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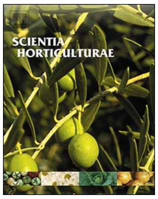

Research paper

Effect of scion and graft type on transpiration, hydraulic resistance and xylem hormone profile of apples grafted on Geneva® 41 and M.9-NIC™29 rootstocks

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ABSTRACT

New highly productive apple rootstocks resistant to fire blight and crown rot can improve orchard profitability. However, some of the most promising rootstocks appear to have weak or brittle graft unions that are susceptible to breakage in the nursery and in the field. Flexural strength of graft unions may be related to the amount of vascular connections between graft partners, and poor vascular connections may cause increased hydraulic resistance. We sought to determine if transpiration rate and hydraulic resistance were correlated with graft union strength by comparing Geneva® 41 with the strong graft-forming Malling 9 selection NIC™ 29. The primary goal of this study was to compare weak and strong graft unions between similarly vigorous rootstocks by measuring whole-tree transpiration, as well as hydraulic resistance using the pressure chamber method. The effects of rootstock and grafting method on xylem hormone concentrations were also compared. There was no correlation between graft union strength and whole tree transpiration rate or leaf area-specific transpiration rate. The hydraulic resistance of the weaker forming rootstock was not significantly different than the strong graft union rootstock and was more closely correlated with overall size of the root system. However, hydraulic resistance of the scion and graft union were small relative to root system resistance, making it difficult to detect differences in graft union resistance among scion-rootstock combinations. Conversely, we observed differences in the hormone profile of xylem exudate among rootstock genotypes and among graft types.

1. Introduction

Planting a new apple (*Malus xdomestica* Borkh.) orchard represents a 20-year commitment. Besides cultivar selection, growers are faced with other key decisions that will affect orchard viability and profitability. Over the last 50 years, dwarfing rootstocks have stimulated dramatic changes in tree density and training systems. However, there is a serious threat to high-density apple orchards, since many of them are planted on dwarfing rootstocks such as M.9 and M.26 that are highly susceptible to the bacterial disease fire blight (*Erwinia amylovora* Burill), thus limiting new plantings (Norelli et al., 2003; Russo et al., 2007). In an effort to overcome widespread infection and death of dwarfing apple orchards, the Geneva® apple rootstock breeding program jointly led by Cornell University and USDA-ARS has developed rootstock genotypes which are resistant to fire blight and crown rot (*Phytophthora* spp.) (Cummins and Aldwinckle, 1983; Fazio et al., 2015). However, some of the Geneva® rootstocks, especially G.41, appear to form weak or brittle graft unions that are susceptible to breakage in the nursery and in the field (Adams et al., 2017; Adams, 2016; Tworkoski and Fazio, 2015). This weakness has been problematic in the nursery during strong wind events or during harvest and shipment, suggesting potential graft incompatibility. However, the good performance and disease resistance of these new Geneva® rootstocks (Autio et al., 2011a; Autio et al., 2011b; Fazio et al., 2015; Marini et al., 2014; Norelli et al., 2003; Robinson et al., 2004, 2011; Tworkoski et al., 2016) make further research on the nature of this weakened graft union critically important to the apple industry.

Weak graft unions may be symptomatic of poor vascular connections between graft partners that could increase hydraulic resistance. For instance, Tworkoski and Fazio (2015) suggested that hydraulic

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conductivity might be limited by the root system or graft union. Atkinson et al. (2003) found that vigorous rootstocks increased the conductivity of the whole plant and its parts, and the grafts of the vigorous rootstocks had less resistance than the dwarfing rootstock grafts while correcting for differences in segment length. The increased conductivity of vigorous rootstocks was also related to an increased percentage of stained xylem within the graft segments, suggesting that trees on vigorous rootstocks may have increased conductance due to increased proportion of active xylem (Atkinson et al., 2003). Therefore, most methods of assessing graft incompatibility focus on quantifying vascular differentiation. These methods include break or tensile strength testing, hydraulic conductance, electrical conductance, and anatomical analysis through microscopy, staining or other imaging (Atkinson et al., 2003; Gasco et al., 2007; Gee et al., 1973; Moore, 1983; Solari et al., 2006; Sperry et al., 1988; Tworkoski and Fazio, 2011; Tyree and Ewers, 1991; Tyree et al., 1995).

Although G.41 apple rootstock forms weak grafts, the resulting effect on vascular connection and conductivity is not known. We sought to determine if transpiration rate and hydraulic resistance were correlated with graft union strength by comparing G.41 with the strong graft-forming Malling 9 selection NIC™29 (M.9), which shows approximately the same vigor in the orchard. The scion cultivar ‘Scilate’ was selected due to reports in the nursery that it formed more brittle graft unions than other cultivars such as ‘Fuji’ and ‘Gala’ (Adams, 2016).

The primary goal of this study was to compare weak and strong graft unions by measuring whole-tree transpiration, as well as hydraulic resistance using the pressure chamber method. We hypothesized that the weaker G.41 rootstock would result in poor or irregular vascular tissue, leading to reduced transpiration and greater hydraulic resistance. The effects of grafting method on transpiration, graft union resistance and xylem hormone concentration were also compared.

2. Materials and methods

2.1. Tree propagation and growth

2.1.1. 2014 trials

‘Gala’ and ‘Scilate’ were custom budded on G.41 and M.9 rootstocks at Willow Drive Nursery in Ephrata, WA in August of 2013. In March of 2014, ‘Scilate’ was also saddle grafted and whip/tongue grafted on G.41 to make an incomplete factorial of 2 scions × 2 rootstocks × 3 grafting methods, where only one scion-rootstock combination was included in two of the three graft types (Table 1). In May 2014, 10 trees from all six combinations were selected for uniformity, dug from the nursery field and placed in 8-l pots with the field soil (Timmerman coarse sandy loam) with care taken to keep soil around the roots. Trees were initially grown outside under shade to minimize transplant shock. At the end of May, the trees were transported to the USU research greenhouse facility in North Logan, UT and irrigated automatically for 30 min, 4 d/week with drip emitters (4 L/h). Trees received fertigation on one of the 4 watering days with a 21N-5P-20K fertilizer solution (Peters Excel Multi-Purpose with minor elements; Scotts-Sierra Horticultural Products, Marysville, OH) at 100 mg L^{-1} N. The greenhouse was equipped with metal halide lamps to supplement sunlight and plants were given a 14-h photoperiod. The daytime temperature was 21 °C and the nighttime temperature was 15 °C. Trees were evaluated during three different time periods: July 9–12, July 30–Aug 2, Oct 3–6.

Table 1
Treatment combinations of scion, rootstock and graft type for 2014 and 2015 experiments, representing incomplete factorial treatment structure.

	Scion	Rootstock	Graft Type
2014 Trial	Scilate	M.9	Chip bud
	Gala	M.9	Chip bud
	Scilate	G.41	Chip bud
	Gala	G.41	Chip bud
	Scilate	G.41	Saddle
	Scilate	G.41	Whip/tongue
2015 Trial	Scilate	G.41	Chip bud
	Fuji	G.41	Chip bud
	Scilate	G.41	Saddle
	Fuji	G.41	Saddle
	Scilate	M.9	Saddle
	Fuji	M.9	Saddle

2.1.2. 2015 trials

‘Fuji’ and ‘Scilate’ were custom budded on G.41 at Willow Drive Nursery in August of 2014, and the same cultivars were custom saddle grafted in March 2015 on G.41 and M.9 rootstock making an incomplete factorial (Table 1). Ten trees of each combination were selected for uniformity and then covered in moist sawdust for transportation to the USU research greenhouses in North Logan, UT. Upon arrival, the trees were planted in 8-l pots using a commercial soilless media (Sunshine Mix #4, SunGro Horticulture, Agawam, MA). Trees were then grown in the greenhouse for three months prior to testing. At the beginning of the transpiration study, trees height was 182 ± 11 cm, and did not differ between graft types. Scion cross sectional area (SCSA) did differ by graft type at the beginning of the experiment, with bench graft and chip bud SCSA of 0.60 and 1.31 cm^2, respectively. Greenhouse conditions were the same as described for 2014. Trees were watered by hand at first, and then switched to automatic drip irrigation in May. Drip irrigation ran for 25 min, on daily cycles, but was later adjusted to 20 min to minimize nutrient deficiency. Drip emitters were the same as for 2014. Trees received fertigation on two of the seven days with the same solution as for 2014.

2.2. Experiment 1: transpiration

2.2.1. 2014 trial

In July, eight trees of each scion/rootstock/graft method combination were selected for uniformity and assigned to one of eight rows within the greenhouse. Rows were spaced such that there was 60 cm in between rows with very little space between pots in the row. Pots were watered the night before testing and allowed to drain to field capacity. The pots were then wrapped in white plastic bags to prevent evaporation from the soil surface. Each tree and pot was weighed in the morning and night for each trial. Each morning, each tree and pot was weighed, the weight of water used over the 24-h period was determined, and water was added to bring the pot and tree back to the initial weight. This process was repeated daily for 5–7 days. This method of adding back water each day was discontinued the following year because we noticed that we had significant leaking from the bottom of the pot due to the low infiltration rate of the field soil leading to preferential flow down the sides of the pot. During this time, leaf number and length were measured and recorded for each tree for a non-destructive estimate of total leaf area per tree. This method was calibrated by destructively measuring total leaf area for one tree of each cultivar using a bench-top leaf area meter (model L1-3000 LI-COR, Lincoln, NE). Tree size measurements were also taken for each trial date, including rootstock shank diameter (5 cm below graft union), graft union diameter based on two perpendicular measurements at the widest part of the graft union, scion diameter (5 cm above the graft union) and scion height from the graft union.

2.2.2. 2015 trial

In July, four trees of each rootstock/scion/graft method combination were selected for uniformity and randomly assigned to one of four rows within the greenhouse. Trees were spaced with 60 cm between rows and 30 cm within rows. Pots were watered the night before any testing and allowed to drain to field capacity. The pots were then wrapped in white plastic bags to prevent evaporation from the soil surface. Each tree and pot was weighed in the morning and night for each trial. Bags were then removed at night and trees were watered and allowed to reach field capacity for the next day. Non-destructive leaf area estimates were carried out as described above. The process of weighing and measuring leaf area was repeated at three separate trial periods in July, however one of the trial periods was affected by leaking irrigation from other experiments taking place in the greenhouse and was thus excluded. Tree size measurements were also taken as described above.

2.3. Experiment 2: hydraulic resistance and hormone concentration

Following the completion of transpiration measurements, a pressure chamber designed for a potted tree root system was used to measure the hydraulic resistance through the rootstock, graft union, and a portion of the scion stem with sequential tissue removal to determine resistance of the scion, graft, and root system (Davies and Flore, 1986; Fiscus, 1975). For 2014 all eight trees for each combination were tested row by row. For the 2015 trials, all ten trees from each combination in the transpiration experiment were re-randomized as a completely randomized design for testing in the pressure chambers.

The protocol for testing hydraulic conductance was as follows. First, the trees were watered the night before testing and allowed to equilibrate to field capacity overnight. Leaves were removed and collected for leaf area determination, using the leaf area meter previously described. Potted trees were then placed in the pressure chamber and the trees were topped just below the lowest branch to allow the pressure chamber lid to go over the tree. The chamber lid was then secured and a rubber stopper was secured around the trunk to seal the root system inside the chamber. Pressure was then applied to reach 0.345 MPa (50 psi) in the chamber. The tree was then re-topped about 34 cm above the graft union and 2 cm of bark (assumed to be phloem) was removed just below the cut, and the diameter (assumed to be xylem and pith) was measured. Plastic and rubber tube pieces were fitted over the xylem section and attached to thin plastic tubing used to collect exudate into a centrifuge tube. Exudate was collected for 10 min, and the centrifuge tube and contents were weighed to determine the quantity of exudate collected. The tree was then cut to 6 cm above the graft union and the above process repeated. We then cut the tree to 4 cm below the graft union and followed the same steps as above but collected exudate for 20 min. During this time, lengths were measured for the scion segment, graft segment, and later, the rootstock shank segment. Following testing in the pressure chamber, roots were washed of their soil or potting media and allowed to air dry in the greenhouse for two weeks. Once dry, the weight of the root mass removed from the rootstock shank was determined.

Exudates extracted from 6 cm above the graft union from ‘Scilate’ trees chip-, saddle-, and whip/tongue-grafted on G.41 and chip budded for M.9 were stored at −80 °C until analyzed for hormone content. Five random samples of each rootstock and graft type combination were analyzed by UPLC ESI–MS/MS (Lulsdorf et al., 2013; Zaharia et al., 2005) at the National Research Council Plant Biotechnology Institute (110 Gymnasium Place, Saskatoon, Saskatchewan, S7N0W9, Canada) to identify and quantify hormone content.

2.4. Data analysis

For comparing transpiration rate, the leaf area-specific (LA) transpiration rate was calculated as transpiration (g or ml/min) divided by leaf area (m^2). Transpiration was calculated by taking the difference in potted tree weight (water loss) divided by the time interval of water loss. Although accepted SI units for transpiration are $kg\,s^{-1}\,m^{-2}$, the units $g\,min^{-1}\,m^{-2}$ are used here for ease of reporting. Transpiration and flow measurements were averaged over each trial period in which the size measurements were taken. Diameter measurements of the rootstock, graft union, and scion were used to calculate cross-sectional areas (CSA).

For the pressure chamber experiment, we used the weight of the collected exudate, the pressure head, and the CSA to calculate resistance and CSA-specific resistance. Resistance was reported instead of conductance because many of the data in 2014 and some of the data in 2015 had resistance values at or near zero, which would result in infinite calculated conductance. Resistance ($MPa\,min\,g^{-1}$), and CSA-specific resistance ($MPa\,min\,m^2\,g^{-1}$) were calculate similar to Gasco et al. (2007).

The transpiration data were analyzed with date as a repeated measures, using the GLM procedure in SAS (SAS Institute Inc., Cary, NC). The experiments were designed for two sets of pre-planned comparisons. The first was a 2 rootstock × 2 scion factorial for a single graft type (chip bud in 2014, saddle graft in 2014). The second was a comparison of graft types (chip bud, saddle graft, whip/tongue) for one scion-rootstock combination ‘Scilate’ on G.41 in 2014. In 2015, two graft types (chip bud, saddle graft) and two scion cultivars (‘Scilate’ and ‘Fuji’) were compared on the rootstock G.41. ANOVA was carried out for each set of pre-planned comparisons, and the Tukey honestly significant difference test was performed for comparison of means.

Hormone content in xylem was modeled using linear mixed effect models. Mixed models including either rootstock or graft type as fixed factors and rep as a random factor were built to separate treatment effects for the hormone content depending on rootstock or graft type. P values ≤ 0.05 were considered significant. Residual analysis was performed to insure that model assumptions were met. Hormone concentration means were used in multivariate analysis to generate two-way similarity cluster diagrams based on graft type similarity and variable similarity. The Ward’s minimum variance criterion was used. Data were analyzed using the JMP statistical software package (Version 12; SAS Institute Inc., Cary, North Carolina).

3. Results

3.1. Transpiration

Analysis of variance for both 2014 and 2015 transpiration data showed significant two-way interactions between sampling time and other factors such as scion, rootstock and graft type. These interactions are shown in Figs. 1 and 2.

3.1.1. Scion × Date

In 2014 ‘Scilate’ and ‘Gala’ did not differ in transpiration or leaf area until the October 3–6 date (Fig. 1). By this final date, four of the ‘Gala’/G.41 trees had stopped growing and had set terminal buds resulting in a significant difference in leaf area between cultivars. This leaf area difference accounted for the transpiration rate differences as LA specific transpiration did not differ between cultivars (Fig. 1).

In 2015 ‘Fuji’ was used instead of ‘Gala’, because it was thought to form a stronger graft union (G. Fazio, personal communication). ‘Scilate’ had much higher tree transpiration and leaf area, however the LA specific transpiration rate was lower than ‘Fuji’ (Fig. 2).

3.1.2. Rootstock × Date

In 2014, G.41 and M.9 did not differ in tree transpiration or leaf area at the earliest date (Fig. 1). Over time, both transpiration and leaf area increased more for M.9 than for G.41. The LA specific transpiration differed slightly between rootstocks at the intermediate measurement date, but not at the earliest or latest measurement dates.

In 2015, G.41 grew faster than M.9 resulting in higher rates of tree transpiration and more leaf area at the later measurement time (Fig. 2).

3.1.3. Scion × Rootstock

In 2014, M.9 generally had higher tree transpiration, but this only resulted in a higher LA specific transpiration rate for ‘Gala’ (Fig. 1).

In 2015, G.41 had higher tree transpiration rates and higher leaf

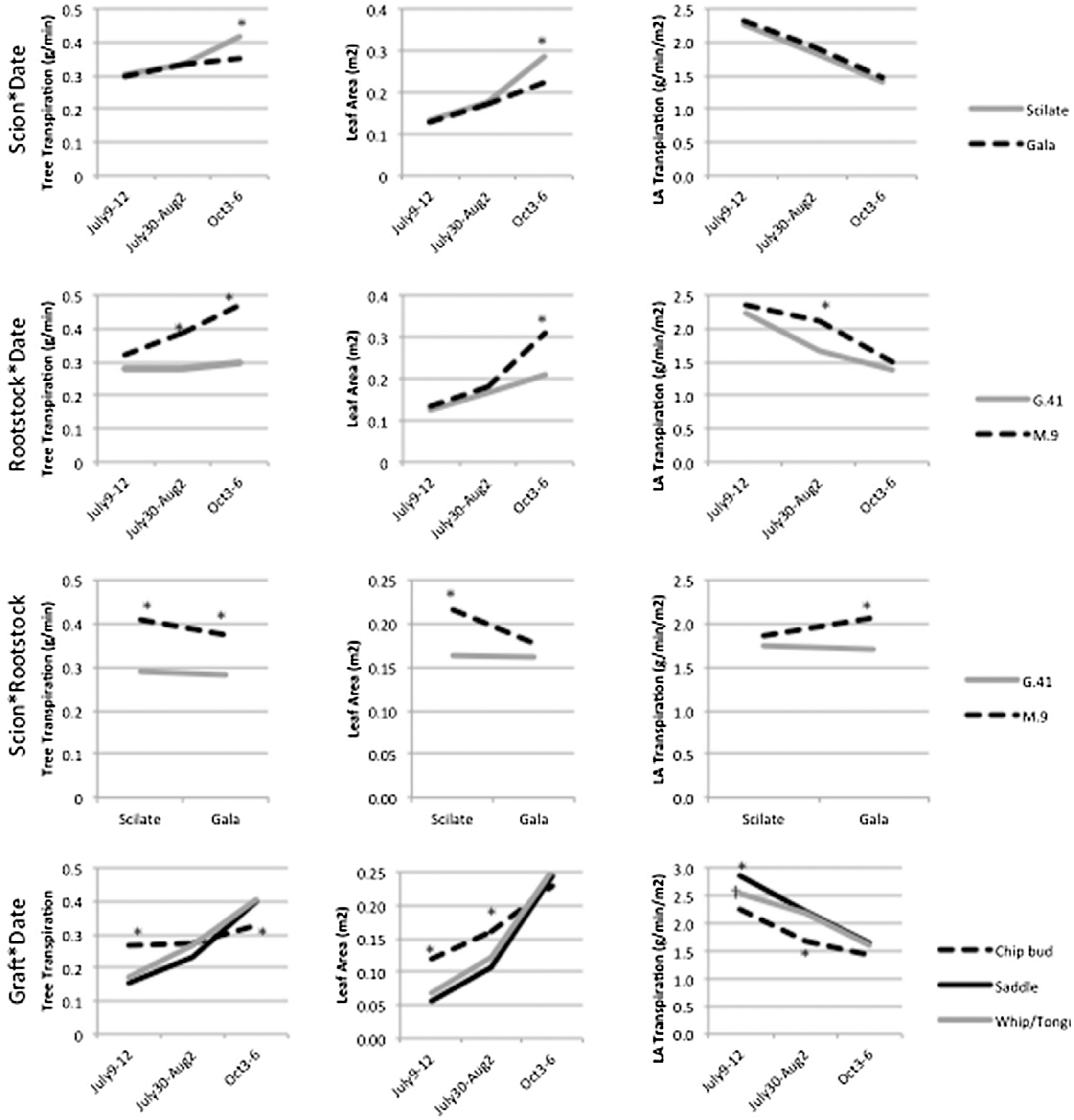

Fig. 1. Tree transpiration (g/min), leaf area (m^2), and leaf area specific (LA) transpiration rate ($g/min/m^2$) for scion × date, rootstock × date, scion × rootstock, and graft × date interactions in the 2014 experiment. An * indicates significant differences between lines within x-axis variables. A † indicates no significant differences from other lines within x-axis variables.

area than M.9 for both scions (Fig. 2), which resulted in higher LA specific transpiration rates for G.41 than for M.9.

3.1.4. Graft × Date

In 2014 chip budding was compared to two types of bench grafting: saddle graft and whip/tongue grafts (Table 1). For the comparison of graft types, 'Scilate' and G.41 were used as scion and rootstock in all cases. On the first measurement date, chip buds had significantly higher tree transpiration and leaf area than both bench grafts (Fig. 1), but LA specific transpiration rates were similar (Fig. 1). For the July 30–August 2 period, chip buds still had a higher leaf area but there was no difference in tree transpiration rate, which translated into a significantly lower LA specific transpiration rate than both bench graft types (Fig. 1). For the final measurement (October 3–6) the leaf areas were not significantly different among graft types, but chip buds had lower tree transpiration (Fig. 1). This resulted in a LA specific transpiration rate for chip buds that was numerically slightly lower than that of bench grafts, but the difference was not statistically significant.

Because there was no difference between bench grafts in 2014, only one bench graft method was used in 2015. This allowed for comparison of two different scions for each graft method. For both measurement dates, chip buds had higher tree transpiration and leaf area than saddle grafts but the magnitude of the effect was scion cultivar specific. Bench grafted 'Fuji' had much lower tree transpiration and leaf area than chip budded 'Fuji', or either of the 'Scilate' combinations (data not shown). The net effect of this reduction in tree transpiration and leaf area was that bench-grafted trees had higher LA specific transpiration for both scion cultivars (data not shown, see Adams, 2016).

3.2. Regression comparisons

Although care was taken in both years to select trees of uniform initial size, the scion and rootstock cultivars, as well as graft type, contributed to some differences in growth rate and leaf area. Much of the difference in whole tree transpiration among treatments and times was the result of this difference in final leaf area. To compare tree transpiration across relative tree sizes, we compared LA specific transpiration for each of the treatment combinations. Plotting a regression of tree transpiration relative to leaf area allowed for comparing transpiration rates independent of tree size. Regressions were calculated for

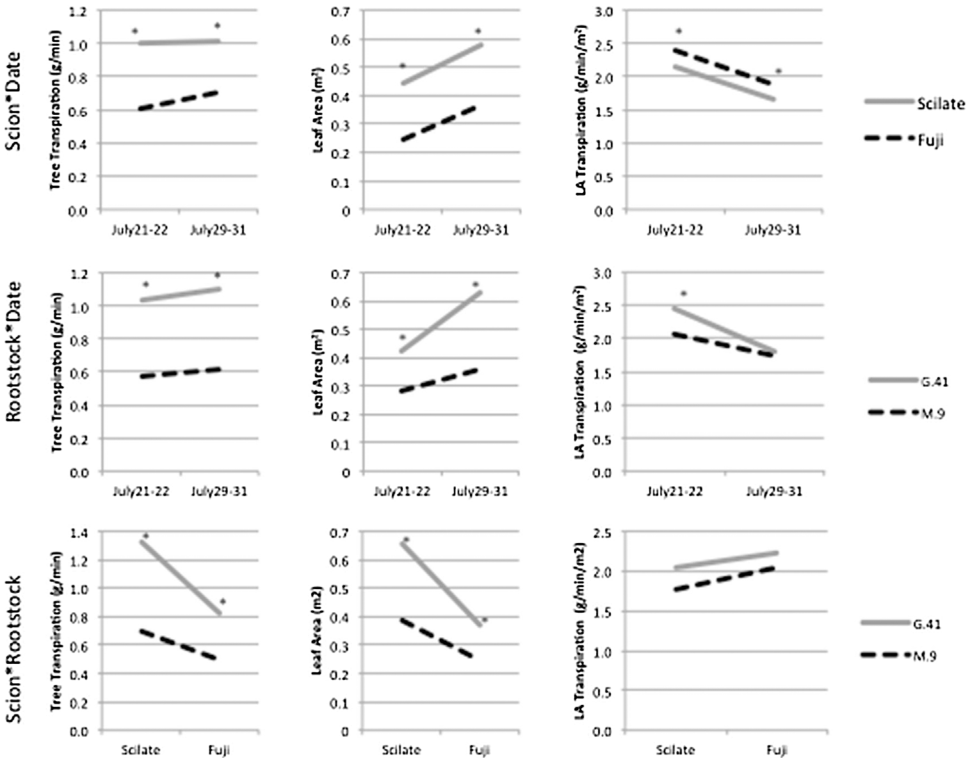

Fig. 2. Tree transpiration (g/min), leaf area (m^2), and leaf area specific (LA) transpiration rate (g/min/m^2) for scion × date, rootstock × date, and scion × rootstock interactions from the 2015 pre-planned comparison of rootstock × scion interactions for chip budding (comparison 1). An asterisk indicates significant differences between lines within x-axis variables.

treatment means in both the 2014 and 2015 studies (Fig. 3). In the 2014 study, trees chip budded on G.41 generally tended to have transpiration rates lower than would be predicted based on leaf area (Fig. 3A), which was particularly pronounced for the later measurement dates with the ‘Gala’ scion. In the 2015 study however, G.41 trees tended to have transpiration rates that were similar to or higher than other combinations with similar leaf area (Fig. 3B). The 2014 study was conducted using field soil and M.9 had higher growth rates (Fig. 1). Using soilless media in 2015, G.41 had faster growth (Fig. 3).

3.3. Hydraulic resistance

3.3.1. 2014 trials

Data from 2014 the pressure chamber experiment had many calculated resistance values that were near zero, and in some cases, negative. Since resistance cannot be negative, we set any negative values to zero for the analysis. However, the majority of the measurements in the scion segment and the graft union segment resulted in zero or negative values (data not shown). These values are likely the result of the serial tissue removal method we used. Because the overall hydraulic resistance of the root system in wet field soil was high relative to that of graft union and scion segments, it was not possible to detect the resistance in these tissues. The rootstock resistance values were relatively high for all treatments, ranging from 1.42 to 1.78 MPa min mL^{-1}, and did not differ among treatment combinations (Table 2). This despite significant differences in rootstock shank cross sectional area (RCSA) and root biomass per cross sectional area.

3.3.2. 2015 trials

As in 2014, there were some negative calculated resistance values, but much fewer than in 2014. Saddle grafted ‘Fuji’ on M.9, in particular, had negative values when calculating graft resistance. These negative values were set to zero, and the data transformed by adding a constant to facilitate statistical analysis. Because of this transformation, we were unable to calculate CSA-specific resistance for the scion and graft union segments. With ‘Fuji’, resistances through the graft segment, were significantly lower than with ‘Scilate’ (Table 3). Part of this effect was skewed in favor of ‘Fuji’ because when it was saddle grafted on M.9, 80% of the samples yielded negative resistance. ‘Fuji’ also had less resistance than ‘Scilate’ in the scion segment when comparing saddle grafts and chip buds (Table 3).

The root system of G.41 had less resistance than M.9 for saddle grafts, and chip buds had less resistance than saddle grafts (Table 3). G.41 roots systems also has a larger RCSA and larger root mass per SCA. The net effect was CSA-specific rootstock resistance values that were similar across all bench grafted rootstock × scion combinations. Chip-budded trees had slightly lower rootstock resistance values, which resulted in significantly higher CSA-resistance due to the much larger root systems (Table 3).

Final root mass was divided by scion cross-sectional area (SCSA) to provide a root mass relative to tree size (similar to a root to shoot ratio) to account for potential differences in biomass partitioning that may have contributed to water stress (Table 2). In 2014, a higher root mass per scion cross-sectional area was observed on G.41. All of the other combinations had a similar root mass per cross-sectional area, and a similar LA specific transpiration rate when accounting for differences in leaf area (Fig. 2). In 2015 the root mass per scion cross-sectional area was much lower, yet G.41 still had a bigger root system (Table 3). For both years, higher root mass was observed on trees that were chip budded.

3.4. Hormone concentrations

Different metabolites of abscisic acid (ABA), auxins, cytokinins, and gibberellins were detected in xylem extracts (Fig. 4). Biologically active ABA was found in larger amounts compared to its catabolites. The most abundant catabolite appeared to be PA (formed through the 8′-hydroxylation pathway), followed by ABAGE (formed through conjugation). *Trans*-ABA is formed through the isomerization of natural ABA under UV light exposure. Overall, higher levels of ABA metabolites were found in saddle grafts, followed by whip/tongue grafts and then chip buds, however, no significant differences were found. Auxins were represented by biologically active indole-3-acetic acid (IAA) and its conjugates with aspartic acid IAA-Asp. Among all the bioactive free

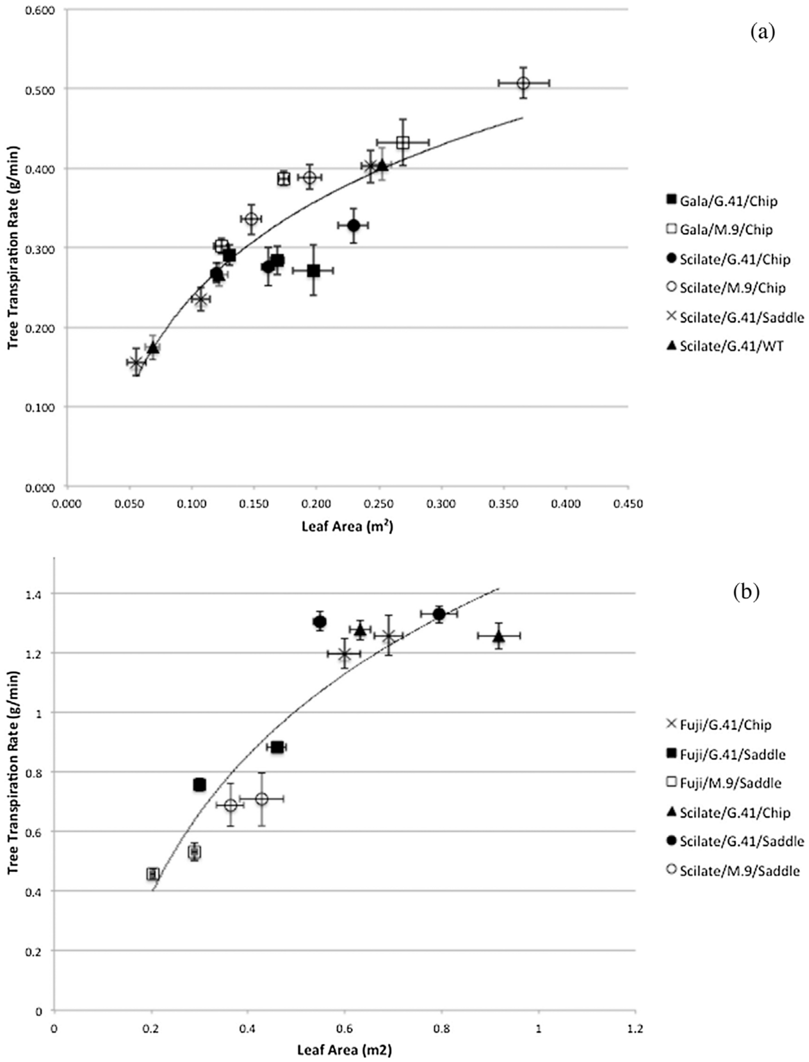

Fig. 3. Regression of tree transpiration rate(g/min) in relation to leaf area (m2) for all treatments and measurement times in the 2014 (a) and 2015 (b) experiments. Each data point represents the average of 8 replicate trees in 2014 and 4 replicate trees in 2015, and includes standard error bars. Regression lines were fitted using logarithmic equations.

base cytokinins (Z, dhZ, iP), only Z (*trans*-isomer) was found. The biosynthetic precursors ZR (both *cis*- and *trans*-isomers, the latest significantly more abundant) and iPR were detected as well. No significant differences among graft types were observed for auxins and cytokinins, but there was a tendency of higher levels for saddle grafts, followed by chip buds, and whip/tongue grafts having the lowest levels. GAs on both the 13-hydroxylation (GA53 → GA44 → GA19 → GA20 (→GA29) → GA1 → GA8) and non-hydroxylation (GA12 → GA15 → GA24 → GA9 (→GA51) → GA4 → GA34) pathways were present. The presence of GA34 and GA51 indicates that biologically active GA4 and GA9 respectively, must have been previously produced. No significant differences regarding GAs were observed either; however, there was also a trend for higher content with saddle grafts, followed by whip/tongue grafts, and then chip buds.

Table 2
Root mass and hydraulic resistance for the 2014 study. Values are means of 8 replicate trees and represent root mass per stem cross-sectional area (Root/SCSA, g/cm^2), rootstock resistance (MPa min mL^{-1}), rootstock shank cross-sectional area (RCSA, cm^2), and RCSA-specific resistance (RCSA-Resistance, MPa min cm^2 mL^{-1}).

Treatment			Root/SCSA	Resistance	RCSA	RCSA-Resistance
Gala	G.41	Chip	89.5	1.52	1.73	2.63
Gala	M.9	Chip	47.9	1.49	1.97	2.89
Scilate	G.41	Chip	97.1	1.78	1.50	2.60
Scilate	M.9	Chip	53.0	1.56	3.06	4.73
Scilate	G.41	Saddle	37.0	1.48	1.16	1.71
Scilate	G.41	WT	42.9	1.42	1.30	1.86
ANOVA *p* values						
	Comparison 1 – Rootstock × Scion for chips					
	Scion		n.s.[a]	n.s.	n.s.	0.0809
	Rootstock		< 0.0001	n.s.	0.0004	0.0002
	Scion × Rootstock		n.s.	n.s.	0.0075	0.0047
	Comparison 2 – Graft Type for ‘Scilate’/’G.41’					
		Graft	< 0.0001	n.s.	n.s.	0.0188

[a] Non-significant effect at $p < 0.1$.

Table 3
Root mass and hydraulic resistance for the 2015 study. Values are the means of 10 replicate trees and represent root mass per stem cross-sectional area (Root/SCSA, g/cm^2); scion, graft and rootstock resistance (MPa min mL^{-1}); rootstock shank cross-sectional area (RCSA, cm^2), and RCSA-specific resistance (RCSA-Resistance, MPa min cm^2 mL^{-1}).

Treatments			Root/SCSA	Resistance			RCSA	CSA-Resistance
				Scion	Graft	Rootstock		Rootstock
Fuji	G.41	Saddle	10.4	0.182	0.100	1.01	0.886	0.90
Scilate	G.41	Saddle	19.2	0.328	0.150	0.91	0.951	0.87
Fuji	M.9	Saddle	7.5	0.160	0.026	1.30	0.666	0.87
Scilate	M.9	Saddle	12.8	0.257	0.143	1.41	0.849	1.19
Fuji	G.41	Chip	26.9	0.087	0.065	0.66	2.538	1.68
Scilate	G.41	Chip	28.9	0.213	0.115	0.84	1.887	1.58
ANOVA p values								
	Comparison 1 – Rootstock × Scion for graft							
		Scion	< 0.0001	n.s.[a]	0.0415	n.s.	0.0623	n.s.
		Rootstock	0.002	n.s.	n.s.	0.0036	0.0192	n.s.
		Scion × Rootstock	n.s.	n.s.	n.s.	n.s.	n.s.	0.083
	Comparison 2 – Graft Type × Scion for 'G.41'							
		Scion	0.0028	0.0558	n.s.	n.s.	n.s.	n.s.
		Graft	< 0.0001	n.s.	n.s.	0.0655	< 0.0001	0.0003
		Scion × Graft	0.0115	n.s.	n.s.	n.s.	0.0323	n.s.

[a] Non-significant effect at $p < 0.1$.

The multivariate analysis used to generate two-way similarity cluster diagrams showed that graft types were clustered within three different groups for hormone content of the scion 'Scilate' on G.41 (Fig. 5). Higher content of ABA, cytokinins, gibberellins and auxins was observed when trees were saddle grafted (Fig. 5). Chip budding induced the lowest content of ABA and gibberellins, whereas cytokinins and auxins were at the lowest level when scions were whip/tongue grafted (Fig. 5).

Higher content of ABA metabolites was observed when 'Scilate' was chip budded on G.41 than on M.9 (Fig. 6). On the other hand, auxins were higher for M.9 than for G.41. No significant differences were observed regarding cytokinins or gibberellins.

4. Discussion

Transpiration measurements were made comparing scion and rootstock combinations in order to test the hypothesis that weaker graft unions would result in reduced vascular connection, which would then limit transpiration. In both years, differences in LA specific transpiration rate between scions were minimal, particularly when accounting for differences in leaf area. One of the general trends was of decreasing LA specific transpiration rates over time, due to an increase in leaf area without a proportional increase in transpiration. The most likely explanation for this is that as trees put on more leaf area, the older leaves remain on the tree but contribute less to the overall transpiration of the tree partly due to internal shading. These observations coincide with results from Flore and Lakso (1989), who reported that leaf exposure to light plays a critical role regarding the continued function of the leaf. Hence, the best comparisons of LA specific transpiration rates were seen when leaf areas were similar, or a regression could be generated to account for non-linear relationships between transpiration rate and leaf area. Transpiration rate graphs should be interpreted with this in mind.

M.9 tended to have higher tree transpiration than G.41 in 2014, and the opposite was observed in 2015. This may have been due to water stress in trees on G.41 and thus lower than expected tree transpiration rates. Root systems have relatively low hydraulic conductivity which can cause a significant effect on transpiration (Jones et al., 1985), and this may be compounded in young potted trees that have limited root systems. Lauri et al. (2014) observed xylem conductivity in shoots decreased when they were water-stressed. As mentioned, in our 2014

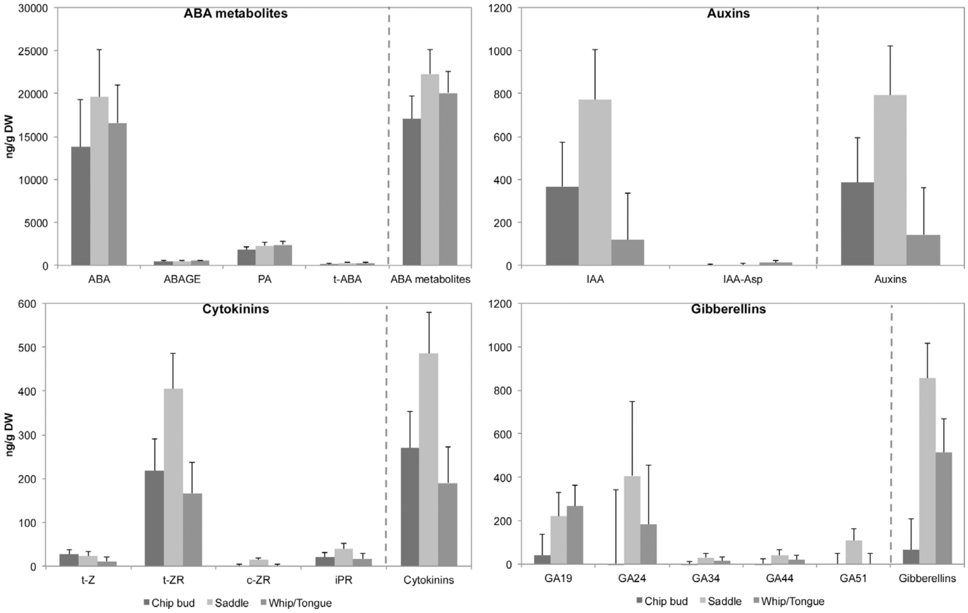

Fig. 4. Average concentration (ng/g dry weight) and standard error for detected hormones in xylem of 'Scilate' scion either chip bud, saddle, or whip/tongue grafted on G.41. Analyzed hormones were *cis*-abscisic acid (ABA) and metabolites abscisic acid glucose ester (ABAGE), phaseic acid (PA), *trans*-Abscisic acid (t-ABA) and total ABA metabolites; auxins and associated metabolites indole-3-acetic acid (IAA), N-(Indole-3-yl-acetyl)-aspartic acid (IAA-Asp), and total auxins; cytokinins and metabolites (trans), trans Zeatin (t-Z), trans Zeatin riboside (t-ZR), (cis) Zeatin riboside (c-ZR), Isopentenyladenosine (iPR) and sum of all cytokinins; gibberellins (GA) 19, 24, 34, 44, 51, and sum of all gibberellins.

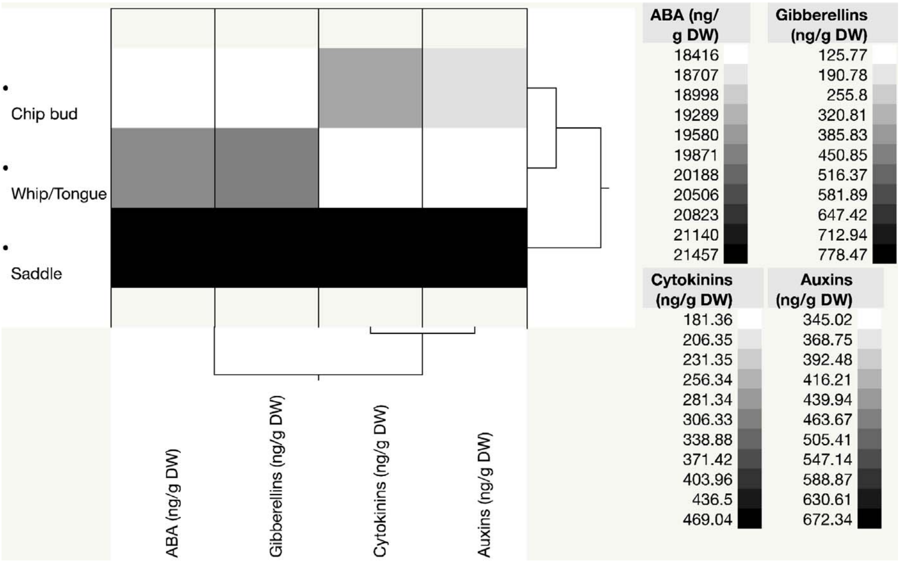

Fig. 5. Clustering of the three graft types based on Abscisic acid (ABA), gibberellins, cytokinins, and auxins concentration (ng/g dry weight) in xylem of 'Scilate' scions grafted on G.41.

study, some of the 'Gala' on G.41 had set terminal buds by the later trial period, which may indicate they went through significant stress. This stress appears to have affected the G.41 chip buds the most resulting in reduced tree transpiration rate for the later measurement periods. Plant water stress in G.41 may also be indicated by the significantly higher root mass per scion cross-sectional area, suggesting more partitioning to roots for water uptake. Alternatively, in 2015, G.41 had higher tree transpiration rates and higher leaf area than M.9 for both scions, which resulted in higher LA specific transpiration rates at a given leaf area. Similar results were observed with 'Honeycrisp', when Lordan et al. (2017) observed that G.41 had higher transpiration rates than M.9. Tworkoski and Fazio (2015) reported larger root systems for G.41 compared to M.9. The higher LA specific transpiration rates observed in 2015 for G.41 may be the result of a more extensive root system than M.9, which indicates lower water stress since the soilless media allowed for better water availability. Differences in growth media may also explain the differences in rootstocks between years.

Chip buds had higher transpiration and leaf area than both bench grafts at the beginning of the season. Since chip budding occurred in the fall and bench grafting occurred the following spring, budded trees were further advanced at the beginning of the experiment, which is evidenced by the larger leaf area at the earlier measurement dates. Nonetheless, on the final date, the leaf area and LA specific transpiration rate did not significantly differ. The higher transpiration rate and leaf areas observed in chip buds in 2015 were most likely due to differences between scions in 2015 vs 2014. Looking at leaf area and LA specific transpiration across combinations, saddle grafted 'Scilate' had a comparable leaf area to chip budded 'Fuji', and these two treatments did not differ in transpiration rates. Therefore, we found no correlation between transpiration and weak graft unions based on our results. When the above explanations are considered, the differences between treatment combinations were small and more related to tree and root system size than graft strength.

The pressure chamber experiment was designed to determine the site of hydraulic resistance in the tree. However, much of our graft and scion resistance data for 2014 had to be disregarded since many of the resistance measurements were negative. Negative numbers occur when the flow above the graft union was greater than or equal to the flow of the rootstock only. This resulted from the much higher resistance of the rootstock and soil compared to that of the graft union and scion segment, so that we could not accurately measure differences in the graft and scion resistance when still connected to the rootstock. Other researchers have measured graft resistance by testing a wood segment with the graft attached and then removing the graft portion and retesting the segment (Gasco et al., 2007). This would have removed the possibility of high resistance overshadowing the low resistance in the above ground portions of the tree. It is also possible that flow may decline over time in the pressure chamber. However, we measured the change of flow in five minute increments on an extra tree and found no detectable changes over time (data not shown). We were able to detect

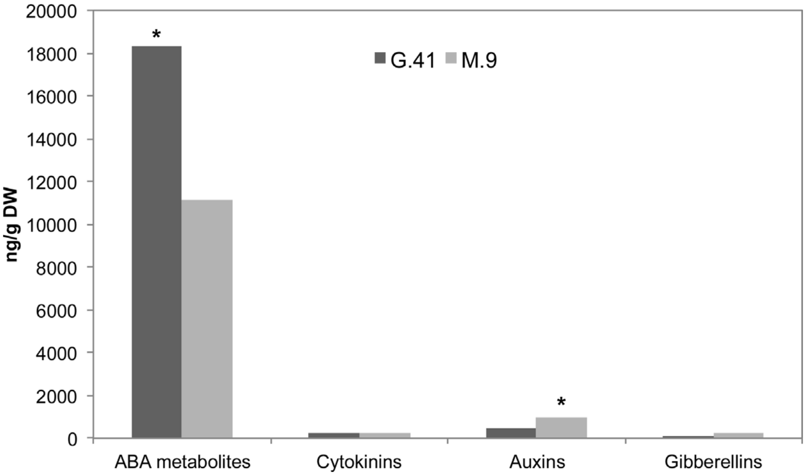

Fig. 6. Average weight (ng/g dry weight) of hormones in the xylem sap of 'Scilate' scions chip budded on G.41 and M.9. Analyzed hormones were ABA metabolites, cytokinins, auxins, and gibberellins. Bars with asterisk denote significant differences between rootstocks ($P \leq 0.05$).

significant differences in rootstock resistance between M.9 and G.41, but these were largely due to differences in the size of the rootstock as CSA-resistance was similar between rootstocks.

We did find a slight difference in graft union resistance between cultivars in 2015 where 'Fuji' had slightly less resistance than 'Scilate' ($p = 0.0415$). Differences between scions may also depend on the leaf area. Lakso (1994) pointed out, hydraulic conductivity of apple root systems can change in relation to the apparent evaporative demand of the top. Rootstock resistance of G.41 was significantly lower than for M.9 ($p = 0.0036$). These results coincide with the observations made by Tworkoski and Fazio (2015) who observed significantly higher hydraulic conductivity on G.41 compared to M.9. Regarding graft types, chip buds had more CSA specific resistance than saddle grafts, but this was primarily due to much larger RCSA than for saddle grafts.

The weakness of the graft union of G.41 previously reported (Adams, 2016; Tworkoski and Fazio, 2015), could be due to alterations in xylem anatomy and hormonal differences such as auxin accumulation (Aloni, 2007; Tworkoski and Fazio, 2011). Coinciding with other studies, we observed that synthesis and movement of hormones might either be affected by the graft, or by the rootstock (Atkinson et al., 2003; Lordan et al., 2017; Sorce et al., 2002; Tworkoski and Fazio, 2016; Tworkoski and Miller, 2007; Van Hooijdonk et al., 2011). In our study no significant differences were found for either cytokinins or gibberellins concentrations. This might be explained because gibberellins are mainly synthesized in shoots and seeds (Davies, 2004), thus, rootstock and graft will have low impact on that. G.41 and M.9 seemed to have a similar profile regarding cytokinins. Coinciding with a previous study (Lordan et al., 2017), similar levels of cytokinins were also reported for M.9 and G.41 with 'Honeycrisp', indicating that this trend is repeated independently of the scion cultivar. On the other hand, we observed higher levels of ABA for G.41 compared to M.9, whereas the opposite was observed for auxins. This follows a similar pattern that we already observed in that aforementioned study (Lordan et al., 2017), where G.41 induced about 25% less auxins in the scion than M.9, whereas ABA tended to be higher for G.41. Auxin moves basipetally and generally in opposite directions within stems and roots (Petrášek and Friml, 2009). Complicating hormone movement is the possibility that seasonal changes in hormone transport are likely associated with rootstock regulation of tree growth and development (Van Hooijdonk et al., 2011). Sorce et al. (2006) suggested that the vigor of a rootstock would depend on ability to induce high auxin levels. Since lower levels of auxins were observed for G.41 in our study, and auxins also tended to decrease when chip and whip/tongue grafting compared to saddle grafting, we suggest that whip/tongue grafting or chip budding on G.41 are the least advisable methods when grafting brittle varieties onto G.41.

The role of synthetic hormones on graft union strength has also been tested. Adams (2016) reported an increase in flexural strength when applying the cytokinin benzyl adenine. In our study, we observed less auxins and cytokinins in the scion when whip/tongue grafting, whereas saddle grafts had the highest rates. In addition, the study by Adams (2016), reported that graft union flexibility was increased when saddle grafting. Hence, since hormones can affect graft strength and scion behavior, new studies should address not just graft union strength solely, but how the interaction of different combinations of grafts and rootstocks can affect scion behavior through the hormone profile.

The main objective of this study was to compare the hydraulic resistance of weak and strong graft forming rootstocks. This was done by comparing measurements of transpiration in a controlled environment to resistance measurements with a pressure chamber. There was no consistent effect of scion or rootstock on transpiration rate. Grafting method had no effect on the LA specific transpiration rate either. Total tree leaf area and water availability may have influenced the results. As discussed, leaf area tends to increase at a faster rate than water flow, thus leading to a general decrease in the transpiration rate over time. Having a higher leaf area may have resulted in reaching water uptake limits, which would also reduce transpiration rates. Regardless, we found that G.41 did not suffer from reduced tree transpiration and LA specific transpiration rates. In the future, larger pots should be used to prevent reaching water availability limits when the trees get larger and fill their space.

Hydraulic resistance of the scion and the graft union was small relative to the root system, making it difficult to measure resistance in the graft union. The hydraulic resistance of the weaker forming rootstock was not significantly different than the strong graft union rootstock and there was no correlation between graft union strength and transpiration rate. On the other hand, although grafting method had no effect on the LA specific transpiration rate and resistance, we observed some effect of grafting method on the hormone profile. Based on these results poor vascular connections were not detected in the weak graft-forming G.41 compared to the strong graft forming M.9. Instead, the results suggest another mechanism for graft weakness in G.41. For future research, measuring resistance through a graft union would be more accurate using wood segments that do not include the root system.

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