

Hormone and growth interactions of scions and size-controlling rootstocks of young apple trees

T. Tworkoski¹ · G. Fazio²

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Abstract Size-controlling rootstocks are critical to grow small, efficient trees that enable early and high yield in plantings of apple. Improved knowledge of rootstock-related size-control processes is important for breeding and cultural practices. The research objective was to determine hormone profiles within trees grafted on Malling rootstocks. Buds from ‘Gala’, ‘Fuji’, M.7, M.9, M.27, and MM.111 were grafted to rootstocks M.7, M.9, M.27, and MM.111 and then grown in a greenhouse and the field. After 2 years, heights of trees grafted to growth-controlling rootstocks decreased in the order M.9 > M.7 > MM.111 in the greenhouse, the reverse trend that was measured in the field. Although soil resources were not measured, ample availability of resources such as water and mineral nutrients in the greenhouse may have been responsible for the reversal of rootstock effects on tree height in greenhouse and field. Elevated abscisic acid (ABA) and ABA metabolites were associated with ‘Gala’, MM.111 and M.9 scion that were grafted on M.9 rootstocks. Abscisic acid and abscisic acid glucose ester were generally greater in root, rootstock stem below the graft union, scion above the graft, and xylem exudate of rootstock M.9 than MM.111. Reduced gibberellin (GA₁₉) was found in roots and xylem exudate of ‘Gala’ grafted to M.9 than MM.111 rootstocks. These results support the hypothesis that hormone signals from rootstocks control tree growth but it is likely that stage of development, time after planting, and environmental resources will also interact to influence growth effects of

size-controlling rootstocks. It is proposed that gene expression associated with hormone metabolism can be developed to further understand the underpinnings of size-controlling rootstocks and assist the selection of rootstocks for size control and, possibly, for other hormone-related characteristics.

Keywords Dwarf fruit trees · Absciscic acid · Auxin · Cytokinin · Gibberellin · Root-shoot communication · Graft union

Introduction

Size-controlling rootstocks of apple are integral to modern, efficient orchard-management systems. New rootstocks are needed as growers contend with resource limitations, abiotic and biotic stress, and mechanization in response to labor shortages (Fallahi et al. 2002; Hoying and Robinson 2006; Parker et al. 1998; Webster 2001). Several mechanisms for size-control by rootstocks have been proposed including root system size, mineral uptake and allocation, carbohydrate distribution, anatomical development at the graft union and hydraulic and hormone communication between roots and shoot (Zeiger and Tukey 1960). Cytokinins (CK) and gibberellins (GA) that are produced in rootstocks can affect bud-break; shoot growth and development of scion (Kamboj et al. 1999; van Hooijdonk et al. 2011). Other evidence suggests that elevated abscisic acid (ABA) may play a role in dwarfing apple rootstocks (Kamboj and Quinlan 1998; Kamboj et al. 1999; Tworkoski and Miller 2007; Yadava and Dayton 1972). Soumelidou et al. (1994) found smaller vessel diameters in dwarfing than in semi-dwarfing apple rootstocks, possibly due to altered auxin transport at the bud union of dwarfing rootstock. Subsequent reduced supply of water and minerals to scion

✉ T. Tworkoski
tom.tworkoski@ars.usda.gov

¹ Appalachian Fruit Research Station, ARS, USDA, 2217 Wiltshire Rd., Kearneysville, WV 25430, USA

² Plant Genetics Resources Unit, ARS, USDA, Cornell University, 630 W. North St., Geneva, NY 14456, USA

could then dwarf the scion (Hussein and McFarland 1994; Rogers and Booth 1960).

ABA 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. 1994). Auxin concentrations were consistently lower, and declined more rapidly with time, in the cambial region of stems from dwarfing apple rootstock than from more vigorous rootstocks (Michalczuk 2002). ABA was elevated in new shoots of citrus scion growing on dwarfing rootstocks and the high ABA was believed to be responsible for reduced growth of scion (Noda et al. 2000). Higher ABA concentrations were in shoot tips of field-grown apple trees that were grafted on M.9 dwarfing rootstocks than on more invigorating seedling 'Antonovka' rootstock (Tworkoski and Miller 2007).

In addition to hormones, research has shown that regulation of water movement can be a significant process of regulation in size-controlling rootstocks. Atkinson et al. (2003) demonstrated that hydraulic conductivity of young trees was less in the scions and the graft unions of shoots grafted on dwarfing than semi-vigorous apple rootstocks. 'Fuji' scions on dwarfing rootstocks generally had higher ABA flux in the transpiration stream and 50 % less water conductance in the xylem (Tworkoski and Fazio 2011). The reduced conductance was attributed to reduced number of large vessel elements that also reduced cross-sectional lumen area and water conductivity. Early work used reciprocal grafts of scions on size-controlling rootstocks to evaluate interactive effects on growth and dry weights but hormone quantities and influences are not clear, in part, because of analytical limitations of physio-chemical assays such as mass spectroscopy (Moore 1975; Vyvyan 1955). While a genetic locus (*Dw1*) conferring the dwarfing effect found in Malling nine rootstock has been identified on Chromosome five of the apple genome, very little is understood about its heritable effects on the scion and whether it is the only genetic factor that causes dwarfing of the scion in apple rootstocks (Pilcher et al. 2008). Improved understanding is needed of root and shoot signals of scion/rootstock combinations to fully utilize the genetic capabilities to efficiently manage orchards and to develop rootstocks that are adapted to new problems such as reduced availability of water.

Rootstocks consist of stems and their associated adventitious roots. They are often propagated clonally from below-ground stems that generate multiple new stems and roots. The capacity of rootstocks to regulate growth of scions is well known but the innate growth capacities of the stems of these rootstocks have been much less studied. In

many fruit trees dwarfing can be obtained by grafting a stem (interstock) from a dwarfing rootstock between a vigorous rootstock and a scion (Fallahi et al. 2002; Webster 2001). This demonstrated that the stem, without roots, from the dwarfing rootstock has capacity for size-control.

Physiological processes of rootstock stems may be critical to the overall size-controlling competency and knowledge of these processes may assist development of new rootstocks. We were interested in the innate capacity of rootstocks to regulate scion growth and the influence of a rootstock, used as scion, on size-control. Toward that end reciprocal grafts of dwarfing and invigorating rootstock stems would illuminate size-controlling processes in different tree components at a single point in time. Evaluating young trees were considered to be a reasonable approach to measuring hormone concentrations in plant parts as previous work determined hormone differences in 1 and 2-year-old apple trees (Kamboj et al. 1999; Li et al. 2012).

The objectives of this work were to determine (1) tree growth, development, and hormone response to size-controlling rootstocks used as scion or rootstock, and (2) the effect of grafting by comparing a scion on its 'own root' whether intact (not grafted) or grafted.

Materials and methods

In this experiment scions of different size-controlling rootstocks and of two cultivars were grafted on size-controlling rootstocks. Tree parts (stem tips, stems near the graft union, and roots) were sampled along with xylem exudate that was forced from stem tips with a pressure chamber and hormone analysis was conducted in tree parts from a subsample of trees (Fig. 1).

Trees

2008

Rootstocks were purchased from Willamette Nurseries, 25571 S. Barlow Rd., P.O. Box 3, Canby, OR, 97013. In March 2008, 100 MM.111 EMLA, M.7 EMLA, M.9 EMLA and M.27 EMLA were planted in 4-L pots with soil-less media (Metromix 360/fine sand, 2.8/1, v/v). In July 2008, scions of 'Gala', 'Fuji', M.7, M.9, M.27, and MM.111 were 'T' budded on to rootstocks M.7, M.9, M.27, and MM.111. There were 6 scion and 4 rootstock treatments with 15 trees of each scion/rootstock combination. Ten additional trees of each rootstock were used to compare the effect of grafting a scion on to its own rootstock (e.g. M.9 bud grafted to M.9 rootstock) with an ungrafted shoot growing from the same rootstock (e.g. an M.9 bud growing from an M.9 rootstock).

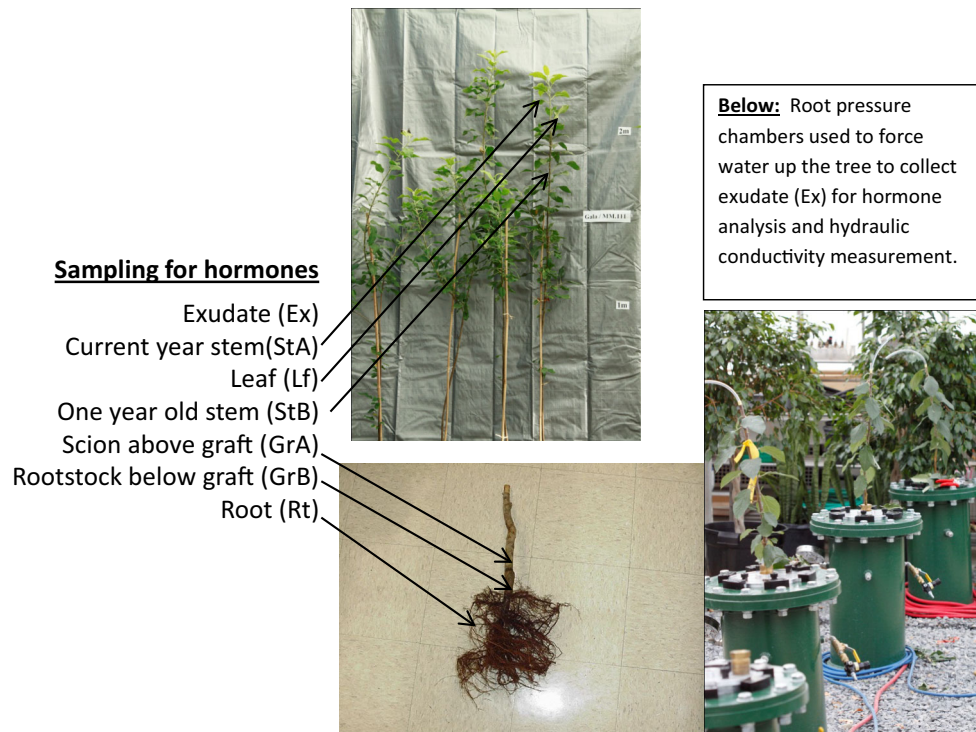


Fig. 1 Sampling components of scion and rootstock and root pressure chambers used to collect exudate and measure hydraulic conductivity

2009

Trees in the greenhouse were allowed to cool to 1–5 °C, with natural photoperiod from October 2008 to April 2009 when trees were transplanted into 8-L pots. Rootstock shoots of all grafted trees were removed above the graft union in May 2009. Ungrafted rootstock trees were also cut above a bud at the same cutting height as trees with grafted buds (approximately 5 cm above the shoot/root transition). In 2009 all scion/rootstock combinations were grown in the greenhouse. During the growing season, trees in the greenhouse were watered each day and fertilized (1.75 g of 20 N-8.8P-16.6 K w/w/w per tree) on a weekly basis. Trees were not pruned and were supported with stakes. Trees were grown for one full season and again maintained over winter in the greenhouse as described above. Average sunlight over the growing season was $511 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation and 23 ± 5 °C.

2010–2012

Of the 15 trees of the same scion/rootstock combination 5 were harvested in 2010 for morphological and hormone measurement, 5 were grown in the greenhouse in 2010 and 2011 for growth measurement, and 5 were planted in the field in 2010. Selected combinations of scion-rootstock combinations were planted in the field at the Appalachian Fruit Research Station in Sept. 2010. Trees were planted at

4.9 m × 4.9 m spacing in a Hagerstown silt loam. In the field, trees included scions of ‘Fuji’, M.9, M.7, and MM.111 on rootstocks of M.9, M.7, and MM.111. Five ungrafted M.9, M.7, and MM.111 on their own roots were also planted.

Measurements, xylem exudate and tree harvest

In the greenhouse during October of 2010–2012 growth was measured, including scion and rootstock diameters, tree height, number of internodes, number and length of branches. No trees on M.27 rootstock were grown in 2012. Also in 2010 and 2011 the number of days to bud break was counted and time to flowering was measured in 2011. Height and diameter of the field-grown trees were measured in Oct. 2011 and 2012.

After 1 month of growth in the greenhouse in 2010 five trees of each scion/rootstock combination were evaluated for hydraulic conductivity and for hormone concentration in xylem exudate. Roots were well-watered the evening prior to collecting exudate and predawn leaf water potential of two distal leaves was measured (Soil Moisture Equipment Corp., Santa Barbara, CA). Trees were placed in root pressure chambers, pruned to 1 m above the graft union and 3 cm of green bark (assumed to include the phloem) was stripped (Fig. 1). In general, trees began to produce xylem exudate from tree cuts when root pressure was 0.34 MPa. Xylem exudate was collected, the volume and

collection time were recorded, and the exudate from each tree was quickly frozen and lyophilized (Tworkoski and Fazio 2011). Hydraulic conductivity (HC) was calculated as derived from Fick's Law (Jones 1983; Hubbard et al. 1999) and normalized for stem diameter:

$$\text{HC} = \frac{\text{exudate volume (mL)}}{\text{collected time (h)}} \times \frac{\text{scion diameter (mm}^2\text{)}}{(\text{leaf water potential} - \text{soil water potential}) \text{ (MPa)}}$$

At the time of measurement the average stem water potential were 0.46, 0.42, 0.48 and 0.45 MPa for trees on rootstock M.27, M.7, M.9, and MM.111, respectively. Soil water potential was assumed to be zero as the trees were well-watered the night before HC measurement.

After collecting exudates, plant components were quickly frozen and lyophilized for measuring hormones. From each tree, plant components included current year growth leaf (Lf) and stem (StA), 1-year-old stems (StB), stems from scion 5-cm above the graft (GrA), rootstock stems from 5 to 10-cm below the graft (GrB) and, after washing away the soil-less media, 10 root subsystems directly off lateral roots of the rootstock (Rt) (Fig. 1). A root subsystem was 1–2 mm diameter at the proximal end where the cut was made and consisted of all roots distal to the cut. Tree components not used for hormone analysis were dried at 80 °C for 7 days and dry weights were measured of all shoots above the graft (Stem & Leaf), the rootstock stem (Rtstk stm) below the graft and roots (Root).

Hormone analysis of tree components

Major hormones (abscisic acid, auxin, cytokinins, and gibberellins) and associated metabolites (Davies 2004) were measured in 'Gala'/MM.111 (G/111) (scion/rootstock combination), MM.111/MM.111 (111/111), M.9/MM.111 (9/111), 'Gala'/M.9 (G/9), MM.111/M.9 (111/9) and M.9/M.9 (9/9). The MM.111 and M.9 scion-rootstock combinations were selected for analysis because they represent two very different dwarfing capacities and were considered most likely to have hormone differences. Many of the hormones were not detected in quantifiable amounts and only those that could be quantified are presented. Specific hormones that could be measured included ABA (*cis*-abscisic acid) and metabolites DPA (dihydrophaseic acid), ABAGE (abscisic acid glucose ester), PA (phaseic acid); t-ZR (*trans* zeatin), iPA (isopentenyladenosine), t-ZOG (*trans* Zeatin-O-glucoside), 2iP (Isopentenyladenine); IAA (indole-3-acetic acid); and gibberellins (GA) 8, 9, 19, 24, 34, and 53. The likely metabolic links of ABA-related compounds presented as are GA-related compounds (Fig. 7).

Specific hormones that were targeted but not found or found in trace amounts included t-ABA (*trans*) Abscisic

acid), 7'-OH-ABA (7'-Hydroxy-abscisic acid); t-Z (*trans*) Zeatin), c-Z (*cis*) Zeatin), dhZ (Dihydrozeatin), c-ZR (*cis*) Zeatin riboside), dhZR (Dihydrozeatin riboside), IAA-Asp (N-(indole-3-yl-acetyl)-aspartic acid), IAA-glu (N-(indole-3-yl-acetyl)-glutamic acid), IAA-Ala (N-(indole-3-yl-acetyl)-alanine), IAA-Leu (N-(indole-3-yl-acetyl)-leucine); and gibberellins (GA) 1, 3, 4, 7, 20, and 51.

Hormones were measured (National Research Council, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, S7N0W9). Briefly, samples were extracted in isopropanol:water:glacial acetic acid (80:19:1, v/v/v) that was spiked with stable isotopes of each hormone, dried, reconstituted in acidified methanol, partitioned with hexane, and the aqueous phase was then dried. Residue was reconstituted in acidified methanol, loaded on a C18 column, and the eluate was dried. Residue was reconstituted in acidified 40 % methanol (v/v) and injected in an HPLC ESI-MS/MS (Chiwocha et al. 2003; 2005). Individual hormones were quantified based upon the peak area of the native hormone and corrected for loss based on recovery of the internal standard (Ross et al. 2004). The limit of quantitation was established where the signal-to-noise ratio dropped below 8.

Experimental design

The experimental design was completely randomized in both the greenhouse and the field. In the greenhouse, 5 reps of the each scion-rootstock combinations were destructively sampled for hormone and dry weight measurements in 2010. An additional 5 reps were maintained in the greenhouse for growth measurements in Oct. 2010 and 2011. In the field there were 5 replications of selected scion/rootstock combinations for growth measurements in Oct. 2011 and 2012. Data were analyzed with GLM procedure to test for significance of main effects and their interactions and with the MIXED procedure and mean separation by the PDIF procedure (SAS Institute, Inc. 2003. The SAS system for Windows. Release 9.1. SAS Inst. Inc., Cary, NC 27513). Main effects are presented where significant scion-by-rootstocks (S × R) interactions were not found.

Results

Growth in the greenhouse and field

Height

In greenhouse-grown trees, scion and rootstock significantly affected height (Fig. 2). Few S × R interactions

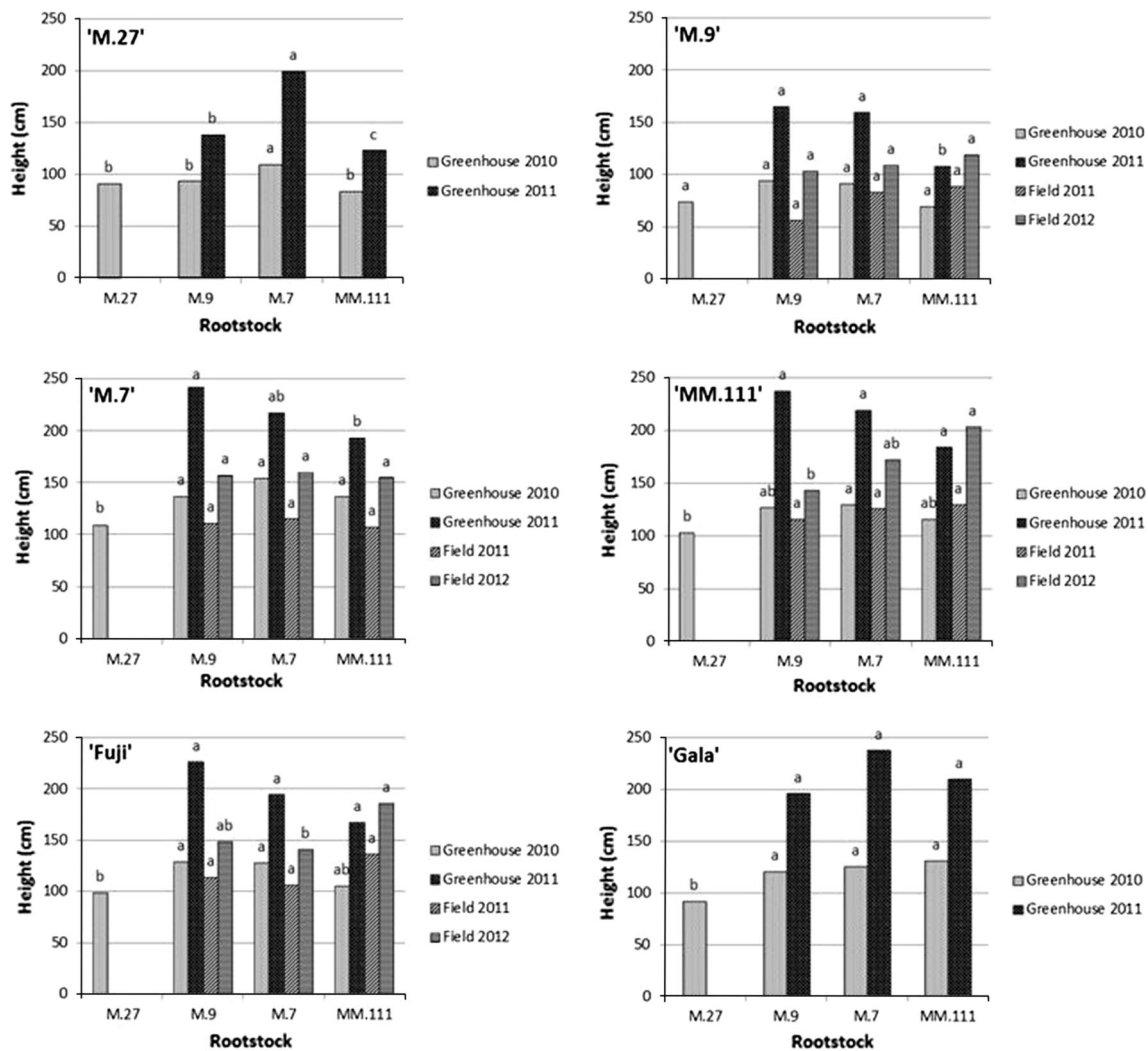


Fig. 2 Effect of rootstock on scion height (clockwise from *top left panel*, M.27, M.9, MM.111, 'Gala', 'Fuji', and M.7) grown in the greenhouse and the field. Within each scion, year, and location bars

with the *same letter* designate no significant rootstock difference at the 0.05 level of confidence

occurred. In general, most scions were shorter if grafted to the very dwarfing rootstock, M.27, but size-control effects were not consistent among the other rootstocks. Unexpectedly, scions grafted to M.9 were numerically taller than those grafted to MM.111, with the exception of 'Gala', when measured in 2010 and 2011 in the greenhouse. Rootstocks did not significantly affect height in field-grown trees after 1 year but differences were found in the second year (Fig. 2). In the field during 2012, heights of 'Fuji' and MM.111 were greater on MM.111 than on M.7 and M.9.

Weight

Dry weight (DW) and DW distribution within the trees differed among scion/rootstock combinations but broad characteristics were observed (Fig. 3). Averaging across rootstocks the percent of dry weight allocated to stem and

leaves was greater with scion MM.111 than with scion M.9 (60 and 42 %, respectively) and the other scions had approximately 55 % of the total dry weight in stems and leaves. Conversely, less dry weight was allocated to roots of trees with scions of MM.111 than M.9 (15 and 23 %, respectively) with the other scions having approximately 18 % of the total dry weight in roots. In addition to having the most percent of the total dry weight allocated to roots, trees with M.9 scions had the driest weight allocated to rootstock stem (34 % compared with approximately 26 % of all others). All trees with M.27 and M.9 scions weighed approximately 44 g; that was significantly less than trees with other scions (approximately 55 g). Trees with MM.111 and M.9 scions averaged an S/R ratio of 4.3 and 2.1, respectively, which statistically differed from each other as well as from the other scions (S/R of approximately 3.2).

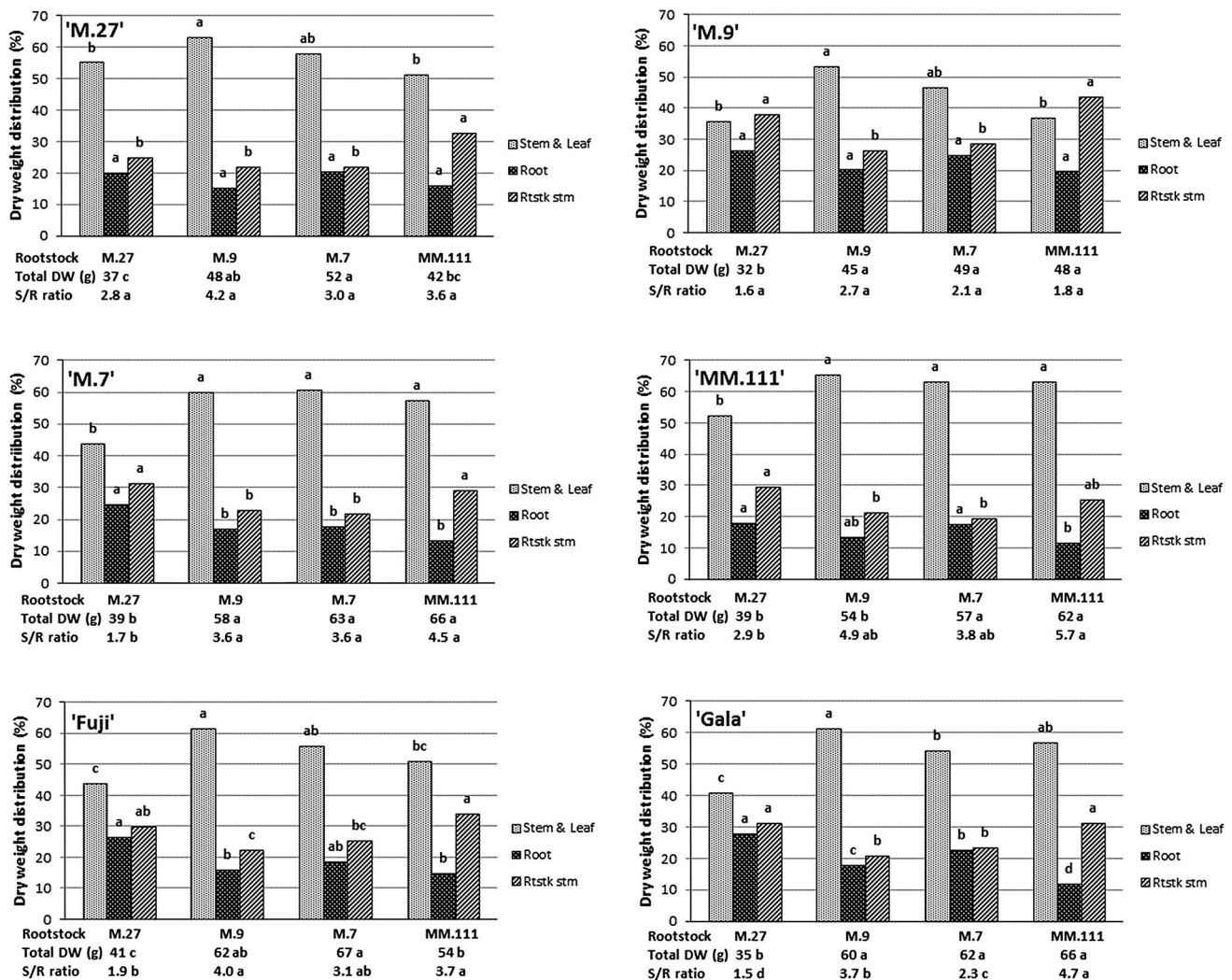


Fig. 3 Effect of rootstock on total tree dry weight and shoot-to-root dry weight ratio (S/R) (left-to-right) below the bars for rootstocks M.27, M.9, M.7, and MM.111 and dry weight distribution within trees of scion (clockwise from top left panel, M.27, M.9, MM.111, 'Gala', 'Fuji', and M.7) grown in the greenhouse. Within each scion, bars of

the same plant component with the same letter (above bars) designate no significant rootstock difference at the 0.05 level of confidence. Within each scion, rootstock effects on DW and S/R are compared within each row. Values with the same letter within a row designate no significant rootstock difference at the 0.05 level of confidence

All scions on M.27 rootstock had the smallest whole-tree dry weights and generally the lowest percent of dry weight partitioned to stem and leaf (Fig. 3). For each scion the S/R was always least in M.27 rootstock and not greatly different among the other rootstocks.

Internodes

In 2010 scion, rootstock, and the $S \times R$ interaction significantly affected internode length in greenhouse trees. In 2011 scion but not rootstock and the $S \times R$ interaction were significant. Internode lengths of scions of M.7 and MM.111 were not affected by any rootstock. Within each rootstock the average internode length tended to be least in

scions of M.9 and M.27. In general, internode lengths increased from 2010 growth to 2011 growth but number of internodes decreased (Table 1).

Diameter

Scion and rootstock significantly affected scion diameter as measured in the greenhouse in November 2011. Scion-by-rootstock interaction was significant for greenhouse- but not field-grown trees. In the greenhouse, scion diameter was lower only with MM.111/MM.111 (Table 2). In the field, scion diameter was greatest with MM.111 as scion. As rootstocks MM.111 and M.7 had greater diameters than M.9 (Table 2). In the greenhouse and the field there were no

Table 1 Stem internode length and number in annual shoot growth of trees grown in 2010 and 2011 in the greenhouse

Rootstock	Scion	Length 2010 (cm)	Length 2011 (cm)	Number 2010	Number 2011
M.27	Fuji	1.7 a B ^z	no trees	56 a AB	no trees
	Gala	1.8 a B	no trees	51 a B	no trees
	M.27	1.5 ab A	no trees	57 a A	no trees
	M.9	1.3 b B	no trees	55 a A	no trees
	M.7	1.7 a A	no trees	61 a C	no trees
	MM.111	1.8 a A	no trees	56 a A	no trees
M.9	Fuji	2.2 a A	2.3 a A	58 b A	55 ab A
	Gala	2.3 a A	2.4 a A	53 b B	41 bc A
	M.27	1.6 c A	1.7 a A	58 b A	33 c B
	M.9	1.6 c A	2.0 a A	56 b A	40 bc A
	M.7	2.0 b A	2.0 a A	68 a BC	64 a A
	MM.111	1.9 b A	2.0 a A	63 a A	58 a A
M.7	Fuji	2.2a A	2.3 a A	57 c AB	37 b B
	Gala	1.9 b B	2.2 ab A	65 bc A	55 a A
	M.27	1.7 bc A	1.9 b A	64 bc A	49 ab A
	M.9	1.6 c A	1.8 b AB	57 c A	44 ab A
	M.7	1.8 bc A	2.1 ab A	86 a A	37 b B
	MM.111	1.9 b A	2.0 ab A	68 b A	50 ab A
MM.111	Fuji	2.1 a A	2.1 a A	48 b B	36 a B
	Gala	2.1 a A	2.2 a A	62 a A	39 a A
	M.27	1.7 b A	1.6 b A	49 b B	34 a B
	M.9	1.4 c B	1.6 b B	48 b A	40 a A
	M.7	1.8 b A	2.2 a A	73 a B	36 a B
	MM.111	1.8 b A	1.8 ab A	63 a A	49 a A

^z Within columns, means followed by the same lower case letters designate no significant difference at the 0.05 level of confidence comparing scion within a rootstock. Within columns, means followed by capital letters designate no significant difference at the 0.05 level of confidence comparing the same scions on different rootstocks

significant differences in tree height between a scion on its 'own root' whether intact (not grafted) or grafted (Fig. 4).

Branching

Few significant effects of rootstock or scion were found on branching, either in the field or greenhouse and $S \times R$ interactions were found. In 2010, the only significant difference was that branch numbers for all scions on M.27 were significantly less than the other rootstocks. In 2010, the number of branches for scions on rootstocks MM.111, M.7, M.9, and M.27 were 3.0, 2.9, 2.1, and 0.5, respectively. By the end of 2011 scions on rootstock M.7 had significantly more branches than MM.111 (9.3 and 6.1 branches, respectively), and neither differed from scions on M.9 (8.0 branches).

Bud break and flowering

The time to bud break in 2010 was affected by scion and rootstock and $S \times R$ interactions were determined (data

not shown). All scions broke bud more slowly on rootstocks M.9 or M.27 than on other rootstocks. In contrast, scions of M.9 and M.27 broke bud earlier than scions of M.7 and MM.111 on any rootstock. This suggested that shoots of M.9 and M.27 were ready to grow but as rootstocks they delayed growth. However, no differences in time to bud break were found in 2011 or 2012. In 2011 32 % of scions on M.9 rootstock flowered, compared to <5 % of scions on other rootstocks. Yet as a scion M.9 did not produce more trees that flowered.

Hydraulic conductance

Xylem flow and hydraulic conductivity varied by scion but not by rootstock (Fig. 5; Table 3). No interactions were found between scion and rootstock. On their own root, xylem flow from largest to smallest was MM.111 = M.7 > M.9 > M.27. Across all rootstocks, 'Gala' had significantly higher xylem flow than 'Fuji', 3.8 and 2.1 mL/h, respectively. Hydraulic conductivity of scions was greatest for 'Gala' and M.9 (0.93 and 1.05) and least for 'Fuji',

Table 2 Tree diameters and diameter growth of different scion and rootstock combinations in the greenhouse and field

Scion	Rootstock	Stem diameter Nov. 2011 (mm)	Diameter growth 2011 (mm)
<i>Greenhouse 2011</i>			
Fuji	M.9	14.1 a ^z	5.4 b
Fuji	M.7	13.0 a	6.0 ab
Fuji	MM.111	14.5 a	8.1 a
Gala	M.9	11.9 a	4.0 a
Gala	M.7	11.6 a	4.7 a
Gala	MM.111	13.0 a	5.5 a
M.111	M.9	13.6 a	5.3 a
M.111	M.7	13.1 a	4.4 a
M.111	MM.111	9.6 b	2.4 b
M.27	M.9	12.0 a	3.7 ab
M.27	M.7	11.2 a	3.2 b
M.27	MM.111	11.8 a	5.0 a
M.7	M.9	12.3 a	5.2 ab
M.7	M.7	13.2 a	3.9 b
M.7	MM.111	13.2 a	6.1 a
M.9	M.9	11.9 a	4.1 a
M.9	M.7	11.5 a	5.4 a
M.9	MM.111	9.8 a	4.1 a
<i>Field 2011</i>			
Scion	Fuji	14.5 b	
	M.9	14.4 b	
	M.7	12.3 b	
	MM.111	19.2 a	
Rootstock	M.9	12.8 b	
	M.7	16.6 a	
	MM.111	16.0 a	

^z In the greenhouse and within a column, means followed by same lower case letters designate no significant difference at the 0.05 level of confidence comparing the same scions on different rootstocks. In the field and within a column, means followed by same lower case letters designate no significant difference at the 0.05 level of confidence comparing the main effects of scions and rootstocks

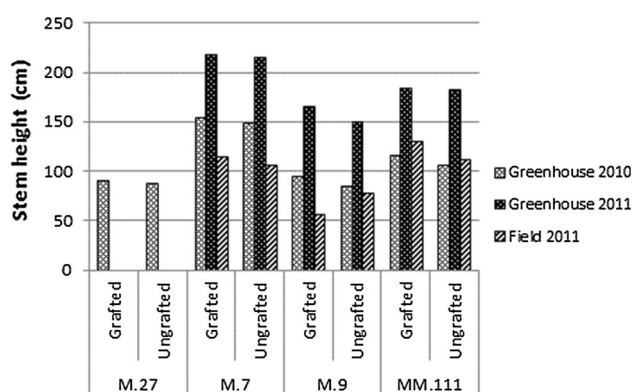


Fig. 4 Stem height of scion grafted on (from left-to-right) M.27, M.7, M.9, and MM.111 grown in the greenhouse and field. For each rootstock, Grafted and Ungrafted are the heights for the rootstock grafted to its own rootstock or left intact

M.27, and MM.111 (0.56, 0.44 and 0.56 mL/mm² stem/h/MPa).

Hormone profiles

'Gala' trees grafted to M.9 and MM.111 rootstocks. Results of scion 'Gala' are presented separately from the reciprocal graft work of scion M.9 and scion MM.111 on M.9 and MM.111 rootstock. As expected, hormone concentrations differed with plant component. ABA and the degradation compound, PA, were prominent in xylem exudate (Ex) and PA was higher in Ex of M.9 than MM.111. Nearly twice as much ABA and ABAGE was found in M.9 as MM.111 in rootstock stem below the graft (GrB) (463 and 211 ng/g DW, respectively, Fig. 6). Trace quantities of 7'-OH-ABA and t-ABA were found in Ex, current-year leaf

Fig. 5 Xylem flow of scion grafted on rootstocks (from left-to-right) MM.111, M.7, M.9, and M.27 grown in the greenhouse. For each rootstock, Grafted and Ungrafted are the xylem flow for the scion grafted to its own rootstock or left intact. Within each rootstock, bars with the same letter designate no significant scion difference at the 0.05 level of confidence

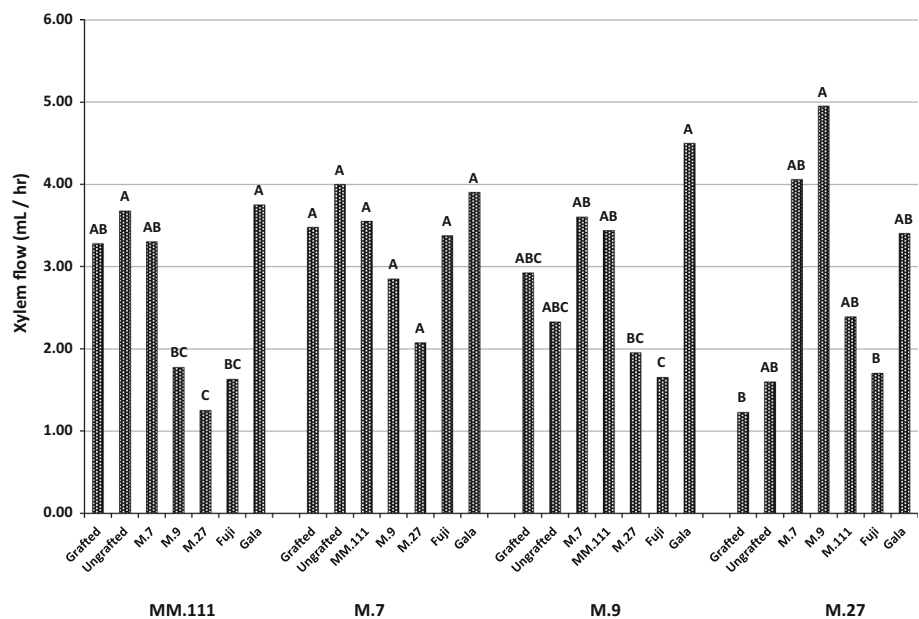


Table 3 Hydraulic conductance (mL/mm² stem/h/MPa) of rootstocks and scion of apple in the greenhouse in 2011

Main effect	Hydraulic conductance
<i>Scion</i>	
'Gala'	0.93 a ^z
'Fuji'	0.56 b
M.27	0.44 b
M.9	1.05 a
M.7	0.70 ab
MM.111	0.56 b
<i>Rootstock</i>	
M.27	0.53 a
M.9	0.75 a
M.7	0.78 a
MM.111	0.61 a

^z Within each main effect, means followed by same lower case letters designate no significant difference at the 0.05 level of confidence

(Lf), and current-year stem (StA) of 'Gala' on both MM.111 and M.9. ABA fluxes in the exudate of 'Gala' trees grafted to M.9 and MM.111 rootstocks were 2.4 and 2.6 pmol/mL/h, respectively.

Gibberellins 9, 19, and 53 were found in quantifiable amounts. GA₁₉ and GA₅₃ were present in nearly equivalent concentrations in StA, Lf and StB of 'Gala' on both rootstocks (Fig. 6). However, GA₁₉ was higher in root (Rt) and Ex of 'Gala'/111 than 'Gala'/9. GA's 19 and 53 are precursors to GA₁ which is an active GA in apple (Croker et al. 2000). Nearly twice the concentration of GA₉ was found in Lf of 'Gala' on MM.111 as M.9 (Fig. 6). With the

exception of GA₁₉ no GA was found in roots (Fig. 6). Generally <10 ng/g of GA₄, GA₇, GA₂₄, GA₃₄ and GA₄₄ was found and with no consistency among plant components of 'Gala' on MM.111 and M.9.

Most of the cytokinins were present in equivalent concentrations in 'Gala' on both rootstocks (Fig. 6). Trace quantities (<10 ng/g) of t-ZOG, t-Z, c-Z, dhZ, c-ZR, dhZR, and 2iP were found in most plant components of 'Gala' on both M.9 and MM.111. No significant differences in most cytokinins were found due to rootstock with the exception of iPA in Ex and GrA (Fig. 6). t-ZR was the most abundant cytokinin in exudate.

IAA was present in StA, Lf and StB of 'Gala' on both rootstocks (Fig. 6). IAA was abundant in exudate and GrB of MM.111 but not M.9. No metabolites of IAA were found in any plant component.

Hormone concentrations were not correlated with any growth parameters including height, number of nodes, diameters, dry weights, or functional parameters such as hydraulic conductivity or leaf water potential (data not shown).

M.9 and MM.111 scions grafted to M.9 and MM.111 rootstocks. ABA and related compounds were affected by scion and rootstock (Table 4). More PA was determined in StA of M.9 than all other scion/rootstock combinations. At least five times more PA was found in GrA and Ex of M.9 than MM.111 as scion. More ABAGE was in StA of MM.111 than M.9 (scion effect). More ABA and ABAGE was in GrB and Rt of 111/9 and 9/9 than other scion/rootstock combinations (rootstock effect). ABA and ABAGE did not differ in any scion-rootstock combination in GrA. ABA and ABAGE were two-times higher in roots

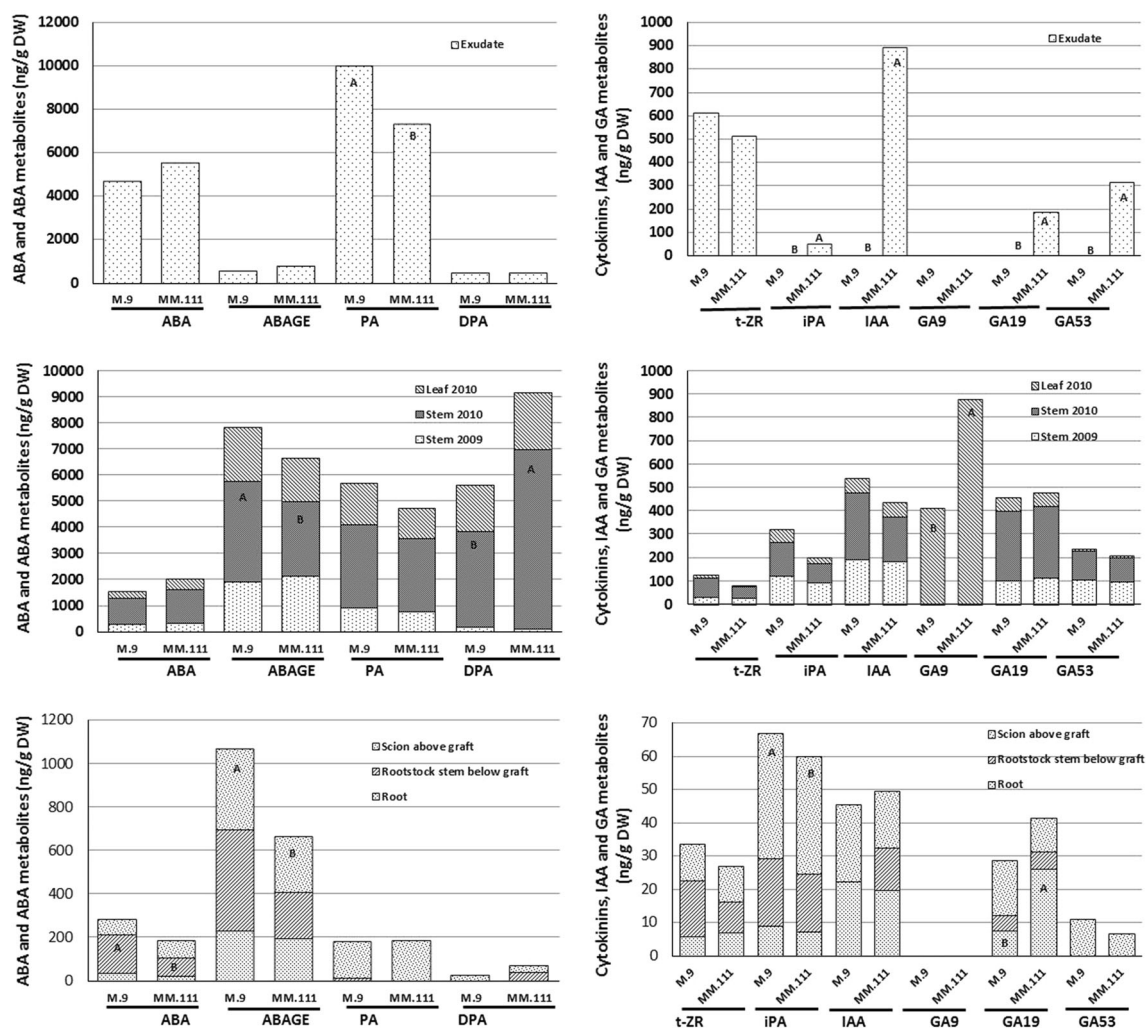


Fig. 6 Hormone profiles of 'Gala' on MM.111 and M.9 rootstocks in 7 plant components with xylem exudate (Ex) in top panels; 1-year-old stem (2009, StB), current-year stem (2010, StA) and current-year leaf (Lf), in the middle panels; scion stem above the graft (GrA), rootstock stem below the graft (GrB), and root in the bottom panels. The *left panels* present ABA (cis-abscisic acid) and metabolites DPA (dihydrophaseic acid), ABAGE (abscisic acid glucose ester), and

PA (phaseic acid). The *right panels* presents t-ZR (*trans* zeatin), iPA (isopentenyladenosine), IAA (indole-3-acetic acid), GA₉ (gibberellin 9), GA₁₉ (gibberellin 19), and GA₅₃ (gibberellin 53). Within each plant component the hormone and hormone metabolite that is annotated by a letter designates differences between M.9 and MM.111 rootstocks at the 0.05 level of significance

of M.9 than MM.111 rootstocks. As in 'Gala'/MM.111 and 'Gala'/M.9, reciprocal grafts of these two rootstocks had trace amounts of 7'-OH-ABA and t-ABA.

Few differences in IAA or cytokinins were found. In GrA and StA IAA was higher in M.9 as scion or rootstock and lowest in MM.111 (Table 5). No metabolites of IAA were found in any plant part. t-ZR and iPA were abundant in exudates of all scion rootstock combinations (Table 5). There were at least two times more t-ZR in Rt and GrB of MM.111 than M.9 (rootstock effect). Higher concentrations of iPA and t-ZOG were in Ex of MM.111 than M.9 rootstocks. Trace quantities (<10 ng/g) of t-Z, c-ZR, dhZR, and 2iP were found in most plant parts and Ex of reciprocal grafts on both M.9 and MM.111. Unlike 'Gala', no evidence of c-Z and dhZ were found.

Only two significant differences in GA were found (Table 4). GA₂₄ was greater in GrA of M.9 than MM.111 (scion effect). As with 'Gala' on M.9 and MM.111, GA₁₉ and GA₅₃ were found; although the abundance of GA₅₃ was much lower (Fig. 6; Table 4). Also like 'Gala' GA₉ was not found in the plant components that were sampled. On average, GA₈ was the most abundant gibberellin in all plant parts but not in exudate.

Discussion

Size-controlling effects of apple rootstocks are well-known and widely studied. There is abundant information that supports the idea that biochemical and hydraulic processes

Table 4 Hormone profiles of M.9 and MM.111 grafted on M.9 and MM.111 rootstocks in 5 plant components including xylem exudate (Ex), current-year stem (StA), scion stem above the graft (GrA), rootstock stem below the graft (GrB), and root (Rt). Main effects of scion (top) and rootstock (bottom) present cis-abscisic acid (ABA)

and metabolites dihydrophaseic acid (DPA), abscisic acid glucose ester (ABAGE), phaseic acid (PA), 7'-Hydroxy-abscisic acid (7'OH-ABA), trans-Abscisic acid (t-ABA) and gibberellins (GA) 8, 19, 24, 34, and 53

Plant component	Main effect	ABA and ABA metabolites (ng/g DW)						Gibberellins (ng/g DW)				
		ABA	DPA	ABAGE	PA	7'OH-ABA	t-ABA	GA ₈	GA ₁₉	GA ₂₄	GA ₃₄	GA ₅₃
Exudate	M.9	3991 ^z	207	76	71844	0	77	0	114	296	405	0
	MM.111	8745	2667	485	14,171	0	408	0	0	687	687	0
Stem 2010	M.9	808	2763 b	819 b	2165	41	42	548	20 b	381	14	10
	MM.111	1364	14967 a	1726 a	3344	37	32	371	37 a	225	5	17
Scion above graft	M.9	75	20	306	350 a	0	5	106	52	52 a	6	11
	MM.111	73	58	313	55 b	0	8	56	13	13 b	0	0
Rootstock stem below graft	M.9	59	8	325	23	0	0	37	8	5	0	0
	MM.111	95	11	376	21	0	0	25	6	5	0	0
Root	M.9	27	0	175	0	0	0	49	0	0	0	0
	MM.111	30	20	217	0	0	14	42	0	0	0	0
Rootstock												
Exudate	M.9	7226	1846	426	51,763	0	0	0	0	72	558	0
	MM.111	5807	1848	310	26,734	0	0	0	0	157	76	0
Stem 2010	M.9	958	12,579 a	1342	3411	34	0	442	27	308	13	18
	MM.111	1214	5152 b	1203	2097	43	0	477	29	298	10	12
Scion above graft	M.9	82	44	325	183	0	0	52	0	31	4	0
	MM.111	65	37	294	222	0	0	109	4	33	7	0
Rootstock stem below graft	M.9	126 a	10	502 a	27	0	0	33	0	8	5	0
	MM.111	27 b	7	198 b	11	0	0	29	0	6	4	0
Root	M.9	39 a	20	266 a	0	0	0	45	0	0	0	0
	MM.111	16 b	0	124 b	0	0	0	47	0	0	0	0

^z Significant differences between M.9 and MM.111 as scion and rootstock are highlighted in bold font and means with different letters designate significant differences between scions (top) and rootstocks (bottom) at the 0.05 level of confidence

and morphological and anatomical structures are likely associated with growth control of apple trees. In the research reported here we evaluated processes of growth control with known size-controlling stems as a rootstock and as a scion. Our focus was on whole-plant growth and endogenous hormones that regulate growth.

In the greenhouse and field trees with M.9 and M.27 scions were shorter than the other four scions when compared within any rootstock (Fig. 2). This suggests that part of the size-controlling effect was stem-related, i.e. the rootstock shank, and was not solely associated with the graft or the roots. Significant size-control of scion can be attained with interstock grafted between the rootstock and scion and the amount of size control is related to interstem length (Webster and Wertheim 2003). Previously, reciprocal grafts of four Malling rootstocks determined that rootstock controls total scion growth but scion controls the distribution of growth (Moore 1975). Interestingly, most scions grafted to more dwarfing M.7 and M.9 rootstocks in the greenhouse tended to be taller than scions grafted to

less dwarfing MM.111. It is possible in the greenhouse that abundant resources and care (water, nutrients, and pest control) may have mitigated some mechanisms of size-controlling rootstocks. It is also possible that pots in the greenhouse may have restricted root growth and thereby had a greater impact on the more vigorous rootstocks such as MM.111. In Nov. 2011 rootstock M.9 reduced scion diameter in the field but not the greenhouse (Table 2). The fact that few rootstock effects were found in the first 2 years in both the field and greenhouse suggests that, in this experiment, size-controlling processes required time for manifestation that may be associated with tree development or environmental co-factors.

The rootstock effect on tree size is generally apparent in the early life of the tree, but the effect on shoot extension growth may be less evident, particularly in the first two or three growing seasons (Fallahi and Mohan 2000; Tukey and Brase 1941). As the tree ages the effect of the more dwarfing rootstock can be seen as reduced shoot (Hirst and Ferree 1996; NC-140 1996) or trunk (Fallahi and Mohan

Table 5 Hormone profiles of M.9 and MM.111 grafted on M.9 and MM.111 rootstocks in 5 plant components including xylem exudate (Ex), current-year stem (StA), scion stem above the graft (GrA), rootstock stem below the graft (GrB), and root (Rt). Main effects of scion (top) and rootstock (bottom) present cytokinins and associated

metabolites (trans) zeatin-O-glucoside (t-ZOG), trans zeatin (t-ZR), (cis) zeatin (c-ZR), dihydrozeatin riboside (dhZR), isopentenyladenine (iP), isopentenyladenosine (iPA), and indole-3-acetic acid (IAA)

	Scion	Cytokinins and associated metabolites (ng/g DW)						Auxin (ng/g DW)
		t-ZOG	t-ZR	c-ZR	dhZR	iP	iPA	IAA
Exudate	M.9	76 ^z	850	888	0	0	36 a	35
	MM.111	408	741	752	0	0	168 b	165
Stem 2010	M.9	41	3	59	14	0	170 a	179 a
	MM.111	33	5	39	11	0	88 b	98 b
Scion above graft	M.9	5	2 b	32	3	0	80	81
	MM.111	8	9 a	25	0	0	45	45
Rootstock stem below graft	M.9	6	6	12	3	0	15	15
	MM.111	6	6	18	0	0	17	17
Root	M.9		10	8	0	0	8	7
	MM.111	14	11	6	0	0	7	7
Rootstock								
Exudate	M.9	66	0	900	0	0	36 b	33
	MM.111	247	0	741	0	0	122 a	122
Stem 2010	M.9	37	5	46	12	2	158	138
	MM.111	38	4	51	13	3	139	139
Scion above graft	M.9	0	5	48 a	3	0	77	96 a
	MM.111	0	7	13 b	3	0	29	29 b
Rootstock stem below graft	M.9	0	4 b	22 a	3	0	18	20
	MM.111	0	9 a	8 b	0	0	13	14
Root	M.9	14	2 b	5	0	0	6	5
	MM.111	0	18 a	9	0	0	9	9

^z Significant differences between M.9 and MM.111 as scion and rootstock are highlighted in bold font and means with different letters designate significant differences at the 0.05 level of confidence

2000) growth compared to the same cultivar on a more vigorous rootstock. Dwarfing rootstocks reduce the number of neoformed nodes and axes that develop in the scion and enhance flowering. These effects may be cumulative and may strongly affect scion architecture with each season in the orchard (Costes and Garcíá-Villanueva 2007; Seleznyova et al. 2003). Enhanced flowering of scion on dwarfing rootstocks may reduce shoot growth by sink competition within the tree canopy (Costes and Garcíá-Villanueva 2007). It is highly likely that factors that regulate tree growth become complex as trees age. Morphological adaptation to the environment may be driven by different factors within the crown and canopy architecture is thus the compilation and integration results of these factors. For example in *Quercus crispula* Blume., Yoshimura (2011) determined that the upper canopy may be influenced more by hydraulic adaptation to reduced water availability but light deprivation may affect development in the lower canopy. The impact of a rootstock and tree age will likely increase the complexity of tree

adaptation to a changing orchard environment that, for example, may be associated with climate change.

The graft union has been identified as a potential site of growth-control activity. Elevated IAA may induce excessive non-conducting vascular tissue at the graft that contributes to size-control (Tubbs 1973; Simons and Chu 1984; Soumelidou et al. 1994; Tworowski and Fazio 2011). In those studies, grafts were between dissimilar genotypes of scion and rootstock. In our study, early stem height growth of ‘own-rooted’ trees in the greenhouse or the field was not affected by grafting, per se (Fig. 4). On their own root, grafted or not, the heights of Malling rootstocks did not differ in 2011 and 2012. In comparison, ungrafted trees have been found to be more vigorous than corresponding trees that were grafted (Hirst and Ferree 1995). Stress and tree development into reproductive phases may work in synchrony of rootstock type to bring about dwarfing effects (Tubbs 1973). In the current field study by 2013 the ungrafted trees grew taller than trees grafted with the same scion/rootstock combination (data not shown). It is possible

that a graft union may affect scion growth after additional time in the field and the trees in this experiment will be monitored in the future.

Dwarfing effects of rootstocks have been attributed to root-shoot chemical messengers such as hormones, environmentally-induced effects such as abiotic stress, capacity for water movement through the soil–plant–atmosphere continuum, or combinations of these and other factors (Atkinson and Else 2001). In greenhouse-grown trees ABA and the ABA conjugate, ABAGE, were significantly higher in the root and the rootstock shank of trees grown on M.9 than MM.111 rootstocks (Fig. 6; Table 4). The main ABA metabolism pathway appeared to be through 8'-hydroxylation (which results in PA that may be further reduced to DPA) as well as conjugation (resulting in ABAGE) (Fig. 7). The abundance of ABA metabolites suggests that significant quantities of ABA were previously biosynthesized in tissue (Fig. 6; Table 4). ABAGE is inactive as a hormone but in roots it may be a source of ABA as it is converted to ABA that can enter xylem for acropetal transport (Hartung et al. 2002). ABAGE also can move acropetally in xylem. ABAGE was prominent above and below the graft of 'Gala' on M.9 rather than on MM.111.

ABA concentrations increased in leaves of *Xanthium strumarium* that were water stressed (Zeevaart 1980). When rehydrated, ABA levels decreased and concentrations of an ABA degradation product, PA, increased. Elevated PA in the xylem exudate and most plant parts of 'Gala' on the dwarfing rootstock M.9 may have been associated with the trees being well-watered (Fig. 6). It is possible that under water-stressed conditions in the field that ABA degradation would be reduced in scions grafted to dwarfing rootstocks. In the field-grown trees ABA concentrations in shoot tips were 46 % greater in scions grafted to M.9 than on seedling rootstocks (Tworkoski and Miller 2007).

In other work, dwarfing rootstocks generally had higher ABA flux in the transpiration stream and 50 % less xylem conductance than the more invigorating rootstocks

(Tworkoski and Fazio 2011). The reduced conductance was attributed to reduced number of large vessel elements that also reduced cross-sectional lumen area and water conductivity. In the current work ABA flux was too variable to confirm the earlier finding. However, in the reciprocal graft experiment, ABA and related compounds were nearly all higher in trees grown on rootstocks M.9 than MM.111 (Table 4). It is possible that ABA concentrations in the transpiration stream increase when grafted apple trees are under drying conditions (Atkinson and Else 2001). Water use was not measured in this experiment but drying can occur during the day and the potential impact of this diurnal cycle on ABA synthesis in roots in different rootstocks is unknown.

Previously, GA's in apple encompassed GAs 53, 12, 20, and 9 (Kusaba et al. 2001); GAs 1, 4, 7, 15, 20, 44, and 53 (Ramirez et al. 2004); and GAs 19, 29, 20, 1, and 8 (Crocker et al. 2000). In the current study gibberellins were detected in most samples, including GAs 53, 44, 19, 20 and 8 of the early 13-hydroxylation pathway, and GAs 24, 9, 4 and 34 of the non-hydroxylation pathway (Figs. 5, 7; Table 4). GA₄ was the only biologically-active GA found, albeit in small quantities but closely associated metabolites were found including GA₃₄, a 2-hydroxylated metabolite from active GA₄ (Fig. 7). GA₃₄ was particularly abundant in Ex of M.9 and MM.111 on M.9 rootstock, suggesting that M.9 rootstocks may have higher capacity for GA degradation than MM.111 rootstock (Table 4; Fig. 7). It is also possible that GA20 and GA3 oxidases may be less active in dwarfing M.9 than MM.111. The enzyme GA20 oxidase catalyzes conversion of 20-carbon GA's (e.g. GA₁₂ and GA₅₃) to GA₁₉ and GA₂₀ that are precursors, respectively, to active GA's GA₄ (which we found) and GA₁ (which we did not find).

It is noteworthy that in 'Gala' GA was found in Ex of MM.111 but not in M.9. GA₉ was found only in Lf and was twofold higher in 'Gala'/MM.111 than 'Gala'/M.9 (Fig. 6). Within each rootstock internode lengths, a trait that may be regulated by GA, were shorter during 2010 with scions grafted to M.9 than MM.111 (Table 1). It is possible that if hormone profiles were quantified over time that a clearer relationship of hormones and growth would emerge. GA₉ converts to GA₄ (active form) via GA3ox (Zhao et al. 2010). GA₅₃ converts to GA₄₄ and then to GA₁₉ via GA20ox; and GA₁₉ to GA₁ (active form) via GA3ox. Seleznyova et al. (2003, 2004) determined that trees on dwarfing rootstocks may have fewer neoformed (current season) nodes that may contribute to shorter internodes and over 2 or 3 years, fewer nodes were produced per branch. Previously, GA₁₉ and GA₂₉ were found to be the most abundant GA's in shoot meristems of genetically dwarf and standard apple grown on their own root (Steffens and Hedden 1992). Between April and June GA₁₉ declined in

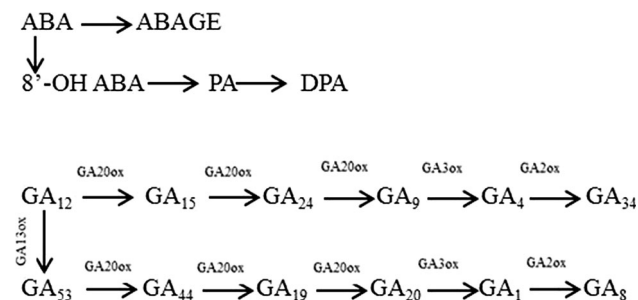


Fig. 7 Metabolic pathways in apple tree components of abscisic acid (ABA) (top) and gibberellins (GA) 1, 4, 8, 9, 12, 15, 19, 20, 24, 34, 44, and 53 (bottom)

standard but not dwarf trees. However, high temperatures decreased sensitivity of Dwarf trees to endogenous GA and growth ceased. In the current work GA₁₉ was higher in Rt and Ex of ‘Gala’ on MM.111 than on M.9 (Fig. 6). In other work, reduced GA₁₉ was measured in M.9 sap as the season progressed suggesting that it was a factor in dwarfing apple rootstocks, possibly in a role of terminating growth earlier than in vigorous rootstocks (van Hooijdonk et al. 2011).

In 2010 internode lengths of scions of M.7 and MM.111 were not affected by any rootstock but internode lengths in scions of M.9 and M.27 were generally shorter (Table 1). As scion, neither M.9 nor MM.111 had consistently higher GA in all plant components but GA tended to be higher above and below the graft with M.9 scion (Table 4). GA’s 9, 19, and 53 were generally more abundant in ‘Gala’ grafted to MM.111 than M.9 (Fig. 6). Richards et al. (1986) found that GA transport through grafts were lower in dwarfing M.9 than more vigorous MM.115. In addition to ABA and GA other plant hormones may be significant in the mechanisms of size control by rootstocks. In our experiment few differences in IAA concentrations were found. IAA was equally abundant in most plant components of ‘Gala’ on M.9 and MM.111 (Fig. 6). However, in GrB and Ex, IAA was abundant in MM.111 rootstock but absent in M.9. In reciprocally-grafted trees IAA was much higher in GrA of trees on M.9 than MM.111 rootstocks but did not differ in GrB or Rt (Fig. 6; Table 5). In our reciprocal grafts only t-ZR was more abundant in Rt and GrB in trees grown on MM.111 than on M.9 (Table 5). In ‘Gala’ t-ZR concentrations did not differ by rootstock in any plant component (Fig. 6). IPA was greater in GrA of plants grown on M.9. With dwarfing rootstocks, the graft union may decrease basipetal transport of auxin and also disrupt acropetal movement of cytokinin and movement of GA (Kamboj et al. 1999; van Hooijdonk et al. 2010). Auxin may not pass through the graft union of a scion grafted to a dwarfing rootstock that, in turn, results in abnormal and reduced xylem formation (Soumelidou et al. 1994; Tworowski and Fazio 2011). The anatomical irregularity may have disrupted water and mineral movement from a dwarfing rootstock. Seasonal changes in hormone transport are likely associated with rootstock regulation of tree growth and development (van Hooijdonk et al. 2011). As the growing season progressed, concentrations of basipetally moving indole-3-acetic acid declined whereas acropetal movement of t-ZR increased and hormone concentrations also varied by rootstock (van Hooijdonk et al. 2010). Kamboj et al. (1999) found higher concentrations of Z and tZR in xylem sap of invigorating rootstocks. In other work cytokinins did not differ significantly with apple rootstocks of different vigor but

the relative abundance of the auxin-to-cytokinin ratio regulated bud break (Tworowski and Miller 2007).

This experiment supported the hypothesis that hormones are signals that likely contribute to size-controlling processes of apple rootstocks. Elevated ABA and reduced GA were associated with the more dwarfing rootstocks. Hormone signals, however, were not clearly the sole size-controlling factors in that height and diameter growth, and internode length were not reduced in young, greenhouse-grown tree. It is possible that biological response to stage of tree development and environmental stress may interact to affect hormone signals and other size-controlling factors. We are currently evaluating effects of environmental stress (e.g. water and nutrient availability) on rootstock size-controlling capacity.

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