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Small Genomes in Tetraploid *Rubus* L. (Rosaceae) from New Zealand and Southern South America

Kim E. Hummer[1] and Lawrence A. Alice[2]

Additional index words: *C* value, flow cytometry, genome, ploidy, *Rubus*, germplasm

Abstract

The genus *Rubus* contains crop wild relatives of raspberries and blackberries. *Rubus* subgenera *Micranthobatus* and *Comaropsis* are endemic to the Southern Hemisphere in trans-Pacific Ocean environments of Australasia, South America, and the Falkland Islands. The United States Department of Agriculture, National Clonal Germplasm Repository (NCGR) houses a *Rubus* genebank of living plants, including representatives of subgenera *Micranthobatus* and *Comaropsis*. Previously, accessions were determined by chromosome counts to be tetraploid. Our objective was to examine the nuclear DNA content (*C* values) of the tetraploid *R. cissoides, R. parvus, R. schmidelioides, R. squarrosus,* and *R. geoides* in contrast with those of diploid and tetraploid black raspberries (*R. occidentalis*) and diploid red raspberry (*R. idaeus* subsp. *idaeus*). Nuclear DNA content was determined using flow cytometry. Surprisingly, the *C* values of these species were significantly smaller than an autotetraploid clone of *R. occidentalis* or other tetraploid genotypes, and numerically equivalent to about the size of triploid raspberries. The small genomes may provide clues concerning the evolution of these subgenera.

Polyploids, especially allopolyploids, are common in *Rubus* L. (Rosaceae; Rosoideae) and are a major factor confounding its taxonomy and evolutionary history. Reports have recognized divergent ploidy levels of *Rubus* species ranging from diploid to dodecaploid (Thompson, 1997) with tetraploids most abundant. The number of species worldwide ranges from ~400 (Focke, 1894, 1910, 1911, 1914) to 700 (Bailey, 1941; Lu and Boufford, 2003; Alice et al., 2008). Focke, in his publications recognized 12 subgenera (subg.) whereas GRIN-Global database (USDA ARS, 2016) recognizes 15 (including two nothosubgenera). The gametic chromosome number in *Rubus*, like other Rosoideae, is $x = 7$. Nondisjunction, whole genome duplication (WGD), interspecific hybridization and apomixis frequently occur in *Rubus* (Alice et al., 2008). The U.S. Department of Agriculture, National Clonal Germplasm Repository (NCGR) maintains a diverse *Rubus* collection preserved as living plants as well as seed (Hummer, 1996; Hummer et al., 2016). The latest counts for the genebank can be found on the GRIN-Global database (USDA ARS, 2016). Besides preservation, NCGR is responsible for characterization of genetic resources including *Rubus*. Ploidy levels for accessions in the collection were determined through chromosome counts (Thompson, 1995a; 1995b; 1997) and flow cytometry (Meng and Finn, 2002; Hummer et al., 2016).

The New Zealand species of subgenus *Micranthobatus* (Kalkman, 1987) commonly called "bush lawyers" are not well known internationally. These species are sprawling vines with prickles useful for climbing on other plants. Many species have unisexual flowers.

Rubus parvus Buchanan, commonly called "creeping lawyer," is a low growing sub-

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shrub. The long narrow, simple leaves are serrate, with red prickles on the mid-vein. It has solitary, perfect (Webb et al., 1988) or in some reports "unisexual" (Cheeseman, 1925), white flowers about 1.8 cm in diameter that produce red to orange drupelets. A clone at the NCGR genebank has perfect flowers (Fig. 1a). The drupelets form aggregate fruit that ripen red and remain attached to the receptacle when harvested, similar to that of a blackberry (Fig. 1b). Other *Micranthobatus* species, *R. cissoides* A. Cunn. and

Fig. 1a: *Rubus parvus* commonly called "creeping lawyer," has long narrow, simple serrate leaves and solitary, perfect white flowers. Photo by Kim Hummer, USDA.

Fig. 1b: *Rubus parvus* drupelets from aggregate fruit that ripen red and remain attached to the receptacle when harvested, similar to a blackberry fruit. Photo by Kim Hummer, USDA.

Fig. 2: *Rubus schmideloides* has trifoliate leaves with small lamina. Leaf scan by Adrienne Oda, USDA.

R. schmideloides A. Cunn. are dioecious lianas, with red prickles on stems, petioles, and leaf midrib, small leaves (Fig. 2) relative to others in the subgenus, white to cream-colored petals on a many-flowered panicle-like cyme from 12 to 60 cm long depending on taxon (Webb et al., 1988). *Rubus cissoides* has 10 or more serrations on each simple leaf margin, while *R. schmideloides* has less than 10. The so-called leafless bush lawyer, *R. squarrosus* Fritsch has slender to stout stems, yellow prickles on the petiole and petiolule, and the trifoliate leaves (Fig. 3) lack significant lamina (~1 cm long). It is a climber with intertwining branchlets. This species has not flowered at NCGR.

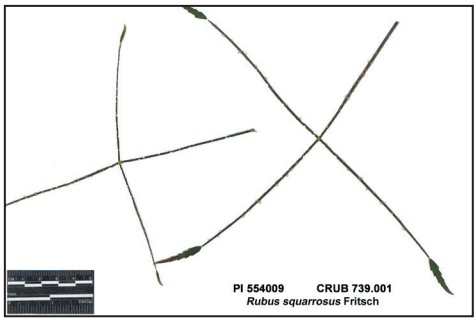

Fig. 3: *Rubus squarrosus* very small trifoliate leaves with prickers on petioles and petiolules. Leaf scan taken by Tyler Young, USDA.

Fig. 4: *Rubus geoides* flower and trifoliate leaves. Photo by Kim Hummer, USDA.

Rubus geoides Sm. (Fig. 4) is a low growing subshrub endemic to southern Argentina, Chile, and the Falkland Islands (Focke, 1910; USDA ARS, 2016). It has trifoliate leaves with small, weak prickles and perfect flowers. It is harvested from the wild for the red raspberry-like fruit. This species was considered for bramble breeding, crossing with species endemic to the northern hemisphere because of hardiness, few prickles, and its ability to produce fruit under windy and extreme environmental conditions; however, crosses between *R. geoides* and northern *Rubus* were unsuccessful and therefore not pursued for commercial development (Haskell and Paterson, 1966). Alice and Campbell (1999) included three members of subg. *Micranthobatus* in their phylogenetic study: Australian *R. moorei* and *R. australis* G. Forst., and *R. parvus* Buchanan from New Zealand. These species form a monophyletic group along with *R. geoides* of subg. *Comaropsis* and Tasmanian *R. gunnianus* Hook. from subg. *Dalibarda*. Hummer et al. (2016) observed that five tetraploid *Rubus* species native to New Zealand and southern South America had relatively small genomes compared to those of other species.

The objective of this study was to determine the amount of nuclear DNA (*C* values) of the tetraploids *R. cissoides, R. parvus, R. schmidelioides, R. squarrosus,* and *R. geoides*. The DNA *C*-value for diploid *R. idaeus* subsp. *idaeus* L. 'Meeker' red raspberry and *R. occidentalis* L. 'Munger' black raspberry, and an autotetraploid 'Munger' produced through tissue culture were determined for comparison.

Materials and Methods

Plant material. Young leaves of *R. cissoides, R. parvus, R. schmidelioides, R. squarrosus, R. geoides*, and diploid and autotetraploid *R. occidentalis* 'Munger' and diploid *R. idaeus* subsp. *idaeus* 'Meeker' growing in greenhouses at the USDA ARS NCGR in Corvallis, Oregon, were collected. Samples were sent overnight to Plant Cytometry Services (Schijndel, The Netherlands) in July 2014. Three leaves (replicates) were analyzed for each accession. Sample leaf material (~1 cm^2/20-50 mg) was combined with leaf material of an internal standard (*Vinca minor* L.). The plant material was chopped with a razor blade in 500 µL of CyStain PI absolute Extraction buffer (Partec GmbH, Münster, Germany) containing RNase, 0.1% DTT (dithiothreitol) and 1% polyvinylpyrolidone (ice-cold), in a plastic Petri dish. After 30-60 s of incubation, 2.0 mL staining buffer containing propidium iodide (PI) as fluorescent dye, RNA-se, 0.1% DTT (dithiothreitol) and 1% polyvinylpyrolidone was added. Remaining cell constituents, large tissue samples, and the internal standard were filtered through a 50 µm mesh nylon filter.

Nuclear DNA determination. After an incubation of at least 30 min at room temperature, the filtered solution with stained nuclei was measured with a CyFlow ML flow cytometer (Partec GmbH, Münster, Germany) with a green diode laser 50 MW 532 nm (for use with PI) and analyzed with Flomax version 2.4 d software. The amount of DNA of the unknown samples was calculated by multiplying the amount of DNA of the internal standard by the DNA ratio of the relative DNA amount of the unknown sample and the internal standard. Flow cy-

tometry determinations were performed by Plant Cytometry Services (AG Schijndel,The Netherlands). The pg/2*C* of nuclear DNA of the *Rubus* samples was calculated based on the value of *Vinca minor* nuclear DNA = 151 pg/2*C* (Bennett and Leitch, 2012). Analysis of variance (ANOVA) was calculated on the pg/2*C*. Least significant difference (LSD) was calculated to separate significantly different means.

Results and Discussion

The amounts of nuclear DNA (pg/2*C*) for the *Rubus* samples are shown (Table 1). The amounts of nuclear DNA of the study group were significantly different as determined by ANOVA ($df = 23$, $F = 850$; $P < 0.01$), therefore LSD was applied for mean separation ($P < 0.01$) and determined three groups (Table 1). The smallest genomes of our samples were diploid 'Meeker' red raspberry, 0.64 pg/2*C* and diploid 'Munger' black raspberry, 0.67 pg/2*C*. These were larger than the genomes reported by Meng and Finn (2002) for *R. illecebrosus, R. crataegifolius*, and *R. nivalis*. The largest genome we sampled was the autotetraploid 'Munger' at 1.39 pg/2*C*, slightly more than twice the amount of diploid 'Munger'. The nuclear DNA amounts for the five tetraploid species from New Zealand and southern South America ranged from 0.89 to 0.93 pg/2*C*, significantly more than the diploids, but significantly less than the autotetraploid 'Munger'.

The amounts of nuclear DNA for the tetraploid species in subgenera *Micranthobatus* and *Comaropsis* were significantly smaller than that of autotetraploid 'Munger', and smaller than that of other tetraploid *Rubus* species, such as *R. alceifolius* Poir. (Amsellem et al., 2001), or cultivated blackberry tetraploids (Hummer et al., 2016). The five *Rubus* species from New Zealand and southern South America had approximately the DNA amount predicted for a triploid, judging from genome size of *Rubus* subg. *Idaeobatus* (raspberry) (Table 1). Gardner (2002) remarked on the small size of bush lawyer chromosomes, and our results were surprisingly low, considering that the species are tetraploid. Whole-genome duplication is widespread in diverse taxa (McGrath and Lynch, 2012) and the combination of genomes through autopolyploidy or allopolyploidy occurs in the plant kingdom at rates comparable to that of point mutations (Lynch and Conery, 2000). When this happens, allopolyploids are expected to have genomes twice as large as their diploid progenitors, and increasing proportionately with ploidy level. The *C* value of the tissue culture-derived autotetraploid 'Munger' was more than

Table 1. Sample identification, mean size (n = 3) of diploid nuclear DNA (pg/2*C*), ± variance, pg/1*C*, and chromosome count. Least significant difference (LSD) was applied to separate means ($P < 0.01$).

Plant Inform. (PI)	Corvallis local identifier	Taxon	Identifier	Mean DNA pg/2*C*	Variance	DNA pg/1*C*	Chromosome Count
553384	989.001	*R. idaeus* L. subsp. *idaeu*	Meeker	0.64a	0.0002	0.32	14
553740	490.001	*R. occidentalis* L.	Munger	0.67a	0.0000	0.34	14
643940	1981.001	*R. geoides* Sm.	Chacao, Chile	0.89b	0.0000	0.45	28
554009	739.001	*R. squarrosus* Fritsch	Hangley Gardens	0.90b	0.0000	0.45	28
553883	741.001	*R. schmideloides* A. Cunn.	SK-NZ-12	0.90b	0.0000	0.45	28
654992	2512.001	*R. parvus* Buch.	rupa576	0.92b	0.0002	0.46	28
654992	772.001	*R. cissoides* A. Cunn.	Lincoln 42	0.93b	0.0002	0.46	28
660944	2573.001	*R. occidentalis* L.	Munger - autotetraploid	1.39c	0.0008	0.69	(28)

twice that of its diploid progenitor consistent with the hypothesis of additivity. In nature *C* values of many polyploid series have DNA amounts less than predicted suggesting that genome reduction can take place immediately following a polyploidization event or can occur over time (Leitch and Bennett, 2004). To get to the tetraploid state, the most recent common ancestor of subg. *Micranthobatus* and subg. *Comaropsis* species must have initially experienced a WGD or allopolyploidization event. The small genomes of these tetraploids may indicate that they were derived from diploid species with small genomes or that genome size has decreased.

Thus, in searching for potential closely related diploids with small genomes, *R. nivalis* Douglas and ancestors of several Asian *Idaeobatus* species, such as *R. illecebrosus* Focke or *R. crataegifolius* Bunge could be considered (Hummer et al., 2016).

The small genomes we observed provide support, in addition to nuclear ITS (Alice and Campbell, 1999) and chloroplast DNA sequences (L. Alice, Western Kentucky University, unpublished data), to the hypothesis that members of the these subgenera likely originated from a single allopoly ploidization event followed by species divergence.

Geographically isolated populations may experience greater speciation rates within polyploid lineages (McGrath and Lynch, 2012). At this time neither the age nor historical biogeography of these taxa is known, therefore dispersal and vicariance, evolution through geographical separation, are viable hypotheses. An alternative is that one or more diploid progenitors with larger genomes were involved in an autopolyploid event followed by genome reduction.

Genome size of polyploids could be expected to be the sum of the genomes inherited from progenitor species.Differences from the expected DNA amounts could be the result of genome size decreases or increases. Increases in genome size following polyploidization are rare (Leitch and Bennett, 2004). Given that our results show smaller DNA amounts than expected for other *Rubus* tetraploids, we can rule out that possibility. Another possibility is the complete additivity of the genomes of diploid progenitors. This is more likely to occur in autopolyploids than allopolyploids.The diploid ancestors of the *Rubus* tetraploids we examined are unknown and may be extinct. Progenitor candidates could include individuals similar to *Rubus nivalis* from northwestern North America which appeared closely related to these *Micranthobatus* and *Comaropsis* taxa (Alice and Campbell, 1999).

Other progenitor candidates might be diploid blackberries which grouped as a sister clade to *R. nivalis* and the Southern hemisphere lineages. Based on flow cytometry data, DNA amounts of subgen. *Rubus* diploids vary from 0.59 to 0.75 (Meng and Finn, 2002). However, doubling the genome size of the blackberry possessing the smallest genome sampled yields a value too large.

Another possibility might be found among the basal members of the *Rubus* phylogeny, such as *R. lasiococcus* Focke or *R. pedatus* Sm. A doubling of the size of those species or *R. crataegifolius* would be close to the size of these New Zealand tetraploids.

The genome size of raspberries in subg. *Idaeobatus* is likely too large to consider as progenitor diploids for *Micranthobatus*, unless significant genome "downsizing" occurred.

We suggest that likely progenitor species for *Micranthobatus* and *Comaropsis* had small genomes initially, such as those for *R. crataegifolius* or *R. lasiococcus*, then moderate downsizing occurred during the development to the modern day species. Molecular phylogeny of *Rubus* species is under investigation and will provide insight to this phylogenic question.

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The Effect of Plant Growth Regulators on Apple Graft Union Flexural Strength and Flexibility

Stuart Adams[1,2], Brent L. Black[1,3], Gennaro Fazio[4] and Nicholas A. Roberts[5]

Additional index words: *Malus,* graft strength, benzyl adenine, NAA, prohexadione

Abstract

The apple rootstock 'Geneva® 41' ('G.41') forms weak graft unions with some scions. Exogenous plant growth regulators (PGR) can influence vascular differentiation and wood formation, and thus may improve graft union strength. A series of commercial and experimental PGR formulations were applied to trees on 'G.41' rootstock over two seasons in May and June, and graft union strength and flexibility were measured. Treatments included abscisic acid (S-ABA), 1-napthaleneacetic acid (NAA), prohexadione-calcium (PCa), and benzyl adenine (BA) as dilute sprays; and a concentrated formulation of BA applied in a latex paint solution to the graft union. BA in latex paint significantly increased the flexural strength per scion cross-sectional area and the flexibility of the union. Foliar applications of PCa also increased graft union flexural strength and flexibility, but temporarily limited scion extension growth. Applying PGRs in the nursery to more brittle rootstock-scion combinations may be an option for improving graft union strength and preventing tree losses. However, more efficient methods of application are needed for this approach to be commercially viable.

The United States Department of Agriculture - Agricultural Research Services (USDA-ARS), in conjunction with Cornell University has developed a series of apple rootstocks with resistance to the bacteria *Erwinia amylovora* (Norelli et al., 2003), the causal agent of fire blight (Robinson et al., 2007; Russo et al., 2007). These rootstocks are identified as Geneva® rootstocks and are given a unique number designation (e.g. 'Geneva® 11', 'Geneva® 41', 'Geneva® 935'). Geneva® rootstocks also have resistance to crown and root rots from *Phytophthora*, and induce high yield efficiency and good fruit size (Fazio et al., 2013). However, some of the Geneva® rootstocks appear to have weak or brittle graft unions that are susceptible to breakage. Some scions on 'Geneva® 41' have had losses of 20-40% in a single wind event in the nursery (R. Adams, personal communication). Due to the disease resistance and economic potential of these new Geneva® rootstocks, research to understand and remedy this brittleness problem is of great importance to the apple industry.

Application of exogenous plant growth regulators (PGRs) may provide an avenue for increasing graft union strength through improved callusing, vascular differentiation, or wood formation. However, studies on plant growth regulators and grafting can result in variable results due to differences in hormone balance among species and between graft partners. Several plant hormones have been suggested for influencing graft union development and wood strength, including: auxin, cytokinin, gibberellin inhibitors, and abscisic acid (S-ABA).

Auxin has been shown to increase callus proliferation and vascular differentiation in graft unions of vegetable and cactus grafts (Moore, 1983; Parkinson and Yeoman, 1982;

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Shimomura and Fuzihara, 1977; Stoddard and McCully, 1980). In a study with grapes, auxin application resulted in reduced or inhibited callus formation (Kose and Guleryuz, 2006). However, the grape study used concentrations that were 5 to 20 times higher than that of other studies, which may have been too high to induce a favorable response. Regardless, auxin may be a possible avenue for increasing graft success.

In the presence of auxin, cytokinins promote callus proliferation and differentiation of vascular tissue when many cell divisions are occurring (Aloni, 1995; Kose and Guleryuz, 2006; Parkinson and Yeoman, 1982). Exogenous cytokinins have also activated thickening growth in stems of cytokinin-deficient Arabidopsis mutants, including increased vessel number, number of cells in the phloem, and number of xylem cells with some of increased size (Matsumuto-Kitano et al. 2008).

Little research has investigated the effects of gibberellins (GA) on graft formation. Parkinson and Yeoman (1982) found that GA decreased the number of vascular connections when applied to grafted internodes in a petri dish. This negative effect suggests that GA inhibitors could be beneficial to improving graft success. Prohexadione-calcium (PCa) is a common GA inhibitor widely used for apple trees to reduce shoot growth and improve fire blight resistance. In apples, foliar PCa applications increased cortical parenchyma cell wall thickness of youngest leaves and shoots (Sundin, 2014). It is not clear to what extent this cell wall thickening would affect graft union strength.

Few studies have been published on the effect of S-ABA on the graft union. Parker et al. (2012) treated drought stressed peach trees with a soil drench of S-ABA and found that future drought tolerance was increased. S-ABA applications were also associated with increased trunk diameter, fresh weight, dry weight, and root growth. More recently, Murcia et al. (2016) found that S-ABA application to grapevines increased phloem area, but it is unclear how this would influence wood formation or strength. In poplar, exogenous S-ABA increased radial number of undifferentiated cambial cells and the formation of longer fiber cells, as well as fewer but larger, vessel cells (Arend and Fromm, 2013). S-ABA has also been shown to be synergistic with IAA and BA in promoting callus formation at the abscission zone of leaf petioles on citrus bud explants (Altman and Goren, 1971).

The objective of this study was to determine if exogenous plant growth regulator applications would have a positive effect on the growth characteristics and break strength of apple graft unions. More specifically, comparisons were made among growth regulators, and application methods. Results were compared based on both scion size (height and stem cross sectional area) and graft strength and flexibility.

Materials and Methods

2014 Study

Experiment Design. Rootstock liners of 'G.41' were chip budded in Aug. of 2013 with 'Scilate' and 'Gala' scion cultivars in a commercial apple nursery (Willow Drive Nursery, Ephrata, Washington). Within each scion, 22 blocks of 10 trees were selected for uniformity in Spring 2014 and assigned to one of 22 treatments. Treatments were not randomized within each row.

Plant Growth Regulator Application. The PGR and control treatments used in this preliminary experiment are described in Table 1. A single application of each PGR was made on 18 June. For those treatments receiving a second application, treatments were made on 15 July. Foliar applications were in dilute sprays until leaf drip, using a 4-L hand-pump spray bottle. Latex trunk paint treatments all contained 50% water and latex paint (v/v) and the PGR concentration shown in Table 1. Paint solutions were applied using 1 mL disposable pipettes so that every tree received ~ 2 mL.

Growth Measurements. Following harvest, four growth measurements were taken: root-

Table 1. Plant growth regulator treatments used in 2014. The commercial formulations, concentrations, application method, and number of applications are shown. ACC provided as experimental formulation from Valent BioSciences (Libertyville, IL).

Chemical Name	Trade Name	Concentration ($mg \cdot L^{-1}$)	Application method	# of Applications
Untreated control	–	NA	NA	NA
Painted control	Water+Paint	50:50 (v)	Graft Paint	1
NAA	Fruitone® N	20	Foliar Spray	1
NAA	Fruitone® N	20	Foliar Spray	2
NAA	Fruitone® N	250	Graft Paint	1
NAA	Fruitone® N	250	Graft Paint	2
IBA	Water+Ethanol	2600	Graft Paint	1
IBA	Water+Ethanol	2600	Graft Paint	2
ACC	Experimental	200	Foliar Spray	1
ACC	Experimental	200	Foliar Spray	2
ACC	Experimental	2500	Graft Paint	1
ACC	Experimental	2500	Graft Paint	2
Ethephon	Ethrel®	2500	Graft Paint	1
Ethephon	Ethrel®	2500	Graft Paint	2
S-ABA	ProTone® SG	320	Foliar Spray	1
S-ABA	ProTone® SG	320	Foliar Spray	2
S-ABA	ProTone® SG	4000	Graft Paint	1
S-ABA	ProTone® SG	4000	Graft Paint	2
BA	MaxCel®	2500	Graft Paint	1
BA	MaxCel®	2500	Graft Paint	2
GA_{4+7}	ProVide®	2500	Graft Paint	1
GA_{4+7}	ProVide®	2500	Graft Paint	2

stock shank diameter (5 cm below the graft union), two perpendicular graft union diameter measurements at the widest part of the graft union, scion stem diameter (5 cm above the graft union and scion height above the graft union.

Sample Preparation. In November, trees were harvested mechanically using standard commercial practices and kept in cold storage for later graft strength analysis. When ready for analysis, trees were topped to an overall length of about 70 cm and the roots, leaves and lateral shoots were removed. Trees were then bundled according to tree number, packed in ice and transported to a laboratory at Utah State University in Logan, Utah.

Break Strength Testing. In the laboratory, each specimen was loaded to failure using a 3-point bend apparatus with a 16 cm separation (Fig. 1). The apparatus was used in conjunction with a Bench Testing Machine (Tinius Olsen H50KS, Horsham, PA) 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. Force measurements were acquired through the equipment software (Tinius Olsen Test Navigator) at 1-second intervals throughout the measurement until a failure condition was achieved. Upon achieving the failure condition, the fracture strength was obtained from the data based on the geometry of the 3-point

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

bend apparatus and the specimen. For each treatment, five replicate samples were broken with the chip bud proximal to the displacement force (bud up), and five replicate samples were broken with the chip bud distal to the displacement force (bud down).

Each sample was categorized according to the nature and location of the resulting break. A clean break at the graft union was categorized as a 1st order break. A break just above the graft union but that included part of the graft union was categorized as a 2nd order break, as was a break just below the graft but including part of the graft union. A break at the graft union but with significant scion and rootstock tissue remaining attached was categorized as a 3rd order break. Finally, trees that broke well above or below the graft union, or that did not break under maximum test displacement were categorized as 4th order.

Data Analysis. Means were calculated and ranked for 2014 growth and break strength data. The following variables were analyzed: force (F), graft cross-sectional area (GCSA), scion cross-sectional area (SCSA), F/GCSA, and F/SCSA and height. Some of the trees had the top few centimeters broken during commercial harvest, so height measurements in 2014 may not be accurate.

Table 2. The plant growth regulators treatments used in 2015, their concentration, application method, and number of applications.

Chemical Name	Trade Name	Concentration a.i. ($mg \cdot L^{-1}$)	Application method	Application #
Control paint	Water+paint	50:50 (v)	Graft paint	2
BA	MaxCel®	5000	Graft paint	2
Control spray	Water+surfactant	NA	Foliar spray	2
Prohexadione-Ca	Apogee®	250	Foliar spray	1
Prohexadione-Ca	Apogee®	500	Foliar spray	1
NAA	Fruitone® N	20	Foliar spray	2
S-ABA	Protone® SG	400	Foliar spray	2

2015 Study

Experiment Design. Rootstock liners of 'G.41' chip budded with 'Scilate' and 'Gala' in Aug. of 2014 were selected in a commercial apple nursery (Willow Drive Nursery, Ephrata, Washington) in Spring 2015. Four adjacent rows were selected for each scion . Within each row, 96 trees were selected for uniformity and divided into 8 groups of 12 consecutive trees. The eight blocks in each row were then randomly assigned one of the eight treatments described in Table 2, such that each cultivar received all eight treatments with four replications, making a split plot design where the main plot treatments were scion cultivar and the sub-plot treatments were PGR.

Plant Growth Regulator Application. The PGR and control treatments are summarized in Table 2. For abscisic acid (ProTone® SG, Valent USA, Walnut Creek, CA), NAA (Fruitone® N, AMVAC Chemical, Newport Beach, CA), and the controls, the commercial non-ionic surfactant Regulaid® (Kalo, Inc. Overland Park, KS) was included at a concentration of 0.1% (v/v). A single application of PGR was applied on 14 May. A second application was made on 4 June for all treatments except PCa, due to concern that a second application of PCa could result in unacceptable reductions in tree height. Foliar applications were made in the same manner as 2014. Trunk spray was applied in a similar manner to foliar application except the spray was directed at the trunk, graft union, and about eight cm of scion stem until thoroughly coated and allowed to drip. For the first latex paint application, one-mL disposable pipettes were used to apply paint so that every tree received about two mL. Paint treatments were mixed such that half of the solution volume was latex paint. However, when BA (MaxCel®, Valent USA, Walnut Creek, CA) was mixed with the paint, the mixture was too thick to be applied with the pipettes, so the paint was applied using a paintbrush such that 5 cm of the rootstock, the graft union, and 1-2 cm of the scion stem were evenly coated. Although this did not allow for precise metering of the quantity of solution applied, it was estimated that approximately 2 mL was applied per tree. The second application of each paint treatment was then applied using just the paintbrushes to apply an even coat over the previous treatment area.

Growth Measurements. Rootstock, graft and scion diameters and stem height were measured 8 May (pre-treatment), 13 July (mid-season), and 12 Oct. (end of season), as described for 2014.

Sample Preparation. In Nov., trees were dug mechanically and kept in cold storage for later analysis. Six trees from each treatment group within each row were selected and topped to an overall length of 70 cm and the roots, leaves and lateral shoots removed. Diameters were re-measured to account for any changes during storage. Trees were then bundled according to replication number, packed in ice and transported to Utah State

University in Logan, Utah.

Break Strength Testing. Break strength was measured in the same manner as described for 2014. However, for 2015 only six trees were sampled per treatment group and replication, with three samples broken with the chip bud proximal to the displacement force and three samples broken with the chip bud distal to the displacement force. Deflection, or the maximum displacement of the testing machine between contact with sample and graft failure, was acquired in addition to the fracture strength described above. This measure was included to determine if any PGR treatments affected the flexibility of the graft union.

Data Analysis. Final CSA, deflection, and break strength data were analyzed in SAS using the GLIMMIX procedure and the Tukey-Kramer adjustment for multiple comparisons with nesting for each treatment per block. Height data showed a significant sampling time×PGR interaction and were analyzed by sampling time using the GLM procedure. For break type categorization, the GLIMMIX procedure was used for a multinomial analysis to determine the probability of lower order break types to occur based on the numeric order described above, where a clean break at the graft union was categorized as 1st order, and an unbroken sample or a break on the rootstock or scion not involving the graft union was categorized as 4th order.

Results and Discussion

2014 Study. Due to the lack of randomization or true replication, results from 2014 should be considered preliminary, but were used to identify PGR treatments that warranted further investigation in the subsequent study in 2015. Generally, few large numerical differences were measured for force, GCSA, SCSA, F/GCSA, or F/SCSA (Table 3). However, there were some interesting numerical trends. NAA foliar2, ABA foliar1, and BA latex2 tended to require greater force than the respective controls, regardless of scion or break direction. ACC foliar1 was the weakest treatment and lower than the untreated control.

NAA foliar2 tended to have a larger GCSA, while ABA foliar1 was only slightly larger than the control. Since ABA foliar1 did not increase the GCSA, there may be a stronger connection in the graft union relative to the graft union area. This is confirmed with F/GCSA, which shows that ABA foliar1 had break strength 24% higher than the untreated control. NAA foliar2 had essentially the same F/GCSA as the untreated control, which suggests that the greater strength could simply be due to tissue proliferation at the graft union, as indicated by increased GCSA.

BA latex2 on the other hand appeared to more directly affect the cross-sectional areas at the graft and the scion. As seen in Table 3, both BA treatments were among the largest for SCSA, with repeat applications resulting in the highest per-tree break strength. This suggests that the increase in strength of these trees is due to an increase in size or an expansion of the union rather than a strengthening of the tissue. This is confirmed in both the F/GCSA and F/SCSA being at an intermediate level.

Trends in this preliminary data suggested that an S-ABA foliar spray might actually increase the strength of the wood tissues in or around the graft union. On the other hand, NAA applied as a foliar spray, or BA applied in latex may increase the graft size, which leads to an increase in force required to break the tree.

2015 Study. Based on preliminary results in 2014, the 2015 treatments focused on S-ABA, NAA, and BA, with the addition of PCa. In 2015, there were no significant main effects on break force (Table 4), and only the scion cultivar had an effect on the GCSA. Also, no significant differences in break type were detected between PGR treatments. However, for SCSA, F/SCSA, and deflection there were significant PGR main effects, with SCSA showing a significant scion×PGR interaction. The PGR treatments that were among the highest in flexural strength cor-

Table 3. The effect of plant growth regulator (PGR) treatments on flexural strength (Force in Newton), graft cross-sectional area (GCSA), scion cross-sectional area (SCSA), force per graft cross-sectional area (F/GCSA), force per scion cross-sectional area (F/SCSA), and height of 'Scilate' and 'Gala' apples grafted on 'Geneva 41' rootstock. Values are averaged over scion cultivar, and treatments are ranked for each parameter.

PGR	Application	Force (N)		GCSA (cm^2)		SCSA (cm^2)		F/GCSA ($N \cdot cm^{-2}$)		F/SCSA ($N \cdot cm^{-2}$)		Height (cm)	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
BA	Latex2	566	1	8.56	1	1.94	1	65.9	7	292	4	204	4
S-ABA	Foliar1	511	2	6.78	12	1.71	9	75.3	1	301	2	204	5
GA_{4+7}	Latex1	483	3	7.27	6	1.93	2	65.2	9	249	17	196	11
NAA	Latex1	468	4	7.29	5	1.63	11	63.1	12	289	6	206	1
S-ABA	Latex1	461	5	6.89	10	1.81	6	66.4	6	252	16	205	2
Ethephon	Latex1	460	6	7.70	2	1.77	7	60.3	19	264	12	200	6
BA	Latex1	451	7	6.90	9	1.84	4	65.2	8	242	20	193	19
Control	Latex1	451	8	6.73	13	1.60	12	67.1	4	287	8	196	12
NAA	Foliar2	445	9	7.49	4	1.74	8	62.9	13	261	13	195	14
S-ABA	Foliar2	444	10	6.23	20	1.53	15	70.7	3	290	5	193	17
S-ABA	Latex2	441	11	7.57	3	1.92	3	57.9	20	229	21	199	7
GA47	Latex2	439	12	7.04	7	1.83	5	62.3	15	242	19	197	8
IBA	Latex1	428	13	6.02	22	1.44	20	73.3	2	302	1	205	3
ACC	Latex1	428	14	6.98	8	1.69	10	62.4	14	257	14	194	16
ACC	Foliar2	428	15	6.69	14	1.47	18	63.7	10	293	3	190	21
NAA	Foliar1	417	16	6.26	18	1.50	16	66.5	5	280	9	193	18
Ethephon	Latex2	404	17	6.23	19	1.48	17	63.5	11	271	10	192	20
ACC	Latex2	396	18	6.43	16	1.58	13	61.0	17	252	15	196	10
IBA	Latex2	394	19	6.86	11	1.42	22	56.6	21	270	11	194	15
NAA	Latex2	394	20	6.49	15	1.42	21	61.1	16	288	7	197	9
Control	Untreated	362	21	6.05	21	1.46	19	60.5	18	247	18	190	22
ACC	Foliar1	345	22	6.26	17	1.56	14	55.9	22	224	22	195	13

Table 4. A comparison of scion cultivar ('Scilate' and 'Gala') and PGR main effects for 2015 treatments. Comparisons are for flexural strength (Force), graft cross-sectional area (GCSA), scion cross-sectional area (SCSA) force per scion cross-sectional area (F/SCSA), and deflection. Main effect means followed by the same letter are not significantly different at $p < 0.05$. A dash indicates $p > 0.1$. Deflection is a measure of flexibility where greater deflection prior to failure indicates greater flexibility.

Effect		Force (N)	GCSA (cm^2)	SCSA (cm^2)	F/SCSA ($N \cdot cm^{-2}$)	Deflection (cm)
Scion	Gala	518	9.24 a	2.54 a	208 b	0.344
	Scilate	496	8.36 b	2.24 b	228 a	0.433
PGR	Control - paint	525	8.78	2.61 a	208 b	0.363 b
	BA paint	531	9.51	2.21 cd	250 a	0.601 a
	Control - water	514	8.95	2.50 ab	209 b	0.337 b
	BA spray	533	8.60	2.47 abc	226 ab	0.426 ab
	PCa 250	477	8.92	2.28 bcd	213 ab	0.403 ab
	PCa 500	498	8.63	2.15 d	236 ab	0.415 ab
	S-ABA	492	8.46	2.44 abcd	206 b	0.314 b
	NAA	486	8.48	2.48 abc	199 b	0.373 ab
Direction	Down	495	8.86	2.42	209 b	0.445 a
	Up	519	8.72	2.36	228 a	0.354 b
ANOVA p-values	Scion	–	0.006	0.002	0.083	–
	PGR	–	–	0.019	0.013	0.014
	Scion×PGR	–	–	0.033	–	–
	Direction	–	–	–	0.059	0.031
	Scion×Direction	–	–	0.006	–	–
	PGR×Direction	–	–	–	–	–
	Scion×PGR×Direction	–	–	–	–	–

rected for SCSA were BA applied as graft paint, BA as a trunk spray, and the high rate of PCa. The other PGR treatments, S-ABA, NAA and the low rate of PCa, showed little difference in F/SCSA compared to the controls (Table 4).

BA applied as a latex paint increased F/SCSA compared to both controls. However, break force per tree was the same as the painted control, indicating that the difference was due to a reduction in SCSA. Although the SCSA showed a significant scion×PGR interaction (Table 5), the BA paint treatment was smaller than the paint control for both scions. Kose and Guleryuz (2006) reported that cytokinin increases callus proliferation at the graft union. Although the paint applications of BA resulted in the largest measured GCSA in both years, these differences were not statistically significant.

Table 5. Interaction effects of plant growth regulator and scion treatment on scion cross-sectional area (SCSA) in the 2015 study. Separated by scion, main effect means followed by the same letter are not significantly different at $p < 0.05$.

PGR	SCSA (cm^2) 'Gala'		SCSA (cm^2) 'Scilate'	
Control - paint	2.90	a	2.3	ab
BA paint	2.50	abc	1.9	b
Control - water	2.67	abc	2.33	ab
BA spray	2.43	abc	2.51	a
PCa 250	2.30	bc	2.26	ab
PCa 500	2.26	c	2.04	ab
S-ABA	2.77	ab	2.11	ab
NAA	2.51	abc	2.45	ab

In addition to increased F/SCSA, BA paint

also had a significantly higher deflection, or maximum lateral displacement before fracturing, than both controls. This indicates greater flexibility, which would contribute to reduced risk of breaking in the field. Part of this could be due to the reduced SCSA, however, the high rate of PCa had a similar reduction in SCSA without any increase in flexibility.

The high rate of PCa had a F/SCSA that was numerically higher than the control, but this difference was not significant (Table 4). Further, PCa temporarily reduced shoot growth by shortening internodes. The high rate PCa trees averaged 37 cm shorter than the control at the July measurement date, representing a 29% reduction in growth (Table 6). However, by harvest, these trees were only 13 cm shorter than the control, a difference less than 7% and not statistically significant. However, PCa treated trees continued to have a smaller SCSA and a section of shortened internodes that may be undesirable to growers. PCa also had a 23% increase in deflection compared to the control, which may help reduce damage in windy conditions.

This temporary reduction in scion growth is not surprising as PCa is a GA inhibitor used commercially to reduce vegetative growth in apple (Evans et al., 1997). How this reduction in stem elongation affects nursery tree value is not known. It is not clear whether or not PCa had any strengthening effect on the graft union.

Although F/SCSA for BA in a dilute trunk spray did not differ significantly from the water control, this treatment may merit further investigation. Compared to BA paint, BA in a directed aqueous spray could be more easily adopted by growers due to ease of application. The main challenge of any PGR use is efficient delivery of active ingredient to the appropriate plant tissue. Over both seasons, BA applied to the graft union appeared to be the most effective for increasing break strength. Additional work to improve delivery may make this approach the most commercially viable method of increasing graft union strength and flexibility.

Conclusion

These results indicate a possible strengthening to the graft union through the use of PGRs. In particular, results from both 2014 and 2015 showed BA applied in a latex paint increased GCSA leading to an increased break force requirement. However, BA paint did have reduced SCSA, which may be undesirable to the nursery. Applications in latex paint were more effective than aqueous trunk

Table 6. A comparison of tree height (cm) over three sampling times in 2015. PGR effect means followed by the same letter for each measurement period are not significantly different at $p < 0.05$.

	Height (cm)		
PGR	May	July	October
Control - paint	18.8 a	129 ab	196 a
BA paint	19.3 a	128 b	181 b
Control - water	18.5 a	136 a	192 ab
BA water	18.0 a	133 ab	190 ab
PCa 250	19.6 a	109 c	178 b
PCa 500	18.5 a	99 d	179 b
S-ABA	18.2 a	133 ab	190 ab
NAA	17.6 a	129 ab	188 ab
ANOVA p-values			
Scion	–	–	–
PGR	–	<.0001	0.0007
Scion×PGR	–	–	–

application, indicating that better methods for delivery are needed. PCa at higher rates may be another good option to increase strength per SCSA. However, reduced scion growth could reduce the value of the nursery tree. Increased flexibility of the graft would also allow more movement in the wind, and both BA and PCa increased graft flexibility as indexed by lateral displacement.

Lastly, while S-ABA and NAA treatments were among the strongest in 2014, these results did not occur in 2015. Results in previously published studies suggest that NAA has greater effect on graft strength. Our results may again highlight the difficulty of PGR delivery in a field application setting. However, our results from 2015 follow more of the results of Kose and Guleryuz (2006) who found cytokinin had more of a positive effect on the grape graft union than auxin. Additional research is needed to find more efficient methods of PGR delivery, and also to determine whether there is any long-term effects of the PGR treatments on subsequent orchard establishment before this approach can be recommended for nurseries to increase graft union strength.

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Potential Anatomical Methods for the Determination of Weak Wood in Apple

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Additional index words: Geneva rootstock, graft compatibility, Honeycrisp

Abstract

Two experiments were performed to study the anatomical traits related to the development of graft unions of relatively weak ('Honeycrisp'/'M.26 EMLA', 'Cripps Pink' cv. Maslin/'Geneva® 41', 'Scilate' (Envy™)/'Geneva® 41' and strong ('Honeycrisp'/'M.7 EMLA', 'Zestar!'/'M.26 EMLA', 'Zestar!'/'M.7 EMLA', 'Cripps Pink' cv.Maslin/'M.9 NAKB T337', 'Scilate' (Envy™)/'M.9 NIC29') scion/rootstock combinations of apple. The objective was to determine the cause of the weak unions so it may be used to develop a rapid screening tool to identify new potentially weak combinations. Fiber cell walls were thinner below and at the union in 'Honeycrisp' and 'Zestar!' when propagated on 'M.26 EMLA'. 'Honeycrisp' had significantly thicker cell walls at the union than 'Zestar!' combinations. 'Cripps Pink' and 'Scilate' combinations were thinner below and above the graft union on 'G.41' rootstocks. Trees propagated on 'M.26 EMLA' produced significantly less fiber tissues than those propagated on 'M.7' EMLA', and 'Honeycrisp' produced significantly less fiber and conductive tissues than 'Zestar!'. Laser ablation tomography (LAT) revealed weak and strong combinations both contained areas of poor xylem differentiation at the graft union. Xylem tissues at the graft union are highly variable, making it difficult to determine the strength of a scion/rootstock combination based off of anatomical features of the union alone.

The formation of a mechanically weak graft union in young nursery trees is a problem associated with some scion/rootstock combinations of apple. Recently, commercial nurseries have been losing large numbers of newly budded trees of 'Cripps Pink' and 'Scilate' on 'G.41' (N. Manly, personal communication). Other combinations are prone to weakness in the nursery and throughout their life in the orchard, including 'Honeycrisp'/'M.26 EMLA' (Privé et al., 2011), and 'Gala/'G.30' (Robinson et al., 2003).

Graft failure may be caused by many factors, including poor environmental conditions, poor propagation practices, or by an incompatibility between the rootstock and scion (Andrews and Serrano Marquez, 1993). Fiber cells of apple xylem provide much of the mechanical strength to the tree (Winandy and Rowell, 2013), as their secondary cell walls are heavily lignified (Déjardin et al., 2010). This suggests differences in the anatomical characteristics of the fiber cells may lead to the structural weaknesses of the union.

Strong, mechanically resistant wood is characterized by having dense, thick-walled fiber cells. The secondary cell walls of fiber cells are heavily lignified, and the lignified layer provides tensile strength to the wood. Apples propagated to a dwarfing interstem produced thinner fiber cell walls (Doley, 1974). Trees with thin-walled fiber cells may bend more easily under high winds (Déjardin et al., 2010). If the stems bend while being attached to a rigid stake or support post, the tree may be more likely to break.

In addition to fiber cells, the secondary xylem of apple wood consists of ray parenchyma, axial parenchyma, fiber-tracheids, and vessel elements (Pratt, 1990). The relative proportions of these cell types vary between

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rootstock cultivars in both the roots (Beakbane and Thomsen, 1947) and in the trunks below the graft union (Komarofski, 1947). The relative proportions of each cell type is partially related to the vigor of the rootstock, as more vigorous rootstocks tend to produce more fiber cells and less parenchyma cells than dwarfing rootstocks.

While fewer fibers are generally found in dwarfing rootstocks, an underproduction of fiber cells has been observed in scion/rootstock combinations exhibiting incompatibility at the union, and incompatibility may play a role in the formation of some weak graft combinations (Simons, 1987). Incompatibility has been defined by Andrews and Serrano Marquez (1993) as "the failure of a graft combination to form a strong union and to remain healthy due to cellular, physiological intolerance resulting from metabolic, developmental, and/or anatomical differences." Rather than differentiating into fiber cells, the callus tissues produced at the graft union differentiate into irregularly oriented ray parenchyma cells (Mosse, 1962). Unions of the combination 'Jonagold/Mark' had regions of poorly differentiated parenchyma, and some of these trees broke along a line of this parenchyma tissue (Warmund et al., 1993). A decreased proportion of fiber cells at the union may lead to weaknesses of young nursery trees.

Visualizing a large portion of the union may allow for further understanding of the causes of structural weaknesses between scion/rootstock combinations. Anatomical work to visualize the entire graft union has been performed on apple (Warmund et al. 1993) and grape (Milien et al., 2013) using magnetic resonance imaging (MRI) and X-ray computed tomography (CT-Scan) respectively. In laser ablation tomography, a laser beam ablates samples while images are simultaneously captured. These images are then layered back together to form a three-dimensional model of the sample (Chimungu et al., 2015). Laser ablation tomography is a method that may also allow for the imaging of a large section of the union, and may help to determine the cause of weakness in young trees.

The purpose of this study was to investigate the cause of weak unions in three scion/rootstock combinations that are known to be prone to graft failure ('Honeycrisp'/'M.26 EMLA', 'Cripps Pink'/'G.41', and 'Scilate'/'G.41') and to evaluate anatomical methods for determining union strength that may be employed to identify weak combinations in the future.

Materials and Methods

Sample Preparation. In Feb. 2014, finished chip-budded apple trees were received from Willow Drive Nursery, Ephrata, WA. These were budded in 2012, and included six trees each of 'Cripps Pink' on the rootstocks 'G.41' and 'M.9 NAKB T337' and 'Scilate' on the rootstocks 'G.41' and 'M.9 NIC29'. In Apr. 2014, additional chip-budded trees were received from Adams County Nursery, Aspers, PA. These included ten trees each of the cultivars 'Honeycrisp' and 'Zestar!' on the rootstocks 'M.26 EMLA' and 'M.7 EMLA'. All trees were kept at 6 ° C until sampling. Weak combinations consisted of 'Cripps Pink' and 'Scilate' on the 'G.41' rootstocks, and 'Honeycrisp' on the 'M.26 EMLA' rootstock. Strong trees included 'Cripps Pink' and 'Scilate' on the 'M.9' rootstocks, 'Honeycrisp' on 'M.7 EMLA', and 'Zestar!' on both the 'M.26 EMLA' and 'M.7 EMLA' rootstocks.

Beginning in May 2014, trees were cut using a circular saw to 10.0cm in length from 7.0cm below to 3.0cm above the union, and then sectioned to 3.0-4.0mm thick longitudinal sections using a band saw. Two longitudinal sections from the center of the tree were kept for use in the following studies (Figure 1).

Fiber Cell Walls. Six trees of each combination were utilized in the experiments. Following the initial sample preparation, sections were placed in water for three to seven days to soften the wood tissue for hand sec-

Fig. 1: Initial cuts of nursery trees produced 10cm long, 4mm thick longitudinal sections from 3cm above the top of the union to 7cm below the union. The longitudinal sections closest to the center of the tree were kept for the experiments. Sections were then cut transversely, and hand sectioned from 7cm below, at, and 3cm above the top of the union for microscopy studies.

tioning. Two replicates from the Pennsylvania nursery were kept in 70% ethanol for 38 and 27 days before being moved into water for five and six days, respectively.

After softening, the longitudinal sections were hand sectioned transversely to 12.0mm^2 from three different areas of the section: 7.0cm below the union, at the union, and 3.0cm above the union. The phloem tissue was removed from the outer edge of these blocks to facilitate hand sectioning of the xylem. Sections were placed in two drops of distilled water on glass microscope slides. Sections were then stained with 1% toluidine blue for one minute and rinsed with distilled water before cover slips were applied.

Sections were examined at 400x magnification with an Olympus® CX-41 compound microscope (Olympus Inc., Tokyo, Japan). Photomicrographs were taken using an Olympus® DP-72 digital camera connected to the microscope and Olympus® Cellsens Standard software was used for image capture and data gathering. Fifty radial fiber cell walls were measured from the middle lamella to the lumen of the cell using a measuring tool in Cellsens. Cell walls were measured from each area of the tree section (below, at, and above the union) and were subsequently averaged.

Statistical analysis was performed using the aov command in R (R Foundation for Statistical Computing, Vienna, Austria). Data from the different nurseries were considered different experiments and were analyzed separately. Each experiment was analyzed as a 2 x 2 factorial in a completely randomized design, with two cultivars and two rootstocks. A two-way ANOVA was performed, to test main effects and the interaction. For

cell wall thickness above the graft union of the Washington nursery trees, the interaction was significant. In this case the testInteractions function from the R package "phia" (Martinez, 2015) was used to compare rootstocks within each cultivar and to compare cultivars within each rootstock.

Xylem Cell Proportions. Six replications of the 'Honeycrisp' and 'Zestar!' combinations were utilized in this experiment. Samples were sectioned, stained, and imaged at 200x magnification using the same microscope/camera/software system previously described. Xylem cells were divided into three tissue types based on their function within the wood: fibrous tissue, parenchymatous tissue, and conductive tissue. Percentages of the three types of tissue were determined using ImageJ image analysis software (National Institutes of Health, Bethesda, Maryland) (Rasband, 2014). The parenchymatous and conductive cells were traced manually, while fibrous tissues were estimated by subtracting the two former measurements from the total area of the photomicrograph. Statistical analysis was performed using the aov command in R as previously described.

Laser Ablation Tomography. Four replications of each of the 'Honeycrisp' and 'Zestar!' combinations were used. After the initial sample preparation procedure, sections were cut to a width of 2.5cm to fit within the field of the laser beam. Sections were stored in 70% ethanol for at least one week, and were ablated using an AVIA 7000 355mm pulsed laser (Coherent Inc., Santa Clara, CA). Images were taken at 100.0µm intervals to either 2.5cm or 3.0cm in length from top to bottom. Images were captured using a Canon® T3i camera (Canon Inc., Tokyo, Japan) with a Canon MP-E 65mm 5x micro lens, reduced to 1x zoom to capture a greater field of view.

Images were stacked to create 3D models of the sections using Avizo™ imaging software, (FEI Company, Hillsboro, OR). Samples were visually inspected for the development of callus parenchyma tissue, irregularly oriented xylem, and areas of necrosis.

Results and Discussion

Fiber Cell Walls. In the Pennsylvania trees, the type of rootstock and cultivar had a significant effect on cell wall thickness in different regions of the tree, and the interactions were not significant (Table 1). Tree combinations on 'M.26 EMLA' had thinner cell walls than those on 'M.7 EMLA' below and at the graft union (Table 2). 'Honeycrisp' combinations had thicker cell walls than 'Zestar!' at the union.

For the Washington nursery trees, the type of rootstock significantly affected cell wall thickness (Table 1). Trees grafted to 'G.41' had thinner cell walls below and above the graft union. There were no significant differences at the graft union. Cell wall thickness differed significantly between cultivar treatments above the graft union, as trees of the 'Scilate' cultivar produced thinner fiber cell

Table 1. *P*-values from analysis of variance for rootstock (R) and cultivar (C) effects on fiber cell wall thickness 7cm below, at, and 3cm above the graft union in tree combinations from Pennsylvania and Washington nurseries.

Nursery	Treatments and Interactions	Below the Union	At the Union	3cm Above the Union
Pennsylvania	R	0.004**[z]	<0.001***	0.938
	C	0.412	0.029*	0.110
	R*C	0.186	0.422	0.875
Washington	R	<0.001***	0.163	0.017*
	C	0.158	0.324	0.021*
	R*C	0.911	0.569	0.021*

[z] Significant statistical differences are indicated by asterisks: *p<0.05, **p<0.01, ***P<0.001.

Table 2. Mean fiber cell wall thicknesses (µm) 7.0cm below, at, and 3.0cm above the unions of Pennsylvania nursery graft combinations by rootstock and cultivar.

	7cm Below	At Union	3cm Above
Rootstock			
'M.7 EMLA'	3.81a[z]	3.97a	3.88
'M.26 EMLA'	3.50b	3.66b	3.87
Cultivar			
'Zestar!'	3.61	3.72b	3.79
'Honeycrisp'	3.69	3.91a	3.96

[z] Means followed by different letters within a column indicate significant differences as determined by the ANOVA F-value at p=0.05.

Table 3. Mean fiber cell wall thicknesses (µm) 7.0cm below, at, and 3.0cm above the unions of Washington nursery graft combinations by rootstock and cultivar.

	7cm Below	At Union	3cm Above
Rootstock			
'M.9'	3.81a[z]	3.58	3.69a
'G.41'	3.31b	3.34	3.33b
Cultivar			
'Cripps Pink'	3.47	3.54	3.68a
'Scilate'	3.65	3.38	3.33b

[z] Means followed by different letters within a column indicate significant differences as determined by the ANOVA F-value at p=0.05.

Table 4. Analysis of interaction means for rootstock and cultivar effects on mean fiber cell wall thickness (µm) 3cm above the graft unions of Washington nursery trees. *P*-values are from ANOVA tests of each rootstock within each cultivar, and each cultivar within each rootstock.

Rootstock	'Cripps Pink'	'Scilate'	P-value
M.9	4.04	3.34	0.004*
G.41	3.33	3.33	0.992
P-value	0.003*	0.946	

[z] Significant statistical differences are indicated by asterisks: *p<0.01.

walls than 'Cripps Pink' (Tables 1 and 3). There was an interaction between rootstock and cultivar in the cell wall thickness above the graft union (Table 4). The fiber cell walls in the scion wood of 'Cripps Pink were thinner when grafted on 'G.41' compared to 'M.9', while the fiber walls of 'Scilate' did not differ when propagated on different rootstocks.

In a previous study (Doley 1974), the wall thickness of fiber cells within the scions of the combination 'Cox's Orange Pippin'/'MM.104' were significantly thinner when trees were grafted to the very dwarfing interstock 'M.20'. Our results support the findings that rootstock differences could lead to anatomical changes within other regions of the tree, as fiber cell wall thickness varied above the unions of 'Cripps Pink' when propagated on differing rootstocks.

'M.26 EMLA' produces a more dwarfing tree than 'M.7 EMLA', and is consistent with Doley's findings that dwarfing rootstocks may produce thinner fiber cell walls.

However, 'G.41' produced thinner cell walls than 'M.9', even though these rootstocks are in a similar size category (Marini et al., 2014).

While differences in wall thickness existed above and below the unions, there were few clear trends in the data between cell wall thickness and the combinations that have been reported weak in the field. Combinations on the weaker rootstock 'M.26 EMLA' had thinner cell walls below and at the union, and combinations on 'G.41' had thinner walls below and above the union, but combinations of 'Honeycrisp' had thicker cell walls than 'Zestar!' at the union, even though 'Honeycrisp' is considered the weaker cultivar. These findings suggest cell wall thickness may not be an appropriate measure of union strength in young trees.

Xylem Cell Proportions. Significant differences in the distribution of fiber and parenchyma tissues were observed between rootstock treatments (Table 5). 'M.26 EMLA' combinations contained significantly less fiber and more parenchyma tissue than 'M.7 EMLA' combinations (Table 6). Previous studies have found that more dwarfing rootstocks tend to have higher proportions of parenchyma and fewer fiber cells within their wood (Beakbane and Thompson, 1947), and our results with new cultivars agree with these findings.

Cultivar significantly affected the percentages of wood tissues (Tables 5 and 6). 'Honeycrisp' combinations contained significantly more parenchyma tissue and less fiber and conductive tissues than 'Zestar!' combinations. Like dwarfing rootstocks, the 'Honeycrisp' cultivar is considered a weak growing cultivar (Robinson et al., 2011), and may help to explain its decreased production of fiber cells at the union compared to trees of the 'Zestar!' cultivar.

The combination of 'Honeycrisp'/'M.26 EMLA' had the most parenchyma tissue and the least fiber (47.11 and 46.08 percent respectively), whereas the combination of 'Zestar!'/'M.7 EMLA' had the least parenchyma and most fiber (22.29 and 65.65 percent, respectively). The ratio of parenchyma to fiber cells in the 'Honeycrisp'/'M.26 EMLA' combination was 1.02, while

Table 5. *P*-values from analysis of variance for rootstock (R) and cultivar (C) effects on the proportions of parenchymatous, fibrous, and conductive tissue at the unions of tree combinations from Pennsylvania nurseries.

Treatments and Interactions	Parenchymatous	Fibrous	Conductive
R	0.021*[z]	0.041*	0.362
C	0.001**	0.012*	0.017*
R*C	0.967	0.775	0.517

[z] Significant statistical differences are indicated by asterisks: *p<0.05, **p<0.01.

Table 6. Percentages of wood tissues by rootstock and cultivar in the graft unions of the Pennsylvania nursery trees.

	Parenchyma	Fiber	Conductive
Rootstock			
'M.7 EMLA'	29.78b[z]	59.61a	10.61
'M.26 EMLA'	39.79a	50.98b	9.23
Cultivar			
'Zestar!'	27.38b	60.76a	11.85a
'Honeycrisp'	42.19a	49.83b	7.98b

[z] Means followed by different letters within a column indicate significant differences as determined by the ANOVA F-value at p=0.05.

other combinations varied from 0.34 to 0.70.

An increase in the amount of parenchyma relative to fiber cells at the union may create a weak point at the union where trees are more likely to break (Warmund et al., 1993). However, since dwarfing rootstocks are prone to producing less fiber cells, this may have caused the difference we saw between our study trees. This complication suggests this method may not be useful when comparing rootstocks across different size and vigor categories. Our subsequent study also found that tissues at the union can be very variable, making this method unlikely to be useful for determining future weak scion/rootstock combinations.

Laser Ablation Tomography. Callus parenchyma tissue was present in all combinations between the rootstock and scion (Figure 2 & 3). Swirling tissue was

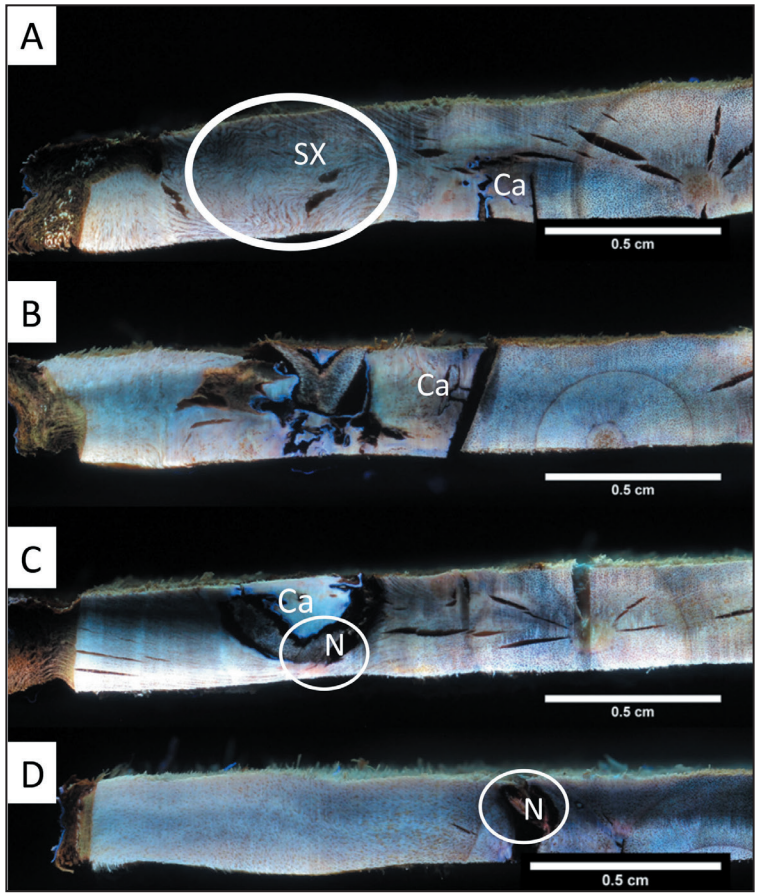

Figure 2. Transverse sections of wood from 'Honeycrisp'/'M.26 EMLA' (A) 'Honeycrisp'/'M.7 EMLA' (B) 'Zestar!'/'M.26 EMLA' (C) and 'Zestar!'/'M.7 EMLA' (D) with the scions on the left and rootstocks on the right. The wood tissue of 'Honeycrisp'/'M.26 EMLA' shows a large area of swirling xylem (SX) tissue within the subsequent year of growth. In 'Honeycrisp'/'M.7 EMLA', necrotic wood (N), callus tissue (Ca), and bark-like tissue can be seen. In 'Zestar!'/'M.26 EMLA', an area of necrosis surrounded by callus tissue can also be observed. 'Zestar!'/'M.7 EMLA' also shows a small section of bark-like necrotic tissue. Fragments of the callus tissue that initially bridged the gap between the rootstock and scion can be seen within the unions of 'Honeycrisp'/'M.26 EMLA' and 'Honeycrisp'/'M.7 EMLA'.

commonly observed in the scion adjacent to the union and in areas of callus parenchyma proliferation. A very large section of swirling xylem extended into the following season's growth in one sample of 'Honeycrisp'/'M.26 EMLA' (Figure 2A).

For 'Honeycrisp'/'M.7 EMLA', 'Zestar!'/'M.26 EMLA', and 'Zestar!'/'M.7 EMLA', one sample of each contained a large area of necrotic tissue. For 'Honeycrisp'/'M.7 EMLA', the tissue around this necrotic wood consisted mostly of callus tissue, which extended towards the outer growth of the union. 'Honeycrisp'/'M.7 EMLA' also appeared to have a few large areas of parenchyma tissue. Tissue that resembled bark was also

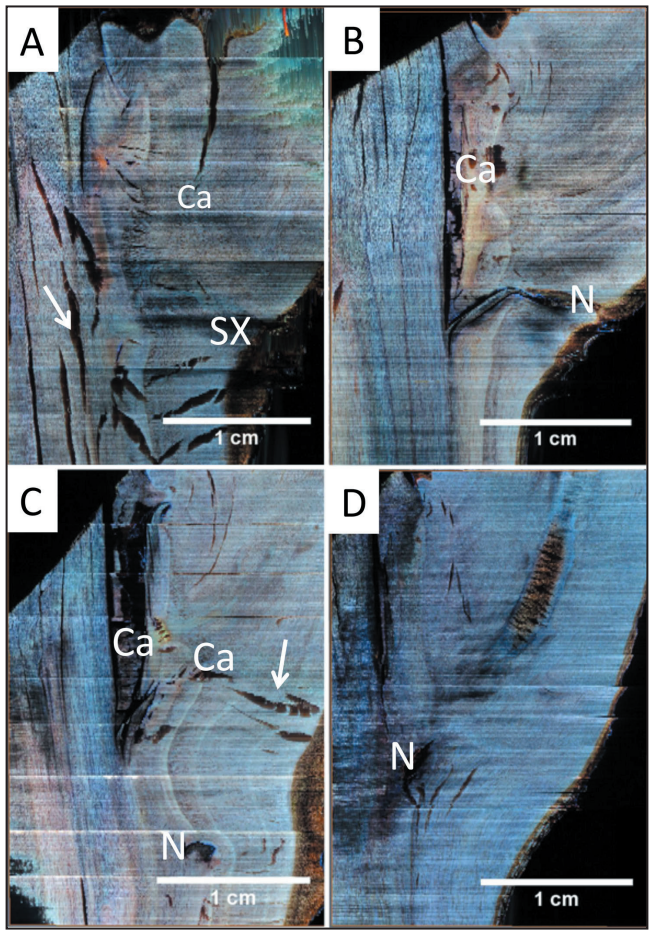

Figure 3. Unions of 'Honeycrisp'/'M.26 EMLA' (A), 'Honeycrisp'/'M.7 EMLA' (B), 'Zestar!'/'M.26 EMLA' (C) and 'Zestar!'/'M.7 EMLA' (D) in longitudinal view with the rootstock on the left and the scion portions on the upper right. Swirling xylem (SX) appears at the middle of the union extending towards the bark in 'Honeycrisp'/'M.26 EMLA'. 'Honeycrisp'/'M.7 EMLA', 'Zestar!'/'M.26 EMLA', and 'Zestar!'/'M.7 EMLA' appear to have isolated areas of necrosis (N). Callus tissues (Ca) and empty spaces surrounding them between the rootstock and scion can be easily distinguished in 'Honeycrisp'/'M.7 EMLA' and 'Zestar!'/'M.26 EMLA'. The wood tended to split at this callus layer during the ablation process, producing these gaps. An additional small area of callus is seen in 'Zestar!'/'M.26 EMLA'. Open spaces further down the union of 'Honeycrisp'/'M.26 EMLA' and in 'Zestar!'/'M.26 EMLA' (arrows) were very thin gaps also likely caused by the ablation process.

present (Figure 2B and Figure 3B). In one 'Zestar!'/'M.26 EMLA' sample, the vascular system had a small region of callus disrupting the xylem at the union, though normal xylem growth soon began to differentiate from it (Figure 3C). A region of necrotic tissue surrounded by wound callus was also observed further down the union as well (Figure 3C). A sample of 'Zestar!'/'M.7 EMLA' had a necrotic zone where new wood tissue was growing around what appeared to be remnant bark material (Figure 2D).

In terms of previous descriptions of incompatibility provided by Mosse (1962) and Andrews and Serrano Marquez (1993), we found a large area of swirling xylem tissue within the wood of one sample of 'Honeycrisp'/'M.26 EMLA', but also found regions of poor differentiation in the other combinations that are not prone to breaking in the field. Warmund et al. (1993) and Milien et al. (2012) found regions of vascular discontinuity within poor growing graft unions of apple and grape, but our observations suggest it may be difficult to determine union continuity and strength based on anatomical observations alone when trees are young in the nursery, as the tissues are still very variable across the scion/rootstock combinations, and irregularities in the wood can be found in weak and strong combinations.

We were unable to achieve cellular resolution using laser ablation tomography due to the size of our samples. While cellular level traits can be determined on small samples, such as maize roots (Chimungu et al., 2015), the size of the unions and the woody tissue made samples difficult to ablate and image to achieve cellular resolution.

Conclusions

The anatomical features of weak wood in three commercially important scion/rootstock combinations were investigated using light microscopy, laser ablation tomography, and imaging software. This is the first such report for a Geneva rootstock and for three new cultivars.

Fiber cell wall thickness varied between rootstocks below, at, and above the graft unions, and varied between cultivars at the union. Trees on 'M.26 EMLA' had thinner fiber cell walls below and at the union, and trees on 'G.41' rootstocks had thinner fiber cell walls below and above the union. However, the weak cultivar 'Honeycrisp' had significantly thicker fiber cell walls at the union than the strong variety 'Zestar!', suggesting that fiber cell wall thickness may not be useful for determining weaknesses in young nursery trees.

Scion/rootstock combinations tended to have less fiber cells at the graft union when propagated on 'M.26 EMLA' rootstocks and when 'Honeycrisp' was the cultivar. However, since we did not have a strong graft combination on a dwarfing rootstock to compare against, it is difficult to determine if strong, more dwarfing combinations would have more or less fiber cells. Additionally, as our laser ablation study suggests, tissues at the graft union can be extremely variable at a young age, making this method an unlikely candidate for determining graft strength of future scion/rootstock combinations.

Laser ablation tomography provided a larger view of the union, and showed that characteristics commonly described as features of weak combinations could be observed in some combinations not prone to graft failure in the field. Laser ablation tomography appears to be an unsuitable method for observing the cellular level anatomy of large samples of woody tissue.

The proceeding experiments suggest that while many anatomical variables have been associated with the development of weak unions, these factors may be difficult to interpret due to the variability of the tissues at the graft union in young nursery trees.

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About The Cover:

Rubus parvus Buch and *Rubus* Hybrid 'Triple Crown' Blackberry

Rubus parvus Buchanan fruit and leaves in the center. Rubus hybrid 'Triple Crown' blackberry on left and right. Both are tetraploid (2n = 4x = 28) chromosomes, though *R. parvus* has a much smaller genome.

Photo by Kim Hummer.

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Effect of the Seedlessness (Fs) Gene in Fruit Quality Traits in Mandarin Segregating Populations

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Additional index words: Kishu, Mukaku Kishu, seedless, triploid, dominance, SSC, xenia, metaxenia

Abstract

Xenia and metaxenia effects can be responsible for variation in fruit size, fruit shape, and sugar content in fruit. In the process of developing new mandarin citrus hybrids, the University of Florida Fruit Tree Breeding Program produced four populations segregating for the seedlessness gene *Fs*. The objective of this research was to determine if the presence or absence of seed had xenia-like effects on the mandarin hybrids. The four populations contained a total of 213 trees. The fruit produced by these trees were evaluated by sampling three random fruit and measuring the soluble solids concentration (SSC) of each fruit. Additionally, the fruit were scored for the presence or absence of a fruit neck at the stem end. There were no statistically significant differences between seedless and seeded offspring in the four hybrid populations for fruit weight (g) or SSC (% w/w). The "neck" phenotype also appears to be controlled by a single locus and follows a Mendelian segregation ratio of 3:1 (neck: flush). These results support the use of the seedless gene *Fs* without negative effects on fruit size and sugar concentration in the resulting progeny.

Seedlessness is an important trait in many fresh fruit crops. Consumers desire seedless fruit in a number of fruit crops including grapes, watermelon, and citrus. The seedless trait has been induced in citrus using several techniques, including chromosomal variation, triploidy, self-incompatibility, and mutants affecting seed development (Khan, 2007).

Self-incompatibility coupled with parthenocarpy has been used in citrus to produce seedless cultivars. One such notable example is 'Clementine' mandarin *Citrus reticulata* Blanco.'Clementine' plants must be grown in isolated blocks to minimize the number of seed per fruit (Spiegel-Roy and Goldschmidt, 1996). Another method to produce seedlessness is to apply gibberellins 1-14 days after flowering (DAF) (García-Martínez and García-Papí, 1979). In some hybrids, such as 'Orlando' Tangelo and 'Imperial', the reduction in fruit size is so severe that the fruit is unmarketable (Wallace and Lee, 1999; Wallace et al., 2002).

The effects of seedlessness on other fruit characteristics such as fruit size are due to xenia, or the effect of the pollen source on the seeds of the fruit. In addition, seedlessness could also be due to metaxenia, which refers to the effect that the pollen source may have on any structure outside of the embryo and endosperm. This means any tissues derived entirely from the mother plant (Denney and Martin, 1990). These effects have been shown to occur in several citrus interspecific crosses. 'Ellendale' tangor experienced changes in fruit set, fruit size, and seed count depending on the pollen donor cultivar (Vithanage, 1991). Similar changes occurred in cultivars such as 'Minneola', 'Orlando', 'Page', and 'Robinson' (Futch and Jackson, 1993; Hearn et al., 1968). For example, the use of specific pollinators increases fruit set in Clementines and is associated with greater early ovary growth due to increased size of fertilized ovules (García-Papí and García-Martínez,1984).

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Another technique for obtaining seedless mandarin hybrids is the creation of triploids, such as 'Tahoe Gold' from University of California Riverside (Chao, 2005). Important problems with many citrus triploids include low fruit set and thorniness (Khan, 2007).

Citrus kinokuni 'Mukaku kishu' is a completely seedless bud sport of the seedy kinokuni mandarin (Nesumi et al., 2001). Seedlessness was produced by female sterility resulting from arrested embryo development. Two genes were responsible for the abortion of the zygote, a *Fs* dominant gene and an *Is* repressor gene. The *Is* repressor gene inhibits the expression of the seedless trait (Yamasaki et al. 2009; Chavez and Chaparro, 2011).

At the Fruit Tree Breeding Program at the University of Florida (Gainesville , FL), *C. kinikuni* 'Mukaku kishu' has been crossed with two advanced breeding lines of seedy citrus. The objective of this research was to understand if there are any xenia-like effects for fruit size and soluble solids content (SSC) between seeded and seedless individuals in the populations.

Materials and Methods

Plant material. In fall 2013, a total of 213 ten year-old F_1 individuals from two breeding populations segregating for genetic seedlessness *Fs* were used in this study. Breeding selections Robinson OP 'GS' and 'G96-01' were used as female parents in crosses with *Citrus kinokuni* 'Mukaku kishu' PI539530 at the Fruit Tree Breeding Program at the University of Florida, Gainesville, FL. Segregating populations were planted, maintained, and grown following standard commercial production practices in Florida.

Phenotypic studies. Populations were evaluated for a period of 3-4 fruiting seasons to confirm presence or absence of seeds [as previously reported by Chavez and Chaparro (2011)]. Additional fruit phenotypic characteristics, fruit weight (g), SSC (%), and presence (neck)/absence of a neck (flush) at the fruit stem end, were evaluated in at least three fruit per genotype for one season. Fruit was harvested and evaluated on-site using a handheld refractometer (Cat. no. FS1394621, Thermo Fisher Scientific, Waltham, MA) and a portable OHAUS™ Scout™ Pro Series Electronic Toploading Balances (OAHUS corporation, Parsippany, NJ) to measure SSC and fruit size, respectively.

Data analysis. The Mendelian segregation ratios for seedlessness and the presence/absence of neck in the F_1 progeny were calculated using the Chi-square 'goodness-of-fit' test. Analysis of variance (ANOVA) was performed using SAS's PROC GLM procedure (Statistical Analysis System Version 9.1, SAS Institute, Cary, NC). Means for weight and SSC were compared with Tukey's test (*p-value* <0.05). Correlations between fruit weight and SSC were calculated using the PROC CORR procedure of SAS.

Results

For the Robinson OP 'GS' × *C. kinokuni* segregating population, seedless (*Fsfs*) fruits had higher SSC than seedless/seeded (leaky) fruit and seeded (*fsfs*) fruit were intermediate (Table 1). For the 'G96-01' × *C. kinokuni* family, both seedless and seeded genotypes

Table 1. Fruit weight and soluble solids concentration of Robinson OP 'GS' × *C. kinokuni* segregating population for genetic seedlessness *Fs* as separated by presence (*Fsfs*) or absence (*fsfs*) of seeds.

Phenotype	Genotypes (no)	Fruit (no)	Weight (g)	SSC (%)
Seedless *Fsfs*	82	227	96.9 a[z]	9.1 a
Seeded *fsfs*	84	241	102.8 a	8.9 ab
Seedless/Seeded[y]	12	36	106.4 a	8.8 b

[z] Similar letters within a column indicates means not significantly different, Tukey's test, α=0.05.

[y] Seedless/Seeded represented genotypes that contain one or traces of seeds.

Table 2. Fruit weight and soluble solids concentration of 'G96-01' × *C. kinokuni* segregating population for genetic seedlessness *Fs* as separated by presence (*Fsfs*) or absence (*fsfs*) of seeds.

Phenotype	Genotypes (no)	Fruit (no)	Weight (g)	SSC (%)
Seedless *Fsfs*	18	49	91.4 a[z]	10.0 a
Seeded *fsfs*[y]	17	44	127.6 a	9.9 a

[z] Similar letters within a column indicates means not significantly different, Tukey's test, α=0.05.
[y] Seedless/Seeded genotypes were not included in the analyses because only one was identified in this population.

had similar SCC (Table 2). Seeded/seedless (leaky) individuals were hybrids that presented minute traces of seeds or one/two small seeds in the flesh. The average SSC of the seedless (*Fsfs*) was higher than that of the seedless/seeded (leaky) individuals for the Robinson OP 'GS' ×*C. kinokuni* segregating population (Table 1). There was no difference in fruit weight between the three types.

A histogram showing the distribution of the fruit weight from the Robinson OP 'GS' × *C. kinokuni* 'Kishu' family (Fig. 1) showed that there was little difference between the averages of the seeded (101.0g) and seedless (98.4g) types. Additionally, SSC was similar for the two types in this family (Fig. 2).

The presence or absence of the neck at the stem end of the fruit did not deviate from a Mendelian segregation of 3:1 (neck/no neck; Fig. 3) for both segregating populations (χ^2=0.31). It is difficult to determine the nature of the allelic gene composition for both parents because no additional test crosses were made.

The presence or absence of the enlarged neck in progeny of the Robinson OP 'GS' x *C. kinokuni* 'Mukaku kishu' PI539530 progeny did not affect in the overall fruit size and

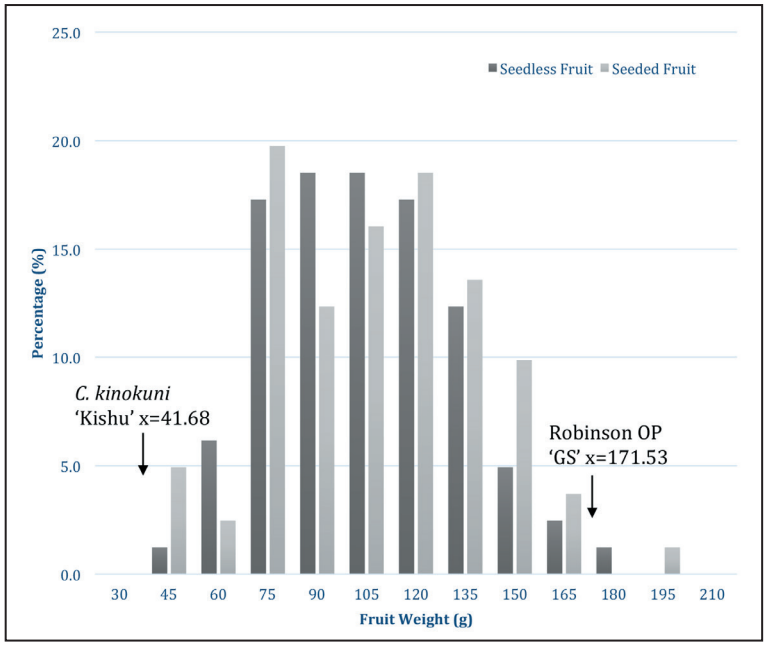

Fig. 1. Fruit size (g) distribution in segregating population between breeding selection Robinson OP 'GS' and *Citrus kinokuni* 'Mukaku kishu' PI539530.

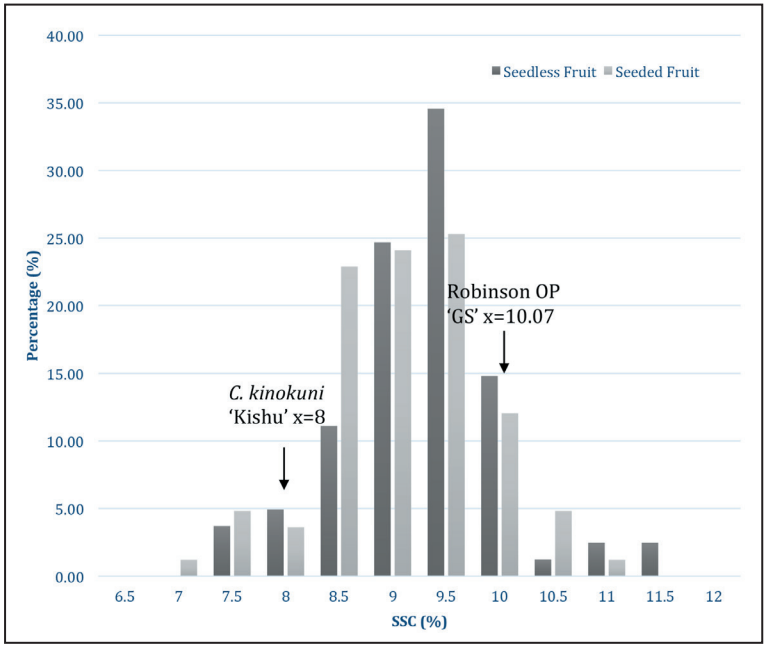

Fig. 2. Soluble solids concentration (%) distribution in segregating population between breeding selection Robinson OP 'GS' and *Citrus kinokuni* 'Mukaku kishu' PI539530.

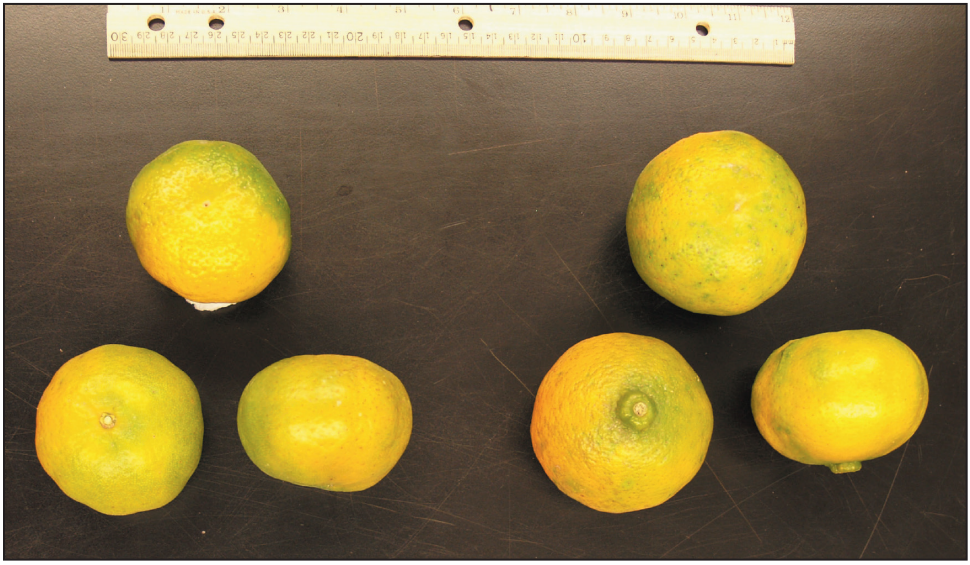

Fig. 3. Absence (left) or presence (right) of a neck at the stem end of fruit from progeny of the segregating population of Robinson OP 'GS' and *C. kinokuni* 'Mukaku kishu' PI539530.

Table 3. Fruit weight and soluble solids concentration of Robinson OP 'GS' × C. kinokuni segregating population for genetic seedlessness Fs as separated by presence or absence of fruit neck.

Phenotype	Genotypes (no)	Fruit (no)	Weight (g)	SSC (%)
Neck	138	396	100.31 a[z]	8.95 a
Flush (no neck)	40	108	100.70 a	9.05 a

[z]Similar letters within a column indicates means not significantly different, Tukey's test, α=0.05.

SSC (Table 3). However, this characteristic was associated with the mean fruit weight in the 'G96-01' ×*C. kinokuni* 'Mukaku kishu' PI539530 segregating population, with fruit from genotypes with enlarged neck having an average weight of 90.7g in comparison with their flush counterparts of 137.9g. Similarly, fruit from genotypes with a neck had a SSC of 9.7 in comparison with fruit with no neck with an average SSC of 10.2. For all the segregating populations SSC was not correlated with fruit weight.

Conclusions

In the pursuit of developing a seedless citrus cultivar with a heritable seedless trait, it is important to identify and understand any affects that this trait may have on fruit size and sugar content. The research presented shows that the four families of F1 breeding populations segregating for the seedless (*Fsfs*) trait, have no significant difference in fruit weight (g) or SSC (%) from their seeded counterparts. In addition, the presence of a neck at the stem segregated in a 3:1 (+/-) fashion among these populations. This trait had no consistent effect on the measured parameters in this study.

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NC-140 Multi-State Research Project: Improving Economic and Environmental Sustainability in Tree-Fruit Production Through Changes in Rootstock Use

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Abstract

The North Central Project 140 (NC-140) was established in the mid-1960s to facilitate evaluation of apple rootstocks and interstem trees in the north central region of the U.S. over the years, the project has grown to include cooperators from more than 20 states, four Canadian provinces and one Mexican state. The project played a major role in the rapid adoption of intensive orchard systems by the North American apple industry. This paper summarizes the history, accomplishments, participants, and potential future of the project.

History U.S. Rootstock Research Related to NC-140. The Morrill Act of 1862 established land grant universities to teach agriculture, mechanics, military science and classical studies.In 1887, the Hatch Act provided funds to the land grant institutions to establish agricultural experiment stations. The Research and Marketing Act of 1946 was passed by the U.S. Congress and signed into law by President Truman. The Act earmarked 25% of Federal Hatch funds to state experiment stations specifically for regional research. Effectively, this act resulted in the organization of the four regional experiment station associations: South (SAAED – 1946), Northeast (NERA – 1947), North-Central (NCRA – 1947), and Western (WAAESD – 1948). All Regional (now Multi-State) Projects are proposed, approved, and administered by one of the regional associations cooperatively with the Cooperative State Research, Education and Extension Service of the U.S. Department of Agriculture (CSREES, formerly CSRS).

The North Central Project 140 (NC-140) is one of many Multi-State projects authorized by CSREES. This project began in the mid 1960's when several scientists formed NC-78, a North-Central Region study to evaluate rootstocks for horticultural plants. NC-78 was approved for two cycles. However in 1970, the experiment station directors were concerned about approving projects knowing the proposed cooperative trials would extend well beyond the project period. Those researchers interested in rootstocks continued to meet under the structure of a North-Central Region coordinating committee, NCR-82, Stock/Scion Relations in Horticultural Plants, while working on a new project proposal. For six years, scientists from Alaska, Illinois, Indiana, Iowa, Kansas, Kentucky, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, Vermont, and Wisconsin continued to meet annually. They also worked with the International Dwarf Fruit Tree Association (IDFTA) to further rootstock research

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through the development of an IDFTA Rootstock Research Committee and annual funding in support of rootstock research.

NCR-82 initiated the first cooperative apple rootstock/interstem research trial planted at 10 locations in 1976. This lead to a successful proposal in 1977 for a full project called NC-140, entitled "Scion/Rootstock and Interstem Effects on Apple Tree Growth and Fruiting."

Dr. Richard Hayden from Purdue University chaired the first meeting of the NC-140 committee in August 1977 with Dr. James Cummins from Cornell University hosting at the New York State Agricultural Experiment Station in Geneva, NY. Members included scientists from Illinois, Indiana, Iowa, Kansas, Kentucky, Massachusetts, Michigan, Missouri, New York, Ohio, and Wisconsin. Arkansas, Minnesota, Oregon, and Vermont participated at the beginning but became members in subsequent years. Cooperators from Ontario and Quebec, Canada, also participated at the beginning of the project. See Table 1 for committee membership throughout its history.

The NC-140 committee coordinated the trial established under NCR-82. It included 'Delicious' and 'Empire' on M.9 interstems with Antonovka, MM.111, and Ottawa 11 as rootstocks. Uniform protocols for tree management and data collection were developed, and all data were compiled and analyzed by Drs. David Ferree and Bert Bishop at The Ohio State University.

At the first meeting in 1977, planning began for a uniform apple rootstock trial, scheduled for planting in 1980. It was successfully implemented and has led to 20 additional apple rootstock/interstem trials under the direction of the NC-140 committee. The first renewal of the NC-140 proposal (1982-87) expanded the objectives to include stone fruit, with the first uniform NC-140 peach trial planted in 1984. Four additional peach trials have been established. Uniform sweet and sour cherry rootstock trials were planted in 1987, a pear rootstock trial was planted in 1988, and a plum rootstock trial was planted in 1990. Four additional uniform cherry and three additional pear trials have been established. The NC140 project, to date, has established 38 uniform trials over the 30 years of its existence.

The current NC-140 project, "Improving Economic and Environmental Sustainability in Tree-Fruit Production Through Changes in Rootstock Use," has 40 regular participants from 22 states, 2 USDA facilities, 2 Mexican locations, 3 Canadian provinces, and Chile joined in 2015. In 1987, two NC-140 members edited and led a group of authors in writing a book on rootstocks where much of the information was a culmination of knowledge gained from NC-140 trials (Rom and Carlson, 1987). Seven of the 15 authors contributing to this book, titled "Rootstocks for Fruit Crops", were NC-140 members.

NC-140 Objectives at the Beginning and Now. Prior to the first NC-140 project, knowledge of rootstock performance was based upon unrelated studies. Results often varied from state to state, and there was little chance of isolating the influences of climate, soil and tree management. NC140's founders wished to shorten and greatly enhance the evaluation process through the uniform testing of rootstocks over a wide range of climatic and soil conditions. They recognized a burgeoning interest among orchardists in trees on dwarfing rootstocks; however, they were particularly interested in finding a rootstock or interstem that would result in a free-standing, semi-dwarf to dwarf sized tree. They also were looking for rootstocks that were easy for the nursery to propagate and ones that tolerated biotic and abiotic stresses in the orchard. The first NC-140 project (1977-82) had three specific objectives:

1. To evaluate the production efficiency of rootstock and interstem materials now available and any additional such materials which may become available which are potentially precocious, dwarfing, free standing, easy to propagate, disease re-

sistant, and adapted over the wide range of climatic conditions which exist in the many fruit areas of the United States.
2. To determine the propagation practicability of new rootstock and interstem material and to ascertain the anatomical factors in plant material combinations that are associated with compatibility.
3. To ascertain the cause and prevent the decline of apple trees on new and existing rootstocks and interstems and to evaluate the influence of various cultural practices on rootstock survival and performance.

In 30 years, the orchard industry has changed dramatically. Utilization of full-dwarfing rootstocks with support is commonplace and the desire for free-standing dwarf trees has diminished. Orchardists have embraced new training and management systems and are interested in fine-tuning rootstock choices to best fit those systems. NC-140 objectives are similar to the earlier ones, but have changed as orchard management has evolved. Further, objectives on rootstock development and on the physiology of the rootstock/scion interaction have been added. Still, the uniform testing of rootstocks under different climatic and soil conditions remains the backbone of NC-140's research effort. Objectives of the current NC-140 project (2012-2017) are as follows:

1. To evaluate the influence of rootstocks on temperate-zone fruit tree characteristics grown under varying environments using sustainable management systems.
2. To develop improved rootstocks for temperate-zone fruit trees using state-of-the-art genomic tools in breeding programs.
3. To accelerate adoption of new rootstocks (a) by improving propagation techniques and (b) by acquiring new rootstocks from worldwide sources.
4. To better understand the impacts of biotic and abiotic stresses on scion/rootstock combinations in temperate-zone fruit trees.
5. To enhance the sustainability of temperate fruit farming through development and distribution of research-based information utilizing eXtension.

Specific Accomplishments of NC-140. During the past 30 years 38 trials have been conducted by NC140. Upon completion of a project, the data are published in peer-reviewed and trade journals. Approximately 125 peer-reviewed articles have resulted directly from NC-140 trials, and more than 1,500 related articles have been published by NC-140 cooperators. Below is an abbreviated list of information resulting from the project:

- The length of time required to evaluate rootstocks has been reduced tremendously. The uniform trials expose a new rootstock to an extremely wide range of climates and soils, so a new rootstock can be recommended for commercial trial in less than 10 years. Before NC-140 different researchers used different cultivars, tree spacings, training systems, and collected different types of data to evaluate rootstocks, so it was impossible to compare rootstock performance from one location to another. For these reasons, M.9 was still being evaluated in the 1970s although it was brought to North America in the 1920s.
- MARK rootstock was identified as a potential dwarfing rootstock in certain regions of North America, but it performed very poorly in hot arid regions (NC-140, 1991; Marini et al., 2006).
- Budagovski 9 (B.9) was identified as a possible replacement for M.9. Final tree size varies depending upon location. B.9 is quite resistant to fireblight and imparts some resistance to the scion. This led to additional research on genetic control of rootstock/scion interactions (Ferree et al., 2002; Jensen et al., 2012; Gardener et al., 2012)
- Fireblight screening to gauge resistance has been modified. At one time, the bacterium was injected into growing shoot

tips and researchers assumed the amount of dieback was indicative of relative susceptibility. This test indicated that B.9 was quite susceptible, but field observations gave contradictory results. More recently we have learned that young shoots collected from the stool bed and older budded trees may not always respond similarly to inoculation tests. As a result, fireblight screening protocols have been modified (Johnson et al., 2000).

- Seven M.9 clones have been evaluated with clones varying in vigor control. Nic 29 and Pajam 2 are nearly as vigorous as M.26, but Fleuren 56 is more dwarfing than NAKBT337, which is the most widely planted clone of M.9. Therefore growers need to know which clone they are ordering. Additionally, obtaining a range of tree sizes can be accomplished by using various clones of M.9 thus avoiding the use of M.26, which has higher tree mortality in most trials Marini et al., 2006; Autio et al., 2008).
- Nineteen rootstocks from the Cornell-Geneva (G) program have been evaluated. G.30 requires more support than most other rootstocks in that size category. If support is not adequate the trees break at the bud union, especially with brittle cultivars such as 'Gala.' G.41 and G.35 also produce weak bud unions when budded with brittle cultivars.
- The Vineland (V) series may have commercial potential, especially in the southeast because tree survival was much better on V.1 and V.3 than on the Malling (M) rootstocks. This was surprising, because they were selected for northern growing conditions (Marini et al., 2006).
- Apple cultivar-by-rootstock interaction is small. The relative tree size differences among rootstocks are similar regardless of the scion. Therefore, cultivar selection for rootstock studies need not be limited to those varieties which are grown in a specific region (Autio et al., 2001).
- The Gisela series of cherry rootstocks was first tested by NC-140 and Gisela 6 has become the most widely-planted sweet cherry rootstock in the Pacific Northwest. Research results by NC-140 members have been used to develop the information used by growers interested in producing cherries in high tunnels.
- NC-140 research guided propagation of fruit trees by nurseries, allowing them to tailor their production to grower demands and to avoid problematic rootstocks. As an example, a series of cherry rootstocks from Russia were gaining a great deal of interest, but NC-140 workers found them to be hypersensitive to *Prunus* Necrotic Ringspot virus, reducing their suitability for U.S. production.
- Through experience, we have modified the protocols, experimental designs and statistical analyses of our trials to enhance efficiencies in rootstock evaluation.
- Extension and outreach is integral to the NC-140 project. Therefore, research plantings serve as the focus of field days, and results are disseminated quickly and widely as soon as they are available. As an example of the outreach effort, nearly 200 grower-oriented publications were developed, about 450 talks were given, nearly 150 field days were conducted, and more than 50,000 grower contacts were made in the last 5 years to disseminate information from NC-140 projects. The NC-140 website (NC140.org) is another vehicle for distributing rootstock information, and attracts over 20,000 hits per year. Because of the extensive output of NC-140 and the widespread participation, all modern North American recommendations regarding rootstocks, tree training and orchard systems for fruit crops have their basis in NC-140.
- NC-140 has become an important organization for training future generations of pomologists. Graduate students often attend the annual meeting of the NC-140 technical committee and often collect and analyze data associated with NC-140 tri-

als. These activities provide a unique opportunity for young pomologists to network with more experienced pomologists and to learn about fruit production and research activities at the international level.

Impacts of NC-140. It is difficult to quantify impacts of a large project such as NC-140, particularly since they touch every state where temperate tree fruit are grown, the southern Canadian provinces and some areas in Mexico. Further, NC-140 is a major source of rootstock information worldwide. Reasonable estimates of NC-140 impacts are:

- Overall, the work of NC-140 resulted in recommendations and educational programs which guided planting of 170,000 acres of fruit trees over the last five years in the U.S.
- Growers have realized significantly earlier returns on investments related to tree establishment.
- Yields have increased on average 20% per acre in mature orchards, fruit size has improved by10%, and the percentage of fruit meeting the highest grade category increased by 20%.
- The financial benefit to U.S. fruit growers from earlier returns, greater yield, and higher fruit quality was $200,000,000 over the 5-year period.
- Because most new plantings have been primarily in the dwarf category (with a substantially reduced canopy volume per acre), pesticide usage on the new acreage was reduced by nearly 40%, with the associated environmental benefit plus $100,000,000 saved over the 5-year period in pesticide cost and application.
- Tree losses declined by 10% over the 5-year period due to the introduction and planting of disease-resistant rootstocks.
- Individuals from Canada and Mexico are integral to NC-140, therefore expanding its influence throughout the Americas. The project and its output, however, are valued worldwide.
- NC-140 continues to develop advanced experimental design approaches to reduce the costs of rootstock research. Recently we learned that six to seven years are required to accurately assess rootstock vigor rather than the 10-year period that was formerly used (Marini et al. 2016).
- NC-140 cooperators introduced molecular approaches to the breeding programs, enhancing the efficiency of development and selection of the next generations of fruit tree rootstocks.
- Cumulative state and federal investment in NC-140 for the last 5 years was about $5,000,000. Cumulative, measurable benefits to the U.S. temperate tree-fruit industries were more than $300,000,000. Less easily measured benefits, such as averted losses and enhanced environmental quality, certainly increase the financial value of NC-140 to well beyond $300,000,000 in the last 5 years.
- Through links to cooperative extension programs, information generated by NC-140 is rapidly available to fruit growers. Many of the technical committee members have extension appointments and provide information to stakeholders in their states and provinces. In 2013 alone, NC-140 members presented information related to the project at more than 140 grower meetings (http://www.nc140.org/2013/annualreport.pdf). NC-140 has a long-standing close relationship with the International Fruit Tree Association (formerly International Dwarf fruit Tree Association). Many members of the NC-140 have presented updates on rootstock performance at annual meetings of IFTA and have received funding for uniform trials (tree costs) and support of critical research for independent studies on rootstock issues. NC-140 developed a website (http:///www.nc140.org/) more than 15 years ago to make results from the project widely available. The eXtension website (http://www.extension.org/pages/60760/apples-community-

information#,VGzEBckXLlg) was developed by NC-140 members to archive information from NC-140 and apple cultivar trials to make research-based information available to the general public.

In recognition of NC-140's exceptional collaboration and research impacts, NC-140 received the 2015 Experiment Station Section Excellence in Multistate Research Award from the Experiment Station Committee on Organization and Policy.

Future of NC-140 and Other Pomological Research. Due to declining state and federal support for state agricultural experiment stations, applied agricultural research is in jeopardy. Land grant university colleges of agriculture around the country now expect faculty members to externally fund their research. About 10 years ago NC-140 members estimated the cost of maintaining an acre of rootstock plantings at about $4,000 per year. This value was probably conservative because it did not include costs for office space, salaries and fringe benefits, office supplies, staff support, creation and maintenance of the NC-140 website, transportation of cooperators to meetings, and page charges for publishing. Without support from national and international organizations, such as the International Fruit Tree Association (formerly IDFTA), applied research on fruit crops will decline rapidly. As college and department administrators consider replacing vacated pomology positions, one criterion that will be used is the ability to attract grant funding for a world-class research program. If support is not deemed adequate then faculty positions focusing on more basic research may be considered. These positions might be of little immediate help to the industry. Over the next decade we will likely see the number of pomologists decline across the United States, particularly in states where the fruit industry is small or fail to provide substantial research support. States with relatively small fruit industries provide the variety of climatic conditions needed to rapidly test rootstocks. If rootstock research is limited to the major fruit-growing regions, evaluation of new rootstocks to withstand environmental stresses as the climate changes will take much longer.

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Rom, R.R. and R. F. Carlson. 1987. Rootstocks for fruit crops. Wiley-Interscience, ????

Table 1. Administrative advistors and NC-140 participants.

Location	Name of Members	Location	Name of Members
CREES Advisors		*North Central Administrative Advisors*	
		MN	K.A. Huston
USDA	D.R. Tompkins	MI	Ian Gray
USDA	C. Stushnoff	MN	D.A. Holt
USDA	S.C. Wiggans	MN	R.G. Gast
USDA	T. Bewick	MN	R.W. Hougas
		OH	B. Randal
		MI	R. Perry
Participants for more than 3 years			
AL	E. Coneva	NC	S. McArtney, E. Young, M. Parker
AR	R. Rom, C. Rom	NE	W.A. Gustafson
CA	T. DeJong, S. Johnson, R. Elkins, W. Micke	NJ	R. Marini, W. Cowgill, R. Belding, E. Durner, D. Ward
CO	A. Gaus, H. Larsen, K.S. Yu, G. Litus	NM	S. Yao
GA	K. Taylor, T. Beckman, S.C. Myers, D. Chavez	NY	G. Fazio, J. Cummins, R.L. Anderson, T. Robinson
IA	P. Domoto, D. Cochran	OH	D.C. Ferree, D. Miller
ID	E. Fallahi	OR	A. Azarenko, M.N. Westwood, P. Lombard, E. Mielke, T. Einhorn
IL	M. Kushad, R. Simons, D.B. Meador	PA	G. Green, L.D. Tukey, R. Crassweller, R. Marini, J. Schupp
IN	R. Hayden, P. Hirst	SC	W. Olien, G. Reighard
KY	G. Brown, D. Wolfe	SD	A. Fennell
KS	F. Morrison, A. Erb	TN	C.A. Mullins, D. Lockwood
MA	W. Lord, W. Autio, J. Clements	TX	J. Worthington
MD	C.S. Walsh, M. Newell	UT	L. Anderson, B. Black, T. Lindstrom, D.R. Walker
ME	W. Olien, J. Schupp, R. Moran	VA	J. Barden, R. Marini, G. Peck, K. Yoder
MI	R. Carlson, R. Perry, G. Lang	WA	B.H. Barritt, G. Lang, S. Muscchi
MO	T. Hopfinger, M. Warmund	WV	T. Baugher
MT	N.W. Callan	WI	F.A. Gilbert, T. Roper, M. Stasiak
International Participants			
B.C., CA	C. R. Hampson, H Quammer, F. Kappel, D. Neilsen	N.S., CA	C. Embry, S. Blatt
N.B., CA	J.P. Prive	QUE, CA	G.L. Rousselle, R.L. Granger
ONT., CA	Hutchinson, D. Elfving, G. Tehrani, R.E.C. Layne, J. Cline	CAEF, Chile	C. Munoz, F. Gainza
CHIH, Mexico	R. Quezada		

Table 2. NC-140 trials, including the name of the trial, the trial coordinator and cooperating locations.

Year	Name of Trial	coordinator	Collaborating locations
1977	1976 Apple Interstem	David Ferree	IA, IL, IN, KS, KY, OH, MA, MO, MI
1980	1980-81 Apple Rootstock	David Ferree	AR, CA, GA, IL, IN, WI, KS, KY, MA, MI, MN, NC, NY, OH, OR, ON, PA, QUE, TN, UT, SC,VA, WA, WI
1984	1984 Apple Rootstock	David Ferree	
1984	1984 Peach Rootstock	Ronald Perry	AR, CA, GA, VA, MO, NY, MI, UT, PA, OH, KY, CO, NJ, KS, IL, ON
1987	1987 Tart and Sweet Cherry Rootstock	Ronald Perry	*Bing* -- BC, CA, CO, OR, UT, WA; *Hedelfingen* -- MD, MI, NY, ON; *Montmorency* -- AR, KS, MI, NJ, NY, ON, OR, PA, UT, WI
1988	1988 Pear Rootstock	Anita Miller/ Eugene Mielke	AR, BC, CO, KY, MD, NS, NY, OH, ON, OR, WA, WV
1990	1990 Apple Orchard Systems	Bruce Barritt/Richard Marini	IL, MI, MN, NC, NY, ON, QUE, VA, WA
1990	1990 Gala Apple Rootstock	Bruce Barritt/Richard Marini	IL, MI, MN, NC, NY, ON QUE, VA, WA
1990	1990 Apple Cultivar/Rootstock	Wesley Autio	AR, CO, GA, IA, IN, KS, KY, MA, ME, MI, NC, OH, PA, QUE, TN, UT, VA
1990	1990 Plum Rootstock	Gus Tehrani/Robert Anderson/Joseph Masabni	CA, GA, KY, IN, MI, NY, ON, OR, QUE
1992-93	1992-93 Apple Rootstock	James Cummins/Terrence Robinson	AR, CA, CO, IA, IN, ME, MI, NC, NY, OH, PA, WA
1994	1994 Peach Rootstock	Gregory Reighard	AR, CO, GA, IL, IN, KS, KY, MD, MS, MI, MO, NJ, NY, OH, ON, SC, TN, UT
1994	1994 Dwarf Apple Rootstock	Richard Marini	AR, CO, GA, IA, IL, IN, KY, MA, ME, MO, NY, OH, ON, OR, PA, WA, MI, MN, NC, NJ, NS, QC, SD, WI, VA, SC
1994	1994 Semidwarf Apple Rootstock	Richard Marini	AR, CO, GA, IA, IL, IN, KY, MA, ME, MO, NY, OH, ON, OR, PA, WA, MI, MN, NC, NJ, NS, QC, SD, WI, VA, SC
1998	1998 Apple Rootstock	Terrence Robinson	BC, MA, MI, MO, NC, NJ, NS, NY, OH, UT, WA

Table 2. *continued*

1998	1998 Tart Cherry Rootstock	Frank Kappel/Gregory Lang	MI, NY, ON, PA, UT, WI
1998	1998 Sweet Cherry Rootstock	Frank Kappel/Gregory Lang	*Hedelfingen* -- MI, NY, ON, PA, SC *Bing* -- BC, CA, OR, UT, WA
1999	1999 Semidwarf Apple Rootstock	Wesley Autio	*Fuji* -- CA, IN, KY, MO, NC, OH, PA, UT, SC, WA; *McIntosh* -- MA, MI, MN, NS, NY, ON, PA, VT
1999	1999 Dwarf Apple Rootstock	Wesley Autio	*Fuji* -- CA, IN, KY, MO, NC, OH, PA, SC, UT, WA *McIntosh* -- MA, MI, MN, NY, NS, ON, PA, VT
2001	2001 Peach Rootstock	Gregory Reighard	*Redtop* -- CA, GA, MD GA *Redhaven* -- IN, MI, MO, NJ, ONT, UT *Cresthaven* – CO, IL, WA, TX
2002	2002 Apple Rootstock	Wesley Autio	AR, BC, IL, IN, KY, MA, MI, NJ, NY, OH, Mexico
2002	2002 Peach Physiology	Scott Johnson	CA, GA, MD, NJ, NY, SC, WA
2002	2002 Peach Rootstock	Scott Johnson	*Redhaven* – CA, GA, MA, MD, MX, MO, OH, ONT, PA, SC; *Cresthaven* – CO, IL, MO, NJ, NY, TX, UT, WA, MX
2002	2002 Pear Rootstock	Eugene Milke/ Steve Castagnoli	WA
2002	2002 New Jersey-Massachusetts Rootstock	Winfred Cowgill	NJ, MA
2003	2003 Apple Rootstock	Richard Marini	AR, BC, CA, GA, IA, KY, ME, MI, NY, OH, PA, UT WI
2003	2003 Apple Physiology	Richard Marini	AR, BC, CA, GA, IA, IN, KY, MA, ME, MI, MX, NJ, NY, NS, OH, ONT, PA, UT, WI
2004	2004 Pear Rootstock	Steve Castagnoli	NY, WA, NS
2005	2005 Pear Rootstock	Steve Castagnoli	CA, MX, NY, OR, WA
2006	2006 Apple Replant	Terence Robinson	CA, NY, OR, WA, Mexico
2009	2009 Peach Rootstock	Greg Reighard	SC & MA
2010	2010 Sweet Cherry Rootstock 7 Training Systems	Greg Lang	BC, MI, NY, NC
2010	2010 Apple rootstock	Wesley Autio	*HoneyCrisp*-- BC, CO, IL, IN, IA, MA, MN, MI, MX, NJ, NS, NY-G, OH, UT, WI. *Fuji* -- ID, KY, MX, NC, NY-HV, PA, UT
2013	Pear Training/Spacing/Rootstock	Todd Einhorn	NY, OR, CA
2014	2014 Apple Rootstock	John Cline	AL, ID, IN, MA, ME, MN, NC, NJ, NY, ON, PA, SC, UT, VA, WA, WI
2015	Organic Apple Rootstock	Terence Robinson & Wesley Autio	CA, CO, ID, IA, MA, MI, NJ, NM, MX, NS, NY, VA, VT, WI

Table 3. Refereed journal articles resulting from NC-140 trials.

Autio, W. R., J. A. Barden, and G.R. Brown. 1991. Rootstock affects ripening, size, mineral composition, and storability of 'Starkspur Supreme Delicious' in the 1980-81 NC-140 cooperative planting. Fruit Var. J. 45:247-251.

Autio, W., R. Hayden, W. Micke, and G. Brown. 1996. Rootstock affects ripening, color and shape of Starkspur Supreme Delicious' apples in the 1984 NC-140 cooperative planting. Fruit Va. J. 51:45-53.

Autio, W.R., J.L. Anderson, J.A. Barden, G.R. Brown, R.M. Crassweller, P.A. Domoto, A. Erb, D.C. Ferree, A. Gaus, P.M. Hirst, C.A. Mullins, and J.R. Schupp. 2001. Performance of 'Golden Delicious,' 'Jonagold,' 'Empire,' and 'Rome Beauty' apple trees on five rootstocks over ten years in the 1990 NC-140 cultivar/rootstock trial. J. Amer. Pomol. Soc. 55:131-137.

Autio, W.R., J.L. Anderson, J.A. Barden, G.R. Brown, R.M. Crassweller, P.A. Domoto, A. Erb, D.C. Ferree, A. Gaus, and P.M. Hirst. 2001. Location affects performance of 'Golden Delicious', 'Jonagold', 'Empire', and 'Rome Beauty' apple trees on five rootstocks over ten years in the 1990 NC-140 cultivar/rootstock trial. J. Amer. Pomol. Soc. 55:138-145.

Autio, W., T. Robinson, D. Archbold, W. Cowgill, C. Hampson, R. Parra Quezada, and D, Wolfe. 2013. 'Gala' apple trees on supporter 4, P.14, and different strains of B.9, M.9 and M.26 rootstocks: final 10-year report on the 2002 NC-140 apple rootstock trial. J. Amer. Pomol. Soc. 67:62-71.

Autio, W. R.,T.L. Robinson, B.H. Barritt, J.A. Cline, R. M. Crassweller, C.G. Embree, D.C. Ferree, M.E. Garcia, G.M. Greene, E.E. Hoover, R.S. Johnson, K. Kosola, J. Masabni, M.L. Parker, R.L. Perry, G.L. Reighard, S.D. Seeley, and M. Warmund. 2005. Performance of 'Fuji' and 'McIntosh' apple trees after 5 years as affected by several semi dwarf rootstocks in the 1999 NC-140 apple rootstock trial. J. Amer. Pomol. Soc. 59:192-201.

Autio, W. R., T.L. Robinson, B.H. Barritt, J.A. Cline, R. M. Crassweller, C.G. Embree, D.C. Ferree, M.E. Garcia, G.M. Greene, E.E. Hoover, R.S. Johnson, K. Kosola, J. Masabni, M.L. Parker, R.L. Perry, G.L. Reighard, S.D. Seeley, and M. Warmund. 2005. Performance of 'Fuji' and 'McIntosh' apple trees after 5 years as affected by several dwarf rootstocks in the 1999 NC-140 apple rootstock trial. J. Amer. Pomol. Soc. 59:202-214.

Autio, W.R., T.L. Robinson, B. Black, T. Bradshaw, J.A. Cline, R.M. Crassweller, C.G. Embree, E.E. Hoover, S.A. Hoying, K.A. Iungerman, R.S. Johnson, G. Lang, M.L. Parker, R.L. Perry, G.L. Reighard, J.R. Schupp , M. Stasiak, M. Warmund, and D. Wolfe. 2011. Performance of 'Fuji' and 'McIntosh' apple trees after 10 years as affected by several dwarf rootstocks in the 1999 NC-140 apple rootstock trial. J. Amer. Pomol. Soc. 65:2-20.

W.R. Autio, T.L. Robinson, B. Black, T. Bradshaw, J.A. Cline, R.M. Crassweller, C.G. Embree, E.E. Hoover, S.A. Hoying, K.A. Iungerman, R.S. Johnson, G. Lang, M.L. Parker, R.L. Perry, G.L. Reighard, M. Stasiak, M. Warmund, and D. Wolfe. 2011. Performance of 'Fuji' and 'McIntosh' apple trees after 10 years as affected by several semidwarf rootstocks in the 1999 NC-140 apple rootstock trial. J. Amer. Pomol. Soc. 65:21-38.

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Kappel, F., G. Lang, A. Azarenko, T. Facteau, A. Gaus, R. Godin, T. Lindstrom, R. Nuñez-Elisea, R. Pokharel, M. Whiting and C. Hampson. 2013. Performance of sweet cherry rootstocks in the 1998 NC-140 regional trial in Western North America. J. Amer. Pomol. Soc. 67:186-195.

Marini, R. P., J. L. Anderson, B. H. Barritt, G. R. Brown, J. Cline, W. P. Cowgill, Jr., P. A. Domoto, D. C. Ferree, J. Garner, G. M. Greene, C. Hampson, P. Hirst, M. M. Kushad, E. Mielke, C. A. Mullins, M. Parker, R. L. Perry, J. P. Prive, G. L. Reighard, T. Robinson, C. R. Rom, T. Roper, J. R. Schupp, E. Stover, and R. Unrath. 2000. Performance of 'Gala' apple on four semi-dwarf rootstocks: a five-year summary of the 1994 NC-140 semi-dwarf rootstock trial. J. Amer. Pomol. Soc. 54:84-91.

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NC-140. 1990. Early performance of 'Starkspur Supreme Delicious' on 16 rootstocks in the NC-140 cooperative planting. Fruit Var. J. 44:225-235.

NC-140. 1991. Performance of 'Starkspur Supreme Delicious' on 9 rootstocks over 10 years in the 1980 NC-140 Cooperative Planting. Fruit Var. J. 45:192-199.

NC-140. 1991. Performance of 'Starkspur Supreme Delicious' on 9 rootstocks at 27 sites over 10 years. Fruit Var. J. 45:200-208.

NC-140. 1991. Abnormalities in 'Starkspur Supreme Delicious' on nine rootstocks in the 1980-81 NC-140 cooperative planting. Fruit Var. J. 45:213-219.

NC-140. 1996a. Performance of the NC-140 cooperative rootstock planting: I. Survival, tree size, yield and fruit size. Fruit Var. J. 50:6-11.

NC-140. 1996b. Performance of the NC-140 cooperative rootstock planting: II. A 10-year summary of TCA, yield and yield efficiency at 31 sites. Fruit Var. J. 50:11-18.

NC-140. 1996c. Horticultural characteristics of 'Starkspur Supreme Delicious' apple on 15 rootstocks at 24 sites. Fruit Var. J. 50:18-24.

NC-140. 1996d. Rootstock and scion cultivar interact to affect apple tree performance: A five-year summary of the 1990 NC-140 cultivar/rootstock trial. Fruit Var. J. 50:175-187.

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Effect of Precocious Grapevine Fruiting on Subsequent Year's Growth and Yield

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Additional index words: interspecific hybrid, overcropping, Ravaz index, vine vigor

Abstract

Vineyard managers are often advised to remove reproductive growth components of vines in the first two years of growth to better establish the root system. In general, this is good advice as it will lead to a stronger vine; yet, there is a lack of research information on the effects of producing an early harvest on vigorous vines. Two locations (Oklahoma and Mississippi) were used to evaluate three wine grape cultivars at each location for fruiting in the second year of growth with subsequent effect on third year vegetative growth and reproductive yields. Reproductive component removal treatments had little effect on fruit yield components. In Oklahoma, there were no differences in caliper in the first two data measurements during the year of treatment. In the following year, vines that were allowed to go to harvest were smaller than the vines that had inflorescences removed in the previous year. Similar results for pruning weights were seen in Mississippi with the veraison (color change) and harvest treatments weighing less than the inflorescence removal treatment. The Ravaz index indicated that all cultivars in Oklahoma ('Cynthiana', 'Rubaiyat', 'Traminette') were within the recommended range of 5-10. In Mississippi, 'Blanc Du Bois' was slightly below the recommended range, indicating that the vines could have supported a heavier crop, whereas 'Villard blanc' was near the upper limit indicating that it was probably overcropped. 'MissBlanc' was in the acceptable range. These results suggest that vineyard managers can allow vigorous, well-managed, fully-trained vines to fruit in the second year without causing irreparable damage. The caveat is in marginally adapted and/or less vigorous cultivars, where lack of cold hardiness, disease susceptibility, or overcropping may lead to dieback or loss of vigor, as was seen in 'Villard blanc'.

Both Mississippi and Oklahoma have relatively small grape industries, therefore room for expansion exists. Neither state is considered a prime growing region for bunch grapes (*Vitis* spp.), yet they can be grown successfully with the proper site, cultivar selection, and cultural management. In fact, considerable research on bunch grapes has been conducted in both states for over a century (Stafne, 2006, 2016a) that has provided a solid base of information for possible industry growth. Currently, nearly all grape growers in these two states have small-scale vineyards. Thus, justifying the expense of infrastructure, labor, equipment, and plant material is a critical decision.

Establishment of a vineyard is a capital intensive endeavor. Cost estimates range from $17,290 to $49,400 per ha based on many factors and the break-even point may not achieved within a decade or even longer (Poling and Spayd, 2015). Therefore, early vine production would help to begin the process of recouping start-up costs faster. One option is to train vines to the trellis system in the first year to support fruit in the second year. In some areas, and for some cultivars, this is not possible due to difficult growing

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conditions or lack of vine vigor. However, in regions with long, hot growing seasons and vigorously growing cultivars, vine establishment is not difficult.

Often vineyard managers are advised to remove reproductive growth components (flowers and clusters) in the first two years of growth to better establish the vine root system, or to retain a very few clusters. This helps to prepare the vine for the stress of producing a crop in its third year (Dami et al., 2005; Poling and Spayd, 2015; Zabadal, 1997). Intrinsically this should lead to a stronger vine and root system, yet there is little information concerning early cropping on vigorous vines that may have the capacity to carry a sizeable crop. Zabadal (1997) stated that cropping in year three could be 4 t/ac (9.8 t/ha) with large vine size or even up to 7 t/ac (17.3 kg/ha) for 'Niagara' (*V. labruscana*) in non-limiting growth conditions with proper cultural management. However, the research results demonstrate that large crop loads may not adequately mature or could reduce vine size. Vines that are overly vigorous can benefit from a governing of their growth (Costello, 2010; Dami et al., 2005) because vines that grow too fast may produce weak wood that may be cold sensitive, break easily, and/or produce poor quality fruit in the subsequent year. One way to mitigate this issue is to allow vines to produce fruit to reduce rank vegetative growth.

Previous research has examined the effect of crop thinning on fruit quality and vine growth (Ames et al., 2016; Ferree et al., 2003; Keller et al., 2005; King et al., 2015). However, these techniques are usually applied on mature vines and not those that are newly established. Complete removal of vine reproductive components is also not done, but rather targeted thinning of blooms and/or clusters to achieve a particular desired crop load. Dami et al. (2005) recommended removing all flowers and fruit prior to 30 cm of growth in the first and second growing seasons unless vines were very vigorous, but even then only one or two clusters per vine maximum should be allowed. Much of the information on crop control of vines comes from regions that grow different cultivars in different environments than the southern United States; thus, there is a need to test the effects of crop control in non-traditional, but expanding, grape growing regions.

The purpose of this study was to determine the effect of full crop loads in the second year on six wine grape cultivars of varying vigor and capacity in two southern locations with long growing seasons. The hypothesis was that vines completely trained to the trellis system in the first year will fruit in the second year and will not induce evidence of damage or injury in the subsequent (third) year.

Material and Methods

Two locations were used for this study. The first location was at the Cimarron Valley Research Station, Perkins, OK (35.97° N lat., 97.03° W long). The soil was Konawa loamy fine sand with Teller fine sandy loam intrusions. At this location, 3 interspecific hybrid cultivars were used: 'Cynthiana', 'Rubaiyat', and 'Traminette'. 'Cynthiana' and 'Rubaiyat' vines were not grafted to a rootstock while 'Traminette' was grafted to 101-14 Mgt rootstock. Vines were planted in spring 2009. Plants were spaced 2.4 m apart in-row with a between-row spacing of 3.7 m on a high cordon trellis system 1.8 m high. Four treatments were applied in 2010 at targeted growth stages based on Eichorn and Lorenz (1977): removal of inflorescences, EL 17; removal of clusters at bb-sized berry stage, EL 29; removal of clusters at beginning of veraison, EL 35; and, full harvest, EL 38. Inflorescences and clusters were removed and counted. Cluster weights and berry weights were from an average of 10 clusters and 20 berries per vine, respectively. All vines were allowed to fully fruit without crop load modification in 2011. The experimental design was a completely randomized design with four treatments and three replications per treatment with two replicate vines per treatment. Maintenance practices recommended

by Oklahoma State University Cooperative Extension Service were followed throughout the growing season (Stafne, 2010), with regular irrigation and fungicide applications. Vines were spur pruned in early to mid-March and fresh pruning weights were taken in the field with a Rapala digital scale (Normark Corporation, Minnetonka, Minn.). Approximately 40 to 50 nodes were left on each vine after pruning. Grape vine trunk diameter was measured at 30 cm above the soil line with a Mitutoyo Absolute Digimatic (Mitutoyo Corp., Kawasaki, Japan). Sugar concentration (SSC) was measured using a Digital Pocket Refractometer ATAGO PAL-1(Atago Co., Ltd., Tokyo, Japan).

The second site was located at the United States Department of Agriculture-Agriculture Research Service, Thad Cochran Southern Horticultural Laboratory, Poplarville, MS (30.84°N lat., 89.53°W long.). The soil was Ruston fine sandy loam. At this location, 3 cultivars were used: 'Blanc Du Bois', 'MissBlanc', and 'Villard blanc'. Vine spacing was 2.1 m x 3 m on a high cordon trellis system at 1.8 m. Vines were planted in spring 2013, three treatments (removal of inflorescences EL, 17; removal of clusters at beginning of veraison, EL 35; full harvest, EL 38) were applied in 2014. Cluster weights and berry weights were from an average of 10 clusters and 20 berries per vine, respectively. All vines were allowed to fully fruit without crop load modification in 2015. Vines were drip irrigated and cultural management, including fungicide sprays, followed recommended practices for Mississippi (Stafne, 2016b). The experimental design was a randomized complete block with six blocks, three treatments and three sample vines per treatment in each block. Vines were spur pruned in late February and early March and fresh pruning weights were taken on an Ohaus Explorer Pro model EP12001 balance scale (Ohaus Corp., Pine Brook, NJ). Approximately 40 to 60 nodes were left on each vine after pruning. Trunk diameter was measured at 30 cm above the soil line with a Mitutoyo Absolute Digimatic (Mitutoyo Corp., Kawasaki, Japan). Sugar concentration was measured in °Brix using a Reichert (Leica) AR200 Digital Refractometer (Reichert, Inc., Depew, NY). Data were analyzed by two-way analysis of variance ($P \leq 0.05$) using the FIT MODEL procedure in JMP 12.2 (SAS Institute, Cary, NC, USA) with cultivar and treatment as main effects and cultivar*treatment as the interaction. Main effect means were separated by Tukey's HSD ($P \leq 0.05$) where the interaction was non-significant. Due to differences in location, time, and cultivar, location were analyzed separately and not compared.

Results and Discussion

'Blanc Du Bois' had more inflorescences than 'MissBlanc' and 'Villard blanc'. The total number of clusters removed was not significantly different; however, 'Blanc Du Bois' had almost twice as many as 'MissBlanc' and 2.5 times as many as 'Villard blanc' (Table 1). 'Blanc Du Bois' is known to have a vigorous growth habit (Mortensen, 1987) and to be highly productive. 'MissBlanc' was reported to have excellent vine

Table 1. Reproductive component removal treatments on three interspecific hybrid grape cultivars in second year of growth (2014) in Mississippi.

Cultivar	Inflorescences Removed (no.)	Clusters Removed (no.)	Cluster Weight (g)	Berry Weight (g)
Blanc Du Bois	79.8 a[z]	38.4[y]	41.0 b	1.78
MissBlanc	17.3 b	20.0	27.6 b	1.52
Villard blanc	17.3 b	15.2	57.9 a	1.50

[z] Means within a column not followed by the same letter are significantly different as determined by Tukey's HSD ($P<0.05$).
[y] Means within columns without letters are not significantly different.

Table 2. Second year yield components of six interspecific hybrid grape cultivars at two locations, Oklahoma (2010) and Mississippi (2014).

Cultivar	Berry Weight (g)	Cluster Weight (g)	Harvested Clusters (no.)	Soluble Solids Conc. (%)	Yield (kg•vine^{-1})
			Oklahoma		
Cynthiana	1.08 c[z]	35.8 b	20.8[y]	18.6 c	0.6 b
Rubaiyat	1.84 a	18.4 b	29.7	19.5 b	0.8 b
Traminette	1.47 b	95.2 a	39.7	20.9 a	3.2 a
			Mississippi		
Blanc Du Bois	3.04 a	65.5 a	42.4 a	18.0 a	3.1 a
MissBlanc	2.27 b	29.2 b	13.7 b	15.9 b	0.4 b
Villard blanc	2.46 b	71.1 a	24.5 ab	16.1 b	1.2 b

[z] Means within a column and location not followed by the same letter are significantly different as determined by Tukey's HSD ($P \leq 0.05$).
[y] Means within columns without letters are not significantly different.

vigor when released (Overcash et al., 1982) is able to produce up to 20 kg per mature vine. 'Villard blanc' has more moderate vigor, but well established vines can be very productive (Clark, 1997). 'Villard blanc' had the highest mean cluster weight at almost 58 g (Table 1). Mean berry weight did not differ among the cultivars in Mississippi in the second year. These data were not collected in Oklahoma.

There were significant difference in berry and cluster weights, SSC, and yield in the Oklahoma grape cultivars. In Mississippi, significant differences were observed among the cultivars for all yield components (Table 2). Trunk diameter was smallest for 'Cynthiana' at the beginning of 2010, but 'Traminette' was the largest in fall of 2010 and 2011 (Table 3) in Oklahoma. Pruning weight was highest for 'Traminette' in spring 2011. By the end of the subsequent year vines that were allowed to go to harvest were significantly smaller than vines that had inflorescences removed (Table 3). One concern about early cropping is potential impairment of the root system growth (Poling and Spayd, 2015). In studies of other plants, trunk diameter was positively correlated with root growth (Pool et al., 2012; Drexhage and Gruber, 1999), although this may only relate to structural roots rather than fine roots (Ammer and Wagner, 2005). Lakso and Eissenstat (2012) reported that once 'Concord' vines were cropped only 10 to 20% of growth went to production of new roots. In addition, heavy crop loads may reduce medium-sized roots but not fine roots. The smaller trunk diameter in the harvest treatment when compared to the inflorescence removal treatment indicates that the root system could be likewise affected. However, none of the cultivars tested in this study had suppressed trunk growth from year two to year three (Table 3, 4).

In Oklahoma, fresh pruning weights were highest when inflorescences or EL 29-stage clusters were removed (Table 3). Pruning weight results were similar in Mississippi with the veraison and harvest treatments having less weight than the inflorescence removal treatment (Table 4). Vegetative measurements were not affected by cluster thinning treatments on 'Blanc Du Bois' (Ames et al., 2016), something also noted by Ferree et al. (2003) on 'Vidal blanc' and 'Chardonnay'. In this study there was a significant cultivar*removal interaction at both locations; yet, these interactions were not extremely informative, largely following the main effect results. The following year (2016) results in Mississippi revealed no differences among treatments for prun-

Table 3. Trunk diameter and pruning weights of three interspecific hybrid grape cultivars and four reproductive component removal timings in Oklahoma.

Treatment	Trunk diameter			Pruning weight
	Sp 2010 (mm)	Fall 2010 (mm)	Fall 2011 (mm)	Sp 2011 (kg•vine^{-1})
Cultivar				
Cynthiana	8.2 b[z]	17.4 b	22.8 c	0.63 b
Rubaiyat	9.6 a	18.7 b	25.4 b	0.52 b
Traminette	9.4 a	23.2 a	29.7 a	1.00 a
Removal Timing				
Inflorescence (EL 17)	9.1[y]	20.6	27.7 a	0.97 a
BB-sized (EL 29)	9.3	20.3	26.5 ab	0.85 a
Veraison (EL 35)	9.2	19.1	25.1 ab	0.53 b
None (EL 38)	8.6	19.0	24.6 b	0.51 b
Significance (P-value)				
Cultivar	0.0035	0.0001	0.0001	0.0001
Removal	0.5892	0.0784	0.0309	0.0001
Cultivar*Removal	0.9075	0.0219	0.1455	0.0001

[z] Means within a column and category not followed by the same letter are significantly different as determined by Tukey's HSD ($P \leq 0.05$).
[y] Means within columns without letters are not significantly different.

Table 4. Trunk diameter and pruning weights of three wine grape cultivars and three reproductive component removal timings in Mississippi.

Treatment	Trunk diameter			Pruning Weight	Pruning Weight
	Sp 2014 (mm)	Fall 2014 (mm)	Fall 2015 (mm)	Sp 2015 (kg•vine^{-1})	Sp 2016 (kg•vine^{-1})
Cultivar					
Blanc Du Bois	9.3	22.9 a	28.6 a[z]	1.09 a	2.49 a
MissBlanc	9.1	19.2 b	27.5 a	0.36 b	1.56 b
Villard blanc	8.8	18.7 b	24.3 b	0.51 b	0.71 c
Removal Timing					
Inflorescence (EL 17)	9.3[y]	21.6	28.3	0.84 a	1.79
Veraison (EL 35)	8.9	19.8	25.7	0.61 b	1.51
None (EL 38)	9.1	19.3	26.4	0.50 b	1.46
Significance (P-value)					
Cultivar	0.3527	0.0002	0.0023	0.0001	0.0001
Removal	0.5258	0.0610	0.0873	0.0026	0.3304
Cultivar*Removal	0.4973	0.4858	0.8156	0.0163	0.5645

[z] Means within a column and category not followed by the same letter are significantly different as determined by Tukey's HSD ($P \leq 0.05$).
[y] Means within columns without letters are not significantly different.

ing weight. In Oklahoma, no difference were observed in fruit yield components from the applied treatments (Table 5). Removal treatments had little effect on fruit yield components aside from third-year mean cluster weight in Mississippi, where removal of

Table 5. Third year (2011) yield components of three wine grape cultivars and four reproductive component removal timings in Oklahoma.

Treatment	Berry Weight (g)	Cluster Weight (g)	Yield (kg•vine^{-1})	Ravaz Index (kg•kg)	Soluble Solids Conc. (%)
Cultivar					
Cynthiana	0.48 b[z]	28.1 c	3.0 b	5.31 b	18.0 a
Rubaiyat	1.35 a	54.7 b	3.1 b	7.20 ab	18.7 a
Traminette	1.27 a	77.1 a	6.7 a	8.17 a	16.2 b
Removal Timing					
Inflorescence (EL 17)	1.06[y]	53.2	4.1	4.14 b	18.0 a
BB-sized (EL 29)	1.02	55.3	4.7	5.75 b	16.5 b
Veraison (EL 35)	1.07	54.9	4.4	8.84 a	18.1 a
None (EL 38)	0.99	49.8	3.9	8.83 a	18.0 a
Significance (P-value)					
Cultivar	0.0001	0.0001	0.0001	0.0001	0.0080
Removal	0.2136	0.5548	0.3419	0.0117	0.0001
Cultivar*Removal	0.1440	0.4073	0.4019	0.1622	0.5059

[z] Means within a column and category not followed by the same letter are significantly different as determined by Tukey's HSD ($P \leq 0.05$).
[y] Means within columns without letters are not significantly different.

Table 6. Third year (2015) yield components of three wine grape cultivars and three reproductive component removal timings in Mississippi.

Treatment	Berry Weight (g)	Cluster Weight (g)	Yield (kg•vine^{-1})	Ravaz Index (kg•kg)	Soluble Solids Conc. (%)
Cultivar					
Blanc Du Bois	3.07	151.6	11.1 a[z]	4.97 b	17.8 a
MissBlanc	2.59	151.8	8.3 b	5.66 b	15.8 b
Villard blanc	3.00	132.5	5.3 c	9.75 a	16.2 b
Removal Timing					
Inflorescence (EL 17)	2.91[y]	163.0 a	9.5	6.42	16.9
Veraison (EL 35)	2.91	139.5 ab	7.7	6.50	16.4
None (EL 38)	2.84	133.4 b	7.3	7.47	16.5
Significance (P-value)					
Cultivar	0.0007	0.1956	0.0001	0.0007	0.0001
Removal	0.8057	0.0400	0.1270	0.6421	0.3072
Cultivar*Removal	0.8216	0.6680	0.6887	0.3736	0.8889

[z] Means within a column and category not followed by the same letter are significantly different as determined by Tukey's HSD ($P \leq 0.05$).
[y] Means within columns without letters are not significantly different.

inflorescences led to higher cluster weights than removal at veraison or the control (no removal) (Table 6).

While most of the cultivars used were well-adapted to the local climate, both 'Traminette' and 'Villard blanc' are more marginally adapted for different reasons. 'Traminette' was susceptible to damage from low temperatures in New York, and 'Traminette' in Oklahoma is not as cold hardy as 'Cynthi-

ana' or 'Rubaiyat' (Reisch et al., 1996; E.T. Stafne, personal observation). 'Villard blanc' in Mississippi is tolerant of Pierce's disease (PD) (*Xylella fastidiosa* subsp. *fastidiosa* Wells et al.), but not as resistant as 'Blanc Du Bois' and 'MissBlanc', and can succumb to the disease after a decade or so (Hegwood, Jr., 1987). During this study, 'Villard blanc' exhibited terminal die-back of cordons that was not observed in the other two cultivars. Although diagnostically unconfirmed, this could be related to PD or another stress issue such as overcropping in the second year. Evidence could be seen in relatively poor increase of second year pruning weights as they grew only 39% compared to 128% for 'Blanc Du Bois' and 333% for 'MissBlanc' (Table 4).

The Ravaz index (Ravaz, 1903), the balance between reproductive growth (fruit yield) and vegetative growth (pruning weight), indicated that all cultivars in Oklahoma were within the recommended range of 5-10 (Smart and Robinson, 1991). 'Traminette' was significantly higher than 'Cynthiana', which was on the lower end of the acceptable range (Table 5). Rootstocks can play a role in above-ground response as well (Smart et al., 2006) and this could be the case with 'Traminette'. 'Cynthiana' is a low yielding cultivar, but was on par with 'Rubaiyat' in this study. In Oklahoma, 'Cynthiana' can be a slow grower in the first few years before becoming more vigorous around year five and beyond (E.T. Stafne, personal observation). In Mississippi, 'Blanc Du Bois' was slightly below the recommended Ravaz index range, indicating that the vines could have supported a heavier crop than was harvested (Table 6). Since 'Blanc Du Bois' was very vigorous with a high capacity for fruit production, it should be closer to the high end of the Ravaz index range. On the other hand, 'Villard blanc' was near the upper limit indicating that it may have been overcropped, a conclusion that is supported by the small pruning weight increase and winter dieback.

Removal treatment significantly affected Ravaz index in Oklahoma (Table 5) but not Mississippi (Table 6). Early removal of reproductive components at stages EL 17 (4.14) and EL 29 (5.75) were significantly less than those at EL 35 (8.84) and EL 38 (8.83). The removal at the inflorescence stage led to undercropped vines, whereas the other treatments were within the recommended range of 5 to 10. All treatments in Mississippi resulted in Ravaz indices between 5 and 10.

Conclusions

Overall, the vine reproductive component treatments affected vines in the year of treatment. However, in the subsequent year, except for 'Villard blanc', most vines continued to grow normally after allowing a full harvest in the second year. This suggests that vineyard managers can allow vigorous, well-managed, fully-trained vines to fruit in the second year without causing irreparable damage. The caveat to this is in marginally adapted and/or less vigorous cultivars, where lack of cold hardiness, disease susceptibility, or overcropping may lead to dieback or loss of vigor. Soluble solids levels obtained in the second year were acceptable for wine making, but other parameters such as anthocyanins and phenolics were not measured so the overall physiological maturity of the fruit may or may not be at desirable levels. Grapevine breeders can also use the results of this study to understand how precocious fruiting can be useful in developing new cultivars. The southern U.S. is in dire need of new PD-resistant bunch grape cultivars for commercial markets (Stafne, Sleezer, and Clark, 2015) and cultivars that satisfactorily bear an early crop can assist growers in recouping the upfront costs of production.

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Dr. Susan Brown - 2016 Wilder Medal Recipient

Dr. Susan Brown was recognized by the American Pomological Society with the Wilder Medal for her outstanding work in tree fruit breeding at the New York State Agricultural Experiment Station (NYSAES) over 30 years. She has released four apple and 14 cherry cultivars during her career. Among these releases are 'SnapDragon' and 'RubyFrost' patented and trademarked cultivars which are being planted on 900 acres. These two cultivars have exceptional qualities and will make a major impact on the apple industry.

Dr. Brown grew up in East Haven Connecticut and received her B.S. degree in Plant Science in 1978 at the University of Connecticut. She received an MS in Horticulture in 1980 from Rutgers University with Drs. Fred Hough and Catherine Bailey, then a Ph.D. from the University of California, Davis in 1984 studying Genetics.

Susan's father introduced her to the field of genetics with his passion for improving racing pigeons. Brown's Mom had a "green thumb" and people visited her gardens from miles around. Brown merged those two trainings in her love of both plants and genetics.

Brown has been employed at Cornell University since October,1985 moving through the ranks of assistant, associate and full professor. Her current research involves the genetics and identification of molecular markers of morphological, architectural, physiological, resistance and quality traits in apples. She joined with other collaborators to obtain USDA-AFRI funding for *Rosaceae* mapping research (www.RosBreed.org). This culmination of this work will significantly accelerate apple breeding and allow breeders to more easily pyramid genes for multiple desirable traits.

Brown has more than 60 peer-reviewed research publications in journals such as Genetic Resources and Crop Evolution, Genomics, HortScience, Journal of the American Society for Horticultural Science, Molecular Breeding, International Journal of Food Properties, Plant Physiology, and the Journal of the American Pomological Society. She has also written key chapters for books including Handbook of Plant Breeding, Biotechnology in Flavor Production, Temperate Fruit Crop Breeding, Biotechnology of Fruit and Nut Crops and Apples: Botany, Production and Uses.

Besides her peer-reviewed scientific work, she has also written extensively for the fruit industry through extension and outreach publications including Compact Fruit Tree, New York Fruit Quarterly, Fruit Notes, and the Fruit Varieties Journal. She has made numerous presentations and guest lectures to both fruit industry groups, clubs, and other groups. Her work has been featured in numerous popular magazines including: Prevention magazine, National Geographic, Good Fruit Grower, American Fruit Grower, and the Wall Street Journal. She has also been interviewed twice on National Public Radio. Dr. Brown has been an active advisor of graduate students (7 M.S., 4, Ph.D.) and has hosted visiting scientists from Serbia, India, Italy, Japan and Korea.

Brown served as associate chair of the Department of Horticulture during a time of great change, and then was asked to serve as associate director and then director (and associate dean) of the NYSAES in 2015. During this time she has maintained a very active research program, has continued to mentor graduate students, and has connected with growers, legislators, consumers and school children.

Brown is widely respected among growers who appreciate her hard work and dedication to the industry. She played a vital role in the

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The Pioneering Horticulturist Marshall Pinckney Wilder

John R. Clark

Additional index words: pomology, breeding, pears, fruit, awards

Abstract

Marshal Pinckney Wilder was an outstanding horticulturist who was a founding member of the American Pomological Society. He served as president from its founding in 1848 through 1885. He was born in 1798 and died in 1886. He was a leader in his community and his state of residence, Massachusetts. His love of horticulture was extensive, as he tested and conducted breeding on several ornamental species. However, his greatest love was fruits, particularly pears. He was honored by the Society with the establishment of the Wilder Medal in 1873, its highest honor.

Marshall Pinckney Wilder is best known to pomologists as a founding member of the American Pomological Society (APS). He was the first APS president, being selected for this position when the Society was founded in 1848. He served as president through 1885. He was a very enthusiastic horticulturist, and once stated "I think I can truly say that, from the day my sainted mother took me into the garden to help dress and to keep it, I have never seen the time when I did not love the cultivation of the soil." We have all gained from his skills as a horticulturist, pomologist and leader.

Marshall Pinckney Wilder was born in 1798 in Rindge, NH but lived the majority of his life in Dorchester, MA. He did not pursue higher education, but rather was interested in business and farming, joining his father's store and farm at age 21. He was involved with the dry goods firm Parker, Blanchard and Wilder until 1872. He had 14 children by three wives. Wilder died in Dorchester in 1886. He is not to be confused with his great nephew by the same name born in 1859, who was an actor, humorist and sketch artist.

Wilder always had a strong interest in local affairs, and was active in the State Militia of New Hampshire and in Boston's "Ancient and Honorable Artillery Co" where he was known as "Colonel" Wilder. Further, he was interested in state affairs also, serving as a member of the Massachusetts legislature both as a senator and representative. He was a 33rd degree Mason. He was a leader in many other organizations than APS, including:

- Founding member, New England Horticultural Society, 1829
- President, Massachusetts Horticultural Society, exhibiting at its annual meeting 1833-1886, president, 1840-1848
- President, Massachusetts Agricultural Club
- President, Norfolk Agricultural Society
- President, US Agricultural Society
- President, New England Historic Genealogical Society, 1868-1886

Although he did not receive formal college education, Wilder was a strong advocate for the establishment of an agricultural college in Massachusetts, and later was a trustee for 23 years of the Massachusetts Agricultural Col-

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Special thanks to Dr. Duane Greene, University of Massachusetts, for researching materials in the Marshall P. Wilder Collection in the Special Collections and University Archives, U. Mass Amherst Libraries. Additional thanks to Andrew Jecmen, program associate in fruit breeding at the University of Arkansas, for assembling information for this presentation and manuscript.

lege (now the University of Massachusetts, Amherst). He addressed the first graduating class at the Massachusetts Agricultural College. He was also involved with the founding of the Massachusetts Institute of Technology, and supported it when it was agreed that the institution would provide instruction in pomology and horticulture.

He had broad interests as a horticulturist. He conducted camellia testing as well as breeding. He also had a substantial azalea trial. He also bred a California double poppy. His strongest horticultural interest was in fruits, however. He imported fruit trees from England, France, Belgium and Germany. The pear was his crop of highest interest. At one time, he had 404 pear cultivars under trial in his orchard. In his APS presidential address, he shared his passion for pears when he stated: "Give us pears! The most exquisite sorts, where we can grow them – by all means give us, pears! Pears for ourselves, for our families, for the millions who are about us, and who are to come after us."

He worked tirelessly to make APS a strong organization with a national scope. In his last APS presidential address in 1885, he highlighted the major achievements of the Society since its inception:

- "Brought in close communion of interest, and concert of action, the most experienced pomologists of our country"
- "Raised the standards of excellence by which fruits are judged", including rules on how shown and judged
- Catalogue of Fruits, published biennially, reporting from all states
- Giving of American Pomology "a high character as a science"

The Society further honored Wilder with the establishment of the Wilder Medal in 1873 at its 13th "session" or annual meeting held in Boston. The medal was designed by John J. Thomas. Wilder bequeathed $5,000 in his will to fund the medals. This award in modern day is "conferred on individuals or organizations which have rendered outstanding service to horticulture in the broad area of pomology". However, when the award was first founded, it had four classes of awards: 1) promising new fruits, 2) collections of fruits illustrating horticultural advantages, 3) seedling fruits which may have value as parents for improvements of traits through "judicious hybridizing", and 4) individuals who distinguish themselves by some area of work in horticulture. Due to these broad categories, 43 awards were given in 1873. And, they were awarded as silver or bronze. Over the years the numbers of awards decreased, although those that received the awards were exhibitors such as Wilder, L.H. Bailey Jr. of Michigan, T. V. Munson of Texas, and Luther Burbank of California. As exhibits and collections were reduced in emphasis, the number of awards was reduced, with usually only one award presented annually from 1941 onward. Further, Wilder Medals have been given to cultivars such as 'Campbell Early' grape and 'Golden Delicious' apple along with many others. Significant locations contributing to improvement of fruits have been awarded the Wilder Medal, including in 1926 the New York Agricultural Experiment Station, Geneva, and the New Jersey Agricultural Experiment Station, New Brunswick.

Wilder addressed the Society each year as president, and in one his last and most significant addresses, he shared his love of fruits with this statement: “Fruits are the overflow of nature’s bounty; gems from the skies dropped down to beautify the earth, charm the sight, gratify the taste, and minister to the enjoyment of life; and the more we realize this, the more we shall appreciate the Divine goodness to us, and the duty of providing them for others.” He often included poems in his addresses, and one of his last is presented here:

Like morning’s first light, that gladdens the sight,

So may the best fruits spread over the earth.

And when we shall reach that still fairer land,

And round the life-tree in mercy shall stand,

May each pluck its fruit, and nevermore feel

The serpent’s sharp tooth, once close at his heel.

Robert C. Winthrop, US Senator and Representative (Speaker of the House) of Massachusetts, said of Marshall Wilder: "He deserves grateful remembrance as long as a fine pear is relished or a brilliant bouquet admired."

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Dr. Susan Brown *continued from page 55*

nell and a grower cooperative to commercialize her apple selections.

Brown has received many honors and awards including the NY State Senate Woman of Distinction (2014), SUNY Chancellor’s Award for Faculty Service (2013), CALS Alumni Association Outstanding Faculty Award (2012), a Horticulture Commendation from the Garden Club of America. (2009), and the Massachusetts Horticulture Society’s Jackson Dawson Award (2005).

Brown has “rendered outstanding service to horticulture in the area of pomology” for three decades, has been a longstanding member of the American Pomological Society (APS) and is recognized as an international leader in apple breeding and genetics. She delivered the keynote address at the APS annual meeting in 2015 in New Orleans, and was given the Wilder Medal on 11 Aug. 2016 at the annual meeting of APS in Atlanta, GA. She has cemented a legacy within the fruit industry and academy, and her contributions continue to grow.

Journal of the American Pomological Society 71(1): 59-61 2017

George M. Darrow: The Dean of Small Fruits[1]

Marvin P. Pritts[2] and Alyssa A. Pritts

Additional index words: photoperiod, polyploidy, strawberry, blueberry, breeding

Abstract

George Darrow was one of the leading pomologists of the mid-20th century having a 46-year career with the United States Department of Agriculture. During his appointment as small fruit breeder in Glendale and Beltsville, Maryland, he released a number of significant fruit cultivars. 'Blakemore' strawberry and 'Bluecrop' blueberry dominated the industry after their releases. Darrow also wrote the definitive work at the time entitled "The Strawberry" - a valuable reference book to this day. The introduction to this book was written by Henry Wallace, the vice-president of the United States. Darrow did pioneering work on photoperiodism and perfected techniques to propagate and distribute virus-indexed strawberry plants. Darrow received many awards including the Wilder Medal (1948) and the Liberty Hyde Bailey Award from the American Horticultural Society (1960). He was elected fellow of the American Society for Horticultural Science in 1965 and served as its president in 1949. He was inducted posthumously into the Maryland Hall of Fame in 1996. Darrow retired in 1957, but his joy of working with plants remained steadfast. He started a pick-your-own strawberry farm with his sons in Maryland and began a robust daylily breeding program which produced 59 cultivars. His failing eyesight ultimately deterred his fieldwork. Darrow died at age 94 after a stellar career.

George M. Darrow (1889-1983) was recognized as the foremost American authority on strawberries during the 20th century. The year 2016 marks the 50th anniversary of the publication of his most well-known work "The Strawberry: History, Breeding, and Physiology" (Darrow, 1966). The book is a comprehensive and illustrative work in which Darrow "acquaints the reader with the strawberry, its origin and appearance, the structure of its fruit and plant, where and how it was developed and by whose hands, who is working with it now, and what can be expected of it." He sets out to answer the questions: "Will it continue as a major fruit? What are its weaknesses and its strong points? Is it worthwhile? How can we best take advantage of the present ease of interchange of ideas and germplasm?" The book is filled with historical paintings from as early as 1400, hand-drawn and painted illustrations of maps and cultivars, and an extensive narrative about the history and evolution of the plant and associated culture both in the field and the lab. The Vice-President of the United States and former Secretary of Agriculture, Henry Wallace, encouraged Darrow to write this book. Wallace then wrote the introduction. The first printing of 5,000 copies sold out almost immediately. *The Strawberry* is still used by teachers and researchers as a reference guide.

Darrow was born 2 Feb 1889 on a dairy farm in Springfield, Vermont. He was described by his colleague F.F. Cullinan, as a "genuine Yankee from southern Vermont" with a strong work ethic from a young age (USDA, NAL, Special Collections). To generate off-farm income for the family, Darrow held several miscellaneous jobs selling ice, eggs, hay, medications and phones. He was always interested in plants, and while attending Middlebury College, assisted the president with maintaining his *Viola* collection. Darrow received a Bachelor's degree in Botany from Middlebury College in 1910.

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[1] Much of the information contained in this paper was gleaned from boxes of the personal correspondence and field notes of George Darrow located in the National Agriculture Library in Beltsville, MD. The authors are grateful for the assistance of the librarians in procuring this information.

[2] Horticulture Section, School of Integrative Plant Science, Cornell University, Ithaca NY

Darrow went on to earn a Master's degree in Pomology from Cornell University in 1911 where he studied orchard systems. After graduating from Cornell, he married and began employment with the USDA where he worked for 46 years (1911-1957). His employment with the USDA was temporarily interrupted while he served in the army during World War I from 1918-1919. He was among the first team of researchers to study the strawberry chromosome, and in 1927, his work on strawberry physiology earned him a doctorate at Johns Hopkins. He obtained his Ph.D. while still employed at the USDA and raising six children.

Darrow's accomplishments in strawberry crop breeding and berry crop physiology included the introduction of the cultivar Blakemore, which set a new standard for firmness and productivity for 20 years and was once planted on 30% of the U.S. strawberry acreage. He went on to develop 28 cultivars of strawberry over the course of his career (e.g. Fairfax, Albritton, Surecrop, Redglow). Darrow pioneered work on photoperiodism in strawberry, documenting the need for short days and cool temperatures to induce flowering in most genotypes. His early work on virus-indexing through graft inoculation was among the first for any fruit crop. Darrow realized that breeding efforts would be compromised if parental lines were infected. He worked with nurserymen to propagate clean stock in isolation from other plantings and to use aphicides to control the virus vector. He established collaborative breeding programs throughout the country, but those in North Carolina and Oregon were particularly strong. Darrow also made a strawberry collecting trip to Chile, Ecuador, and Colombia in 1957.

Darrow's contributions were not limited to strawberries. He earlier worked with cranberries in Massachusetts, post-harvest storage of berries in Oregon, citrus in Florida, strawberries in Tennessee and an array of berry crops in Maryland. He released seven cultivars of blueberries (Bluecrop, Earliblue, Blueray, Berkeley, Coville, Wolcott, and Tifblue), and a number of raspberries, blackberries, dewberries, gooseberries, and beach plums. 'Bluecrop' may have been the most widely-planted blueberry cultivar in the world at one time. He curated extensive collections of native American fruit species and he understood the genetic barriers to breeding across ploidy levels. Darrow became a leading contributor to scientific strawberry literature over the course of his career, including 230 books, articles, and bulletins.

While conducting research, he built relationships with farmers and breeders across the United States and around the world. He was recognized for his close relationship with farmers which helped him better refine breeding objectives. He cooperated with experiment stations in the United States and Scotland to develop cultivars that would withstand disease.

He occasionally became embroiled in what would today be considered intellectual property disputes, notably the renaming of cultivars that he named and released. The following personal correspondence details one such incident.

"I have a question to put to you in regard to a nursery changing the name of the Cameron dewberry. Monrovia Nurseries has been advertising and selling this plant under a new name, called "Victory Berry." Now the question is, what should be done about it, if anything. Should it be ignored? Should we ask them why they changed the name? Should we request that they not do this? Should we inform the Departments of Horticulture on the West Coast that they are doing this?" – C.F. Williams, 11 Dec. 1944

"I think it would be well worth while for you to write to Monrovia, stating that it is not good horticultural practice to rename varieties and that you would appreciate a statement in regard to this." – G.M. Darrow, 16 Dec. 1944

Darrow received a number of prestigious awards and promotions, including: Administrative head of Small Fruit Breeding (1945), Wilder Medal (1948), President of the American Society for Horticultural Sci-

ence (ASHS) (1949), Liberty Hyde Bailey Award from the American Horticultural Society (1960), ASHS Fellow (1965), Janick and Moore book dedication (Magness, 1975) and Prince Georges County Maryland Hall of Fame (1996). He was awarded an honorary doctorate from North Carolina State University in 1963 for improving the strawberry and blueberry industry in that state. It was about this time that his colleagues began referring to him as the "Dean of Small Fruits" (Fusonie, 1990).

Darrow retired in 1957, but his joy of working with plants remained steadfast. He continued to participate in making selections at Beltsville. He started a pick-your-own strawberry farm with his sons in Maryland, considered to be the first in the state. He began a robust daylily breeding program which produced 59 cultivars. His friend Henry Wallace states, "He associates joyously with his plants - he is a rare individual, a genuine plantsman. This title in my opinion is far beyond that of any Ph.D. (Wallace, 1966)."

"Darrow was one of those rare individuals whose keen intellect, considerable energy and broad professional interests enabled him to master and help define a field as diverse as 20th century pomology. His personal charisma, utter trustworthiness, boundless enthusiasm, love for young people and excellent communication skills enabled him to talk to farmers, write, plan and execute research programs. He is one of the horticultural giants of the 20th century (Galletta, 1993)." Galletta participated in honoring Darrow on the 100th anniversary of his birth at a meeting of the North American Strawberry Growers Association in Beltsville, MD.

Eventually, Darrow lost his vision and was no longer able to assist with the farm or with making selections. Although he passed away in 1983 at the age of 94, his daylily farm in Vermont continues to be managed by his grandson. Darrow's legacy endures well into the 21st century.

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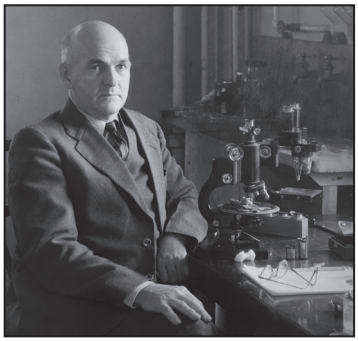

Figure 1. George Darrow working in his laboratory in Beltsville, MD, circa 1940.

Figure 2. George Darrow, John Watson and George Slate examining strawberry selections possibly in Geneva, NY circa 1960.

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Instructions to Authors

Journal of the American Pomological Society

The prime purpose of the Journal of the American Pomological Society is to provide a repository for information on all aspects of fruit and nut crops. The long-term emphasis of the journal on cultivars and rootstocks continues, but manuscripts reporting original research on a wide range of fruit and nut crops are welcomed. Acceptable areas of research including pruning, nutrition, growth regulators, cultural practices, economics, and pest control. Studies involving the interaction of one or more of these aspects with either cultivars and/or rootstocks are particularly appropriate. If in doubt about the suitability of a particular manuscript, please contact the Editor.

Reports on field studies are expected to contain data from multiple years. Reports are to be the result of adequately replicated trials and the data should be subjected to appropriate statistical analysis. Manuscripts submitted for publication in the Journal must not have been previously published, and submission implies no concurrent submission elsewhere.

Scientific names and authorities for plants, disease organisms, and insects should be included parenthetically when the organism is first mentioned. American spelling conventions and SI units should be used. Manuscripts should be double spaced throughout. Typical organization is as follows: Title, Authors, Abstract, Introduction, Materials and Methods, Results, Discussion, Literature Cited, Tables, Figures. The Results and Discussion sections are often combined. Author addresses, email adresses and acknowledgements are in footnotes on the first page. More detailed instructions for manuscript preparation can be found at: http://www.americanpomological.org/journal/journal.instructions.html

Before submission, manuscripts should be reviewed by at least two colleagues and revised accordingly. At the time of submission, the corresponding author must attest in the covering letter to the Editor that all coauthors on the paper have had the opportunity to review it before to submission, that it has not been published previously, and that it is not presently under consideration for publication elsewhere. In addition, the names and full contact information (mailing address, e-mail and telephone numbers) for three potential reviewers should be provided. Submit manuscripts electronically to the Editor: Dr. Richard Marini, 203 Tyson Building, Department of Plant Science, University Park, PA 16802-4200 USA; E-mail: richmarini1@gmail.com. Acceptable format is MSWord.

Manuscripts are sent to two reviewers competent to evaluate scientific content. Acceptance for publication depends upon the combined judgement of the two reviewers and the Editor. In unusual circumstances the Editor, without further review, may return a manuscript, which obviously does not meet Journal standards, to the author.

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Call for Wilder Silver Medal Nominations

The Wilder Committee of the American Pomological Society (APS) invites nominations for the 2017 Wilder Silver Medal Award. All active members of APS are eligible to submit nominations. The award was established in 1873 in honor of Marshall P. Wilder, the founder and first president of APS. The award consists of a beautifully engraved medal which is presented to the recipient at the annual meeting of APS, held during the ASHS Annual Meeting.

The Wilder medal is presented to individuals or organizations that have rendered outstanding service to horticulture in the area of pomology. Special consideration is given to work relating to the origination and introduction of meritorious fruit cultivars. Individuals associated with either commercial concerns or professional organizations will be considered if their introductions are truly superior and have been widely planted. Significant contributions to the science and practice of pomology other than through fruit breeding will also be considered. Such contributions may relate to any important area of fruit production such as rootstock development and evaluation, anatomical and morphological studies, or noteworthy publications in any of the above subjects. Information about the award, past recipients, etc. can be found on the APS website at:

http://americanpomological.org/wilder1.html

To obtain nomination guidelines, please contact committee chairperson,

Dr. John R. Clark
Dept. of Horticulture, University of Arkansas
phone: 479-575-2810
fax 479-575-8619
e-mail: jrclark@uark.edu

Nominations must be submitted by 1 May 2017.

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