Rheological Basis of Splitting in Carrot Storage Roots

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ABSTRACT. Tissue properties may strongly influence the occurrence of harvest splitting in carrot (*Daucus carota* L.) storage roots, a disorder generally assumed to be triggered by a high water status in the storage root. Strain within the root, as well as extensibility of root tissue by using a materials testing instrument was measured. Strain was estimated after incubation of transverse root slices in water. Measurements of the gap that developed as a result of a radial cut into the center of the slice were then used to estimate strain within the root. Extensibility of strips of carrot tissue was measured through two cycles of extension and relaxation, which allowed both elastic and plastic extensibility to be determined. Strain assessment demonstrated that carrot cells have considerable potential to increase in volume when placed in water. In some roots, phloem parenchyma adjacent to the cambium expanded to a greater extent than tissues at the periphery of the root, indicating that rigidity of cells varied across the carrot radius. Tissue extensibility was predominantly elastic, indicating the cells are unlikely to dissipate some of the strain that occurs during periods of rapid water uptake through plastic deformation. However, these measurements of extensibility were related to the properties of cells along the entire 20-mm length of the tissue strip that was used. Because we demonstrated that mechanical properties can vary within a small distance, it is concluded that future studies into the mechanical properties of carrot storage root tissue will rely on empirical strain measurements.

Harvest splitting is a serious disorder in carrot (*Daucus carota*) production as it affects episodically a large proportion of the crop. The disorder is characterized by occurrence of a sudden radial, longitudinal fracture in the phloem parenchyma of the storage root at harvest and during handling (McGarry, 1993). Harvest splitting occurs by cell wall breakage (McGarry, 1993) and is initiated easily in the brittle parenchymatous tissue of the storage root that has little resistance to fracture (Vincent, 1990). Split roots have to be discarded, but as splitting is likely to continue during the handling process (Millington, 1984), the final product will often contain split roots. While there have been several studies into the causes of splitting in carrot storage roots (Dickson 1966; McGarry 1993; McGarry 1995), no definitive explanation has been established.

By comparing splitting-resistant and splitting-susceptible carrot genotypes, McGarry (1993) found no relationship between tissue strength and the incidence of splitting. The same study concluded that water status did not account for differences among genotypes in susceptibility to splitting. Within genotypes, however, the incidence of splitting was inversely proportional to the water status of the root (McGarry, 1993).

Processes that lead to the splitting include 1) the uptake of water and associated increase in cell volume which in turn places increasing stresses within the root tissues; 2) this increase in cell volume is influenced by the plastic and elastic properties of the cell walls; and 3) the stress and strain that occur as part of this process are supported in part by the strength associated with cells being part complex structure of root tissues, e.g., xylem parenchyma, cambium, and phloem parenchyma. These processes are integral to root growth as well as being involved in splitting. To

understand the physiological basis of harvest splitting, approaches are needed that allow strain development to be estimated in storage roots subjected to a high availability of water and tissue strength independent of tissue water status.

In fruit, tissue splitting occurs at a high availability of water to the tissue, when stress in the periphery of the fruit exceeds the tensile strength of the cell walls (Considine, 1982). A method to estimate stress generation is important for understanding of growth stress in the tissue, as it will enable evaluation of the rheological properties of the tissue. Tissue stress has so far been assessed by estimating strain (Cosgrove, 1993) as stress calculations involve measurements of force and load-bearing area that are complicated in heterogeneous biological materials (Vincent, 1990).

Strain in the periphery of apples [*Malus sylvestris* (L.) Mill. var *domestica* (Borkh.) Mansf.] during growth has been estimated by measuring the size of gaps that occurred when cuts were made to the center of the apple (Skene, 1980). Strain in the outer apple tissue was found to depend on the developmental stage of the fruit, and rain often increased the strain. This method provides a principle for estimating strain in carrots, allowing storage roots to be investigated in their natural circular arrangement.

To avoid fluctuations in root water status affecting cell walls and tissue properties (McGarry, 1993; McGarry, 1995), investigations of tissue extensibility must be carried out by methods controlling or excluding changes in water status. Testing the tissue extensibility in a universal testing machine, the Instron technique, has made it possible to differentiate plastic and elastic extensibility (Cleland, 1967). Plastic extensibility (PE_x) correlated with growth rate (Bagshaw and Cleland, 1990; Cleland, 1967) and elastic extensibility (EE_x) was associated with reversible elasticity of the tissue (Cleland, 1967). The Instron technique has been used to study the distribution of growth in leaves (Gardner et al., 1995; Schultz and Matthews, 1993; Taylor et al., 1993). However, care must be taken when relating these measure-

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ments to the true cell wall extensibility of living tissue (Cosgrove, 1993).

The objectives of this work were to study where strain generates in carrot storage roots subjected to high availability of water and to characterize extensibility properties in carrot storage roots at edible maturity. The study included development of methods for strain assessment and evaluation of the Instron technique (Cleland, 1967) for measurements of tissue extensibility.

Materials and Methods

PLANT MATERIAL. Carrot storage roots, narrowly oblong with a rounded apex, were purchased in a local supermarket in Auckland, New Zealand for all experiments, except the extensibility experiment testing the influence of dehydration on EEx and PEx. A new carrot lot was acquired for each experiment, with respect to special root size requirements. After purchase, the lots were stored for <7 d at 4 °C before being used for experiments. The experiments were conducted at 21 ± 1 °C.

STRAIN ASSESSMENT. The swelling reaction of tissue from the cambium to the periphery was determined by image analysis (Fig. 1a). Transverse radial tissue samples were cut $2 \times 2 \times >20$ mm and each sample was divided manually at the cambium, a technique that allowed the youngest cambial cells to be retained, and incubated in distilled water for 4 h before measurements were conducted. Remaining in water, the samples were placed in an image analysis system Optima 4.2 (Optimus Corp., Bothal, Wash.) at 400 $\times$ magnification. A grid with 1-mm squares was used to calibrate tissue dimensions. Width of a sample was measured at 1-mm intervals in transverse tangential direction starting 1 mm from the cambial end of the sample.

The influence of water uptake on the stresses that develop within the tissue was examined using transverse slices of carrot tissue. The method was developed by combining approaches based on water uptake into tissues (Brown et al., 1996; Christensen, 1972), with the use of a radial cut that allowed stress to be assessed from the size of the resulting gap (Skene, 1980). By removing different zones of the carrot cross-section, it was possible to determine the influence of root structure on the stresses within tissues. Strain in the periphery of the storage root was estimated from the gap size in a transverse carrot slice subjected to a high availability of water. On a 5-mm-thick transverse slice, two perpendicular diameter measurements were taken, $d1$ and $d2$, a radial longitudinal split cut to the center of the slice by a scalpel immediately after washing, and the slice incubated in distilled water for 4 h. The width of the gap, w , was measured at the periphery of the slice by using digital calipers (± 0.01 mm). A gap index, I_g , as a measure of strain, was calculated as $I_g = w / ((d1 + d2) / 2)$.

Transverse slices from the crown and distal parts were tested with and without the xylem parenchyma present (Fig. 1b and c). In addition, an experiment was done to test phloem parenchyma situated near the cambium, the inner phloem, and near the periphery, the outer phloem (Fig. 1d). For this experiment, a single slice from the middle of nine roots was used. A corkborer was used to remove xylem parenchyma and to separate inner and outer phloem parenchyma.

EXTENSIBILITY MEASUREMENTS. Cell water capacity and tissue strength are ultimately determined by cell wall properties. While a large number of mechanical tests can be used to measure tissue strength (Harker et al., 1997), we concentrated on measurements that can be used to characterize the elastic and plastic components

Fig. 1. Diagram showing test pieces used for determination of swelling properties by image analysis (a), stain assessment of root slices with (b) and without (c) xylem parenchyma present, evaluation of inner and outer phloem parenchyma (d) and extensibility tests (e and f). Tissue sample dimensions are given in the text.

of strength. Such measurements have not been attempted previously using carrot tissue. Tissue extensibilities were determined by the Instron technique (Cleland, 1967) modified to excised carrot samples.

Rectangular tissue samples $2 \times 2 \times 10$ mm, taken in transverse tangential direction (Fig. 1e), were cut with a double-bladed knife for all experiments. The samples were wrapped in aluminium foil, frozen in liquid nitrogen, and thawed in distilled water at 21 °C for 5 min. The rehydrated sample was attached to two metal pins with cyanoacrylate adhesive (Loctite 401; Loctite Australia Proprietary Ltd, Sydney, Australia). Extensibility tests were carried out in an Instron universal testing instrument (model 4301; Instron Ltd, High Wycombe, U.K.) equipped with a 100-N static load cell. Each tissue sample was extended at a rate of 5×10^{-5} m·s⁻¹ to 30 g load, after which the clamps were returned quickly to the original position and the sample reextended to the same load level. Chord-modulus was determined at 20 g, with 15 and 25 g as the lower and upper limits for each determination, and converted to extensibility by inversion. The first analysis provided values of the combined PEx and EEx, and the second extension yielded EEx only (Cleland, 1967). PEx was calculated as the difference.

To determine the effect of tissue type and orientation on EEx and PEx, samples were excised from the xylem and the phloem parenchyma in the transverse tangential (Fig. 1e) and longitudinal (Fig. 1f) direction. The samples were prepared and extended as stated above. The influence of dehydration on EEx and PEx was tested by placing phloem parenchyma samples up to 60 min in ambient relative humidity (>50%) after thawing. For this

Fig. 2. Swelling of the phloem parenchyma as effected by distance from the cambium at the (A) apex and (B) crown region of the carrot storage root. The samples had a width of 2 mm before incubation in water. Tissue dimensions of roots ($n = 4$) with profound swelling near the cambium (○) compared with root ($n = 1$) with uniform swelling (●) are presented.

Table 1. Effect of tissue location and presence of xylem on strain release in carrot storage tissue with incision to center. The gap index, I_g , indicates the width of the split relative to the root diameter. Values are means of nine replications.

Tissue location	Gap index after (h)	
	4	6
Xylem not removed		
Crown	0.13	0.18
Apex	0.14	0.18
Xylem removed		
Crown	0.54	0.63
Apex	0.41	0.48

experiment, storage roots of 'Futuro' carrot were obtained directly from a grower in Gisborne, New Zealand. Samples were mounted and analyzed at 15-min intervals. Finally, the effects of water status and load on extensibility properties were studied by allowing thawed samples to dehydrate in 0.2 mL polyethylene glycol (PEG) 4000 (osmotic potential -0.69 MPa) for 5 min at 21 ± 1 °C. Extensibility properties were determined for fully hydrated and PEG-incubated samples in 10-g intervals at ≈ 20 , 30, or 40 g load.

The influences of agronomic, climatic, and genetic factors were minimized by using individual storage roots for all treatments within one experiment, i.e., one root represented one replication. Statistical analyses were carried out using GLM procedures of SAS (SAS Inst., Inc., Cary, N.C.)

Results

Swelling of the phloem parenchyma depended on the storage

root. Samples from 4 roots expanded predominantly near the cambium, whereas tissue from one root had a uniform swelling from the cambium to the periphery (Fig. 2). Within each root, the distal and crown portion showed the same swelling reaction from cambium to periphery, i.e., a gradient in swelling or a uniform swelling was observed for individual roots.

Strain release, measured by gap, in slices with the xylem parenchyma removed was higher than in full discs ($P < 0.0001$) (Table 1). A later evaluation, 6 h after the start of the experiment, illustrated that release of strain took place over time ($P < 0.01$). In full slices, similar levels of strain were released in the crown and apex ($P = 0.80$), whereas slices with the xylem parenchyma removed released more strain in the crown section than the apex ($P < 0.0001$). In a few full slices, the phloem broke away from the xylem in the cambium during incubation (data not presented). Time-dependent release of strain (Fig. 3) showed a significant ($P < 0.05$) increase in strain released over the test period for all three types of tissue. Rings of inner and outer phloem parenchyma released more strain than slices not divided ($P < 0.0001$), as especially the inner phloem parenchyma exhibited a large strain release.

EEx remained constant ($P = 0.54$) during the 60 min period used to study the effect of dehydration on extensibility properties, while PEx increased ($P < 0.05$) (Fig. 4). EEx was high relative to PEx. Tissue treated with PEG had significantly increased EEx ($P < 0.05$) and PEx ($P < 0.001$) in comparison with fully hydrated tissue (Fig. 5). EEx at the 20-, 30-, or 40-g load decreased ($P < 0.0001$) with increasing load for both PEG-treated and fully hydrated tissue. In contrast, no changes were found in PEx ($P = 0.26$) when the load was increased on fully hydrated samples, but PEx of PEG-treated samples changed with increasing load ($P < 0.001$).

A reduced EEx was found in the longitudinal xylem parenchyma compared with the transverse tangential section ($P < 0.001$) (Table 2). In the phloem parenchyma, however, tissue orientation did not affect EEx ($P = 0.48$). Of the four tissue and orientation combinations tested, a reduced PEx ($P < 0.05$) was observed for the phloem parenchyma in transverse tangential sections.

Fig. 3. Strain release, measured by gap, in full slices (■), outer phloem parenchyma (●), and inner phloem parenchyma (○) of carrot storage roots depending on time incubated in distilled water. Values are means of nine replications. Vertical bars represent SE.

Discussion

CELL WATER CAPACITY. The increase in cell volume (dV) that occurs per unit increase in water potential ($d\Psi$) is known as cell

Fig. 4. Effect of dehydration on plastic (PEx) (○) and elastic (EEx) (●) extensibility of 'Futuro' carrot storage root phloem parenchyma. Tissue samples were placed for 0, 15, 30, 45, or 60 min at 21 °C and ambient relative humidity (>50%). Values are means of nine replications. Vertical bars represent SE.

Fig. 5. Effect of load and hydration on elastic (A) and plastic (B) extensibility of carrot storage root phloem parenchyma. Test pieces were incubated in PEG solution (○) or water (●) before extensibilities were measured at 20-, 30-, or 40-g load. Values are means of four replications. Vertical bars represent SE.

Table 2. The extensibility properties of carrot storage tissue as affected by tissue type and orientation. Values are means of 10 replications.

Tissue type	Elastic extensibility (%)	Plastic extensibility (%)
Xylem		
Longitudinal	0.60	0.29
Transverse	0.79	0.27
Phloem		
Longitudinal	0.68	0.31
Transverse	0.72	0.18

water capacity (C), and is described mathematically by the formula, $C = dV/d\Psi = V/(\epsilon + \pi)$, where V is the initial volume, ϵ is the cell wall elastic modulus, and π is the osmotic pressure (Dainty, 1976; Tomos, 1988). We used an empirical approach to assess cell water capacity. Results indicated that carrot cells have considerable potential to increase in volume when placed in water (Fig. 2). In some roots, the cells expanded evenly as the radial distance from the cambium increased. In other roots, there was a marked expansion of cambial cells (up to a 35% increase in tissue width), but as the distance from the cambium increased, the cells expanded to a lesser degree. This swelling pattern (Fig. 2) indicates variations in the pliability and rigidity of the cell wall that occur across the carrot radius. Clearly the biomechanical properties of cells vary considerably even when separated by small distances within a single root as well as between different roots. We speculate that the most severe form of strain may develop at the root perimeter when cells from the cambium and inner parts of the phloem parenchyma expand to a proportionally greater extent than cells in the outer zones of the phloem parenchyma and periderm (open circles in Fig. 2). However, the complexity of whole-tissue interactions makes this difficult to test.

INFLUENCE OF ROOT STRUCTURE ON STRESS. The large gaps in slices with the xylem removed (Table 1) indicate that the phloem parenchyma has a great capacity to swell, but the presence of the xylem counteracts this expansion. The observation of tissue fractures between the xylem and the phloem parenchyma in a few transverse slices of root tissue suggests a high level of strain in the phloem parenchyma. Such fracturing at the cambium supports findings that fractures related to harvest splitting only penetrate the phloem parenchyma and do not cross the cambium (McGarry, 1993; Millington, 1984). Dividing the phloem parenchyma in an inner and outer section revealed a large potential swelling of the inner phloem parenchyma when excessive water was available (Fig. 3). This observation is confirmed by results demonstrating the higher water capacity of cells close to the cambium (Fig. 2). The inner phloem parenchyma consists of the immature cells most recently developed by the cambium (Esau, 1940). Such cells are expected to possess a high extensibility as they are in a phase of rapid growth.

BIOMECHANICAL PROPERTIES OF CELL WALLS. Development and validation of the methodology were critical parts of this study. The water content of the sample influences the extensibility properties (Cosgrove and Sovonick-Dunford, 1989; Taylor and Cosgrove, 1989). In this experiment, the extensibility was not affected by the water loss occurring in 60 min (Fig. 4). In fully hydrated samples, values for plastic extensibility will relate to movement of cell sap and residual water out of the tissue by exoosmosis. Thus, water is often removed by pressing the tissue before the testing of hypocotyl and epicotyl tissues (Cosgrove and Sovonick-Dunford, 1989; Taylor and Cosgrove, 1989). The Instron extensibility technique, however, has been used successfully without removing residual water or cell sap from the tissue samples (Bagshaw and Cleland, 1990; Cleland, 1967, 1984; Keyes et al., 1990). Varying amounts of cell sap and residual water are likely to be removed by pressing the tissue with a constant weight, as the tissues may differ in cellular structure and stiffness due to experimental treatments, nutritional status, genotype, or stage of development.

The EEx was load- and tissue-state specific for water incubated and PEG-treated tissue samples (Fig. 5), indicating that a standard protocol for the experiments is needed. Cleland (1967) demonstrated that extensibility properties vary significantly with

the applied load, the rate of extension, and the state of the tissue. A decrease in EEx with increasing load was found within the 20- to 40-g load range in an experiment that also shows a smaller change in PEx with increasing load (Cleland, 1967). The reduced water content of the PEG-incubated samples affected both EEx and PEx suggesting that, although no turgor pressure existed in the cells, the water content of the cell played an important role in the extensibility properties measured. However, it cannot be concluded that a decreased water content in the samples treated with PEG reduced the extensibility properties as argued by Cosgrove (1993). PEG was chosen as osmotic agent because mannitol solutions may diffuse into the cell lumen (Cram, 1984) due to the small size of the mannitol molecules (Carpita, 1979).

The values for EEx and PEx determined for carrot root tissues provide several insights into the biomechanical properties of cells. The high EEx relative to PEx in the storage root tissues (Table 2) indicates the tissues have low growth potential. Growing plant tissues generally have a high PEx, while mature tissues have high EEx (Cosgrove, 1993). The dominance of EEx in carrot root tissue suggests that cell expansion during water uptake (Fig. 2) and the associated increase in strain (Fig. 3) were largely the result of reversible processes in which little plastic deformation occurred. A reduced EEx in the longitudinal sections of the xylem parenchyma was due presumably to the presence of secondary xylem vessels that have a limited extensibility (Esau, 1940).

That PEx of the phloem parenchyma varies with tissue orientation has wide implications if PEx is considered a true measurement of the growth potential in vivo. PEx correlates with the growth rate in coleoptiles of oats (*Avena sativa* L.) (Cleland, 1984) and leaves of grape (*Vitis vinifera* L.) (Schultz and Matthews, 1993). The larger PEx in longitudinal direction compared with transverse direction suggests that growth potential is greatest parallel to the root axis. Thus, radial growth may be compromised by the capacity of the root to tolerate increased strain.

The Instron technique requires rectangular or cylindrical samples which are relatively long (10 mm in this study). The dimensions of the sample, particularly the length, preclude the use of certain tissue orientations. In particular, analysis of EEx and PEx in the circular secondary growth of the storage root is excluded. This is considered a serious limitation to the Instron technique.

POSSIBLE USE OF METHODS IN GENETIC STUDIES. Carrot cultivars have well established differences in susceptibility to splitting (Dickson, 1966; McGarry, 1993; Michalik et al., 1997; National Institute of Agricultural Botany, 1993). This genetic variability is one of the strongest tools for investigating causes of splitting in carrots. One of our aims was to identify potential methods for measuring biomechanical properties of carrot tissues during screening of different cultivars.

Empirical measurements of strain and swelling of thin tissue blocks were completed easily. Measurements were rapid and quantifiable, and large numbers of samples may be processed. Despite the empirical nature of the measurements, the data provided valuable insights into cell water capacity and to stresses and strains that develop within root tissues during water uptake. Conversely, measurements of EEx and PEx were laborious, and will not be suitable for evaluating a sufficient number of roots per treatment or cultivar. Furthermore, having established that EEx is the dominant characteristic in carrot tissue, it is likely that empirical measurements of cell water capacity will indicate the extent of cell extension during swelling. For these reasons, future studies should use empirical approaches to assess the mechanical properties of the carrot tissue.

Literature Cited

- Bagshaw, S.L. and R. Cleland. 1990. Wall extensibility and gravitropic curvature of sunflower hypocotyls: Correlation between timing of curvature and changes in extensibility. *Plant Cell Environ.* 13:85–89.
- Brown, G.S., A.E. Kitchener, W.B. McGlasson, and S. Barnes. 1996. The effects of copper and calcium foliar sprays on cherry and apple fruit quality. *Scientia Hort.* 67:219–227.
- Carpita, N. 1979. Determination of the pore size of cell walls of living plant cells. *Science* 205:1144–1147.
- Christensen, J.V. 1972. Cracking in cherries. III. Determination of cracking susceptibility. *Acta Agr. Scand.* 22:128–136.
- Cleland, R. 1967. Extensibility of isolated cell walls: Measurement and changes during cell elongation. *Planta* 74:197–209.
- Cleland, R. 1984. The Instron technique as a measure of immediate-past wall extensibility. *Planta* 160:514–520.
- Considine, J.A. 1982. Physical aspects of fruit growth: Cuticular fracture and fracture patterns in relation to fruit structure in *Vitis vinifera*. *J. Hort. Sci.* 1:79–91.
- Cosgrove, D.J. 1993. Wall extensibility: Its nature, measurement and relationship to plant cell growth. *New Phytol.* 124:1–23.
- Cosgrove, D.J. and S.A. Sovonick-Dunford. 1989. Mechanism of gibberellin-dependent stem elongation in peas. *Plant Physiol.* 89:184–191.
- Cram, W.J. 1984. Mannitol transport and suitability as an osmoticum in root cells. *Physiol. Plant.* 61:394–404.
- Dainty, J. 1976. Water relations of plant cells, p. 12–35. In: U. Lüttge and M.G. Pitman (eds.). *Encyclopedia of plant physiology*. vol. 2A. Transport in plants. Springer-Verlag, Berlin/New York.
- Dickson, M.H. 1966. The inheritance of longitudinal cracking in carrots. *Euphytica* 15:99–101.
- Esau, K. 1940. Developmental anatomy of the fleshy storage organ of *Daucus carota*. *Hilgardia* 13:175–226.
- Gardner, S.D.L., G. Taylor, and C. Bosac. 1995. Leaf growth of hybrid poplar following exposure to elevated CO₂. *New Phytol.* 131:81–90.
- Harker, F.R., R.J. Redgwell, I.C. Hallett, S.H. Murray, and G. Carter. 1997. Texture of fresh fruit, p. 121–224. In: J. Janick (ed.). *Hort. Rev.* Wiley, New York.
- Keyes, G., M.E. Sorrells, and T.L. Setter. 1990. Gibberellic acid regulates cell wall extensibility in wheat (*Triticum aestivum* L.). *Plant Physiol.* 92:242–245.
- McGarry, A. 1993. Influence of water status on carrot (*Daucus carota* L.) fracture properties. *J. Hort. Sci.* 68:431–437.
- McGarry, A. 1995. Cellular basis of tissue toughness in carrot (*Daucus carota* L.) storage roots. *Ann. Bot.* 75:157–163.
- Michalik, B., R. Baranski, S. Sleczeck, A. Zabaglo, and E. Zukowska. 1997. Effect of harvest date on carrot yield structure and root splitting. *J. Appl. Genet.* 38A:165–171.
- Millington, S. 1984. Quality of carrots in the United Kingdom with respect to harvester and packhouse damage. *Acta Hort.* 163:127–136.
- National Institute of Agricultural Botany. 1993. Descriptive list of varieties of early and maincrop carrots grown in NIAB trials. *Veg. G. Lfl.* 5. Natl Inst. Agr. Bot., Cambridge, U.K.
- Schultz, H.R. and M.A. Matthews. 1993. Growth, osmotic adjustment, and cell-wall mechanics of expanding grape leaves during water deficit. *Crop Sci.* 33:287–294.
- Skene, D.S. 1980. Growth stresses during fruit development in 'Cox's Orange Pippin' apples. *J. Hort. Sci.* 55:27–32.
- Taylor, A. and D.J. Cosgrove. 1989. Gibberellic acid stimulation of cucumber hypocotyl elongation. *Plant Physiol.* 90:1335–1340.
- Taylor, G., A.J.S. McDonald, I. Stadenberg, and P.H. Freer-Smith. 1993. Nitrate supply and the biophysics of leaf growth in *Salix viminalis*. *J. Expt. Bot.* 258:155–164.
- Tomos, A.D. 1988. Cellular water relations of plants, p. 187–277. In: F. Francks (ed.). *Water science reviews*. Cambridge Univ. Press, Cambridge, U.K.
- Vincent, J.F.V. 1990. Fracture properties of plants. *Adv. Bot. Res.* 17:235–287.