

Physiological and Morphological Effects of Size-Controlling Rootstocks on 'Fuji' Apple Scions

T. Tworkoski
Appalachian Fruit Research Station
USDA-ARS
Kearneysville, WV
USA

G. Fazio
Plant Genetics Resources Unit
USDA-ARS
Geneva, NY
USA

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Abstract

The size-controlling effects of apple (*Malus × domestica*) rootstocks were characterized in greenhouse experiments. One-year-old 'Fuji' scions on 25 size-controlling rootstocks, from the USDA apple rootstock breeding program in Geneva, NY, were grown for one season and shoot development was measured each month. Discriminant analysis associated the rootstocks into three clusters based on similarity of shoot length and diameter growth and tree height. Total shoot growth for one season was 367, 283, and 149 cm for rootstock cluster (RC) A, B, and C, respectively. RC-A had significantly greater photosynthesis and transpiration rates than RC-C (18.3 and $12.3 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and 4.2 and $3.1 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively). At the end of the first growing season, trees were placed in controlled cold environments to provide chilling. At the beginning of the second growing season, trees were removed from chilling, the roots were progressively pressurized up to 1.5 MPa in a pressure chamber, shoots were removed 100 cm above the graft union, and the hydraulic conductivity of roots was measured. Xylem exudate was collected and analyzed for cytokinin, abscisic acid (ABA), and inole-3-acetic acid. Lower hydraulic conductance was associated with the more dwarfing, RC-C, compared with the more invigorating RC-A rootstocks (0.58 and $1.41 \text{ ml} \cdot \text{cm}_{\text{Stem}}^{-1} \cdot \text{h}^{-1} \cdot \text{MPa}_{\text{Leaf}}^{-1}$, respectively). Abscisic acid flux was higher in xylem exudates from dwarfing RC-C than vigorous RC-A rootstocks (2.28 and $0.23 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$, respectively). The concentrations of cytokinin and indole-3-acetic acid were variable but rootstock-related differences were not determined. Stem samples of scion and rootstock from above and below the graft were collected and anatomical differences of stem cross-sections were analyzed using scanning electron microscopy. Results indicated that vessel diameter was less in 'Fuji' scion on RC-C than RC-B and RC-A rootstocks. It is possible that elevated ABA altered xylem development and reduced hydraulic conductance in size-controlling processes of apple rootstocks.

INTRODUCTION

Small, efficient trees are critical for early and high yielding apple trees in high density plantings. Apple tree size has been successfully controlled by budding desirable scions to dwarfing rootstocks but new rootstocks are needed for tree size management under a variety of environmental conditions. To assist in developing new rootstocks, improved knowledge of the dwarfing process is needed. The mechanisms for dwarfing rootstocks are not fully known but may include hydraulic and hormone communication between root and shoot.

Reduced growth in dwarfed apple trees has been attributed to altered shoot concentrations of endogenous hormones, including auxin, gibberellin, abscisic acid (ABA) and cytokinin (Grochowska et al., 1984; Jaumien and Faust, 1984; Kamboj et al., 1999a; Looney et al., 1988; Steffens and Hedden, 1992). Cytokinin concentrations in xylem sap were greater from apple rootstocks that invigorated, rather than dwarfed, apple scion growth (Kamboj et al., 1999b). Elevated ABA has been associated with dwarfing apple and citrus rootstocks (Kamboj and Quinlan, 1998; Kamboj et al., 1999b; Noda et al., 2000; Tworkoski and Miller, 2007; Yadava and Dayton, 1972). Changes in hormone

concentrations may effect size control by directly influencing cell processes or by indirectly altering the capacity for transport of resources. For example, altered transport of auxin at the bud union may have resulted in smaller vessel diameters of scion grown on dwarfing than semi-dwarfing apple rootstocks (Soumelidou et al., 1994a). Reduced movement of water and minerals from rootstocks could dwarf the scion (Hussein and McFarland, 1994; Rogers and Booth, 1960). Hydraulic conductivity of roots was lower in dwarfing (M.27) than semi-vigorous (MM.106) rootstocks (Atkinson et al., 2003). Alterations in xylem anatomy may have reduced the hydraulic conductivity in scion ('Queen Cox') of stems grafted to dwarfing than to semi-vigorous rootstocks.

Improved understanding of dwarfing processes will help develop and select rootstocks that provide trees with targeted sizes and that are adapted to specific environments. In this experiment 'Fuji' scion was used to assess root-produced hormonal and hydraulic signals and anatomical changes from a range of size-controlling rootstocks. The objective was to compare hormonal, hydraulic, and anatomical effects of genetically different rootstocks on one genotype of apple scion.

MATERIALS AND METHODS

Trees

Trees consisted of one-year-old 'Fuji' scion on three-year-old rootstocks from the USDA/Cornell Apple Rootstock Breeding Program at Geneva, NY in April 2005. Rootstocks included Ottawa (O), Malling (M), Malling-Merton (MM), and Cornell-Geneva (CG). Two trees were made on each of the following rootstocks: O.3, M.7, M.9, M.27, MM.106, CG.2022, CG.2034, CG.3007, CG.4002, CG.4003, CG.4013, CG.4088, CG.4214, CG.4814, CG.5179, CG.5202, CG.5463, CG.5935, CG.6001, CG.6006, CG.6157, CG.6253, CG.6874, CG.7707, and CG.8534. The first digit in the testing number of the CG series indicates the size potential of that rootstock on a scale of 1 to 10 (10 being 100% of seedling and 1 being 10% of seedling) so that rootstock CG.2022 would give a tree 20% of the seedling size, and rootstock 6874 would give 60%. Rootstocks were started as a rootstock liner in 2002, planted in the field in 2003, and scion was budded at the end of the growing season in 2003, with scion shoot developing in 2004. All of the Geneva[®] rootstocks were selected for fire blight resistance and also for collar rot resistance. They were also selected for precocity and high yield efficiency. Trees were planted in 37.8 L pots in a Hagerstown silt loam/sand/perlite mix (3/1/1, v/v/v) on April 11, 2005. Trees were grown in the greenhouse at 23±5°C without supplemental light during 2005. Trees were watered daily throughout the experiment and fertilized weekly with 0.5% 20N- 8.8P-16.6K solution.

In November 2005, leaves were collected, dried and weighed from each tree and trees were then placed in a cold room maintained at 5°C until May 2006. A subsample of all leaves from ten trees was measured for both leaf area and leaf dry weight so that a regression could be established to estimate leaf area per tree. After removal from the cold room the trees grew for approximately one month in a greenhouse without supplemental light and xylem conductivity and hormone contents were measured.

Seasonal Growth

During 2005, trunk diameter (15 cm above the graft union), the number and length of short (<3 cm) and long (>3 cm) lateral shoots were measured each month. In August tree height and canopy diameter (in a horizontal plane, the average of longest dimension and the dimension perpendicular to the longest dimension) were measured. In August, photosynthesis, transpiration, and stomatal conductance were measured on three leaves from each tree on alternate days for two weeks (CIRAS-1, PP Systems, Haverhill, MA). Average gas exchange values were calculated. Anatomical development of scion and rootstock for 2005 was measured as described below after the xylem exudate was collected.

Xylem Exudate

Trees were removed from the cold room in May 2006, allowed to grow for approximately one month and the hydraulic conductivity and hormone concentrations of xylem exudate were measured. Roots were well-watered the evening prior to collecting exudate and predawn leaf water potential was measured (Soil Moisture Equipment Corp., Santa Barbara, CA). Shoots were then pruned to 1 m above the graft union and green bark (assumed to include the phloem) was stripped from approximately 3 cm of the cut stem. Tygon tubing was fitted over the exposed stem xylem and the detopped tree was placed in a root pressure chamber with the stump protruding. Each rootstock/scion combination was exposed for one hour to each of the following pressure conditions: 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0 and 1.5 MPa. Xylem exudate was collected for each pressure, the volume recorded, and the exudate from each tree was pooled and frozen in liquid N. Xylem conductivity was calculated as the exudate volume collected per hour divided by the scion length and leaf water potential. The frozen exudate was lyophilized and analyzed for auxin, abscisic acid, zeatin, and zeatin riboside (Tworkoski et al., 2006).

After the final xylem exudate sample was collected, the tree was removed from the pressure chamber and an anatomical comparison of stem cross-sections from scion and rootstock was conducted. The remaining tree parts (scion, rootstock stem, and rootstock roots) were dried and weighed.

Anatomical Measurements

Stem cross-sections were taken 100 cm above and 5 cm below the graft union. These sections were fixed in FAA solution (ethanol, acetic acid, formalin, water, 50/5/10/35, v/v/v/v) and stored at room temperature. At the time of measurement, the FAA solution was removed and samples were rinsed 3 times with 0.05 M potassium phosphate buffer followed by ethanol dehydration, 15 minutes each, in a series of 10, 25, 50, 75, 90 and 100% ethanol for final storage. A wedge of tissue, including the pith to the cambial layer was cut from each stem cross-section followed by transverse sectioning with a box plane blade mounted in a manual press. Samples were then placed on a lyophilizer for a minimum of 4 hours and sputter-coated for measurement by scanning electron microscopy (Stereoscan 120, Cambridge Instruments Ltd., Cambridge, England). The thickness of the 2005 year growth ring was measured. Four rectangular regions (0.2 mm²) were selected randomly from within that growth ring and within each area the vessel xylem elements were counted and the average diameter of 10 vessel elements was measured. The cross-sectional area of vessel lumens was calculated by multiplying vessel density by the average area per vessel which was estimated using the area of a circle.

Data Analysis

At the end of the 2005 season, rootstocks were classified as one of three size-controlling rootstock groups based on Proc Cluster analysis of trunk diameter growth, total shoot growth, and tree height (SAS, 2001). Rootstock effects on scion growth, hydraulic conductivity, hormone flux, and xylem vessel traits were analyzed by Proc GLM followed by Duncan's Mean Separation. Trees on rootstock CG2034 died and were dropped from the analysis. Relationships among continuous variables were tested by Proc Corr and Proc Reg in SAS. All statistical tests were considered significant if the $P > F$ was at the 0.05 level.

RESULTS

Stem and branch growth of scion generally followed the anticipated pattern of growth associated with invigorating and dwarfing rootstocks (Fig. 1). However, there was significant variation in growth among rootstocks with the same putative degree of growth control as illustrated by a low regression coefficient ($R^2 = 0.20$). Cluster analysis of individual rootstocks based on size-controlling effects on scion height, trunk diameter, and shoot growth, separated rootstocks into three separate groups. Twenty-three size-controlling rootstocks were grouped by cluster analysis as: Cluster A (vigorous), 4088,

5463, 5179, 6006, 5935, O.3, M.7, 6874; Cluster B (semi-vigorous), 4814, 2002, 8534, 3007, 4214, M.9, 6157, MM.106, 6253; Cluster C (dwarfing), 5202, 4003, 4013, M.27, 6001, 7707. These clusters were composed of at least 12 replications and subsequent analyses utilized these groups.

The grouping of rootstocks based on the cluster analysis was validated by differences in first-year shoot growth and total shoot length (Table 1). The most dwarfing rootstocks (Cluster C) grew more short and fewer long branches than the invigorating rootstocks (Clusters A and B), although statistical differences could not be detected. The most dwarfing rootstocks (Cluster C) also had the lowest photosynthetic and transpirational rates (Table 1).

Concentrations and flux of indole-3-acetic acid and cytokinin in xylem exudates were variable (averaging approximately 5 and 21 pmol/ml/h, respectively) and differences associated with rootstocks could not be detected (data not shown). Concentration of total ABA per gram root did not differ among rootstocks but ABA concentration per mL xylem exudate per hour (i.e. ABA flux) was significantly greater in the dwarfing rootstocks of Cluster C than the more invigorating rootstocks (Table 2). Greater ABA flux was negatively correlated with vessel cross sectional area ($r = -0.41$) and was associated with lower xylem flow in Cluster C (Table 2). The xylem flow for Clusters C and A was 15.4 and 33.3 ml/h, respectively with corresponding hydraulic conductance of 0.58 and 1.41 $\text{ml} \cdot \text{cm}_{\text{stem}}^{-1} \cdot \text{h}^{-1} \cdot \text{MPa}_{\text{leaf}}^{-1}$, respectively.

Vessel diameters were lower but vessel density was greater in scion on dwarfing (Cluster C) than invigorating (Cluster A) rootstocks (Table 3, Fig. 2). The resulting total lumen area of vessels in scion was 422,260 and 369,118 μm^2 for Clusters A and C, respectively. Vessel diameters and density in rootstocks did not differ significantly but were numerically less in the more dwarfing cluster (Table 3).

DISCUSSION

In general, ‘Fuji’ scions that were budded to the same rootstock grew similarly (data not shown). However, different rootstocks with the same size-controlling designation did not consistently affect ‘Fuji’ scion branch growth (Fig. 1). In the field, scion growth can be at variance with the predicted size-control from the rootstocks to which they are budded. Such variability in scion growth may be attributed to environmental influences. In the current experiment, the highly uniform, low stress environment should have enabled detection of rootstock-induced effects that were genetically-based. Cluster analysis grouped rootstocks that could likely have similar biological processes that bring about reduced scion growth. Hydraulic conductivity, hormone flux, and stem xylem anatomy were evaluated as possible biological processes associated with rootstock-induced size-controlling effects.

Hydraulic conductivity was lowest in the most dwarfing rootstock, RC-C (Table 2). Reduced xylem vessel diameter of scion stems and elevated ABA levels in xylem exudate also were associated with the most dwarfing rootstocks (Tables 2 and 3). Previous work has correlated decreased hydraulic conductivity with growth control of apple rootstocks. Hydraulic conductivity of roots was lower in dwarfing (M.27) than semi-vigorous (MM.106) rootstocks (Atkinson et al., 2003). Similarly, hydraulic conductivity was lower in scion (‘Queen Cox’) stems grafted to dwarfing than semi-vigorous rootstocks. It was proposed that differences in xylem anatomy may have been central factors for the dwarfing effect. Results from the current experiment indicate that reduced diameters of xylem vessel elements in scion stems were associated with reduced stem hydraulic conductivity.

The role of hormones in size-controlling processes of apple rootstocks has been unclear but may include elevated cytokinin concentrations in xylem sap of invigorating apple rootstocks (Kamboj et al., 1999b). Cytokinin and auxin promote vascular differentiation and disruption of auxin transport at the graft union can alter vascular development, decrease hydraulic conductivity, and limit growth (Michalczyk, 2002; Ye, 2002). In the current study, no clear patterns in auxin or cytokinin concentrations were

found but only xylem exudate was measured and only at one time (data not shown).

High resistance to water movement in stems and roots may contribute to less water use in 'Golden Delicious' on M.9 than on MM.106, even when adequate water for growth was provided by irrigation (Li et al., 2002). Under these conditions, trees grown on M.9 were found to be under water stress. In the current study, ABA levels were elevated in xylem exudate collected from the more dwarfing rootstocks (Table 2). Absciscic acid has been shown to be a root-produced message that regulates shoot growth and development in drying soils (Davies et al., 2005). In addition to direct inhibitory effects on growth, ABA can inhibit auxin translocation (Basler and McBride, 1977) and subsequently reduce cambial activity and xylem development in dwarfing rootstocks (Soumelidou et al., 1994b). Cambial activity is suppressed and secondary wall thickening of fiber cells has been stimulated by ABA or by elevated ABA to auxin ratios (Mswange et al., 2005; You-Ming et al., 2001). It is possible that in dwarfing rootstocks, elevated ABA may reduce growth and development of both primary (apical) and secondary (cambial) meristems.

CONCLUSIONS

The results of the current study provide a snapshot of hormone and hydraulic conductivity in 'Fuji' scion grown on a variety of size-controlling rootstocks. Findings support previous work that hydraulic conductivity is reduced in more dwarfing rootstocks and anatomical measurements indicated that reduced diameters of scion xylem vessel elements may be a component of reduced conductivity. It is possible that elevated ABA levels from dwarfing rootstocks may play a role in altering scion anatomy which, in turn, may reduce hydraulic conductivity. Alternatively, reduced hydraulic conductivity caused by an undetermined factor may lead to water stress and induction of ABA synthesis. The ABA moving up the stems may exert a growth inhibitory effect. The role of ABA in size-controlling effects of apple rootstocks remains to be elucidated.

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Literature Cited

- Atkinson, C.J., Else, M.A., Taylor, L. and Dover, C.J. 2003. Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.) J. Expt. Bot. 54:1221-1229.
- Basler, E. and McBride, R. 1977. Interaction of coumarin, gibberellic acid and abscisic acid in the translocation of auxin in bean seedlings. Plant and Cell Physiol. 18:939-947.
- Davies, W.J., Kudoyarova, G. and Hartung, W. 2005. Long-distance ABA signaling and its relation to other signaling pathways in the detection of soil drying and the mediation of the plant's response to drought. J. Plant Growth Regul. 24:285-295.
- Grochowska, M.J., Buta, G. J., Steffens, G.L. and Faust, M. 1984. Endogenous auxin and gibberellin levels in low and high vigor apple. Acta Hort. 146:125-134.
- Hussein, I.A. and McFarland, M.J. 1994. Rootstock-induced differences in sap flow of 'Granny Smith' apple. HortScience 29:1120-1123.
- Jaumein, F. and Faust, M. 1984. Stem anatomical structure of 'Delicious' and 'Golden Delicious' apple hybrids with various growth dynamics. Acta Hort. 146:69-79.
- Kamboj, J.S. and Quinlan, J.D. 1998. The apple rootstock and its influence on endogenous hormones. Acta Hort. 463:143-152.
- Kamboj, J.S., Blake, P.S., Quinlan, J.D. and Baker, D.A. 1999a. Identification and quantitation of GC-MS of zeatin and zeatin riboside in xylem sap from rootstock and scion of grafted apple trees. Plant Growth Regul. 28:199-205.
- Kamboj, J.S., Browning, G., Blake, P.S., Quinlan, J.D. and Baker, D.A. 1999b. GC-MS-

- SIM analysis of abscisic acid and indole-3-acetic acid in shoot bark of apple rootstocks. *Plant Growth Regul.* 28:21-27.
- Li, F., Cohen, S., Naor, A., Shaozong, K. and Erez, A. 2002. Studies of canopy structure and water use of apple trees on three rootstocks. *Agricultural Water Management* 55:1-14.
- Looney, N.E., Taylor, J.S. and Pharis, R.P. 1988. Relationship of endogenous gibberellin and cytokinin levels in shoot tips to apical form in four strains of 'McIntosh' apple. *J. Amer. Soc. Hort. Sci.* 113:395-398.
- Michalczyk, L. 2002. Indole-3-acetic acid level in wood, bark and cambial sap of apple rootstocks differing in growth vigour. *Acta Physiol. Plant.* 24:131-136.
- Mwange, K., Hou, H., Wang, Y., He, X. and Cui, K. 2005. Opposite patterns in the annual distribution and time-course of endogenous abscisic acid and indole-3-acetic acid in relation to the periodicity of cambial activity in *Eucommia ulmoides* Oliv. *J. Expt. Bot.* 413:1017-1028.
- Noda, K., Okuda, H. and Iwagaki, I. 2000. Indole acetic acid and abscisic acid levels in new shoots and fibrous roots of citrus scion-rootstock combinations. *Sci. Hort.* 84:245-254.
- Rogers, W.S. and Booth, G.A. 1960. The roots of fruit trees. *Sci. Hort.* 14:27-34.
- SAS Institute, Inc. 2001. The SAS system for Windows. Release 8.02. SAS Inst. Inc., Cary, NC.
- Soumelidou, K., Battey, N.H., John, P. and Barnett, J.R. 1994a. The anatomy of the developing bud union and its relationship to dwarfing in apple. *Ann. Bot.* 74:605-611.
- Soumelidou, K., Morris, D.A., Battey, N.H. and Barnett, J.R. 1994b. Auxin transport capacity in relation to the dwarfing effect of apple rootstocks. *J. Hort. Sci.* 69:719-725.
- Steffens, G.L. and Hedden, P. 1992. Comparison of growth and gibberellin concentrations in shoots from orchard-grown standard and thermosensitive dwarf apple trees. *Physiol. Plant.* 86:544-550.
- Tworowski, T. and Miller, S. 2007. Endogenous hormone concentrations and bud-break response to exogenous benzyl adenine in shoots of apple trees with two growth habits grown on three rootstocks. *J. Hort. Sci. Biotech.* 82:960-966.
- Tworowski, T., Miller, S. and Scorza, R. 2006. Relationship of pruning and growth morphology with hormone ratios in shoots of pillar and standard peach trees. *J. Plant Growth Regul.* 25:145-155.
- Yadava, U.L. and Dayton, D.F. 1972. The relation of endogenous abscisic acid to the dwarfing capability of East Malling apple rootstocks. *J. Amer. Soc. Hort. Sci.* 97:701-705.
- Ye, Z. 2002. Vascular tissue differentiation and pattern formation in plants. *Annu. Rev. Plant Biol.* 53L:183-202.
- You-Ming, Y., Chu-Nian, X., Bao-Min, W. and Jun-Zhen, J. 2001. Effects of plant growth regulators on secondary wall thickening of cotton fibres. *Plant Growth Regul.* 35:233-237.

Tables

Table 1. One-year growth, number of branches, photosynthesis, and transpiration of ‘Fuji’ scion on size-controlling rootstocks grown for one growing season in a greenhouse.

Rootstock cluster	Total shoot length (cm)	First season shoot growth (cm)	Branches <3 cm (no.)	Branches >3 cm (no.)	Photosynthesis ($\mu\text{mol CO}_2/\text{m}^2$ leaf area/s)	Transpiration ($\text{mmol H}_2\text{O}/\text{m}^2$ leaf area/s)
A - Vigorous	471 a	367 a	5.2 a	8.9 a	18.3 a	4.2 a
B - Semi-vigorous	383 b	283 b	5.9 a	8.0 a	20.5 a	4.7 a
C - Dwarfing	244 c	149 c	6.4 a	6.2 a	12.3 b	3.1 b

Table 2. Absciscic acid production and flux and xylem flow of ‘Fuji’ scion on size-controlling rootstocks grown for one growing season in a greenhouse.

Rootstock cluster	Abscisic acid production (pmol/g dw root)	Abscisic acid flux (pmol/ml/h)	Xylem flow (ml/h)
A - Vigorous	34.0 a	0.23 b	33.3 a
B - Semi-vigorous	28.0 a	0.39 b	37.6 a
C - Dwarfing	25.7 a	2.28 a	15.4 b

Table 3. Density and diameter of xylem vessel elements of ‘Fuji’ scion and of corresponding size-controlling rootstocks grown for one growing season in a greenhouse.

Rootstock cluster	Vessel density (no. $\cdot \text{mm}^{-2}$)		Vessel diameter (μm)	
	Scion	Rootstock	Scion	Rootstock
A - Vigorous	321 b	294 a	40.7 a	34.8 a
B - Semi-vigorous	306 b	252 a	38.3 a	32.2 a
C - Dwarfing	396 a	265 a	34.5 b	31.1 a

Figures

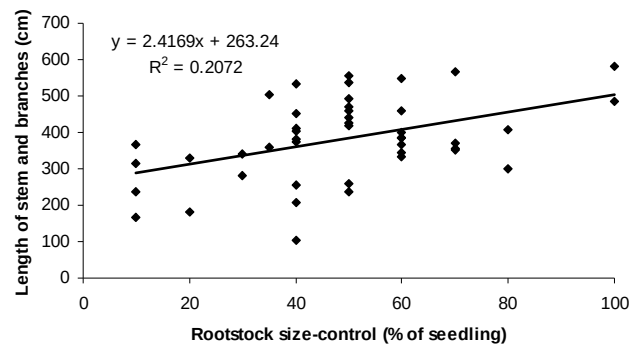


Fig. 1. Stem and branch growth of 'Fuji' scion grown on twenty-five size-controlling rootstocks measured after one growing season in a greenhouse.

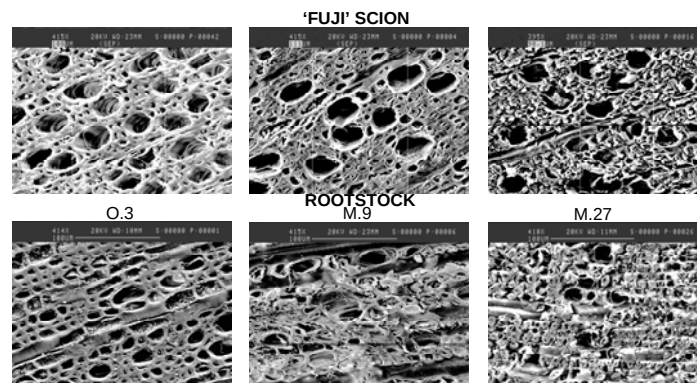


Fig. 2. Scanning electron micrographs (400 x) of three 'Fuji' scion stems (top) and, directly below each scion is the associated rootstock to which it was grafted (bottom from left-to-right: O.3, M.9, and M.27 representing Clusters A, B, and C, respectively). Images are of the annual growth during one growing season in a greenhouse.