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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