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# Rootstock, Scion, and Graft Type Influence Graft Union Flexural Strength of Apple Trees

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## Abstract

Apple tree nurseries utilize various grafting methods to produce numerous combinations of scions and rootstocks joined together ideally to leverage desirable qualities of both. Geneva® apple rootstocks are known for their productivity and disease resistance, however apple rootstock 'Geneva® 41' ('G.41') has been shown to form weak graft unions with some desirable scion cultivars like 'Honeycrisp' and 'Cripps Pink' resulting in breakage during wind, tree harvest in the nursery, and shipment. Other Geneva® rootstocks have also been broken during wind events in the nursery. Flexural strength and flexibility were tested among a combination of six scions ('Fuji', 'Gala', 'Honeycrisp', 'McIntosh', 'Cripps Pink', and 'Scilate') seven rootstocks (G.41, G.935, G.214, G.11, M.9-NIC 29, B.9 and EMLA 26), and two grafting methods (chip bud and saddle graft) in an incomplete factorial design. A sub-sample was selected to measure change in graft union strength over two years. G.41 consistently had the lowest break strength relative to tree size and this persisted into the second year whereas G.214 exhibited the highest strength. G.41 also had the lowest flexibility (as measured by deflection distance to failure) regardless of scion cultivar or grafting method. 'McIntosh' and 'Cripps Pink' formed stronger graft unions, while 'Scilate' formed a weaker graft union relative to tree size. Saddle grafting did not appear to improve graft union flexural strength. Parallel samples of graft unions developed in this study were subjected to micro computed tomography (CT) scans to visualize microstructures within the graft that could be associated with weak grafts. Scans showed that in weak rootstock-scion combinations there were areas of disorderly formation of vascular connections whereas the stronger combinations featured more uniform formation. Therefore, when using G.41 rootstock, care should be taken to select scion cultivars that will form graft unions of sufficient strength to withstand nursery harvest, shipping and initial planting in the orchard.


The US apple industry produces approximately 240 million bushels of fruit worth about $4 billion annually (USApple.org, 2019). Apple production in the U.S. has drastically improved in the last four decades with the conversion from inefficient vigorous rootstocks to yield efficient and resource saving clonal dwarfing rootstocks. These modern dwarfing rootstocks have increased the yield efficiency (quantity of apples produced/ha) of commercial apple orchards, reduced inputs in fertilizer, pesticides, water and labor per unit of apples produced (Autio et al., 2005). This dramatic improvement has been possible with the support of the nursery industry which propagates the rootstocks and trees that are then planted in orchards (Wertheim and Webster, 2003). Many of the common, traditional dwarfing rootstocks that ushered the green revolution for apple production such as Malling 9 (M.9) and Malling 26 (M.26) are highly susceptible to fire blight, a disease caused by the bacteria *Erwinia amylovora* (Burr.) (Norelli et al., 2003). Fire

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blight often enters the plant through blossoms or wounds in stems or leaves and then travels down to the rootstock leading to girdling and eventual tree death. A new series of apple rootstocks bred for fire blight resistance was developed in a joint effort by Cornell University and the United States Department of Agriculture Agricultural Research Services (USDA ARS). These rootstocks are identified as Geneva® rootstocks and are given a unique designation (i.e. G.11, G.41, G.935, etc.). Trees grown on Geneva® rootstocks survive the rootstock phase of fire blight infection, which otherwise would kill the tree and instead allow for pruning of the infected limbs from the tree and quick regeneration of the orchard (Aldwinckle et al., 2004; Russo et al., 2007).

The quality and type of tree being planted in the orchard can also have a significant effect on orchard productivity and economic success (Reig et al., 2019). Tree fruit quality is largely defined by caliper (trunk diameter), feathers (sylleptic branches) and well-developed primary root systems. Their potential impact on orchard productivity has intensified the search for technologies that improve nursery tree quality (Lordan et al., 2017b). Genetic properties of apple rootstocks influence some tree quality attributes like feathering and branch angle as observed in some Geneva® rootstocks like G.935 and G.41 (Fazio and Robinson, 2008a; Fazio and Robinson, 2008b, Lordan et al., 2017b).

Geneva® rootstocks also have resistance to crown and root rots from *Phytophthora* sp., high yield efficiency, and good fruit size (Costa, 2011; Fazio et al., 2015; Norelli et al., 2002; Robinson et al., 2003; Robinson et al., 1999). However, some of the Geneva® rootstocks, such as Geneva 41 (G.41) (Fazio et al., 2005), produce weak and brittle graft unions that are susceptible to breakage in the field (Wallis et al., 2017) with certain scions and in some cases in the tree nursery phase (R. Adams, personal communication). This weakness was observed especially during strong wind events where trees might not have been properly secured or during tree harvest and shipment and may be exacerbated by the induction of large feathers typical of rootstocks like G.935 and G.41 that increase wind resistance relative to less feathered trees on traditional rootstocks. Weakness is described as the reduced capability of the union to endure internal stress due to an external load, while brittleness describes the reduced deformation of the union from strain resulting in sudden brash breaks (Winandy and Rowell, 1984). In the nursery industry these weak graft forming combinations are often called incompatible. However, this definition of incompatibility is different from the incompatibility described in scientific literature (Feucht, 1988; Moore, 1981; Moore, 1984; Musacchi et al., 2000), because it is a broader definition that combines the successful, yet weak graft unions with the grafts that fail to form a proper union or decline with age. The term “affinity” would be better suited for circumstances where the graft union forms successfully, but other growth qualities such as wood formation and growth are not optimal. Regardless of how the term is used, many methods for measuring graft compatibility focus on identifying whether a strong connection is made through the proper formation of vascular tissue. Two such methods are tensile strength and flexural strength testing (Adams et al., 2017).

Testing tensile strength to measure the force to pull a graft apart, has been used extensively for testing grafts of herbaceous species (Lindsay et al., 1974; Moore, 1983; Parkinson et al., 1987) and cherry grafts, but testing is limited since the force required to pull the grafts apart exceeds the machine capacity or ability to adequately hold samples without slipping (Pedersen, 2005). In comparison to tensile testing, flexural testing usually employs a 3-point bend test or some other apparatus that allows for the sample to bend until fracturing at or near the graft union. This method was used with inconsistent results, likely because its primary use was to test for compatibility with seed propagated rootstocks that may have wide variability in strength (Evans and Hilton, 1957; Lapins, 1959). Factors other than vascular tissue, such as wood formation and flexibility, may also affect the outcomes from tensile or break strength tests, so results should be interpreted carefully.

A great difficulty for both types of testing is the method of standardizing or adjusting measured forces to some trunk of graft union size measurement. Due to the nature of many grafting methods, determining an accurate measure for graft union surface area is not viable. Evans and Hilton (1957) related the force to the scion cross-sectional area, but this may not account for swelling at the graft union. The graft union cross-sectional area, or a measure that accounts for the cross-sectional area of the trunk near the graft should also be considered.

In addition to external measurements, the ability to peer into the three-dimensional (3D) structures associated with graft union formation has the potential to reveal potentially weak features in the union. The development of mostly parenchymatic wound tissue between the grafted tissues is usually followed by the formation of longitudinally oriented cambial cells followed by vascular bundles that normally orient themselves in a longitudinal parallel fashion interspersed in parenchyma (Richardson et al., 1996). Watanabe et al. (1988) used a scanning electron microscope (SEM) to observe the initial callus development of the graft followed by the formation of vascular elements during the first year after grafting and found the following progression of tissue development: 1.1nitial adhesion of the xylem tissue of the graft components, 2. union of callus tissue generated from tissues peripheral to the cambiums, 3. vascular element differentiation within the callus at the graft interface and 4. connection between vascular systems of the rootstock and scion. Simons and Chu (1985) using SEM and optical microscopy of stained tissues reported the formation of starch rich regions in M.26/'Red Delicious' graft combinations. Basedow and Crassweller (2017) used laser ablation tomography and optical microscopy to investigate if fiber cell length, thickness, and frequency in connective tissue were associated with weak graft unions and found no clear pattern in the graft combinations tested. However, noting that there was quite a lot of variability between the specimens of the same graft combination, they suggested that the ratio of parenchyma to fiber might be related to weaker regions of the union and observed "swirling xylem" in samples of 'Honeycrisp'/M.26 a well-known weak graft combination. Micro X-ray computed tomography (MicroCT) was used successfully to peer into the micro-structures of plant tissues, plant starch and plant water status (Earles et al., 2018; Klepsch et al., 2018; Mathers et al., 2018). MicroCT data is collected in flat image files that are then stacked by software methods (Parkinson et al., 2018) to produce a rendering of 3D structures based on X-ray scatter density properties of imaged tissues. Water rich parenchyma tissues will assume a different density than lignin rich fibers and show contrasting colors (or variations in grayscale) according to imaging software settings. The ability to reconstruct in 3D also allows to follow fiber structures throughout the graft union and compare visually organization, uniformity, density and distribution.

The aim of this study was to use the flexural testing method to determine the strength of graft unions of apple trees with various scion-rootstock combinations. More specifically, the aim was to determine the strength of graft unions with G.41 as rootstock in relation to other Geneva® selections and industry standard apple rootstocks (M.9, M.26, etc.). The second objective was to compare the influence of scion cultivar on graft union strength, and how the strength of scion-rootstock combinations changes over two-years of growth. As a possible remediation to graft-union weakness, bench grafting was compared to chip budding.

## Materials and Methods

*Tree propagation*

*2014 Trees*. Custom propagated apple trees were produced by a commercial nursery (Willow Drive Nursery, Ephrata, WA) using one budding method and two bench graft methods. Chip budding was performed between Aug. and Sept. of 2013, whereas bench grafting was completed in late March of 2014 using both saddle and whip-and-tongue graft methods. Both methods of bench grafting utilized custom cutting tools to maintain uniformity. After bench grafting, the grafts wrapped with tape were dipped in a custom grafting mix consisting of equal parts paraffin wax, beeswax, and tree resin heated to 70 °C. For the chip budded trees, the apple cultivars 'Gala', 'Scilate', 'Cripps Pink', 'and 'Honeycrisp' were chip budded onto the rootstocks G.11, G.41, G.214, and Malling 9 NIC 29 (M.9-NIC 29) as an incomplete factorial with at least 30 trees per scion-rootstock combination. For the bench-grafted trees, the same four scion cultivars were saddle grafted and whip-and-tongue grafted onto G.11, G.41, and M.9-NIC 29 as an incomplete factorial with at least 30 trees per scion-rootstock-graft combination. Enough trees were bench-grafted so that a full set could be left in the field for testing in the second year. Trees were grown according to standard commercial practices.

*2015 Trees*. For the second year of experiments, custom budding and bench grafting were repeated in Aug. through Sept. of 2014 and in March of 2015 using the same methods as described above except whip and tongue grafting was excluded to accommodate more scion-rootstock combinations, and because 2014 results showed no difference in strength between bench grafting methods. For the chip budded trees, the scion cultivars 'Gala', 'Scilate', 'Fuji', 'McIntosh', 'Cripps Pink', and 'Honeycrisp' were chip budded onto the rootstocks G.11, G.41, G.935, G.214, M.9-NIC 29, East Malling/Long Ashton 26 (EMLA 26), and Budagovsky 9 (B.9), resulting in a six scion by seven rootstock complete factorial, with at least 30 replicate trees per scion-rootstock combination. Additional scion-rootstock combinations were included the second year to provide a complete factorial and to incorporate additional rootstocks and scions that were thought to develop stronger graft unions. For the bench-grafted trees, the same six apple cultivars were saddle grafted onto the same seven rootstocks as in 2014, with the addition of Malling-Merton 106 (MM.106) rootstock. Trees were grown according to standard commercial practices.

*Two-year time course*. Bench grafted trees from the 2014 study were allowed to continue to grow through a second year in the nursery. A complete factorial sub-set of the 2014 saddle grafts consisting of 'Cripps Pink' and 'Honeycrisp' grafted on G.11, G.41, and M.9-NIC 29 was selected for a 2-year study to analyze their change in growth and strength.

*Sampling and Growth Measurements.* Trees were sampled and measured at three different times in each year: mid-June, mid-Aug., and mid-Oct. At each sampling time, 10 trees from each scion-rootstock combination were selected based on visual uniformity. Each tree was tagged and numbered from 1-10 and then several size measurements were recorded, including rootstock shank diameter (5 cm below graft union), two perpendicular measurements of graft union diameter at the widest part of the graft union, and in 2015 a scion diameter (5 cm above point of new growth). The diameter measurements were used to calculate cross-sectional areas (CSA). Trees were then hand dug at each sampling time, all roots were removed, and the scion was cut back so that the total sample length was about 70 centimeters in order to preserve sample moisture and facilitate transportation. Replicates were then bundled together in moist burlap according to replicate number and packed in ice for transportation to laboratory at Utah State University (USU) in Logan, Utah.

*Break Strength Testing.* At USU, each specimen was loaded to failure using a

3-point bend apparatus with a 16-centimeter separation (Figure 1). The apparatus was used in conjunction with a Bench Testing Machine (Tinius Olsen H50KS, 1065 Easton Road, Horsham, PA 19044) operating in compression mode. The tests were performed with a fixed strain rate (25 cm/min) as per the ASTM Standard D790 and D7264, which are commonly used for testing of flexural strength of polymer composites and concrete (ASTM, 2010; ASTM, 2015). A pre-load condition of 10 N was used to bring the crosshead into contact with the specimen at a constant rate of 50 cm/min. For all of the 2014 samples and the June and Aug. 2015 samples, force measurements were acquired through the Tinius Olsen Test Navigator software at 1-second intervals throughout the measurement, until a failure condition (the point when resistance measured by the instrument suddenly ap-

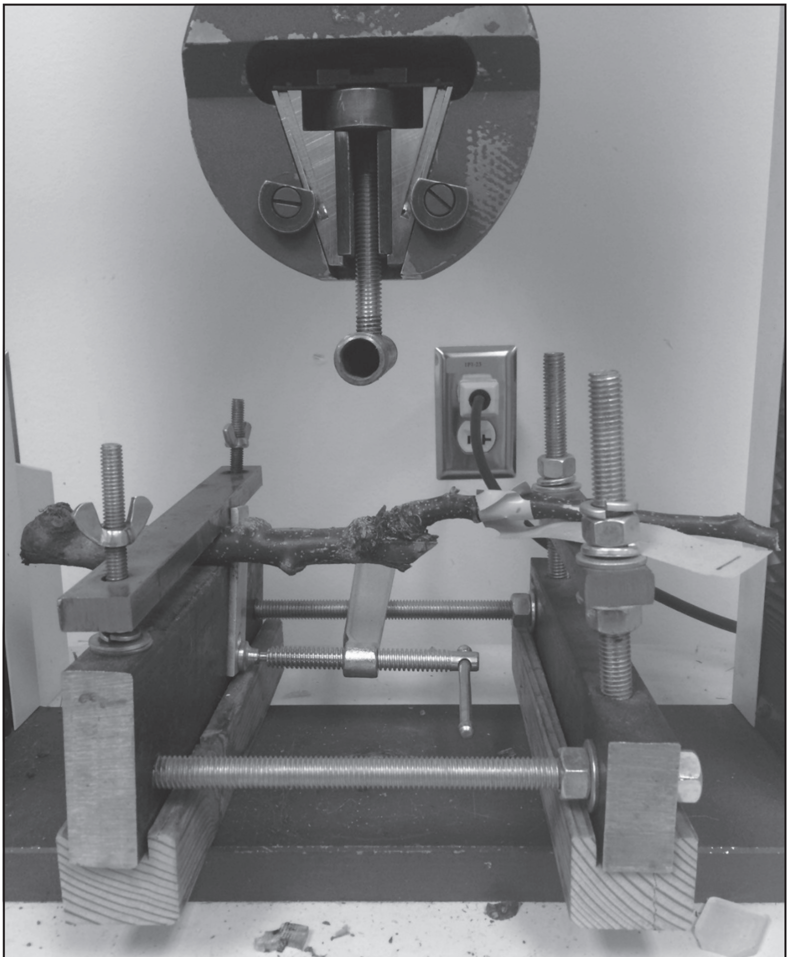

**Fig. 1.** Apparatus used for 3-point flexural strength testing. Sample supported with a 16 cm separation with flexural strength and rigidity measured with a bench testing machine. The sample shown is in the “bud up” position where the chip bud is positioned proximal to the break force.

**Table 1.** Break categorization, category number for analysis in order of average strength, and description of break categories.

| *Notation* | *Numeric Order* | *Description* |
|---|---|---|
| C | 1 | Broke clean at the graft |
| GR | 2 | Broke just below the graft with low/no tissue attached |
| GS | 2 | Broke just above the graft with low/no tissue attached |
| GRNC | 2 | Broke just below the graft but with significant tissue attached |
| GSNC | 2 | Broke just above the graft but with significant tissue attached |
| NC | 3 | Broke at the graft but with significant tissue attached |
| R | 4 | Broke below the graft on the rootstock |
| S | 4 | Broke above the graft on the scion shoot |
| NA | 4 | Did not break under the maximum testing displacement |

proaches zero due to breakage of the sample) was achieved. Upon achieving the failure condition, the fracture strength was obtained from the data based on the geometry of the specimen. For the chip budded trees, half of the samples were broken with the force applied proximal to the chip bud, and the other half were broken with the force distal from the chip bud. For the bench grafts, half of the samples were broken with the graft in a stacked position relative to the applied force, and the other half were broken with the graft in a sideways position. The way each sample broke was categorized according to break type and location of break (Table 1). The categories were later regrouped into orders with assigned numbers as seen in Table 1. During earlier tests, there were apparent differences in sample flexibility, so for the Oct. 2015 tests deflection measurements were also recorded. Additionally, a subset of trees representing a factorial combination of four scions by three rootstocks was tested for the deflection of the scion and the rootstock shank segments only.

*Data Analysis.*

*2014 study*. Response variables measured in 2014 were flexural strength measured as ultimate break force (N), graft cross-sectional area (GCSA, $cm^2$), break force per graft cross-sectional area (F/GCSA, $N/cm^2$) and break type (‘clean break’ or ‘complex break’ depending on the uniformity of the break – sometimes samples would splinter through the graft union causing a complex break). Data were analyzed using the GLIMMIX procedure in Statistical Analysis System (SAS) with blocking by row. Both months were included in one analysis as fixed effects. A separate directional analysis was performed for each graft type for F/GCSA. Samples that did not break (< 0.1% per month) were excluded from analysis for all force, size and deflection measurements. However, those that broke at points other than the graft were included with the assumption that the graft was at least as strong as the measured value. Data from the June sampling were excluded from the analysis due to the high variability in the data, assumed to be due to the immaturity of the growing trees.

*2015 study*. The 2015 experiment was analyzed as a complete factorial with the field location as the blocking factor. GCSA, SCSA, force, graft cross-sectional area specific force (F/GCSA, $N/cm^2$) and scion cross-sectional area specific force (F/SCSA, $N/cm^2$), displacement (deflection), and break type variables from the experiment were analyzed using PROC GLM with log or square root transformations as needed for model assumptions. Like 2014, June data and samples that did not break were excluded (< 0.1% per month). Data were initially combined for analysis, with all sampling months combined as a split plot design with tree numbers as replicates and time (sampling months) and genotype combinations as whole and split plot factors respectively. This first analysis showed significant time interactions, so data were analyzed independently for each sampling time with emphasis on the final Oct. sample. To better understand the interactions in the Oct. sampling, the data were also analyzed separately for each graft type.

*Two-year time course*. The sub-sample used for the 2-year study was analyzed similar to the 2015 break tests with tree as replicate and time as a fixed effect since the samples were harvested destructively. Since GCSA was measured the same across all months, only force, GCSA and F/GCSA were used in the analysis.

*Flexibility*. Deflection or flexibility data were only acquired for the Oct. 2015 sampling time. Data were analyzed using the GLM procedure as a completely randomized design and transformed as needed.

*Categorical Data*. After assigning numerical order to the recorded break types, the GLIMMIX procedure was used to test the probability of lower order categories occurring. In other words, the test determines if a combination in a paired comparison has a higher likelihood of lower order breaks, such as clean breaks compared to non-clean breaks or breaks in locations other than at the graft union.

*Micro Computed Tomography (CT) scans.* Replicated (2-3) apple tree graft specimens of bench grafted 'Cripps Pink' scion on M.9 Nic29 and G.41 rootstocks, 'Gala' and 'Honeycrisp' scions on G.41 rootstock, chip budded 'Fuji' scion on G.41 rootstock, chip budded 'Scilate' scion on G.41 rootstock and 'Gala' scion chip budded on G.41 rootstock were imaged using a Zeiss Versa XRM-520 CT at the Cornell University Biotechnology Resource Center (BRC). Specimens were scanned at 100kV source setting at a 25-30 um/pixel resolution with 1600 frames per scan. DICOM files were loaded in Osirix Imaging Software (Rosset et al., 2004) (https://www.osirix-viewer.com/) and resulting 3D images were adjusted to reflect similar orientation and to maximize contrast of vascular tissues.

## Results and Discussion

*2014 Study*. The 2014 study did not allow for a complete factorial as it was constrained to trees that were available from the 2013 budding season. The analysis adjusted for the missing combinations, but the ability to detect treatment differences was diminished. Break force measurements for 2014 were related to the graft cross-sectional area (GCSA), which is related to the size of the rootstock, but also influenced by proliferation at the graft union. The resulting force per graft cross-sectional area (F/GCSA) provided a variable for comparing combinations that will differ in size. As seen in Table 2, all main effects were significant for F/GCSA, but there were also rootstock*time ($P$ = 0.0087) and graft*time ($P$ = 0.0004) interactions.

G.41 had consistently lower values for F/GCSA than all other rootstocks (Table 2), which was the case for both test dates (data not shown). Although the graft size was similar to M.9-NIC 29, the break force was significantly lower. The other Geneva® selections (G.214, G.11) were as strong as M.9-NIC 29 by Oct. (G.11 was weaker than M.9-NIC 29 in Aug.). Averaged over two dates, there were significant rootstock*graft type interactions for break force and GCSA, but not for F/GCSA., which may be due to differences in G.11 between graft types. G.11 had a larger GCSA accompanied by a larger force as a chip bud compared to both bench grafts. Second, the rootstock*scion interaction was not significant for F/GCSA but there was a significant scion main effect on graft strength. In general, 'Gala' formed stronger unions than 'Honeycrisp', 'Cripps Pink', or 'Scilate' (Table 2).

Comparing graft types, chip buds required greater break force and had larger GCSA at each date. The main effect of graft type averaged over dates, rootstocks and scions was significant and chip buds had significantly higher F/GCSA than the two grafting methods (Table 2), although the differences were not significant within dates and differences

**Table 2.** Break force, graft cross-sectional area (GCSA), and force per graft cross-sectional area (F/GCSA), averaged for Aug. and Oct. 2014, for the main effects of rootstock, scion and graft type, as well as ANOVA *P*-values for the analyses.

| *Variable* | | *Force (N)* | *GCSA ($cm^2$)* | *F/GCSA ($N/cm^2$)* |
|---|---|---|---|---|
| Rootstock | G11 | 268 b[1] | 5.09 a | 52.7 b |
| | G214 | 466 a | 6.34 a | 73.4 a |
| | G41 | 182 c | 4.55 a | 39.9 c |
| | Nic29 | 266 b | 4.53 a | 58.7 b |
| Scion | Gala | 270 a | 4.88 a | 55.3 a |
| | Honeycrisp | 216 a | 4.50 a | 47.9 b |
| | Cripps Pink | 195 a | 4.05 a | 48.1 b |
| | Scilate | 271 a | 5.61 a | 48.2 b |
| Graft Type | Chip | 364 a | 6.87 a | 53.0 a |
| | Saddle | 173 b | 3.69 b | 46.7 b |
| | WT | 172 b | 3.59 b | 47.9 b |
| ANOVA *P*-values | | | | |
| | Rootstock | 0.0004 | >0.1 | 0.0003 |
| | Scion | >0.1 | >0.1 | 0.0428 |
| | Rootstock*Scion | >0.1 | >0.1 | >0.1 |
| | Graft | <0.0001 | <.0001 | 0.0486 |
| | Rootstock*Graft | 0.0264 | 0.0147 | >0.1 |
| | Scion*Graft | >0.1 | >0.1 | >0.1 |
| | Month | <0.0001 | <0.0001 | <0.0001 |
| | Rootstock*month | 0.0507 | >0.1 | 0.0087 |
| | Scion*month | >0.1 | >0.1 | >0.1 |
| | Graft*month | <0.0001 | 0.0017 | 0.0004 |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

between grafting method appeared to decrease over time up to Oct., the final month measured.

*2015 Trial*. In 2015, a wider range of rootstocks and scions were tested to include genotypes thought to represent stronger graft unions and to provide a complete factorial. Scion cross-sectional area (SCSA) was also included as a measure of overall tree size for standardizing the measured break force.

The initial analysis where harvest time (June, Aug. and Oct.) was used as a factor indicated significant interactions with time, so data were analyzed by sampling time separately with a focus on the latest date, which corresponded to nursery harvest. Data for graft types were also analyzed separately at first to determine the effect direction had on the graft union break strength. For the Oct. 2015 tests, there were no significant three-way interactions for either the chip buds or the saddle grafts, but there was a significant rootstock*scion interaction (Table 3). The means and means separations from the rootstock*scion interaction analyses are shown in tables 4-8. We chose to focus on the general trends and use these tables to report and discuss the main effects of rootstock and scion. We will also point out peculiar interactions of note.

The interactions involving direction were also significant and generally show orienting the chip distal to the displacement force will require higher forces to break the graft union, however, this was not the case for every rootstock or scion, or the difference was not sig-

**Table 3.** P-values from Oct. 2015 analysis of variance for break force, scion cross-sectional area (SCSA), force per scion cross-sectional area (F/SCSA), graft cross-sectional area (GCSA), and force per graft cross-sectional area (F/GSA) analyzed by graft type.

| Chip Bud | Force | SCSA | F/SCSA | GCSA | F/GCSA |
|---|---|---|---|---|---|
| Rootstock | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Scion | <.0001 | <.0001 | <.0001 | 0.0012 | <.0001 |
| Rootstock*Scion | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Direction | <.0001 | >0.1 | <.0001 | >0.1 | <.0001 |
| Rootstock*Direction | >0.1 | >0.1 | 0.0509 | >0.1 | 0.0732 |
| Scion*Direction | >0.1 | >0.1 | <.0001 | >0.1 | 0.0775 |
| Roots*Scion*Direction | >0.1 | >0.1 | >0.1 | 0.0536 | >0.1 |
| Saddle Grafts | | | | | |
| Rootstock | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Scion | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Rootstock*Scion | <.0001 | <.0001 | 0.0009 | <.0001 | 0.0144 |
| Direction | 0.0393 | >0.1 | 0.0022 | >0.1 | 0.0055 |
| Rootstock*Direction | >0.1 | >0.1 | >0.1 | >0.1 | 0.0397 |
| Scion*Direction | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 |
| Roots*Scion*Direction | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 |

nificant (data not shown). For saddle grafts, the stacked direction was generally stronger, but the differences were never significant per rootstock or scion. Because the differences in direction were not always significant, we decided to average over the direction. In an actual nursery scenario, the force of the wind will not always be in the exact same direction, so by averaging over direction we obtain a general strength for that tree under variable conditions.

*Force Per Scion Cross-sectional Area.* Break force (Table 4) and scion cross-sectional area (SCSA; Table 5) are presented to

**Table 4.** The influence of grafting method and cultivar within rootstock force (N) required to break the union.

| Chip Bud | *M.9-NIC 29* | *G.214* | *EMLA 26* | *G.11* | *G.935* | *B.9* | *G.41* | *Means* |
|---|---|---|---|---|---|---|---|---|
| *Fuji* | 2043 | 1067 | 1135 | 1036 | 1066 | 780 | 802 | 1133 a[1] |
| *McIntosh* | 1266 | 973 | 992 | 721 | 896 | 950 | 612 | 916 b |
| *Cripps Pink* | 1355 | 831 | 888 | 765 | 679 | 719 | 521 | 823 bc |
| *Gala* | 1368 | 753 | 772 | 787 | 723 | 925 | 414 | 820 bc |
| *Scilate* | 1325 | 598 | 746 | 725 | 630 | 636 | 388 | 721 c |
| *Honeycrisp* | 794 | 777 | 372 | 625 | 500 | 422 | 367 | 551 d |
| *Means* | 1358 a | 833 b | 818 b | 776 b | 749 b | 739 b | 517 c | |

| Saddle Graft | *M.9-NIC 29* | *MM.106* | *EMLA 26* | *G.11* | *G.214* | *B.9* | *G.935* | *G.41* | *Means* |
|---|---|---|---|---|---|---|---|---|---|
| *Fuji* | 563 | 667 | 519 | 422 | 359 | 347 | 299 | 191 | 421 a |
| *McIntosh* | 637 | 392 | 372 | 424 | 418 | 334 | 245 | 312 | 392 ab |
| *Honeycrisp* | 540 | 453 | 416 | 276 | 256 | 269 | 286 | 196 | 337 bc |
| *Cripps Pink* | 481 | 267 | 483 | 418 | 296 | 262 | 289 | 185 | 335 bc |
| *Gala* | 412 | 498 | 323 | 378 | 330 | 331 | 216 | 169 | 332 c |
| *Scilate* | 446 | 314 | 412 | 363 | 266 | 355 | 242 | 136 | 317 c |
| *Means* | 513 a | 432 ab | 421 ab | 380 bc | 321 cd | 316 cd | 263 d | 198 e | |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

**Table 5.** The influence of grafting method and cultivar within rootstock scion cross-sectional area (SCSA; $cm^2$) measured in Oct. 2015.

| Chip Bud | *M.9-NIC 29* | *G.41* | *EMLA 26* | *G.214* | *G.935* | *G.11* | *B.9* | *Means* |
|---|---|---|---|---|---|---|---|---|
| *Fuji* | 4.62 | 3.74 | 2.84 | 2.89 | 3.01 | 2.61 | 2.00 | 3.10 a[1] |
| *Scilate* | 4.10 | 2.18 | 2.44 | 1.87 | 1.67 | 1.89 | 2.05 | 2.31 b |
| *Cripps Pink* | 3.48 | 2.52 | 2.32 | 2.30 | 1.88 | 1.82 | 1.65 | 2.28 b |
| *Gala* | 2.90 | 2.54 | 2.20 | 2.20 | 1.81 | 1.99 | 2.06 | 2.24 b |
| *McIntosh* | 2.49 | 2.27 | 2.06 | 1.84 | 2.00 | 1.65 | 1.97 | 2.04 b |
| *Honeycrisp* | 2.63 | 2.03 | 1.97 | 1.82 | 1.53 | 1.64 | 1.22 | 1.84 c |
| *Means* | 3.37 a | 2.55 b | 2.30 bc | 2.15 cd | 1.98 de | 1.93 de | 1.83 e | |

| Saddle Graft | *MM.106* | *M.9-NIC 29* | *G.41* | *G.935* | *B.9* | *G.11* | *EMLA 26* | *G.214* | *Means* |
|---|---|---|---|---|---|---|---|---|---|
| *Fuji* | 1.92 | 1.52 | 1.34 | 1.47 | 1.53 | 1.16 | 1.22 | 1.33 | 1.44 a |
| *Scilate* | 1.35 | 1.37 | 1.12 | 1.50 | 1.29 | 1.23 | 1.07 | 0.90 | 1.23 b |
| *Gala* | 1.62 | 1.08 | 1.18 | 1.04 | 1.23 | 1.05 | 0.73 | 1.09 | 1.13 bc |
| *Cripps Pink* | 0.87 | 1.06 | 1.17 | 1.15 | 0.92 | 0.92 | 1.10 | 0.85 | 1.01 cd |
| *McIntosh* | 1.11 | 1.19 | 1.45 | 0.82 | 0.85 | 1.02 | 0.77 | 0.82 | 1.00 cd |
| *Honeycrisp* | 1.16 | 1.15 | 1.07 | 0.97 | 1.06 | 0.80 | 0.95 | 0.76 | 0.99 d |
| *Means* | 1.34 a | 1.23 ab | 1.22 ab | 1.16 bc | 1.15 bc | 1.03 cd | 0.97 d | 0.96 d | |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

show how graft strength relative to tree size (F/SCSA) may differ among scion and rootstock combinations. Across all scion cultivars in 2015, G.41 was consistently weaker than all other rootstocks (Table 6). This is a result of having the lowest overall break force (Table 4), despite having a relatively large SCSA (Table 6). For chip bud, M.9-NIC 29 consistently formed the strongest unions. MM.106, which was only included in the saddle grafts, had a significantly lower F/SCSA than M.9-NIC 29, and similar to EMLA 26, and G.11. MM.106 was included because it is classified as a more vigorous rootstock than the rest of the rootstocks included in these studies. The relative break strengths of these rootstocks

**Table 6.** The influence of grafting method and cultivar within rootstock on force per scion cross-sectional area (SCSA; $cm^2$) measured in Oct. 2015.

| Chip Bud | *M.9-NIC 29* | *G.11* | *B.9* | *G.214* | *G.935* | *EMLA 26* | *G.41* | *Means* |
|---|---|---|---|---|---|---|---|---|
| *McIntosh* | 510 | 439 | 484 | 518 | 461 | 492 | 271 | 454 a[1] |
| *Cripps Pink* | 392 | 422 | 433 | 366 | 364 | 392 | 215 | 369 b |
| *Fuji* | 445 | 400 | 386 | 369 | 354 | 404 | 216 | 368 b |
| *Gala* | 464 | 392 | 448 | 340 | 399 | 359 | 173 | 368 b |
| *Scilate* | 326 | 391 | 320 | 312 | 376 | 316 | 183 | 318 c |
| *Honeycrisp* | 298 | 384 | 348 | 430 | 333 | 201 | 186 | 312 c |
| *Means* | 406 a | 405 ab | 403 ab | 389 ab | 381 ab | 361 b | 207 c | |

| Saddle Graft | *EMLA 26* | *M.9-NIC 29* | *G.11* | *G.214* | *MM.106* | *B.9* | *G.935* | *G.41* | *Means* |
|---|---|---|---|---|---|---|---|---|---|
| *McIntosh* | 499 | 546 | 425 | 550 | 382 | 407 | 307 | 222 | 417 a |
| *Honeycrisp* | 453 | 481 | 356 | 343 | 396 | 262 | 304 | 187 | 348 b |
| *Cripps Pink* | 443 | 470 | 461 | 357 | 312 | 291 | 257 | 164 | 344 bc |
| *Gala* | 451 | 389 | 366 | 313 | 318 | 275 | 215 | 146 | 309 cd |
| *Fuji* | 432 | 380 | 375 | 280 | 351 | 229 | 213 | 147 | 301 de |
| *Scilate* | 392 | 342 | 305 | 303 | 243 | 280 | 166 | 129 | 270 e |
| *Means* | 445 a | 435 a | 381 b | 358 bc | 334 c | 291 d | 244 e | 166 f | |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

would indicate that break strength is clearly not correlated with vigor. The relative graft union strengths of the rootstocks included in this experiment differed substantially between graft type, particularly for B.9, G.935, and EMLA 26. B.9 and G.935 were significantly stronger as chip buds than as saddle grafts, whereas EMLA 26 formed a stronger union as a saddle graft than a chip bud.

Scion cultivars also had a significant effect on graft union strength (F/SCSA, Table 6). 'McIntosh' consistently formed the strongest graft union across all rootstocks tested. Conversely, 'Scilate' was consistently one of the weakest graft-forming scions. There were differences for scion strength between graft types, where 'Fuji' and 'Gala' were unusually weak as saddle grafts, and 'Honeycrisp' was unusually strong as a saddle graft.

One of the primary objectives of this study was to determine whether a specific graft type could be used to overcome inherent graft weakness for specific scion or rootstock genotypes. In most cases, F/SCSA did not differ among graft types. The scions 'Fuji' and 'Gala', and the rootstocks B.9 and G.935 formed significantly stronger unions by chip budding than by saddle grafting. Conversely, EMLA 26 formed significantly stronger unions through saddle grafting. In the case of EMLA 26, the greater strength in the saddle graft may have been due to differences in the quality of the rootstock liner. EMLA 26 liners used for bench grafting were from the first cut of a new stoolbed, so the rootstock size was larger than usual. Since this was the only case where saddle grafts appeared stronger than chip buds, switching from the current standard practice of chip budding to bench grafting to improve graft union strength would not be justified. We also observed that some rootstocks in the research plots did not grow as well as they typically would in the nursery. Likewise, some scions grew better than others. 'Honeycrisp' is an example of one scion that grew better in the research plots than generally would be expected.

In addition to general interactions, there were several notable rootstock*scion interactions. First, 'Honeycrisp' chip budded on EMLA 26 was weaker than expected relative to 'Honeycrisp' on other rootstocks, or EMLA 26 with other scions (Table 6). This agrees with anecdotal observations of this

**Table 7.** The influence of grafting method and cultivar within rootstock on graft cross-sectional area (GCSA; cm$^2$) measured in Oct. 2015.

| Chip Bud | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *M.9-NIC 29* | *G.11* | *EMLA 26* | *G.214* | *G.935* | *G.41* | *B.9* | *Means* |
| *Scilate* | 13.84 | 11.29 | 9.75 | 8.13 | 8.06 | 7.07 | 8.24 | 9.48 a[1] |
| *Cripps Pink* | 10.28 | 11.88 | 9.14 | 9.55 | 8.07 | 9.00 | 7.15 | 9.29 a |
| *Fuji* | 11.42 | 11.05 | 8.68 | 9.20 | 9.78 | 8.52 | 5.93 | 9.23 ab |
| *McIntosh* | 9.26 | 10.07 | 9.69 | 9.06 | 8.36 | 7.16 | 7.66 | 8.75 ab |
| *Gala* | 10.59 | 10.08 | 9.32 | 7.72 | 7.87 | 7.70 | 7.70 | 8.71 ab |
| *Honeycrisp* | 11.28 | 9.75 | 10.14 | 7.47 | 7.04 | 8.08 | 5.84 | 8.51 b |
| *Means* | 11.11 a | 10.68 a | 9.45 b | 8.52 c | 8.20 c | 7.92 c | 7.09 d | |

| Saddle Graft | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *G.11* | *M.9-NIC 29* | *EMLA 26* | *G.41* | *B.9* | *G.214* | *G.935* | *MM.106* | *Means* |
| *McIntosh* | 6.65 | 6.24 | 4.38 | 6.50 | 4.51 | 5.15 | 3.81 | 3.13 | 5.05 a |
| *Scilate* | 6.61 | 5.50 | 5.31 | 3.56 | 4.76 | 3.68 | 4.30 | 3.13 | 4.61 ab |
| *Cripps Pink* | 4.97 | 4.90 | 5.26 | 4.61 | 4.05 | 3.98 | 4.13 | 2.54 | 4.30 b |
| *Fuji* | 4.80 | 5.05 | 4.72 | 4.20 | 4.19 | 4.01 | 3.85 | 3.59 | 4.30 b |
| *Honeycrisp* | 5.37 | 4.99 | 5.10 | 4.89 | 3.47 | 3.51 | 3.71 | 3.22 | 4.28 b |
| *Gala* | 5.06 | 4.30 | 4.12 | 4.56 | 3.82 | 4.20 | 3.52 | 3.40 | 4.12 b |
| *Means* | 5.57 a | 5.16 ab | 4.81 b | 4.72 bc | 4.13 cd | 4.09 d | 3.89 d | 3.17 e | |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

**Table 8.** The influence of grafting method and cultivar within rootstock on force per graft cross-sectional area (F/GCSA; N/cm$^2$) measured in Oct. 2015.

| Chip Buds | *M.9-NIC 29* | *B.9* | *G.214* | *G.935* | *EMLA 26* | *G.11* | *G.41* | *Means* |
|---|---|---|---|---|---|---|---|---|
| *Fuji* | 178.5 | 130.2 | 115.7 | 109.2 | 130.5 | 95.4 | 94.4 | 122.0 a[1] |
| *McIntosh* | 137.8 | 124.1 | 105.6 | 107.7 | 103.0 | 73.2 | 85.7 | 105.3 b |
| *Gala* | 128.9 | 120.0 | 98.8 | 91.4 | 81.7 | 77.6 | 53.5 | 93.1 c |
| *Cripps Pink* | 132.0 | 100.7 | 87.2 | 83.8 | 98.0 | 64.6 | 59.2 | 89.4 c |
| *Scilate* | 94.9 | 77.9 | 72.9 | 77.0 | 76.0 | 64.3 | 55.9 | 74.1 d |
| *Honeycrisp* | 68.7 | 72.0 | 103.9 | 71.1 | 37.3 | 64.7 | 45.8 | 66.2 d |
| *Means* | 123.5 a | 104.1 b | 97.4 bc | 90.1 c | 87.8 c | 73.3 d | 65.7 d | |

| Saddle Grafts | *MM.106* | *M.9-NIC 29* | *EMLA 26* | *G.214* | *B.9* | *G.11* | *G.935* | *G.41* | *Means* |
|---|---|---|---|---|---|---|---|---|---|
| *Fuji* | 186.0 | 111.5 | 110.0 | 89.5 | 82.7 | 87.9 | 77.6 | 45.4 | 98.8 a |
| *Gala* | 146.6 | 95.8 | 78.5 | 78.5 | 86.7 | 74.8 | 61.4 | 37.0 | 82.4 b |
| *Honeycrisp* | 140.5 | 108.2 | 81.5 | 72.9 | 77.4 | 51.5 | 77.2 | 40.2 | 81.2 b |
| *McIntosh* | 125.2 | 102.2 | 85.0 | 81.2 | 74.0 | 63.8 | 64.2 | 47.9 | 80.4 b |
| *Cripps Pink* | 105.1 | 98.1 | 91.8 | 74.3 | 64.6 | 84.2 | 70.0 | 40.1 | 78.5 bc |
| *Scilate* | 100.4 | 81.0 | 77.5 | 72.3 | 74.5 | 54.9 | 56.1 | 38.3 | 69.4 c |
| *Means* | 134.0 a | 99.5 b | 87.4 bc | 78.1 cd | 76.6 cd | 69.5 d | 67.8 d | 41.5 e | |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

combination in commercial nurseries. 'Honeycrisp' chip budded on G.214 was unusually strong. This suggests G.214 could be used to increase the strength of the usually weak 'Honeycrisp'. Also, for both graft methods, 'McIntosh' on G.214 was numerically the strongest combination.

*Force Per Graft Cross-sectional Area.* In addition to F/SCSA, force was compared to GCSA to allow for comparison between years, and for the 2-year time course (Table 7). Since GCSA is influenced by tissue proliferation at the graft union, differences compared to F/SCSA should not be surprising.

Consistent with F/SCSA and the results of 2014, G.41 was consistently the weakest rootstock for F/GCSA (Table 8). Likewise, M.9-NIC 29 formed some of the strongest graft unions for both grafting methods. G.214 was a relatively strong graft-forming rootstock (especially for 'Honeycrisp' when chip budded). Unlike for F/SCSA, G.11 was much weaker when compared for F/GCSA. This is a result of having a break force intermediate to other rootstocks (Table 4) but having some of the largest GCSAs and SCSAs. This suggests that the strength comes from an increase in GCSA, and not a stronger union (related to different composition) relative to the CSA. This is an example of one possible mechanism for strengthening the graft union, which was specifically the target of plant growth regulator experiments (Adams et al., 2017; Adams et al., 2018). Another big difference between F/SCSA and F/GCSA was for MM.106, which was the strongest of all the saddle grafts. It had the smallest GCSA and the biggest SCSA (Table 5 and 7). This is possibly due to less callus proliferation and vascular disorganization at the graft union, resulting in a more uniform tree caliper (Soumelidou et al., 1994). Aside from these results, there were consistent graft method differences between rootstocks for B.9, G.935, and EMLA 26.

*Two-year time course*. Since G.41 was consistently weaker during the first year, a subset of bench-grafted trees was sampled in a second year to determine if differences in break strength were reduced with subsequent tree growth. Table 9 provides the analysis of variance for the 2-year time course, where most of the scion*month and rootstock*month interactions were significant. After two grow-

**Table 9.** P-values from analysis of variance pooled over two years for break force, graft cross-sectional area (GCSA) and force per graft cross sectional area (F/GCSA) where trees from the same plots wee sampled twice each year (months) over two years.

| *Effect* | *Force* | *GCSA* | *F/GCSA* |
|---|---|---|---|
| Scion | >0.1 | >0.1 | >0.1 |
| Rootstock | <.0001 | <.0001 | <.0001 |
| Scion*Rootstock | >0.1 | >0.1 | >0.1 |
| Tree | >0.1 | >0.1 | >0.1 |
| Month | <.0001 | <.0001 | <.0001 |
| Scion*Month | 0.0536 | 0.0017 | >0.1 |
| Rootstock*Month | 0.0001 | 0.036 | 0.0005 |
| Scion*Rootstock*Month | >0.1 | >0.1 | >0.1 |

ing seasons G.41 continued to increase in strength, both expressed as force and F/GCSA, but the rate of increase was slower than that of M.9-NIC 29 and G.11 (data not shown). This reinforces the need for a sturdy support system in the fruiting orchard, particularly when using weaker rootstock-scion combinations.

*Flexibility/Brittleness*. As break tests were performed in 2014 and early 2015, there appeared to be differences in the degree to which samples would bend before failing. For Oct. 2015, a measurement of deflection was also included to indicate how far the sample bent before breaking. Deflection measurements of flexibility/rigidity estimate the internal strain or resistance to deformation or displacement (Winandy and Rowell, 2005). Brittleness is related to flexibility/rigidity because it characterizes wood failure that has low deformation or displacement and results in brash or clean break types (Winandy and Rowell, 2005). Deflection was measured for the graft union and independently on a subset of scion and rootstock portions.

There were significant differences for both scion and rootstock flexibility (Table 10). Rootstocks can have a significant influence on scion gene expression (Jensen et al., 2010), phytohormone concentration (Lordan et al., 2017a) and wood fiber formation (Tworkoski and Fazio, 2011) however, rootstock may not have affected overall intrinsic flexibility and strength measured in this experiment, therefore only the scion cultivar had a significant effect on scion deflection, and likewise only rootstock had a significant effect on rootstock deflection. Among scions, 'Fuji' had the highest deflection (greatest flexibility), whereas 'Scilate' had the lowest deflection or the least flexibility. For the rootstock, M.9-NIC 29 and G.935 both tolerated significantly more deflection than G.41. In this case, more flexibility is advantageous

**Table 10.** Deflection of rootstocks pooled over scions and scions pooled over rootstocks measured in Oct. 2015. Values are the amount of deflection (cm) before sample failure.

| *Effect* | | *Scion Deflection (cm)* | *Rootstock Deflection (cm)* |
|---|---|---|---|
| Scion | Fuji | 3.33 a[1] | 1.68 |
| | Honeycrisp | 2.56 b | 1.83 |
| | Gala | 2.19 bc | 1.72 |
| | Scilate | 2.10 c | 1.57 |
| Rootstock | M.9-NIC 29 | 2.51 | 2.03 a |
| | G.935 | 2.66 | 1.82 a |
| | G.41 | 2.47 | 1.26 b |
| ANOVA *p* values | | | |
| | Scion | <.0001 | >0.1 |
| | Rootstock | >0.1 | <.0001 |
| | Rootstock*Scion | >0.1 | >0.1 |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

**Table 11.** Deflection (cm) values averaged over directions for two grafting methods and cultivars within rootstocks, measured in Oct. 2015.

| Chip Bud | G.11 | B.9 | G.214 | M.9-NIC 29 | G.935 | EMLA 26 | G.41 | Means |
|---|---|---|---|---|---|---|---|---|
| *Fuji* | 2.14 | 1.67 | 1.07 | 1.65 | 0.82 | 1.76 | 0.61 | 1.39 a[1] |
| *Gala* | 1.55 | 1.87 | 1.49 | 1.29 | 1.36 | 0.98 | 0.19 | 1.25 ab |
| *Cripps Pink* | 1.59 | 1.18 | 0.86 | 1.02 | 1.23 | 1.22 | 0.38 | 1.07 abc |
| *McIntosh* | 1.47 | 1.11 | 0.51 | 0.89 | 1.16 | 1.02 | 0.61 | 0.97 bc |
| *Scilate* | 1.48 | 0.99 | 1.25 | 0.58 | 0.82 | 0.68 | 0.46 | 0.89 c |
| *Honeycrisp* | 1.74 | 1.40 | 1.08 | 0.61 | 0.66 | 0.30 | 0.37 | 0.88 c |
| *Means* | 1.66 a | 1.37 ab | 1.04 bc | 1.01 c | 1.01 c | 0.99 c | 0.44 d | |

| Saddle Graft | *MM.106* | *M.9-NIC 29* | *B.9* | *G.11* | *EMLA 26* | *G.214* | *G.935* | *G.41* | *Means* |
|---|---|---|---|---|---|---|---|---|---|
| *Fuji* | 3.23 | 1.74 | 1.12 | 2.05 | 1.73 | 1.02 | 1.19 | 0.81 | 1.61 a |
| *Cripps Pink* | 4.26 | 1.61 | 1.45 | 1.62 | 0.95 | 0.97 | 0.97 | 0.52 | 1.54 ab |
| *Honeycrisp* | 2.69 | 1.73 | 1.28 | 1.40 | 1.06 | 1.27 | 1.59 | 0.76 | 1.47 ab |
| *Gala* | 2.45 | 1.30 | 1.36 | 1.08 | 1.57 | 0.93 | 0.87 | 0.65 | 1.28 bc |
| *McIntosh* | 2.13 | 0.97 | 1.57 | 0.83 | 1.04 | 0.91 | 1.00 | 0.81 | 1.16 cd |
| *Scilate* | 1.12 | 1.06 | 1.35 | 0.74 | 0.97 | 1.35 | 0.64 | 0.58 | 0.98 d |
| *Means* | 2.65 a | 1.40 b | 1.36 b | 1.28 bc | 1.22 bc | 1.08 bc | 1.04 c | 0.69 d | |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

because it would allow the tree to bend more before breaking.

The maximum deflection before graft failure was also compared. The analysis of variance for deflection indicated several significant 2-way interactions for both chip buds and saddle grafts. For chip buds, there was a significant difference between directions for deflection, where chip down was significantly more flexible than chip up (data not shown). Differences in direction for saddle graft were not statistically significant. Comparing flexibility in the least flexible direction for each graft type, it appears that saddle grafts have a higher deflection (greater flexibility) than the chip buds in the bud-up position, which is one positive of saddle grafting.

When evaluating the rootstock/scion combinations, G.41 consistently formed the most rigid/brittle unions for both graft types (Table 11). For the chip buds, G.11 and B.9 had the highest deflections, which is a contributing factor to the few to no breaks seen in the nursery. For saddle graft, MM.106 had a much higher deflection than any other rootstock. As noted earlier, MM.106 had a lower break force than expected, and this may be due to the greater flexibility noted here. For the scions, 'Fuji' always had the most flexible union for both graft types and did not differ from 'Cripps Pink'. As noted above, 'Fuji' wood was the most flexible of the scion subsamples tested (Table 10). 'Scilate' was always one of the most rigid unions for both graft types, while 'Honeycrisp' formed the most rigid unions for chip buds. This shows that the flexibility of the scion alone is not the determining factor of the overall graft flexibility, but scion and rootstock flexibility are generally related to the resulting flexibility of the graft combinations.

*Break Type Categorization*. The pattern of graft failure varied from very clean breaks right at the graft union to less precise failures that incorporated significant portions of scion or rootstock tissue. Categories were refined with each of the first two sampling times but were consistently assigned for Oct. 2014 and later times. Categorical data for break type (Table 1) were lumped into orders based on worst to best-case scenarios with order 1 (clean breaks at the graft) being the worst, order 4 (non-graft breaks or no break) being the best and order 2 and 3 having tis-

**Table 12.** Percentage of breaks for each of four order classifications (see Table 1) as affected by rootstock within grafting method or rootstock within direction.

| | Rootstock | % of Breaks Order 1 | Order 2 | Order 3 | Order 4 | |
|---|---|---|---|---|---|---|
| *Chip Buds* | G.11 | 10 | 65 | 14 | 11 | bc[1] |
| | G.214 | 33 | 60 | 4 | 3 | ab |
| | G.41 | 49 | 47 | 2 | 1 | a |
| | M.9-NIC 29 | 3 | 43 | 24 | 30 | c |
| *Whip and Tongue* | G.11 | 1 | 29 | 68 | 2 | b |
| | G.41 | 24 | 67 | 9 | 0 | a |
| | M.9-NIC 29 | 0 | 12 | 83 | 5 | b |
| *Saddle – Rootstock*direction* | | | | | | |
| Sideways | G.11 | 9 | 60 | 29 | 2 | ab |
| | G.41 | 17 | 65 | 17 | 1 | a |
| | M.9-NIC 29 | 14 | 34 | 48 | 4 | b |
| Stacked | G.11 | 93 | 7 | 0 | 0 | a |
| | G.41 | 10 | 61 | 28 | 1 | b |
| | M.9-NIC 29 | 7 | 82 | 11 | 0 | b |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

sue not completely separated between the two genotypes. Samples that did not break were combined with the samples that broke somewhere other than the graft because less than 0.1% of the samples did not break. The statistical procedure tested the probability of having lower order break types. The analyses for these categories for Oct. 2014 are shown in Table 12. The rootstock G.41 consistently had the highest frequency of clean breaks for all graft type except for saddle graft in the stacked position. In general, G.11 and M.9-NIC 29 had a significantly lower probability of clean breaks, except for saddle stacked. G.11 saddle stacked had an unusual percentage of clean breaks compared to all other rootstocks.

For chip buds in Oct. 2015, 'Honeycrisp' had the highest probability of low order (clean breaks) and 'Gala' had the lowest probability of clean breaks (Table 13). Among rootstocks, G.41 was significantly different than all other tested rootstocks, having the highest frequency of clean breaks. Since there was a significant rootstock*direction interaction, rootstocks were analyzed separately for each direction. Regardless of direction, G.41 always had the highest probability for clean breaks. All other rootstocks tended to break less cleanly with G.11 being the least clean or having more non-graft breaks for chip down, while B.9 had more non-clean and non-graft breaks for chip up. For the saddle grafts, G.41 again had the highest probability of having clean breaks followed closely by G.935 (Table 14). The highest proportion of non-clean breaks was for B.9 and MM.106. Break patterns were particularly distinct in MM.106, in part due to the greater flexibility (high deflection); tissue would often tear at locations other than the graft.

*Micro CT scan analysis*. While other techniques (optical and electron microscopes and laser ablation scanning) have been used, this was the first time in our laboratory and to our knowledge in the rest of the rootstock research community that graft unions were imaged using a micro-CT technique. It took several tries to optimize the resolution (between 25-30 microns per pixel) and the con-

**Table 13.** October 2015 percentage of breaks for each of four order classifications (see Table 1) as affected by scion variety within grafting method or rootstock within direction.

| | | % of breaks | | | | |
|---|---|---|---|---|---|---|
| | | *Order 1* | *Order 2* | *Order 3* | *Order 4* | |
| *Scion* | | | | | | |
| | Fuji | 10 | 47 | 34 | 9 | ab[1] |
| | Gala | 5 | 33 | 45 | 18 | b |
| | Honeycrisp | 16 | 54 | 25 | 5 | a |
| | McIntosh | 9 | 46 | 36 | 10 | ab |
| | Cripps Pink | 8 | 43 | 38 | 11 | ab |
| | Scilate | 8 | 44 | 37 | 10 | ab |
| *Rootstock*Direction* | | | | | | |
| Chip Down | G.41 | 34 | 52 | 12 | 2 | a |
| | G.935 | 10 | 48 | 33 | 8 | b |
| | B.9 | 3 | 26 | 47 | 24 | bc |
| | EMLA 26 | 7 | 41 | 40 | 12 | bc |
| | G.214 | 8 | 44 | 38 | 11 | bc |
| | M.9-NIC 29 | 6 | 38 | 42 | 14 | bc |
| | G.11 | 2 | 16 | 45 | 38 | c |
| Chip Up | G.41 | 50 | 42 | 6 | 1 | a |
| | G.11 | 15 | 53 | 26 | 6 | b |
| | EMLA 26 | 9 | 46 | 35 | 9 | bc |
| | G.214 | 11 | 49 | 33 | 8 | bc |
| | G.935 | 6 | 39 | 42 | 14 | bc |
| | M.9-NIC 29 | 7 | 39 | 41 | 13 | bc |
| | B.9 | 4 | 30 | 46 | 20 | c |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

**Table 14.** October 2015 saddle graft percentage of breaks for each of four order classifications (see Table 1) as affected by rootstock within grafting method or rootstock within direction.

| *Effect* | | % of breaks | | | | |
|---|---|---|---|---|---|---|
| | | *Order 1* | *Order 2* | *Order 3* | *Order 4* | |
| *Rootstock* | G.41 | 23% | 50% | 24% | 3% | a[1] |
| | G.935 | 10% | 39% | 42% | 9% | ab |
| | G.214 | 5% | 28% | 51% | 15% | b |
| | EMLA 26 | 5% | 28% | 51% | 15% | b |
| | G.11 | 5% | 27% | 52% | 16% | b |
| | M.9-NIC 29 | 6% | 30% | 50% | 14% | bc |
| | MM.106 | 3% | 18% | 53% | 26% | cd |
| | B.9 | 2% | 11% | 48% | 40% | d |
| *Direction* | Sideways | 7% | 34% | 47% | 11% | a |
| | Stacked | 4% | 24% | 53% | 19% | b |

[1] Main effect means followed by the same letters are not significantly different at $p < 0.05$.

trast needed to cover the union to be able to view the details of the vascular bundles, their orientation and uniformity and identify putative areas of weakness (Fig. 2-4). Like Basedow's findings (Basedow et al., 2017), stronger graft union combinations seemed to have smoother transitions between the rootstock and the scion, whereas the weaker combinations exhibited disorganized patterns for vascular tissue and the presence of water rich parenchyma in the interface between the genotypes. In chip buds, the weaker unions showed a dead area behind the initial placement of the bud (Fig. 2 and 3). Similarly, in

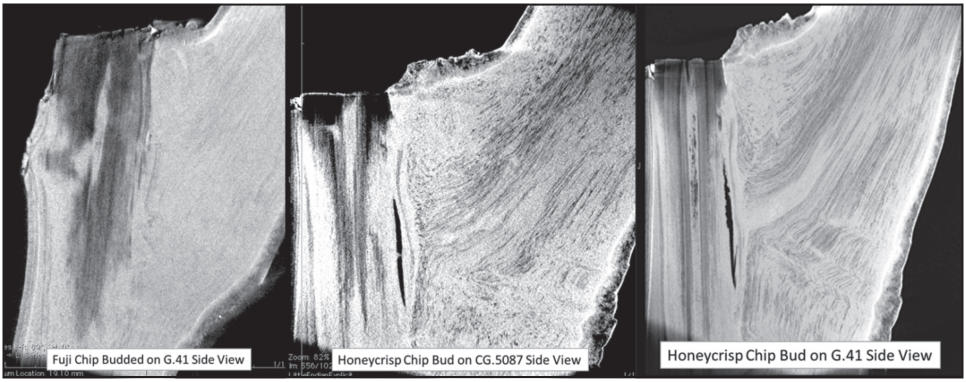


**Figure 2.** Graft unions of a chip budded 'Fuji'scion on 'G.41' (left) and 'Honeycrisp' scion on rootstocks 'CG.5087' (middle) and 'G.41' (right) visualized by 3D X-ray tomography. The stronger graft union combination ('Fuji' on 'G.41') is characterized by a smooth transition between the rootstock and the scion. Both 'Honeycrisp' on 'G.41' and 'CG.5087' exhibit a weak area between the rootstock and the scion where rootstock was cut to force the chip bud. The vascular tissues seem to be more disorganized in both combinations compared to 'Fuji'. The dark region between the scion and the rootstock is where the bud was inserted, not very evident in the 'Fuji'/'G.41' combination. The top portion where the two tissues meet shows less organized wood, dead rootstock tissue and possibly the reason for weaker wood formation.

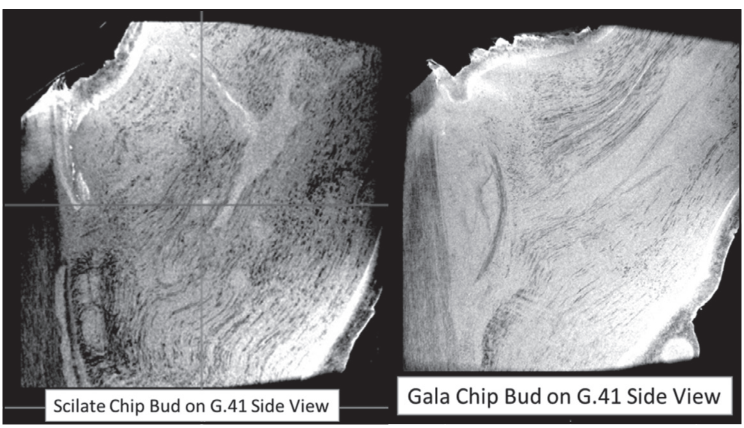


**Figure 3.** Graft unions of a chip budded 'Scilate' scion on 'G.41' (left) and 'Gala' scion on 'G.41' (right) visualized by 3D X-ray tomography. Both 'Scilate' and 'Gala' on 'G.41'show a weak area between the rootstock and the scion where the rootstock was cut to force the chip bud. To a degree, the 'Scilate/G.41' combination features vascular tissues that are more disorganized thank the "Gala/G.41' combination.

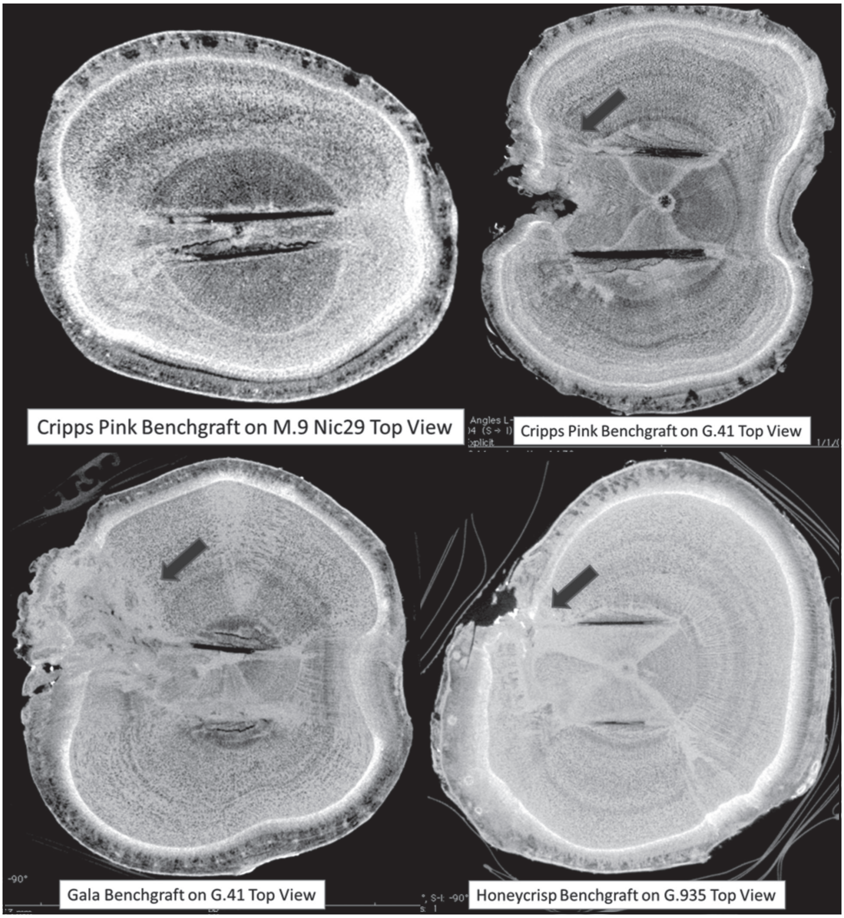


**Figure 4.** Graft unions of bench grafted 'Cripps Pink' scion on 'M.9 Nic29' (top left) and 'G.41' (top right), 'Gala' scion on 'G.41' (bottom left) and 'Honeycrisp' scion on 'G.935' (bottom right) visualized by 3D X-ray tomography. The graft union of 'Cripps Pink/M.9 Nic29' combination shows what would be expected in a normal union where the transition between scion and rootstock is smooth and uniform with regular growth around the initial graft. Weaker areas of disorganized growth around the graft (indicated by arrows) are featured in the other rootstock/scion combinations where normal growth of vascular tissue was inhibited in some portion of the union.

bench grafts, weaker combinations featured areas of reduced and disorganized growth in the circumference around the graft union (Fig. 4). The region above and below the graft union has been shown to feature different hormonal levels (Michalczuk, 2002) and morphologies (Tworkoski and Fazio, 2016). It is possible that normal production of vascular bundles in the proper orientation and density was inhibited by abnormal hormone gradients (like auxins and cytokinins) which govern such processes or by a genotypic difference in the way such signals are interpreted by scions and rootstocks (Zhang et al., 2015).

## Conclusion

Across all studies, G.41 formed a graft

union that was both weak and rigid/brittle whereas M.9-NIC 29 had consistently stronger unions. The break force corrected for both scion and graft size was weaker than other rootstocks over both years. This result was true regardless of scion cultivar or graft method. Although G.41 increases in strength during a second year, the rate of increase remained less than other rootstocks tested, such as M.9-NIC 29. G.41 also had the least deflection or flexibility in the rootstock alone and as part of the graft union. In addition to these results, G.41 had a higher probability of breaking clean at the graft compared to the other rootstocks. Clearly it would be unadvisable to use G.41 as a rootstock for scions that are also known to have more brittle wood or form weaker graft unions such as 'Scilate'. 'Scilate' also produces a large highly feathered tree that is more susceptible to wind damage. 'Honeycrisp' also appears to form a weak union with G.41 but may be less susceptible to wind damage due to reduced growth compared to 'Scilate'. Some cultivars that tended to form stronger graft unions with G.41 include 'McIntosh' and 'Cripps Pink'

Other Geneva® rootstocks appear to be stronger than G.41, especially G.11 and G.214. The rootstock G.935, while stronger than G.41, tended to form weaker graft unions than the remaining rootstocks tested. It also produces a larger tree, so nurseries should carefully select scions to graft onto G.935.

Based on our data, grafting method does not appear to be an effective means of improving graft union strength. Concurrent experiments conducted by our labs showed that the application of auxin compounds and prohexadione calcium have a positive effect on strengthening the G.41/'Scilate' graft union suggesting that hormone status of the rootstock and scion might be manipulated to strengthen the graft union (Adams et al., 2017). However, as with other PGR applications, practical delivery to the target site is likely the limiting factor in commercial use. When comparable scion/rootstock combinations were budded/grafted using juvenile liners from commercial micropropagation production efforts, based on visual/physical observation, the trees from TC propagated liners may form a stronger union, which may be due to their juvenility. Further research needs to be conducted (CT scans, break strength, etc.) to compare similar age/combination type trees before these observations can be verified. However, these observations might indicate the possibility that this problem might be overcome by using more juvenile material or hormone treatment in the nursery. In general, weaker rootstock-scion combinations were also relatively weak when bench grafted. Further, bench-grafting techniques tend to produce less scion growth. Although the resulting smaller trees would be less susceptible to wind damage, growers prefer the larger more feathered trees produced from chip budding.

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