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Anatomical characteristics of roots of citrus rootstocks that vary in specific root length

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SUMMARY

Among citrus rootstocks, higher specific root length (SRL, root length/d. wt) has been linked to several specific morphological and physiological traits, including smaller average root diameter, higher root hydraulic conductivity and higher rates of root proliferation. In this study, thickness of the outer tangential exodermal (hypodermal) wall and its suberin layer, number of passage cells, presence of epidermis, and stelar anatomy were examined and related to variation in root diameter of field roots of known maximum age. We also compared root morphology and anatomy of young roots in the field with those of potted rootstock seedlings in the glasshouse. Fibrous roots were measured separately from pioneer (framework) roots. Among the fibrous roots, only the first-order (root links having a root tip) and second-order (root links bearing first-order roots) laterals were examined. Among first-order field roots, larger root diameter was caused by larger rather than more numerous cells in the cortex. Root diameter of first-order roots was positively correlated with both number of passage cells in the exodermis and thickness of the secondary walls of the exodermis in both field and potted plants.

Exodermal walls were about 80% thicker in field- than pot-grown roots. In the field, in more than 50% of the first-order roots examined less than 30% of the root surface was still covered by epidermis, with few differences among rootstocks. In contrast, in roots of 19-wk-old glasshouse plants generally 70–100% of the epidermis was still intact. There was no evidence of secondary xylem development in second-order fibrous roots in the field; in seedling, pot-grown rootsystems, 75–97% of second-order roots had formed secondary xylem despite their small diameter (<0.8 mm).

It is argued that there can be suites of physiological, morphological and anatomical traits in roots that co-vary with specific root length. Investigations of how root morphology and anatomy are linked to root function, moreover, need to recognize trait variability and the potentially important differences between field- and pot-grown (seedling) roots.

Key words: citrus, root anatomy, exodermis, hypodermis, root diameter, passage cells, specific root length (SRL).

INTRODUCTION

In leaves, suites of morphological, anatomical and physiological traits have been correlated with leaf longevity (Reich *et al.*, 1997). Long-lived leaves are typically tough and thick, with a low specific leaf area (SLA, $cm^2\ g^{-1}$ d. wt) and high tissue density (specific gravity) (Coley, 1988; Garnier & Laurent, 1994; Reich *et al.*, 1997). These morphological and anatomical characteristics are associated with lower shoot-growth rates, lower maximum photosynthetic rates, lower dark-respiration rates and lower concentrations of photosynthetic enzymes. Although the data are much more sparse, similar suites of correlated traits among plants can also occur in the fine, absorptive roots (Eissenstat, 1992).

Among *Citrus* spp. and close relatives (notably *Poncirus* spp.), there is wide variation in average diameter and specific root length (SRL, cm g^{-1} d. wt) of the fine, fibrous root system (Graham & Syvertsen, 1985; Eissenstat, 1991). Secondary growth normally does not occur in the fibrous root system (Schneider, 1968); indeed, fibrous roots can decrease in diameter because of shrinking of cortical cells and loss of the

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epidermis (Eissenstat, 1991). Among trees with genetically identical shoots (scions), root diameter and specific root length of different rootstock genotypes have been correlated with rates of root proliferation in disturbed soil (Eissenstat, 1991). The correlation of root diameter with rates of mycorrhizal colonization was only apparent when adjusted for plants of different mycorrhizal dependency based on phosphorus demand (Graham & Syvertsen, 1985; Graham *et al.*, 1991). Specific root length and average root diameter have also been related to root hydraulic conductivity of seedling genotypes (Graham & Syvertsen, 1985; Eissenstat, 1997). Thus, there is some evidence to suggest that fineness of the absorptive roots might be linked to a suite of traits that influences rates of resource acquisition over the lifetime of the tissue in a manner similar to that recognized in leaves.

In citrus rootstocks, median lifespan of the fibrous roots can exceed 100 d (Eissenstat & Yanai, 1997). During this time, fibrous roots change considerably, both in response to normal development and to environmental conditions. These changes can be biochemical or structural. Biochemically, roots can incorporate polyphenols in the walls of the epidermis that could be involved in disease resistance (Tippet & O'Brien, 1976; Duncan *et al.*, 1993). Structurally, roots can develop thick, cellulosic walls that can be lignified to counter the tendency to collapse under drought stress (Peterson & Waite, 1996). In the exodermis, lignin and suberin layers can develop which protect the root interior after the epidermis is sloughed away (Walker *et al.*, 1984). Thickened exodermal walls impregnated with lignin and suberin can enhance root longevity at the expense of reduced absorptive capacity (Duncan *et al.*, 1993; D. M. Eissenstat unpublished data).

We predict the citrus rootstock genotypes with high SRL and fine average diameter of fibrous roots should exhibit relatively less secondary thickening of their exodermis than those with coarser fibrous roots. As the exodermis becomes more impermeable, there should be greater reliance on passage cells for mineral nutrient and water absorption. In addition, development of the exodermis should be linked to loss of the epidermis, which should enhance development of suberin and lignin layers in the walls of the exodermis. In addition, secondary wall development in the exodermis should reduce cytoplasmic connections between the exodermis and epidermis, thus promoting death of the epidermal cells (Walker *et al.*, 1984; Barrowclough & Peterson, 1994). This study addresses not only mean differences between roots but also the population variability associated with the morphological and anatomical characters. We emphasized fibrous roots of known maximum age for trees in orchard conditions, but because of their frequent use for anatomical studies, roots of seedlings in pots were also examined, permitting contrasts of anatomical differences that arise because of differences in plant age and soil conditions.

MATERIALS AND METHODS

Field experiment

The field site was a citrus rootstock trial located about 7 km southeast of Avon Park, Florida, USA (lat. 27° 34′ N, long. 81° 28′ W) and was the site of previous work on relationships of specific root length (SRL) to root function (Eissenstat, 1991; Graham *et al.*, 1991). Soils were a deep, uniform Astatula fine sand (Typic quartzipsamment) with less than 1% organic matter. The 16-yr-old 'Valencia' sweet orange (*Citrus sinensis* (L.) Osbeck) trees were grown 4.6 m apart in rows 6.2 m apart. Rootstocks were arranged in a randomized complete block design with rootstock cultivars randomly located in three-tree sets within each row (block). To minimize the possibility of sampling roots of neighboring trees, samples were only collected from the middle tree of each rootstock set in each of eight rows. Selected rootstocks were: trifoliate orange (TO, *Poncirus trifoliata* (L.) Raf.), rough lemon (RL, *Citrus jambhiri* Lush), Swingle citrumelo (SC, *C. paradisi* Macf. × *P. trifoliata*) and sour orange (SO, *C. aurantium* L.). These rootstocks represent a wide range of SRL and average fibrous root diameter. Based on past research at this site (Eissenstat, 1991), the ranking of rootstocks in terms of average fibrous root diameter is: TO < RL < SC ≈ SO.

We used an ingrowth approach previously described to examine roots of known maximum age (Eissenstat, 1991). Three soil cores, 7.4 cm in diameter and 14.2 cm deep, were extracted from beneath the canopy of each of 32 trees (four rootstocks × eight replications) at about two-thirds of the distance from the trunk to the canopy dripline. A plastic pipe was snugly fitted into the resultant hole so that the top was flush with the soil surface. To permit roots to grow readily into the plastic pipes, 12 3.6 cm-diameter holes were made in the side of the pipe, and covered with a 3-mm-mesh nylon screen. The soil extracted from beneath the canopy of the four rootstocks in the row was sieved to remove roots larger than 3 mm, mixed to remove any potential nutritional and biotic differences then replaced in the plastic containers. The containers were installed 16 June 1990 and removed 14 wk later (30 Oct. 1990), pooling the roots in the three containers for an individual tree. Roots were divided into three categories: first-order fibrous roots (terminal roots with growing root tips), second-order fibrous roots (portions of the root that bear first-order roots) and pioneer roots (large, straight, generally unbranched roots with prominent tip and large diameter). From each tree, five roots of each

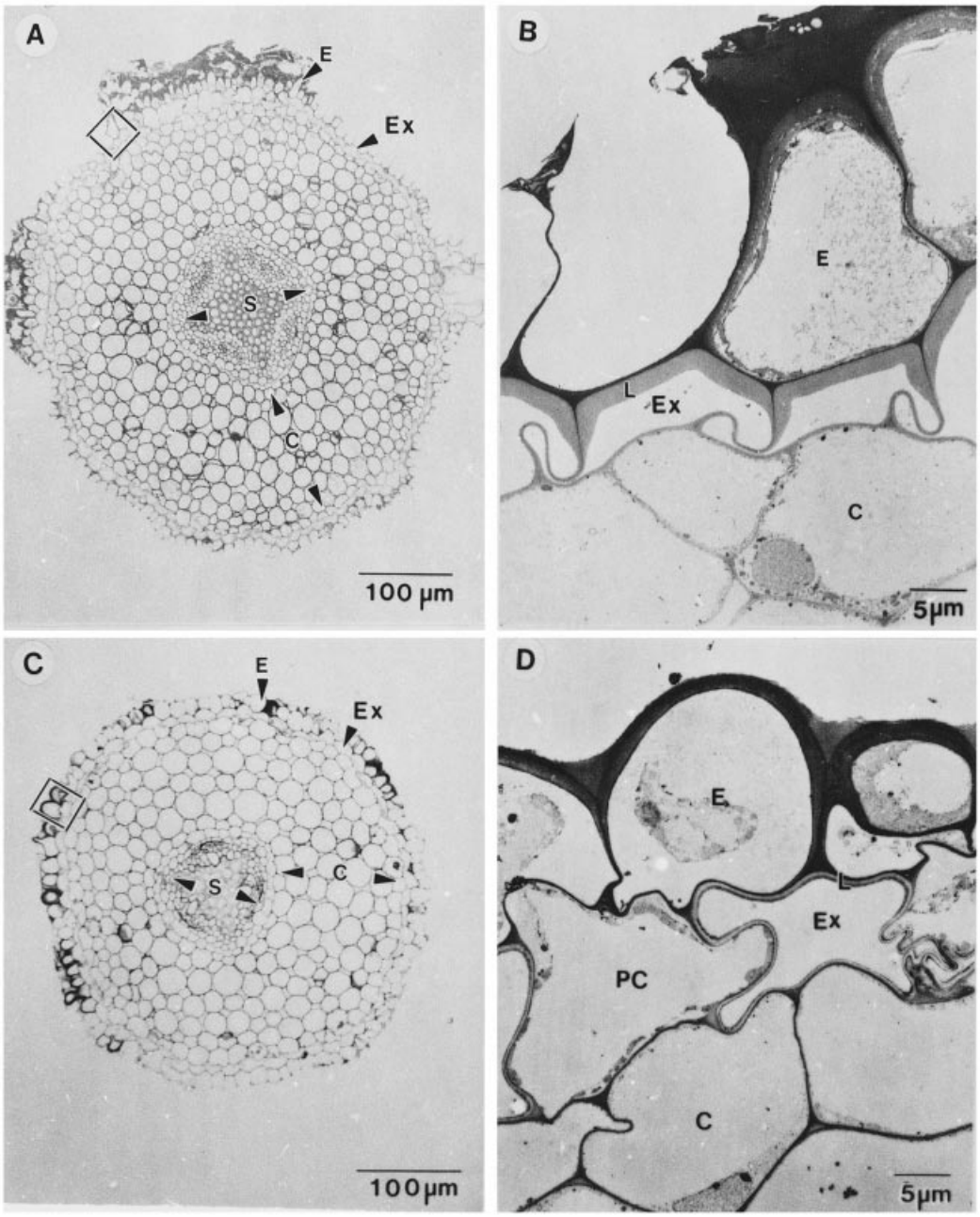

Figure 1. Transverse sections, 1.5 cm from the root tip, of first-order citrus fibrous roots less than 14 wk old collected from field trees. (A) Light micrograph of sour orange root showing epidermis (E), exodermis (hypodermis, EX), inner cortex (C) and stele (S). An enlargement of an area similar to that in the square is shown in (B). (B) Transmission electron micrograph (TEM) of sour orange epidermis (E), exodermis (Ex) and inner cortex (C). Note thickening of outer tangential wall (L) of the exodermal cells. (C) Light micrograph of transverse section of trifoliate orange first-order root showing the areas indicated above. (D) TEM of trifoliate orange root indicated in square in (C) showing the epidermis, exodermis with passage cells (PC) and inner cortex. The outer tangential wall of the exodermal cells is not as thick in trifoliate orange as in sour orange.

category were randomly taken for microscopic examination.

Glasshouse experiment

As part of a study for five rootstock cvs (Graham *et al*., 1997), we grew seedlings of trifoliate orange (TO), Carrizo citrange (CC, *C. sinensis* (L.) Osbeck × *P. triofliata*), Volkamer lemon (VL, *C. volkameriana* Tan. & Pasq.), Swingle citrumelo (SC) and sour orange (SO). In terms of average fine root diameter, these seedlings have the following ranking: TO < CC ≈ VL < SC ≈ SO. Seeds of the five genotypes were germinated in a commercial peat-perlite-bark soilless medium with starter nutrients, and grown in 150-cm^3 plastic containers for 3 months (2–4 true-leaf stage). The medium was thoroughly washed from the roots, and each bare-rooted seedling was transplanted in 1 l of autoclaved (121°C) Candler fine sandy soil (pH 6.8) (soil very similar to that

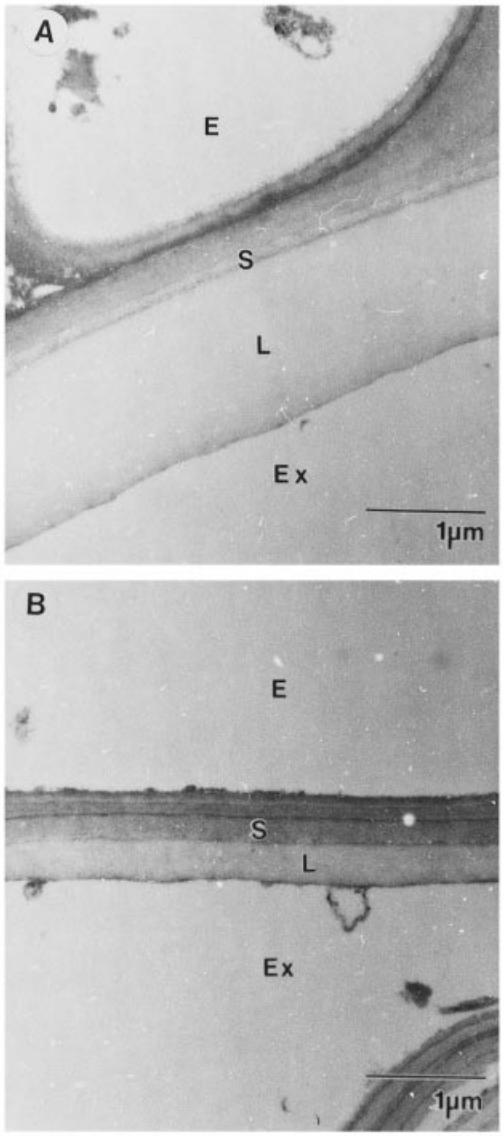

Figure 2. Transmission electron micrographs of transverse sections of field first-order fibrous roots showing the cell wall thickness of the exodermis (Ex). (A) Sour orange. (B) Trifoliate orange. Micrographs taken at same mangification to illustrate the typical widths of the lignified (L) and suberized (S) layers of the outer tangential wall. The area above the wall is a epidermal cell (E) and below the wall is the exodermal cell (Ex).

found at the field site). Plants were inoculated with 0.2 g of Sudan grass (*Sorghum sudanense* (Staph.) Piper) roots containing 1000 propagules (determined by most probable number analysis) of *Glomus intraradices* FL. 208 placed below each seedling at transplanting. Plants were grown from April to August 1991 (19 wk) in a shaded glasshouse with a maximum photon flux density of 1000 μmol m^{-2} s^{-1}. Average day/night temperatures were 33°C/25°C and relative humidity ranged from 60–100%. Seedlings were watered to excess every other day with tap water and fertilized weekly with Hoagland's solution with 5 mM P. Incidence of infection was greater than 71%, except in TO where it was 41% using methods previously described (Graham *et al.*, 1991). For each seedling, five roots of each root category were used for anatomical determinations.

Microscopy

Roots were prepared for light and transmission electron microscopy by first placing them in 3% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.3) for 4–24 h. They were then washed in the same buffer, dehydrated in an acetone series and embedded in Spurr's resin (Spurr, 1969). For first-order roots and pioneer roots, sections were made about 1.5 cm from the root tip. For second-order roots, the link between two first-order roots was sectioned. For light-microscopy measurements, one micrometer sections were made using a glass knife and an ultramicrotome (LKB Huxley) and stained with toluidine blue (O'Brien *et al.*, 1964). For gross measurements and cell counts, an eye-piece micrometer on a compound microscope was used. For finer measurements, we used an oil immersion lens (100×) on a compound microscope (Zeiss) and a Bioquant IV measuring system (R. & M. Biometrics, Inc., Nashville, TN, USA). The following measurements were made on all rootstock genotypes using light microscopy: root diameter, percentage of the surface covered by epidermal cells, number of passage cells in a transverse section of the exodermis, and archy of the stele. Passage cells (short cells) were identified by the absence of a thickened, lignified outer tangential wall and the obvious presence of cytoplasm and cellular organelles, occasionally including the nucleus. Other exodermal cells (long cells) have thickened, lignified outer walls and very little obvious cytoplasm at maturity. In addition, measurements of the thickness of the outer tangential wall of 12 different long cells in the exodermis were made in each root.

For the two rootstocks that differed most in root diameter, TO and SO, we also examined, by light microscopy, aspects of root anatomy that would cause differences in root diameter. We measured radius of the cortical layer from the exodermis to the endodermis (one measurement per root), number of cortical cells in a radial transect from the exodermis to the endodermis (one measurement per root), diameter of each cortical cell (five measurements per root), radius of the stele (one measurement per root), diameter of xylem cells (five measurements per root), number of xylem cells in a radial transect to the center of the root (one measurement per root), and thickness of the cortical and xylem vessel walls (10 measurements per root). Using transmission electron microscopy (×15000, Philips 201), we also measured the thickness of the suberin layer in the outer tangential wall of the exodermal layer (five measurements per root) for comparison with overall wall thickness. Thin sections (90–100 nm) were made with a diamond knife and stained with uranyl acetate

(Stempak & Ward, 1964) and lead citrate (Reynolds, 1963).

Data analysis

All data were analysed using the statistical software, SAS (SAS Inst. Inc., Cary, NC, USA). To assess significant effects of rootstock cultivar, field and glasshouse experiments were analysed separately, using the non-parametric Kruskal–Wallis test with eight replications (trees or seedlings). Medians were compared with the Wicoxon test. Non-parametric tests were chosen for examination for rootstock effects because of serious departures from normality in the data. Simple correlations of root diameter with exodermal wall thickness and with passage cell number across all cultivars were determined separately for field and pot experiments, using Pearson's correlation coefficient.

RESULTS

Representative cross sections of field fibrous roots of citrus are illustrated in Figure 1. The first-order roots of citrus often lose their epidermis after a few weeks. Wall thickness in the exodermis (EX) tends to be thicker in sour orange than in trifoliate orange, except where passage cells are present (PC). The suberin layer in the walls of the exodermis, however, might not covary in a similar fashion to the lignified layer of the exodermal cell wall (Fig. 2). Trifoliate orange, the rootstock with the thinnest exodermal walls, had a suberin layer twice at thick (0.46 ± 0.22 μm, mean $\pm$ SE) as that of sour orange (0.23 ± 0.005 μm).

Root traits such as diameter, wall thickness and number of passage cells do not necessarily exhibit normal distributions (Fig. 3). A frequency distribution of diameter of field first-order roots of TO was distinctly bimodal, with peaks at 400 and 500 μm. Frequency distributions of other rootstocks exhibited first-order root diameters primarily at 500 μm (550 μm for SO) with secondary peaks between 650 and 900 μm. The distributions of the thickness of exodermal walls was fairly symmetrical for TO and SC, but indicated evidence of bimodality in the *Citrus* rootstocks SO and RL. The distribution of number of passage cells visible in an exodermal cross section was strongly skewed to the right, especially in TO.

Root diameters of the first-order roots ranged from 420 to 529 μm in the field experiment and from 386 to 545 μm in the glasshouse experiment (Table

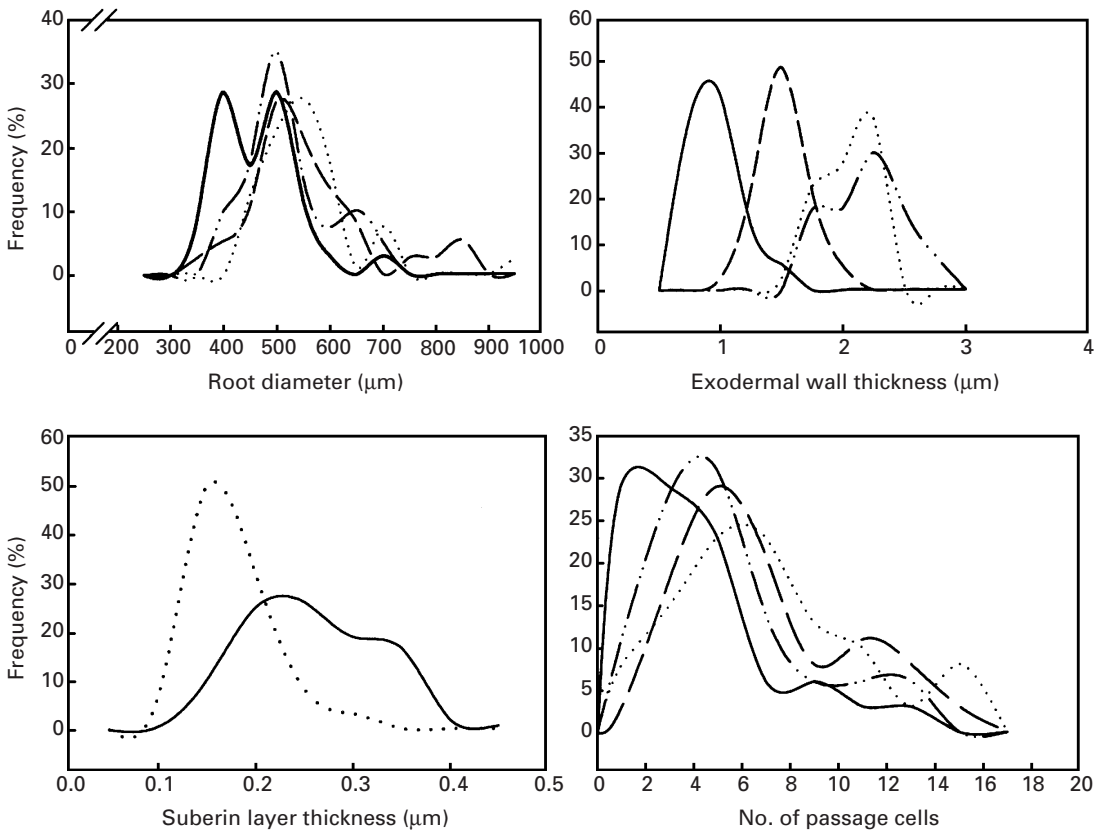

Figure 3. Frequency distribution of root diameter, thickness of the outer tangential wall of the exodermis, thickness of the outer tangential wall of the suberin layer alone, and passage cell number of first-order roots collected from trees in the field. Roots were less than 14 wk old. Trees were Valencia sweet orange on trifoliate orange (TO) (——), sour orange (SO) (· · ·), Swingle citrumelo (SC) (——) and rough lemon (RL) (—· ·) rootstocks. Each frequency distribution represents about 80 roots (10 roots × 8 trees) for each rootstock.

Table 1. *Mean root diameter, thickness of the outer tangential wall of the exodermis, number of passage cells in the exodermis and number of passage cells per unit length of root circumference in citrus roots collected from trees in the orchard (Field) or from seedlings in the glasshouse (Pot)*

	Fibrous roots					
	First order		Second order		Pioneer roots	
	Field	Pot	Field	Pot	Field	Pot
Diameter (μm)						
SO	529 a	545 q	666 a	872 s	952 b	1127 p
SC	522 a	417 p	723 a	691 q	1020 b	1033 pq
RL	487 b		599 a		1069 b	
TO	420 c	386 p	715 a	598 p	1292 a	845 q
VL		502 q		820 s		1213 p
CC		510 q		766 r		1021 pq
Exodermal wall thickness (μm)						
SO	1.93 a	1.25 s	2.16 d	0.74 qr	2.16 a	1.45 p
SC	1.38 b	0.69 q	1.47 b	0.71 r	1.63 c	0.86 q
RL	2.02 a		1.88 c		2.04 b	
TO	0.82 c	0.58 p	0.70 a	0.77 q	0.82 d	0.59 r
VL		0.85 r		0.82 p		0.80 q
CC		0.69 q		0.81 p		0.66 r
Passage cells (no.)						
SO	6.38 a	9.63 pq	4.12 a	4.94 p	3.65 b	5.37 p
SC	6.26 a	6.88 r	7.73 b	4.72 p	7.43 b	8.75 p
RL	4.25 b		4.51 a		3.41 b	
TO	3.55 b	7.06 r	4.07 a	6.28 p	3.72 b	5.46 p
VL		10.97 p		7.84 p		7.18 p
CC		7.81 qr		6.78 p		4.36 p
Relative passage cell frequency (no. mm^{-1})						
SO	4.00 a	5.71 p	2.10 a	1.90 p	1.38 a	1.74 p
SC	3.69 a	5.44 p	3.62 b	2.40 p	2.67 a	2.83 p
RL	3.01 a		2.39 a		1.04 a	
TO	2.72 a	5.88 p	2.04 a	3.44 p	1.06 a	2.13 p
VL		7.12 p		3.12 p		2.26 p
CC		4.94 p		2.89 p		1.58 p

Within a column, statistically significant ($P<0.05$) values are indicated by different letters using a Kruskal–Wallis Test followed by paired-treatment comparisons using the Wilcoxon Rank Test.
Rootstock cultivars were sour orange (SO), Swingle citrumelo (SC), rough lemon (RL), trifoliate orange (TO), Volkamer lemon (VL), and Carrizo citrange (CC).

1). The field roots of SO had the largest diameter, averaging 26% thicker than those of trifoliate orange (TO), the rootstock with the smallest diameter. In potted seedlings, the diameters of first-order roots of SO were 41% greater than those of TO. Second-order roots tended to have larger diameters in potted seedlings than in field fibrous roots, with a notable lack of significant differences in diameter among rootstock cultivars in the field. Over all the rootstock cultivars, root diameter increased from first- to second-order roots an average of 38% in field roots and 59% in potted seedling roots.

Exodermal walls were about 76% thicker in field fibrous roots than in potted, seedling roots (Table 1). Among the three rootstocks common to both experiments (SO, SC, TO), average wall thickness was 1.38 and 1.40 μm in field, first- and second-order roots, respectively. Corresponding wall thicknesses in potted seedling roots were only 0.84 and 0.74 μm. Rootstock ranking of wall thickness was inconsistent between field and glasshouse fibrous roots. Exodermal wall thickness of pioneer roots was similar to that of fibrous roots.

Frequency of passage cells in a transverse section of the exodermis ranged from *c.* 4 to 6 cells in field first-order and from 7 to 11 cells in glasshouse first-order roots (Table 1). There were more passage cells in cultivars with thicker first-order roots than in those with thinner roots. When passage cell frequency was calculated per unit length of root circumference, few significant differences were detected among cultivars (Table 1).

Suberin thickness in the exodermal layer also differed both in mean thickness and in variability of wall thickness between SO and TO, with TO exhibiting both a thicker and more variable suberin layer than SO (Fig. 3). The suberin layer was about 10% of the thickness of the exodermal wall.

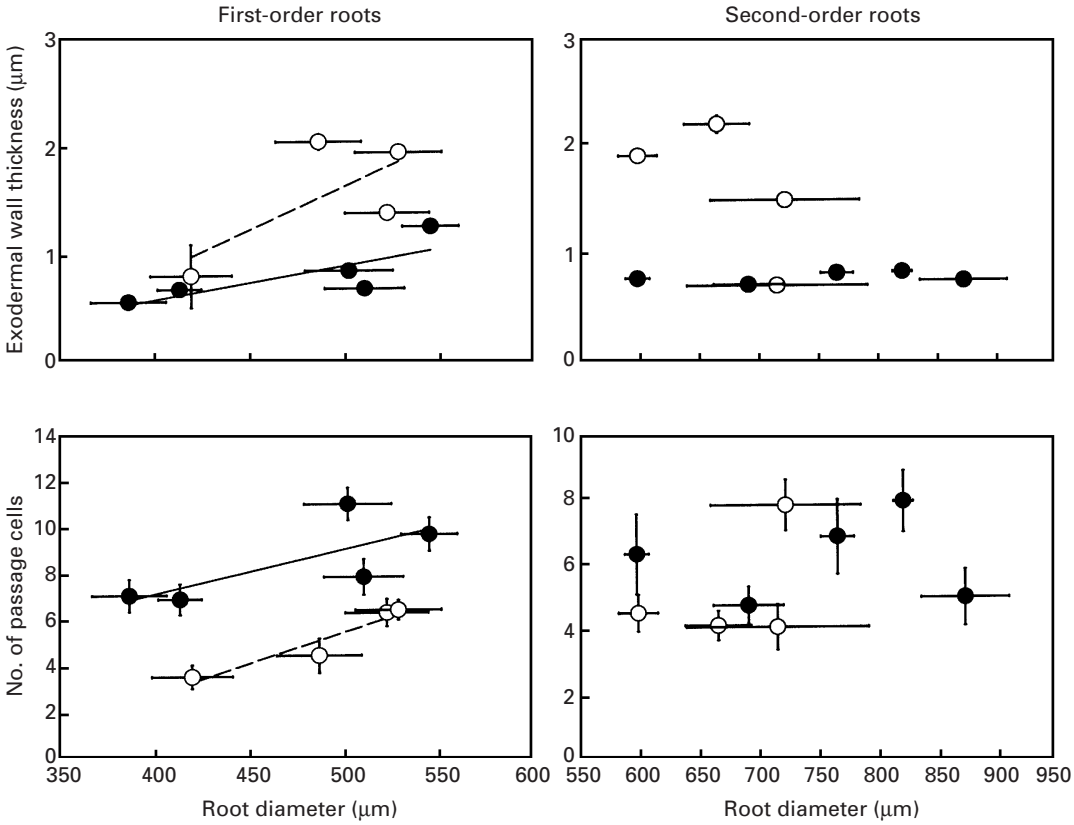

Figure 4. The relationship of root diameter with exodermal outer tangential wall thickness and with number of passage cells in the exodermis in first- and second-order roots. Means (±SE) of fibrous roots of rootstock genotypes from trees in the field (○) and from seedlings grown in the glasshouse (●). Least-squared regression lines presented where the correlation coefficient was significant at $P<0.10$ (see Table 2).

Table 2. *Correlation coefficient (r) of citrus root diameter with number of passage cells in the exodermis and with thickness of outer tangential walls in long cells of the exodermis from field trees or glasshouse-grown potted plants*

	Fibrous roots					
	First order			Second order		
	n	*r*	*P*	*n*	r	*P*
Field tree						
Diameter × passage cells	31	0.3441	0.0581	32	0.1130	0.5377
Diameter × exodermal wall	32	0.4432	0.0111	32	−0.1975	0.2785
Potted seedling						
Diameter × passage cells	40	0.4300	0.0056	40	−0.0273	0.8672
Diameter × exodermal wall	40	0.6352	0.0001	40	0.1121	0.4909

n = number of roots in regression (4 or 5 cultivars × 8 replications).

Rootstocks that tended to have thicker roots with larger diameters also tended to have thicker exodermal cell walls and more passage cells, but only in the first-order roots (Fig. 4, Table 2). No relationship was found in second-order or pioneer roots. When passage cell frequency was calculated per unit length of root circumference, no correlation of passage cells with root diameter was observed (data not shown). Although exodermal walls varied less in pot than field roots, the correlation of wall thickness with root diameter was higher in potted plants.

Loss of the epidermis was a common feature of field roots, but much less common in roots of potted plants (Fig. 5). In first-order roots, less than 30% of the epidermis still was intact in more than half all field roots examined, whereas in potted-seedling roots, more than 70% of the epidermis was intact in

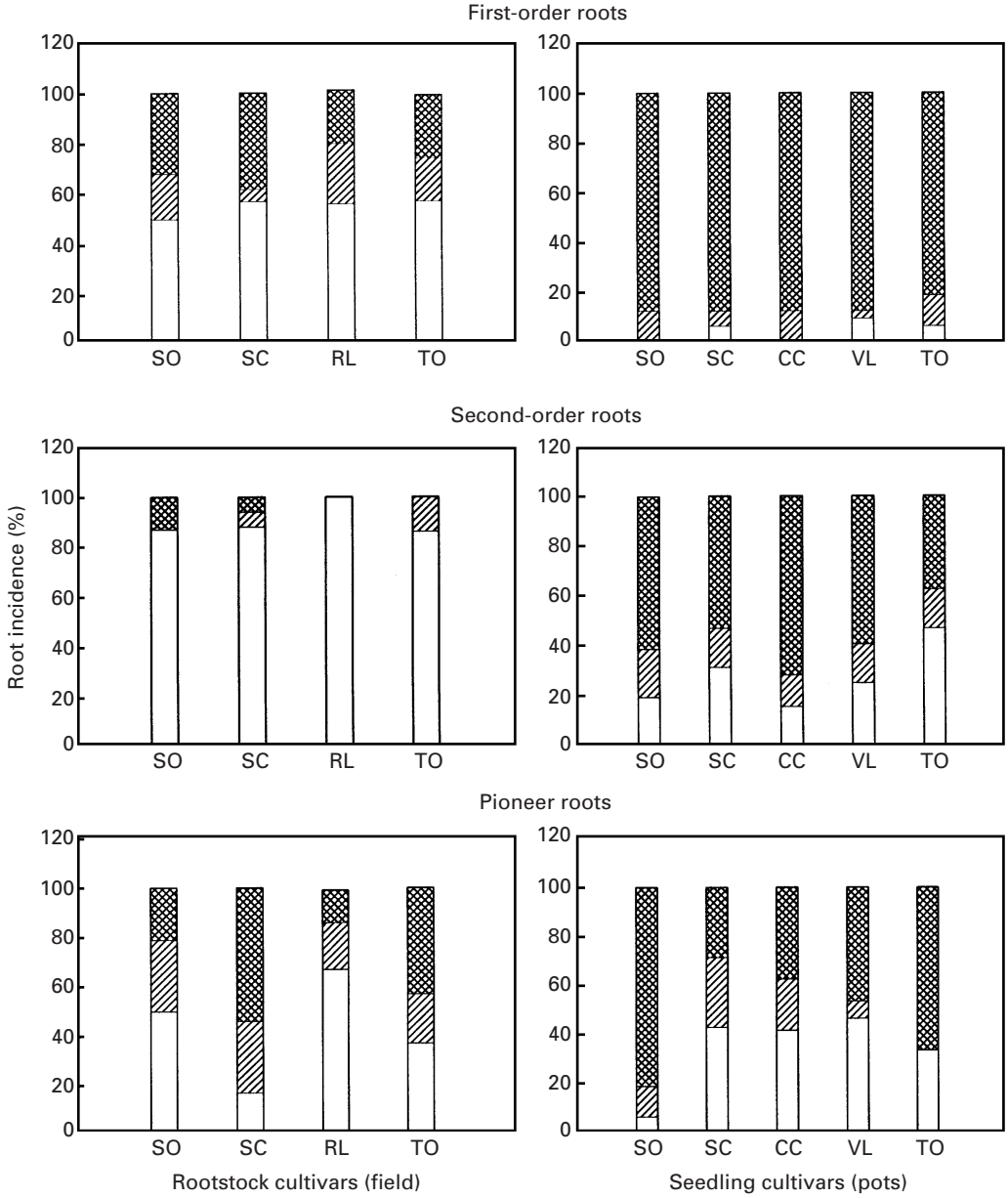

Figure 5. Percentage of roots of each citrus rootstock genotype of which 0–30 (□), 30–70 (▨) and 70–100% (⊠) of epidermis was still intact. Roots collected from field trees and glasshouse potted seedlings were no older than 14 and 19 wk, respectively.

over 90% of the roots. There was generally little variation among rootstock cultivars in the amount of epidermis retained by the roots.

Stele anatomy of field and potted-seedling roots for first-order fibrous and for pioneer roots was fairly similar (Fig. 6). The biggest difference occurred in second-order roots. Although seedling second-order roots were all less than 1 mm in diameter and looked quite similar to those of field fibrous roots, between 75 and 95% of seedling second-order roots exhibited secondary development of the stele (Fig. 6, Table 3). No such evidence of secondary development was found in field-derived second-order fibrous roots.

To determine which aspects of root anatomy accounted for the observed differences in root diameter among the citrus rootstocks, samples of the two extremes, sour orange and trifoliate orange, were examined (Table 4). Along a radial transect, SO had a stele only 8% larger but a cortex 33% larger than that of TO. The larger cortex was primarily caused by size, rather than number, of cortical cells.

DISCUSSION

Costs and benefits of the root exodermis

Earlier studies have hypothesized that there are trade-offs between producing roots of small diameter with low construction costs per unit length and producing fibrous root with relatively large diameter and high construction costs per unit length (Eissenstat, 1992). Previous research has demonstrated that

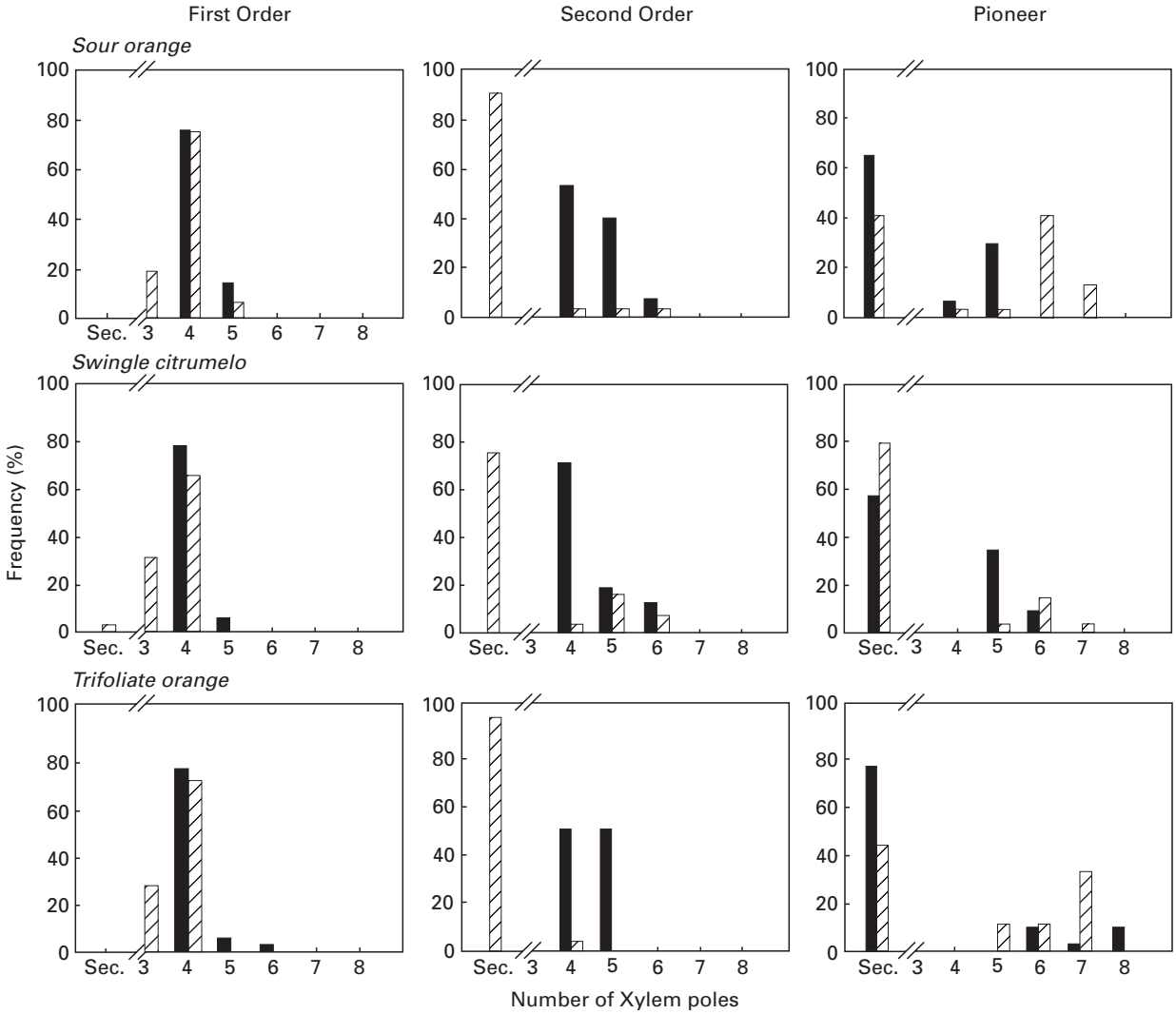

Figure 6. Stelar pattern of roots of three citrus rootstock genotypes, sour orange (SO), Swingle citrumelo (SC) and trifoliate orange (TO), grown in pots in the glasshouse (▨) or in the field (■). Stelar arrangement represents secondary stelar development (Sec) or triarch (3), tetrarch (4), pentarch (5), etc. primary stelar arrangement of first- and second-order fibrous roots and pioneer roots ($n = 80$ for each genotype × growing condition × root-class combination).

Table 3. *Percentage of roots exhibiting secondary xylem development in three citrus cultivars*

	Root type					
	First order roots		Second order roots		Pioneer roots	
Cultivar	Field	Pot	Field	Pot	Field	Pot
Sour orange	0	0	0	90.3	65.0	40.6
Swingle citrumelo	0	3.1	0	75.0	57.0	78.6
Trifoliate orange	0	0	0	96.9	77.0	44.4

Roots were collected from orchard trees (Field) or glasshouse-grown seedlings (Pot).

citrus rootstocks that have small average root diameter and high SRL tend to exhibit high hydraulic conductivity (Graham & Syvertsen, 1985; Eissenstat, 1997) and high rates of root proliferation in favourable soil patches (Eissenstat, 1991). In this study, we show that although the correlations were fairly low, specific anatomical features were significantly correlated with root diameter (Fig. 4, Table 2). Average root diameter of the first-order roots was positively correlated with thickness of the exodermal

Table 4. *Mean cell number, cell size and wall thickness of cortical and stelar tissues in a radial transect of transverse sections of first-order fibrous roots of sour orange (SO) and trifoliate orange (TO)*

	SO	TO	SO/TO
Root radius* (μm)	264.5 (8.0)	216.1 (6.7)	1.22
Cortical tissue			
Cortical radius* (μm)	150.0 (4.9)	112.4 (5.7)	1.33
No. of cortical cells	6.87 (0.15)	6.59 (0.20)	1.04
Cortical cell diameter* (μm)	23.54 (0.40)	19.36 (0.43)	1.22
Cortical cell wall* (μm)	0.15 (0.003)	0.17 (0.003)	0.88
Stelar tissue			
Stele radius* (μm)	56.23 (2.16)	52.09 (2.92)	1.08
No. of xylem cells*	7.30 (0.15)	6.71 (0.16)	1.09
Xylem cell diameter* (μm)	8.75 (0.28)	8.11 (0.20)	1.08
Xylem cell wall (μm)	0.41 (0.007)	0.46 (0.120)	0.89

*Differences in rootstock cultivars significant at $P<0.05$.
Standard error of the mean in parenthesis. The ratio of SO/TO is also indicated.

walls and number of passage cells in the exodermis in both field and glasshouse seedling roots. No correlation was found with second-order fibrous roots. Because fibrous roots of citrus do not increase in diameter with age, correlated ontogenetic changes of root diameter with exodermal development cannot be the cause of the significant correlation. Here, we consider coevolution of suites of traits associated with building a highly absorptive short-lived root vs. a longer-lived root of lower absorptive capacity. Development of a well defined exodermis is likely to be a key determinant of the type of root developed.

Citrus has a dimorphic exodermis with long cells that develop thick tertiary walls and short cells that act as passage cells. In a survey of angiosperm families, Shishkoff (1987) found that 98 of 358 (27%) genera had a dimorphic exodermis with representatives both in evolutionarily 'advanced' families such as the Orchidaceae as well as in 'primitive' families such as the Magnoliaceae. An exodermis with suberin lamellae and a Casparian band can reduce root absorptive capacity by restricting apoplastic water and ion uptake into the inner cortex of the root (Peterson, 1988; Peterson & Enstone, 1996). Lignification of the exodermis can further increase resistance to water and ion flow by blocking plasmodesmata, thereby limiting symplastic access to the plasma membrane of the exodermal cells (Cruz *et al.*, 1992). The thin-walled passage cells in the exodermis, consequently, are the major sites of ion uptake into the symplasm and might serve as key regulators of ion relations in the root (Walker *et al.*, 1984; Peterson & Enstone, 1996).

The finding that citrus rootstock genotypes with small average root diameters and high SRL tend to have less thickening of exodermal walls than thicker fibrous roots is likely to be at least partly related to trade-offs between absorptive capacity and longevity. Although lignification and presence of suberin lamellae might reduce the rate of water and ion uptake, a well developed exodermis should enhance the longevity of the root by helping to maintain ionic relations such as turgor during drought (Cruz *et al.*, 1992), exclusion of Na under salinity stress (Walker *et al.*, 1984; Storey & Walker, 1987; Reinhardt & Rost, 1995) and favourable Mg balance under Mg deficiency (Pozuelo *et al.*, 1984). Besides ionic relations, passage cells might strongly influence both pathogenic (Kamula *et al.*, 1994) and mycorrhizal (Smith *et al.*, 1989; Peterson, 1992) fungal infection of the inner cortex. Moreover, the greatly thickened walls of the long cells of the exodermis might also increase root defences and help deter root-feeding insects, nematodes and pathogenic fungi (Chiang, 1973; Kamula *et al.*, 1994; Cohn *et al.*, 1996). Thus, the evolutionary determinants governing whether or not a plant was more fit by investing in a highly suberized and lignified exodermis probably included trade-offs between short-lived, highly absorptive roots and long-lived roots with lower absorptive capacity.

Although we noted a significant correlation between diameter of first-order roots and wall thickness of the exodermis, there was not a one-to-one correspondence between average SRL or diameter of a citrus cultivar and its average exodermal wall thickness. Many factors besides genotype might influence development of the exodermal wall as well as root longevity. Lignification of citrus fibrous roots associated with root aging has been related to resistance to the citrus nematode (*Tylenchulus semipenetrans* Cobb) and to the root rot fungus (*Phytophthora parasistica* Dastur) (Duncan *et al.*, 1993). General, root resistance to these common pathogens, however, is actually higher in the rootstock cultivar with the thinner exodermal wall, TO, than in SO, the cultivar with notably thick exodermal walls. The differences in pathogen resistance are apparently due

to specific chemicals produced in the cortex of the TO host plant that are absent from SO (Van Gundy & Kirkpatrick, 1964; Widmer *et al*., 1998). Thus, although trade-offs associated with cultivar variation in exodermal wall thickness are generally consistent with variation in citrus root proliferation and root hydraulic conductivity, they are not consistent with rootstock resistance to two important citrus pathogens. Moreover, in the presence of periodic high populations of *Phytophthora*, sour orange roots have a median lifespan similar to that of trifoliate orange roots (about 90 d, Eissenstat & Yanai, 1997). In the absence of major pathogens, however, the median lifespan of sour orange roots is greater than 200 d, even when they are exposed to prolong drought (Eissenstat, 1998), which is probably much longer than that of the more drought-sensitive trifoliate orange.

Peterson (1992) notes the prevalence of species in the Magnoliopsida and Liliopsida that possess an exodermis, indicating that this structural feature evolved very early in angiosperms. Primitive species are also noted for a lack of root hairs, thick fibrous roots, and a high dependency on mycorrhizal fungi (Baylis, 1975). It is interesting that certain more evolutionarily advanced groups, such as Festucoid grasses, are not only noted for small fibrous root diameters and low mycorrhizal dependency, but also for the absence of an exodermis. Similarly to leaves (Reich *et al*., 1997), the evolution of longevity in roots might have involved suites of morphological and physiological traits, but as in leaves, it might be necessary to examine suites of traits across wide groups of plants before clear patterns emerge (Reich, 1993; Reich *et al*., 1997).

Environmental and developmental effects on fibrous root anatomy

Fibrous roots less than 14 wk old that arise from bearing trees in the orchard differed markedly from fibrous roots of potted seedlings grown for 19 wk after transplanting, probably for both environmental (field vs. pot environment) and developmental (seedling vs. tree) reasons. Field fibrous roots tended to have a much thicker exodermis than potted seedlings (Fig. 4, Table 1), probably because of the harsher field environment, where root-feeding soil organisms are more abundant and wetting–drying events in the soil are more frequent (Stasovski & Peterson, 1993; Barrowclough & Peterson, 1994). Secondary and tertiary development of the walls of the long cells of the exodermis were probably linked to the persistence of the epidermis, because plasmodesmata connections can be severed at this stage (Walker *et al*., 1984). Few field fibrous roots had much epidermis intact, in contrast with those of potted-seedling roots, which were well watered and never dried out (Fig. 5). The ephemeral nature of the epidermis has also been noted in *Citrus reticulata* var. *austera* hybrid? and *Citrus medica* L. (Walker *et al*., 1984) as well as other species (reviewed by Peterson & Enstone, 1996). In onion (*Allium cepa* L.), drought accelerated the death of the epidermis and, in some cases, increased the rate of maturation of the exodermis (Perumalla & Peterson, 1986; Stasovski & Peterson, 1993). The more advanced state of the tangential walls of the exodermis would be consistent with the hypothesis that fibrous roots in the field experienced accelerated maturation compared with those of well watered seedlings in pots.

Another major difference between the fibrous roots of potted seedlings and those from trees in the field was the presence of secondary xylem development only in second-order roots of the seedling plants, which typically arose from major laterals (pioneer roots) off the tap root. It seems reasonable that a seedling that still must develop the framework of a large root system might produce fibrous roots with the potential for secondary development, affording an advantage if a major pioneer root were damaged. This difference in root anatomy of fibrous roots of seedlings and trees might be linked to a greater tendency for seedlings than trees to retain fibrous roots under adverse conditions, as has been found for Volkamer lemon under localized drought stress (Espeleta & Eissenstat, 1998). Although it often is considerably more convenient to conduct physiological studies of fibrous roots of seedlings in pots, there is increasing evidence that such studies might not accurately reflect the physiology of fibrous roots of trees in the orchard or forest (Espeleta & Eissenstat, 1998).

Variation in root anatomical features

This study differs from many studies of root anatomy in that variation within as well as between species was characterized. Often only 'representative' photographs of differences in root structures of different species are provided, with little information on how these structures can vary within the species, plant or root. For a given distance from the root tip, root diameter, exodermal wall thickness and passage cell number can differ among species not just in the mean, but also in the variance and shape (e.g., number of modes, skewness) of the distribution (Fig. 3). An understanding of the variation in the trait can be just as informative as the average or 'representative' value.

For example, causes and consequences of variation in first-order root diameter, especially in the distinct bimodality exhibited in TO (Fig. 3), are poorly understood and await further investigation. The diameter of individual fibrous roots has been positively correlated with root longevity (Wells & Eissenstat, 1997) and negatively correlated with root

nitrogen concentration (Pregitzer *et al*., 1997) and respiration rate (Pregitzer *et al*., 1998). Like seeds in a fruit, we suspect that lateral roots along a mother root might have different amounts of vascular connection and different longevity. In apple, we have found individual laterals in close proximity exhibiting different potentials to grow, attract mycorrhizal fungi and prevent infections of sepate fungi (Resendes *et al*., 1997). Further investigation is needed to determine how and why individual roots vary in form and function within a single plant. This underlying variation might have important physiological and ecological consequences that are currently not recognized.

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