesis in tomato leaves. *Journal of Experimental Botany* **27,** 87–97.

Höllriegl H, Koehler H, Franz G. 1986. Über höhermolekulare Polysaccharide aus den Blättern von *Ginko biloba*. *Scientia Pharmaceutica* **54,** 321–330.

Jawanda JS, Bal JS. 1978. The ber, highly paying and rich in food value. *Indian Horticulture* **Oct–Dec,** 19–21.

Jensen WA. 1962. *Botanical histochemistry*. WH Freeman and Co.: San Francisco.

Jeremias K. 1966. Der Einfluβ der Bodentrockenheit auf den Zuckergehalt vegetativer Pflanzenteile. *Zeitschrift für Pflanzenphysiologie* **54,** 237–239.

Kram G, Franz G. 1983. Untersuchungen über die Schleimpolysaccharide aus Lindenblüten. *Planta Medica* **49,** 149–153.

Kram G, Franz G. 1985. Structural investigations on water-soluble polysaccharides of lime tree flowers (*Tilia cordata* L.). *Pharmazie* **40,** 501–502.

Lipp CC, Goldstein G, Meinzer FC, Niemczura W. 1994. Freezing tolerance and avoidance in high-elevation Hawaiian plants. *Plant, Cell and Environment* **17,** 1035–1044.

Mariani P, Rascio N, Baldan B, Paiero P, Urso T. 1988. Epidermal mucilage cells in leaves of *Salix* species. *Flora* **181,** 137–145.

Metcalf CR, Chalk L. 1950. *Anatomy of the Dicotyledons*,Vol. 1. Oxford: Claredon Press.

Morrison WR. 1992. Analysis of cereal starches. In: Linskens HF, Jackson JF, eds. *Seed analysis*. Berlin: Springer Verlag.

Morse SR. 1990. Water balance in *Hemizonia luzulifolia*: the role of extracellular polysaccharides. *Plant, Cell and Environment* **13,** 39–48.

Müller BM, Kraus J, Franz G. 1989. Chemical structure and biological activity of water soluble polysaccharides from *Cassia angustifolia* leaves. *Planta Medica* **55,** 536–539.

Naglschmid F, Kull U, Jeremias K. 1982. Physiologische Untersuchungen über Blattschleime. 1. Untersuchungen an *Verbascum densiflorum*. *Biochemie und Physiologie der Pflanzen* **177,** 671–685.

Nobel PS, Cavelier J, Andrade JL. 1992. Mucilage in cacti: its apoplastic capacitance, associated solutes and influence on tissue water relations. *Journal of Experimental Botany* **43,** 641–648.

O'Brian TP, McCully ME. 1981. *The study of plant structure: principles and selected methods*. Australia: Termarcarphi Pty Ltd.

Pimienta-Barrios E, Nobel PS. 1998. Vegetative, reproductive, and physiological adaptations to aridity of pitayo (*Stenocereus queretaroensis*, Cactaceae). *Economic Botany* **52,** 401–411.

Pollak J, Albert R. 1990. Stoffwechselphysiologische Charakterisierung der Boraginaceen unter besonderer Berücksichtigung ökologischer Aspekte. *Flora* **184,** 151–168.

Richardson KC, Jarett L, Finke EH. 1960. Embedding in epoxy resin for ultrathin sectioning in electron microscopy. *Stain Technology* **35,** 313–323.

Robichaux RH, Morse SR. 1990. Extracellular polysaccharide and leaf capacitance in a hawaian bog species, *Argyroxiphum grayanum* (Compositae-Madiinae). *American Journal of Botany* **77,** 134–138.

Tomoda M, Gonda R, Shimizu N, Yamada H. 1989. Plant mucilages. XLII. An anti-complementary mucilage from the leaves of *Malva sylvestris* var. *mauritiana*. *Chemical and Pharmaceutical Bulletin* **37,** 3029–3032.

Trachtenberg S, Mayer AM. 1981. Composition and properties of *Opuntia ficus-indica* mucilage. *Phytochemistry* **20,** 2665–2668.

Trachtenberg S, Mayer AM. 1982. Biophysical properties of *Opuntia ficus-indica* mucilage. *Phytochemistry* **21,** 2835–2843.

Volk OH. 1938. Untersuchungen über das Verhalten der osmotischen Werte von Pflanzen. *Zeitschrift für Botanik* **32,** 65–149.

Zimmermann U, Zhu JJ, Meinzer FC, Goldstein G, Schneider H, Zimmermann G, Benkert R, Thürmer F, Melcher P, Webb D, Haase A. 1994. High molecular weight organic compounds in the xylem sap of mangroves: implications for long-distance water-transport. *Botanica Acta* **107,** 218–229.