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***Short communication*:**

Calcium-accumulating cells in the meristematic region of grapevine root apices

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Abstract. Apical roots of grapevines were examined by cryo-SEM (scanning electron microscopy) and the intracellular distribution of Ca was demonstrated by X-ray microanalysis in different regions of the primary root. We show that large amounts of Ca are accumulated as raphide crystals in the vacuoles of specialised cortical cells (idioblast cells) of the root apex. These crystal idioblast cells appeared to form a discontinuous cone of cells in the outer region of the root meristem. The raphide crystals within these cells were less apparent in older regions of the root, 10–12 mm basipetal to the root tip. We suggest that the raphide crystals could initially act as another Ca sink involved in the regulation of Ca levels in root apices. In older regions of the root these cells are spaced at intervals around the periphery of the cortex and the subsequent disappearance of the raphides may be indicative of remobilisation, perhaps in the zone of elongation where cell wall synthesis occurs and Ca demand is high. Calcium-accumulating cells were also observed in the older regions of the root, forming endodermal protrusions extending into the cortex. These cells may play a part in regulating Ca delivery to the xylem stream by sequestration of Ca from the radial flow of water at the endodermis. The observed distribution of Ca in root apices was different from the other major cations (e.g. K) and anions (e.g. Cl) because high concentrations were localised to specific cells. We interpret the results in the context of a model of the dynamics of grapevine root growth and cell differentiation, and the temporal balance of solute supply from the protophloem and the external medium.

Keywords: calcium crystals, grapevine, idioblast cells, root meristem, SEM, solute distribution.

Introduction

Calcium ions play an essential role in plants through their role in intracellular signalling (Sanders *et al.* 2002), in a number of important cellular processes such as mitosis (Hepler 1986), cytoplasmic streaming (Takagi *et al.* 1990), responses to environmental change, for example stomatal function (Ruiz *et al.* 1993) and gravitropic responses (Evans and Ishikawa 1997), as well as having an extracellular role as a component of cell walls. Calcium acts as a secondary messenger through oscillations in free cytoplasmic Ca ions at concentrations typically within the micromolar range (White 2000). In other cell compartments Ca is present at much higher concentrations — in the millimolar range in the vacuole, for example.

Numerous observations have been made of calcium oxalate crystals, in the form of raphide bundles, occurring in specialised cells called idioblasts, in many plant species (Arnott and Pautard 1970) including the mature leaves, petioles and roots of *Vitis* (Fabbri *et al.* 1992). It is commonly assumed that such a precipitation mechanism sequesters calcium, avoiding potentially deleterious effects. Precipitation also reflects the low phloem mobility of Ca and a limited capacity to recycle this ion.

Typical soil water concentrations of Ca at the root–soil interface may be 0.1–1 mM and even higher in the bulk soil (Bangerth 1979). At low soil pH (< 4.0), Al is usually the dominant cation on soil cation exchange sites and interferes with Ca uptake possibly by reducing Ca binding to cell walls of root cells (Schroder *et al.* 1988). Under these conditions, plants may be particularly susceptible to Ca deficiency owing to low Ca supply from the soil (Bangerth 1979). In non-acid soils (> 4.0) the dominant cation on the soil cation exchange site is usually Ca, thus excess Ca may then become more of a problem than low Ca supply.

Vitis species grow in a great range of soil types (Northcote 1988; Robinson 1992). The *Vitis* genus includes both calcicole (adapted to alkaline/calcareous soils) and calcifuge (adapted to neutral to acid soils) species. For example, *V. berlandieri* and hybrids with *V. berlandieri* parentage are considered lime tolerant but *V. labrusca* and

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V. riparia are considered lime sensitive (Delas 1992; Hardie and Cirami 1988). Consequently, grapevine genotypes are adapted to both nutrient excesses and deficiencies at the extremes of the soil pH range.

In a histoautoradiographic study of Ca uptake by grapevine (*V. vinifera* L.) roots, Malazian (1965) showed that Ca uptake at the root tip is high and comparable to similar observations in wheat roots (Huang *et al.* 1992*a*, 1992*b*). Thus, if Ca influx into the root apex is high, but the actively growing, cytoplasmic-rich region of the root must maintain low symplastic levels of free Ca while accumulating large amounts of Ca to meet the rest of the plant's needs, the various *Vitis* species need to deal with extremes in Ca supply to accommodate these two conditions. The limited data available suggest that in some *Vitis* genotypes the regulation of Ca uptake and transport across a range of soil pH values varies (Conradie 1983) and that Ca accumulation in leaves is influenced by the rootstock (Pouget and Delas 1982). The basis for this variability is unknown.

In this paper we describe the presence of Ca-accumulating cells in the meristem of grapevine roots and suggest that they might play a role in regulating Ca levels at the root tip and in older regions of the root. The initial accumulation of Ca in these cells and then the subsequent remobilisation of Ca also suggest a role as a temporary sink in modulating Ca availability. We examined cell structure and measured elemental composition of these cells using cryo-SEM and X-ray microanalysis. Our interpretation of the results is consistent with a model of solute supply and distribution in the apical root that depends on contributions from both the protophloem and the external medium (see Oparka *et al.* 1994; Farrar *et al.* 1995; Wyn Jones 1999).

Materials and methods

Full details are given by Storey *et al.* (2003). In brief, vines from a backcross population of *Vitis vinifera* var. Biancone backcrossed to an F_1 hybrid of a cross between *V. berlandieri* and *V. vinifera* var. Sultana (Antcliff *et al.* 1983; Newman and Antcliff 1984) were used in a study of the pattern of ion distributions in the roots of a Cl-excluding genotype (80-23) and a non-excluding genotype (80-15). The results presented in this paper relate to the Cl-excluding genotype 80-23. The lime tolerance of both crosses is unknown but because *V. vinifera* is not lime sensitive and *V. berlandieri* is lime tolerant, then the crosses are likely to be at least moderately lime tolerant (Hardie and Cirami 1988). Vines were clonally propagated by rooting of canes, which were then established in pots containing river sand for 3 weeks and irrigated with a modified Hoagland Solution (Hoagland and Arnon 1938) containing 1 mM Ca and K. Half the plants were treated with 25 mM NaCl for 4 weeks before commencing cryo-SEM and X-ray microanalysis; the remaining plants continued to receive the base nutrient solution. Saline plants were used to examine ion distribution along the longitudinal profile of the apical root.

Root segments were taken from whole plants and mounted in or on aluminium stubs, secured with a small volume of water and immediately frozen in liquid nitrogen or nitrogen 'slush'. Samples were cryo-planed, etched and coated with gold or chromium in a Balzers SCU 102 preparation chamber (Balzers Union, Aktiengesellschaft, Fürstentum, Liechtenstein). The specimens were analysed in a Philips 500 scanning electron microscope (Philips Electron Optics, Eidenhoven, The Netherlands) fitted with an Edax energy-dispersive X-ray detector (Edax International Inc, Illinois, USA) and a Link AN10000 X-ray microanalyser (Oxford Instruments Microanalysis Group, High Wycombe, UK). To examine elemental distributions, multiple elemental maps were collected in real-time mode at a resolution of 128 × 128 pixels. The corresponding secondary image was collected direct to disk at a resolution of 512 × 512 pixels.

Results and discussion

Here we report on Ca-accumulating cells within the meristematic region of *Vitis* roots and address some of the physio-morphological and cultural implications of this observation.

Crystal idioblast cells at the root apex

Scanning electron micrographs and X-ray microprobe analysis of longitudinal sections of the terminal 1 mm of *Vitis* roots show discrete, intense Ca signals in an arc extending distally from the meristem (Figs 1*A*, *B*). At higher magnifications, files of cells containing the characteristic raphide bundles can be seen, interposed between the smaller unvacuolated cells of the meristem (Figs 1*C*, *D*). Comparable idioblast formations were observed in the apical roots of *Yucca whipplei* (Arnott 1962) and *Lemna minor* L. (Franceschi 1989). It should be noted that we did not observe Ca crystals in the cells of the root cap (Fig. 2*F*), in contrast to *L. minor* (Franceschi 1989). In a cross-section of a region of the root, 1–2 mm basipetal to the root tip, the cells were larger than the adjacent cortical cells but the number of raphides appeared to have decreased (Figs 1*E*, *F*). Calcium-accumulating cells have been observed in older roots of *V. vinifera* (Malazian 1965; Richards 1983) but not in the region of active cell division.

Calcium accumulation by idioblast cells at the apex

Malazian (1965) showed that radiolabelled Ca is accumulated mainly in root cells of the terminal 5 mm (inclusive of the root cap). Evidence from pressure probe measurements in a number of species demonstrate clearly that there is no hydraulic barrier or significant resistance to ion fluxes between the external medium and the cell walls of root cells in the region of cell elongation (Pritchard *et al.* 1987). Thus, the typical profile of Ca uptake along the root is highest over the first 2 mm, decreasing basipetally (Huang *et al.* 1992*a*, 1992*b*). Over short distances rates of Ca diffusion and consequently equilibration will be relatively rapid in the root apex. While there are no direct pressure probe measurements or flux data on *Vitis* root tips, it is reasonable to hypothesise that, as in other species, the apoplastic space of growing *Vitis* roots are relatively freely permeable to Ca as well as other ions and water, subject only to some modification by the ion exchange capacity of the cell wall (Bush and McColl 1987) and the electrical charges on the surface of the plasma membrane (Kinraide 2001). Malazian (1965)

detected ^{45}Ca in grapevine root tips after only a 5–20-min uptake period. It is, however, relevant that when growth is interrupted, root tips rapidly suberise in grapevines and the radial hydraulic and nutrient profile of roots might be expected to change dramatically.

The presence of idioblast cells suggests a mechanism for sequestering Ca within this critical but restricted region to regulate cytoplasmic Ca in meristematic cells (Franceschi 1989). This could occur through direct preferential uptake into the idioblasts, possibly decreasing apoplastic Ca

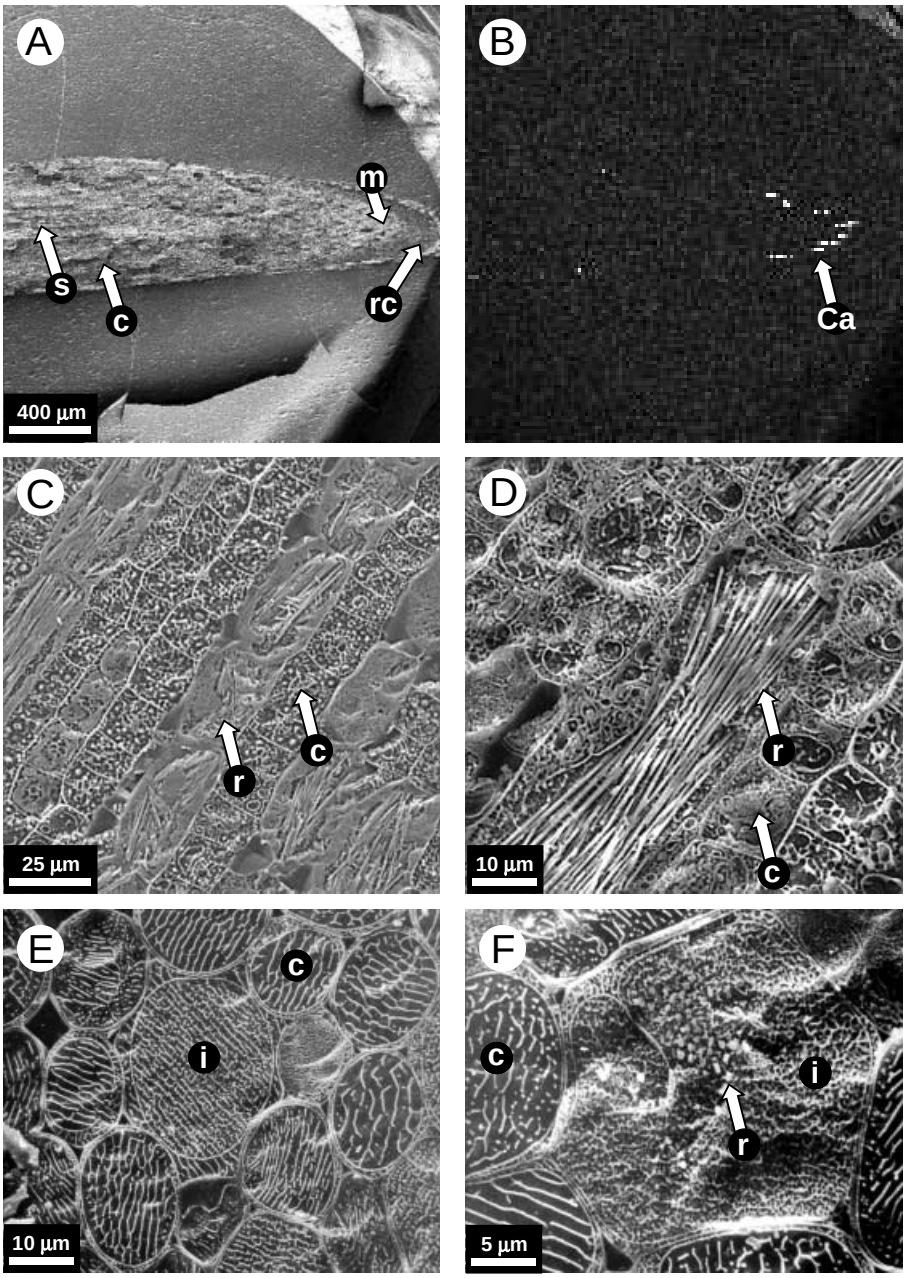

Fig. 1. Scanning electron micrographs and Ca distribution map of cryo-planed frozen-hydrated grapevine roots. Roots were sampled at the root tip (*A–D*) and 1–2 mm basipetal to the root tip (*E*, *F*). (*A*) Secondary electron image of a longitudinal view of the root tip and corresponding Ca distribution map (*B*). (*C*, *D*) Longitudinal views of files of raphide-containing idioblast cells in the meristematic zone. Adjacent cortical cells still appear be dividing. (*E*, *F*) Cross-sectional views of more mature idioblast cells. Raphides were not always visible in the cross-section (*E*) but the cells were apparent by their larger size compared with cortical cells and relative position to the root periphery (not shown). The cytoplasmic compartment is prominent in raphide-containing cells. c, cortex; h, hypodermis; i, idioblast cell; m, meristem; r, raphide bundle; rc, root cap; s, stele.

activity in the immediate vicinity, or by an intracellular symplastic transfer of Ca from the meristematic cells to the idioblasts, which have well-defined cytoplasms in this region. The formation of dense raphide bundles clearly demonstrates that some root cells can transport large amounts of Ca across the symplast from the apoplast to the vacuole over a short period, for example, 1 h (Franceschi 1989). Also, some evidence shows that elevation of apoplastic Ca leads to the induction of raphide formation in the roots of *L. minor* (Franceschi 1989). Paradoxically, any mechanism for precipitating Ca might have the effect of maintaining or steepening the Ca diffusion gradient from the rhizosphere to the meristematic zone. However, any mechanism must operate as a component of a dynamic system of basipetal root growth into unexplored soil, driven at the distal end by cell elongation, accompanied by a rapid

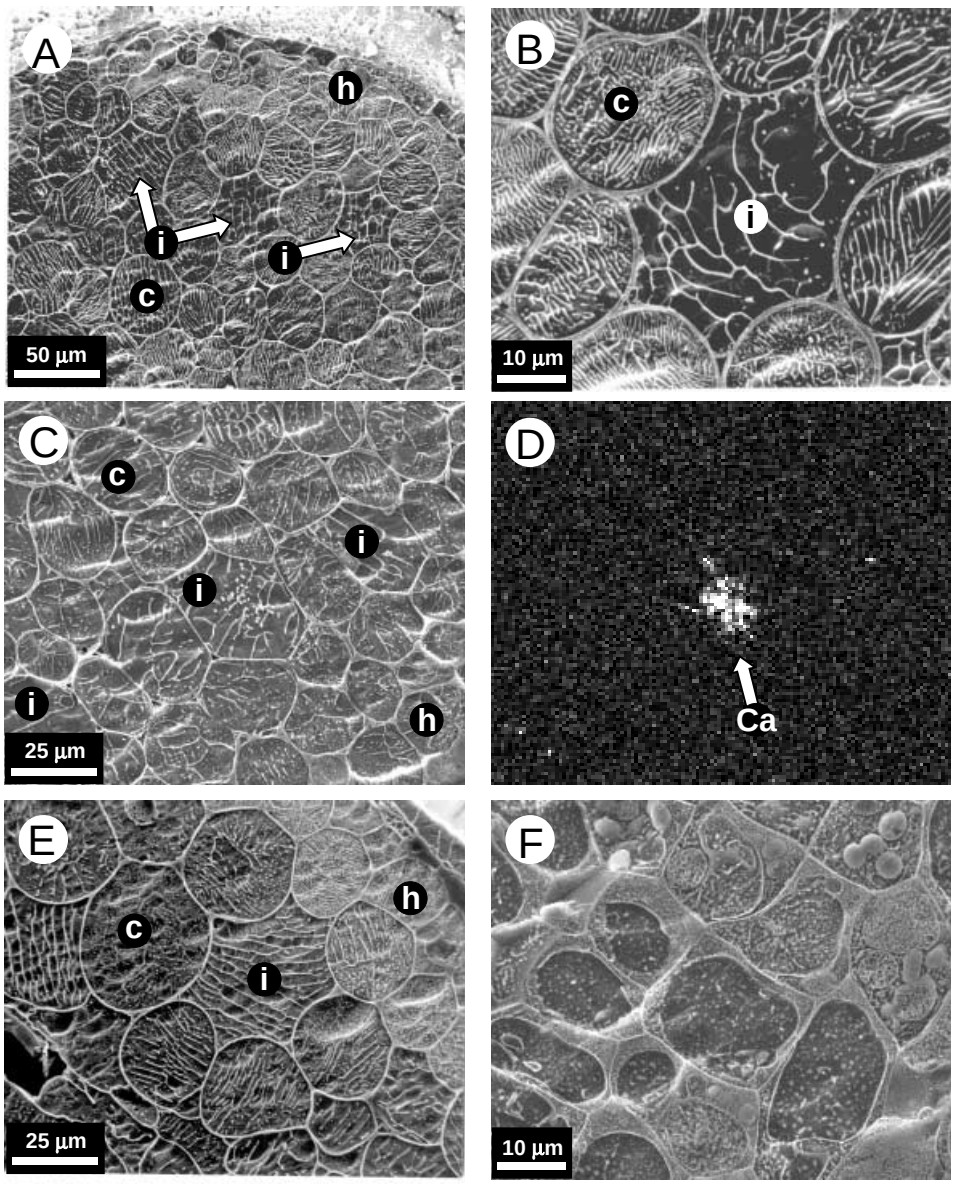

Fig. 2. Scanning electron micrographs and Ca distribution map of cryo-planed frozen-hydrated grapevine roots. Roots were sampled at the root tip (*F*), and from the primary root about 2–3 mm (*C*, *D*), 10 mm (*A*, *B*) and 100 mm (*E*) basipetal to the root tip. (*A*) Cross-section of the root showing idioblast cells spaced at regular intervals in the outer cortex. (*B*) Idioblast cells compressed by the turgor of adjacent cortical cells. (*C*, *D*) Secondary electron image (*C*) and Ca distribution map (*D*) of idioblast cells in the root segment about 2–3 mm behind the root tip. (*E*) Idioblast cell in a root segment *ca* 100 mm behind the root tip. Idioblast cells were identified by their peripheral position in the cortex, cell shape and raphides if present. (*F*) Cells of high cytoplasmic content in the root cap. c, cortex; h, hypodermis; i, idioblast cell.

increase in vacuolar volume and a decline in the cytoplasm : vacuole ratio. These developing vacuoles offer an alternative sink for millimolar quantities of Ca as well as other ions such as Na and Cl in saline environments (Jeschke and Stelter 1976; Storey *et al.* 1983, 2003).

Crystal idioblast cells in older regions of the root

The micrographs from regions 2–3, 10 and 100 mm behind the root tip show idioblast cells to be lying just below the epidermal and hypodermal layers (Figs 2*A–C*, *E*). While high raphide densities characterise the idioblasts in the meristematic zone, at regions 10–100 mm from the tip the picture is varied. Only some cells contained a small number of raphides and only these cells emitted Ca signals (Figs 2*C*, *D*). Also, an examination of the idioblast morphology shows a sequential change from fully rounded, apparently turgid cells with a distinct cytoplasm in the meristematic zone, through hexagonal cells to star-shaped cells with concave walls at and beyond 10 mm (Figs 1*E*, 2*C*, *E*).

Malazian (1965) found that, beyond 10 mm from the root tip, Ca uptake was low in grapevine roots. Our observation that the raphide content of cells appeared to diminish with root maturity suggests these cells may also play a role in providing a readily available source of Ca for cell wall synthesis in a region of the root where net Ca uptake is probably substantially lower than influx at the root tip (Malazian 1965; Huang *et al.* 1992*a*, 1992*b*). In *L. minor*, Franceschi (1989) showed that dissolution of recently formed calcium oxalate crystals can occur, albeit at a slower rate than crystal formation. This remobilisation of Ca might be mediated by changes in the pH of the vacuole, as soil calcium oxalate deposits are solubilised at pH < 4.5 (Cromack *et al.* 1979). More likely, Ca mobilisation could occur by an increase in oxalate oxidase activity localised at the crystal surface (Volk *et al.* 2002).

In addition to these primary observations, another type of idioblast was found in the cell layer adjacent to the endodermis in mature roots (Fig. 3) (Fabbri *et al.* 1992). The radial position and form of these cells suggest that they may have a different function from those of the peripheral cortex. We speculate that they may be acting as 'Ca filters' at the cortex–stelar interface. In mature roots the Casparian band of the endodermis produces a hydraulic separation of the cortical and stellar apoplast and it is suggested that Ca moves across the endodermis in the symplast, possibly as bound rather than free Ca (White 2001). The idioblast cells might accumulate Ca directly from the cortical apoplast or indirectly via symplastic connections with adjacent endodermal cells. However, retrieval from the symplast may be less likely if apoplastic bypass flow is the main route for Ca delivery to the xylem (White 2001). This remains to be resolved, as does the possibility that the cells preferentially develop opposite the xylem poles of the stele.

Solute supply to the meristem

Two apparently contradictory strands of evidence relate to the source of nutrients to root apices. Substantial evidence (Wolf and Jeschke 1987; Sharp *et al.* 1990; Oparka *et al.* 1994; Farrar *et al.* 1995; Marschner *et al.* 1996; see also Wyn Jones 1999) suggests that K, carbohydrate and probably other nutrients are dominantly sourced from the phloem, that is, transported from the leaves or mature roots to the root tips. This transport is preferentially oriented to the meristematic region itself with the elongating cells basipetal to the apical meristem then being back-filled — this is referred to as the 'fountain model' (Oparka *et al.*

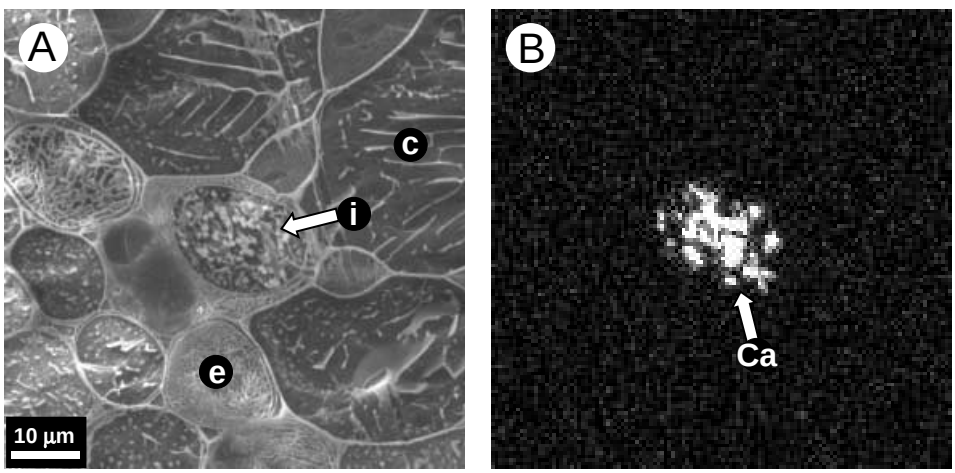

Fig. 3. Scanning electron micrograph and Ca distribution map of a cryo-planed frozen-hydrated grapevine root. The root was sampled about 15 mm basipetal to the root tip. (*A*) Secondary electron image of a cross-sectional view of the root endodermis and inner cortex and (*B*) corresponding distribution map of Ca showing high concentrations in the protruding cell of the endodermis. c, cortex; e, endodermis; i, idioblast cell.

1994; see Wyn Jones 1999). The chemical composition of the meristem and the terminal 1–2 mm of the root may thus be highly buffered against transient changes in the immediate soil solution composition and concentration. The low Na and Cl contents of root apices in halophytes and glycophytes grown in media low in K but enriched with NaCl further illustrate this phenomenon (Jeschke and Stelter 1976; Storey *et al.* 1983). This was again demonstrated by an X-ray map of Na and Cl distribution in the root shown here in Fig. 4, in which Cl levels only started increasing in the elongation zone (Storey *et al.* 2003). This is another example of the pervasive relation between phloem and symplastic solute selectivity and the solute composition of tissues of 'cytoplasmic dominance', for example embryos, apical meristem, pollen, flower petals and so on. The supply of high-energy compounds, carbohydrates, K and other nutrients to meristems is tightly coupled through phloem/symplastic flux and is related to volume increase due to cell division and initial elongation.

In contrast, the explanation offered for the idioblasts found in *Vitis* root meristems, supported by ion uptake studies (Malazian 1965), implies that Ca is rapidly taken up from the external medium into these tissues. Given the evidence on symplasmic and phloem composition, it is unlikely that the

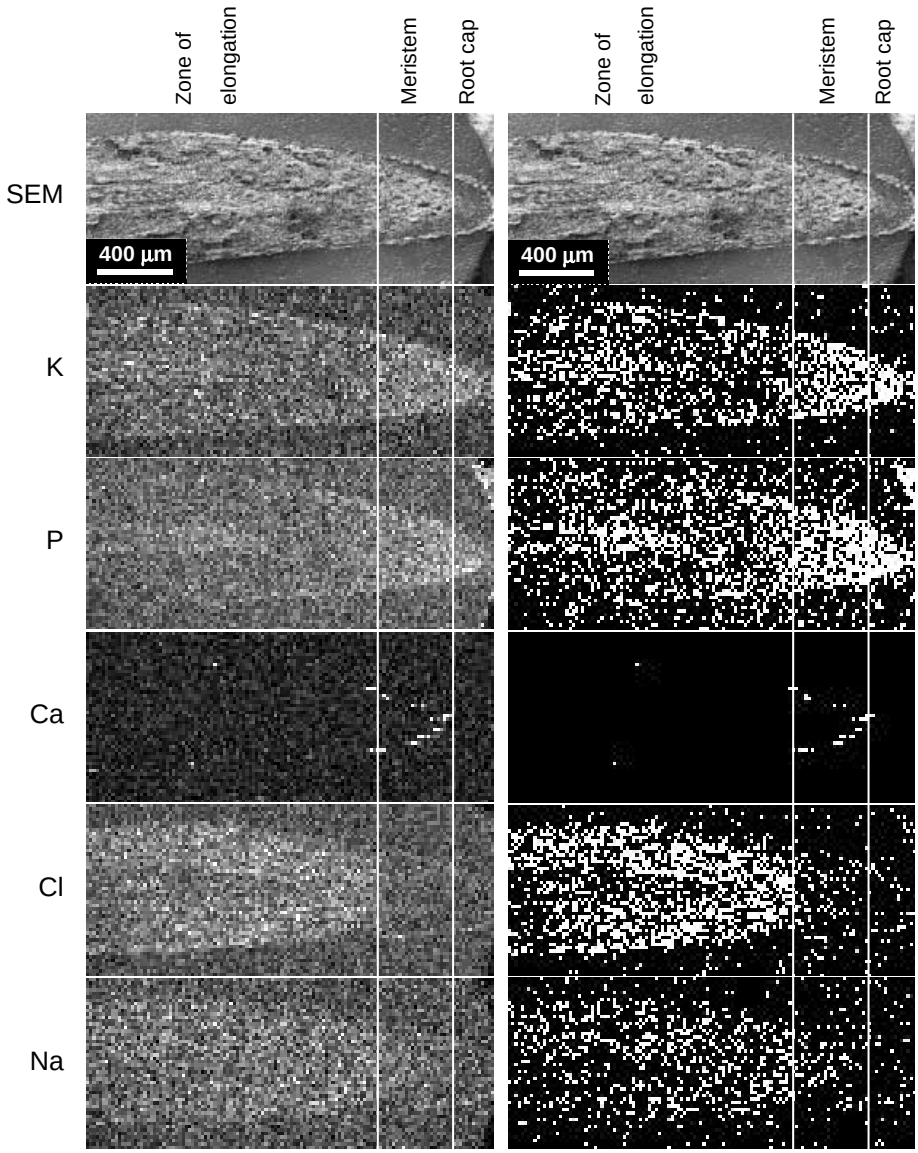

Fig. 4. Scanning electron micrograph (SEM) and longitudinal distribution maps of K, P, Ca, Cl and Na in the apical grapevine root (left). Elemental distribution maps presented as binary images (right). The plants were grown in 25 mM NaCl. The rough appearance of the cryo-planed root surface is an artefact of uneven Cr coating. [Partly based on Storey *et al.* 2003, reproduced with permission of Blackwell Science.]

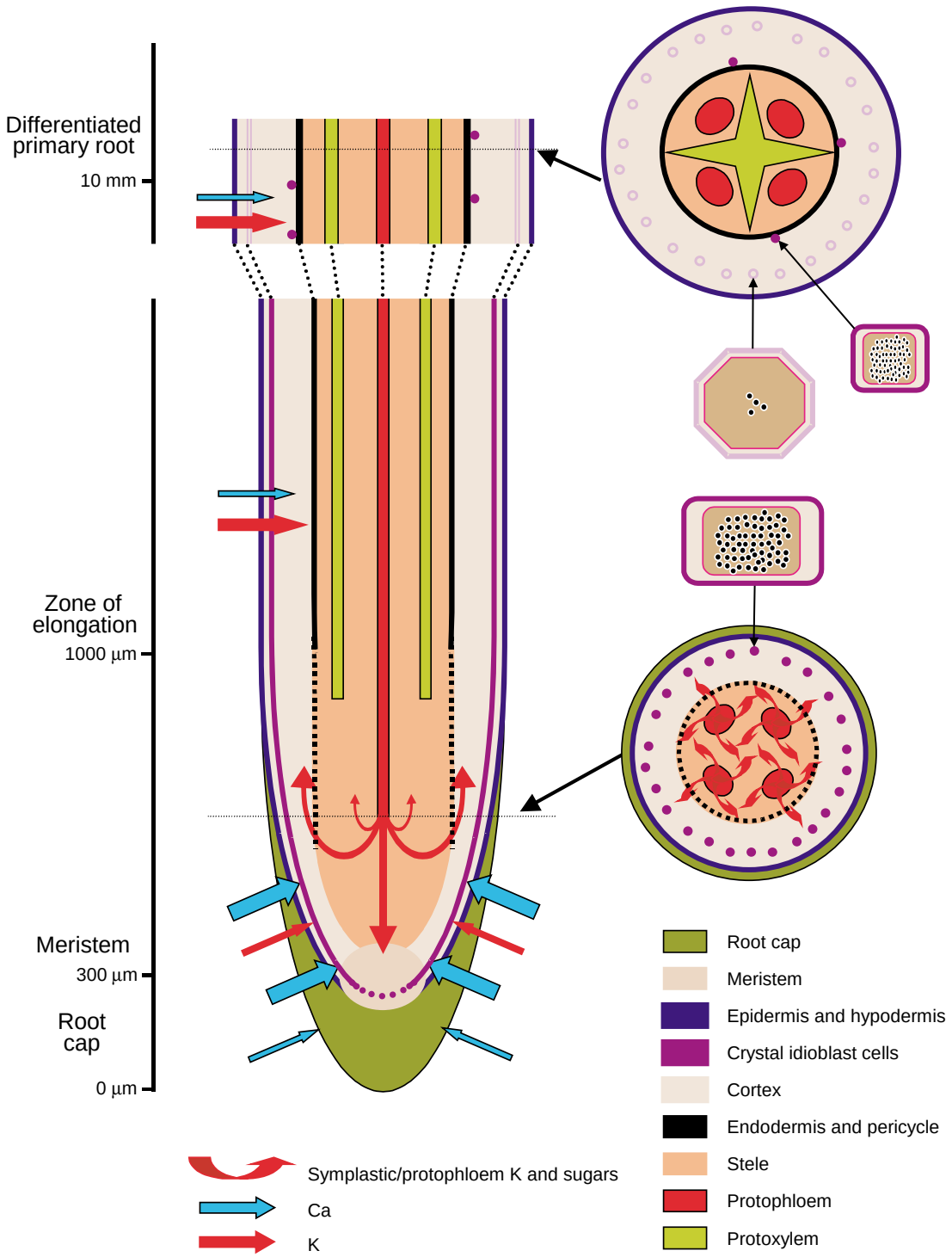

Fig. 5. Model of K supply and Ca uptake by the apical grapevine root. Calcium is sequestered by crystal idioblast cells (specialised cortical cells) of the meristem and by specialised endodermal cells of the primary root. Calcium uptake is greatest in the apical region of the root (Malazian 1965). At 10 mm basipetal to the root tip the epidermis is moribund and the hypodermis forms the outer cell layer of the root. The protoxylem is fully developed with occasional metaxylem vessels. In this region of the root most of the cortical idioblast cells are devoid of Ca raphide bundles but raphide crystals have formed in the specialised endodermal cells.

Ca found in the meristematic idioblasts could be sourced through phloem recycling. Indeed, that the Ca taken up by roots is transported to the shoot with little translocation occurring towards the root tip is frequently reported (Marschner and Richter 1973; Marschner and Richter 1974).

In the root meristem, the driving forces for passive uptake of various ions will differ significantly. In the case of Ca, because of the steepness of the electrochemical gradient, and the extremely low free cytosolic Ca concentration, there is the potential for a large influx of Ca into root meristematic cells. Cells have mechanisms for the tight regulation of Ca influx, active efflux and Ca sequestration. The vacuole appears to act as both a transient source of cytosolic Ca during signal transduction and as a sink for Ca in unstimulated cells (Bush 1995). Over a longer time frame the large Ca sink of idioblast cells may function to regulate apoplastic concentrations of Ca as raphide induction can occur in less than 1 h in the roots of *L. minor* (Franceschi 1989). The distribution of the idioblast cells in *L. minor* led Franceschi (1989) to suggest that the idioblast 'services' a small volume of root tissue. But idioblast cells might also source Ca from adjacent cells by symplastic transfer. Thus, the distribution pattern of idioblast cells in the periphery of the grapevine root may mean that cytosolic Ca levels in the central meristem are regulated by Ca derived directly from the apoplast and/or from the symplast of neighbouring cortical cells.

Model of solute flows at the apical root

So one must conclude that there is probably no inconsistency between our observations of the different distribution patterns of the various ions in the meristematic region of the root. The contrasting behaviour of K and Ca, for example, may be explained by considering the relationships between anatomy, solute flux pathways, growth and electrochemical potential gradients and time (Fig. 5). By forming a discrete layer around the periphery of the root cortex (and given the rapid diffusion of ions over short distances) the crystal idioblast cells might modify the Ca status of the central meristematic region of the root. Increasing Ca content of idioblast cells induces the synthesis of oxalic acid from carbons 1 and 2 of ascorbic acid (Kostman *et al.* 2001) synthesised probably from phloem-derived carbohydrate. The idioblast cells may also serve as a sink for Ca as the cells enter the zone of elongation where there are elevated cytosolic Ca levels (Cramer and Jones 1996). If newly formed raphide bundles can be dissolved within a few hours (Franceschi 1989), this Ca pool could supply the zone of elongation with Ca during cell wall synthesis. With further differentiation of the primary root, in regions of increasing radial water flux, crystal idioblast cells develop adjacent to the endodermis extending into the cortex. These cells may then play a role in regulating Ca movement across the endodermis in a region of the root where translocation of ions to the shoot is significant.

Whole-plant Ca regulation and plant growth

We have presented preliminary data showing the localisation of Ca in grapevine roots and have raised the possibility that there could be an important relationship between specialised Ca-accumulating cells in roots and the calcicole/calcifuge behaviour of grapevine species. Historically, in Australia, and other parts of the world, *V. vinifera*, the dominant species for commercial grape production, was found to be susceptible to soil-born pathogens (phylloxera and nematodes). The problem was addressed by the introduction of American rootstock species such as *V. riparia* and *V. labrusca*, but these genotypes proved to be lime sensitive. Lime tolerance was introduced by making crosses with species like *V. berlandieri*. Although *V. riparia* is ranked as lime sensitive, selections based on *V. berlandieri* and *V. riparia* crosses turned out to be moderately to highly lime tolerant (Hardie and Cirami 1988). Given that the backcross used in this study was based on a parent line from *V. berlandieri*, the expected calcicole behaviour of this genotype might be associated in part with the physiology of the idioblast cells described in this study.

Under conditions of high and prolonged Ca availability, crystal idioblasts in different parts of the plant may function to sequester deleteriously high levels of Ca in vacuoles, leading to localised modulations in symplastic/apoplastic Ca, for example, around stomata, thereby changing stomatal behaviour (De Silva *et al.* 2001). Alternatively, crystal idioblast cells in the roots and other parts of the plant may function in nourishing growing regions with an essential supply of Ca under conditions of low Ca availability. It is possible that the root idioblast cells in lime tolerant grapevines may play a role in the adaptation of rootstocks to calcareous soils in vineyards and offer future opportunities for further genetic improvement of grapevine rootstocks.

References

Antcliff AJ, Newman HP, Barrett HC (1983) Variation in chloride accumulation in some American species of grapevine. *Vitis* **22**, 357–362.

Arnott HJ (1962). 'The seed, germination and seedling of *Yucca*.' University of California Publications in Botany.

Arnott HJ, Pautard FG (1970) Calcification in plants. In 'Biological calcification: cellular and molecular aspects'. (Ed. H Schraer) pp. 375–446. (Appeton-Century-Crofts: New York)

Bangerth F (1979) Calcium-related physiological disorders of plants. *Annual Review of Phytopathology* **17**, 97–122.

Bush DS (1995) Calcium regulation in plant cells and its role in signaling. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 95–122.

Bush DS, McColl JG (1987) Mass-action expressions of ion exchange applied to Ca^{2+}, H^{+}, K^{+}, and Mg^{2+} sorption on isolated cells walls of leaves from *Brassica oleracea*. *Plant Physiology* **85**, 247–260.

Conradie WJ (1983) Liming and choice of rootstocks as cultural techniques for vines in acid soils. *South African Journal of Enology and Viticulture* **4**, 39–44.

Cramer GR, Jones RL (1996) Osmotic stress and abscisic acid reduce cytosolic calcium activities in roots of *Arabidopsis thaliana*. *Plant, Cell and Environment* **19**, 1291–1298.

Cromack K Jr, Sollins P, Graustein WC, Speidel K, Todd AW, Spycher G, Li CY, Todd RL (1979) Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. *Soil Biology and Biochemistry* **11**, 463–468.

De Silva LDR, Mansfield TA, McAinsh MR (2001) Changes in stomatal behaviour in the calcicole *Leontodon hispidus* due to the disruption by ozone of the regulation of apoplastic Ca^{2+} by trichomes. *Planta* **214**, 158–162.

Delas JJ (1992) Criteria used for rootstock selection in France. In 'Rootstock seminar: a worldwide perspective'. (Eds JA Wolpert, MA Walker and E Weber) pp. 1–14. (American Society of Enology and Viticulture: Davis, CA)

Evans ML, Ishikawa H (1997) Cellular specificity of the gravitropic motor response in roots. *Planta* **203**, S115–S122.

Fabbri A, Benelli C, di Collalto G (1992) Calcium oxalate crystals in vegetative and reproductive organs of the grapevine. *Acta Horticulturae* **292**, 107–112.

Farrar JF, Minchin PEH, Thorpe MR (1995) Carbon import into barley roots: effects of sugars and relation to cell expansion. *Journal of Experimental Botany* **46**, 1859–1865.

Franceschi VR (1989) Calcium oxalate formation is a rapid and reversible process in *Lemna minor* L. *Protoplasma* **148**, 130–139.

Hardie WJ, Cirami RM (1988) Grapevine rootstocks. In 'Viticulture. Vol. 1: resources'. (Eds BG Coombe and PR Dry) pp. 154–176. (Australian Industrial Publishers: Adelaide)

Hepler PK (1986) Calcium regulation of mitosis: the metaphase translation. In 'Molecular and cellular aspects of calcium in plant development'. (Ed. AJ Trewavas) pp. 176–177. (Plenum: New York)

Hoagland DR, Arnon DI (1938) 'The water-culture method for growing plants without soil.' University of California, College of Agriculture Circular No. 347, Berkeley.

Huang JW, Grunes DL, Kochian LV (1992*a*) Aluminum effects on the kinetics of calcium uptake into cells of the wheat root apex. Quantification of calcium fluxes using a calcium-sensitive vibrating microelectrode. *Planta* **188**, 414–421.

Huang JW, Shaff JE, Grunes DL, Kochian LV (1992*b*) Aluminum effects on calcium fluxes at the root apex of aluminum-sensitive wheat cultivars. *Plant Physiology* **98**, 230–237.

Jeschke WD, Stelter W (1976) Measurement of longitudinal ion profiles in single roots of *Hordeum* and *Atriplex* by use of flameless atomic absorption spectroscopy. *Planta* **128**, 107–112.

Kinraide TB (2001) Ion fluxes considered in terms of membrane-surface electrical potentials. *Australian Journal of Plant Physiology* **28**, 605–616.

Kostman TA, Tarlyn NM, Loewus FA, Franceschi VR (2001) Biosynthesis of L-ascorbic acid and conversion of carbons 1 and 2 of L-ascorbic acid to oxalic acid occurs within individual calcium oxalate crystal idioblasts. *Plant Physiology* **125**, 634–640.

Malazian GE (1965) Histoautoradiographic studies of calcium uptake and transport across grape (*Vitis vinifera* L.) root cortex in relation to transpiration. University of California, Davis, USA.

Marschner H, Richter C (1973) Accumulation and translocation of K^+, Na^+ and Ca^{2+} supplied to different root zones of maize seedlings. *Zeitschrift für Pflanzenernährung und Bodenkunde* **135**, 1–15.

Marschner H, Richter C (1974) Calcium transport in roots of maize and bean plants. *Plant and Soil* **40**, 193–210.

Marschner H, Kirkby EA, Cakmak I (1996) Effect of mineral nutritional status on shoot–root partitioning of photoassimilates and cycling of mineral nutrients. *Journal of Experimental Botany* **47**, 1255–1263.

Newman HP, Antcliff AJ (1984) Chloride accumulation in some hybrids and backcrosses of *Vitis berlandieri* and *Vitis vinifera*. *Vitis* **23**, 106–112.

Northcote KH (1988) Soils and Australian viticulture. In 'Viticulture. Vol. 1: resources'. (Eds BG Coombe and PR Dry) pp. 61–90. (Australian Industrial Publishers: Adelaide)

Oparka KJ, Duckett CM, Prior DAM, Fisher DB (1994) Real-time imaging of phloem unloading in the root tip of *Arabidopsis*. *Plant Journal* **6**, 759–766.

Pouget R, Delas J (1982) Interaction entre le greffon et le porte-greffe chez la vigne. Application de la méthod des greffages réciproques à l'étude de la nutrition minérale. *Agronomie* **2**, 231–242.

Pritchard J, Tomos AD, Jones RGW (1987) Control of wheat root elongation growth. I. Effects of ions on growth rate, wall rheology and cell water relations. *Journal of Experimental Botany* **38**, 948–959.

Richards D (1983) The grape root system. *Horticultural Reviews* **5**, 127–168.

Robinson JB (1992) Grapevine nutrition. In 'Viticulture. Vol. 2: practices'. (Eds BG Coombe and PR Dry) pp. 178–208. (Winetitles: Adelaide)

Ruiz LP, Atkinson CJ, Mansfield TA (1993) Calcium in the xylem and its influence on the behaviour of stomata. *Philosophical Transactions of the Royal Society of London*. *B* **341**, 67–74.

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signaling. *Plant Cell* **14**, S401–S417.

Schroder WH, Bauch J, Endeward R (1988) Microbeam analysis of Ca exchange and uptake in the fine roots of spruce: influence of pH and aluminium. *Trees*: *Structure and Function* **2**, 96–103.

Sharp RE, Hsiao TC, Silk WK (1990) Growth of the maize primary root at low water potentials. II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Plant Physiology* **93**, 1337–1346.

Storey R, Pitman MG, Stelzer R (1983) X-ray micro-analysis of cells and cell compartments of *Atriplex spongiosa*. II. Roots. *Journal of Experimental Botany* **34**, 1196–1206.

Storey R, Schachtman DP, Thomas MR (2003) Root structure and cellular chloride, sodium and potassium distribution in salinised grapevines. *Plant*, *Cell and Environment* **26**, 789–800.

Takagi S, Yamamoto KT, Furuya M, Nagi R (1990) Cooperative regulation of cytoplasmic streaming and Ca^{2+} fluxes by Pfr and photosynthesis in *Vallisneria* mesophyll cells. *Plant Physiology* **94**, 1702–1708.

Volk GM, Lynch-Holm VJ, Kostman TA, Goss LJ, Franceschi VR (2002) The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. *Plant Biology* **4**, 34–45.

White PJ (2000) Calcium channels in higher plants. *Biochimica et Biophysica Acta*, *Biomembranes* **1465**, 171–189.

White PJ (2001) The pathways of calcium movement to the xylem. *Journal of Experimental Botany* **52**, 891–899.

Wolf O, Jeschke WD (1987) Modeling of sodium and potassium flows via phloem and xylem in the shoot of salt-stressed barley. *Journal of Plant Physiology* **128**, 371–386.

Wyn Jones RG (1999) Cytoplasmic potassium homeostasis: review of the evidence and its implications. In 'Frontiers in potassium nutrition: new perspectives on the effects of potassium on physiology of plants'. (Eds DM Oosterhuis and GA Berkowitz) pp. 13–22. (Potash & Phosphate Institute: Canada)

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