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Phenotyping Protocol for Sweet Cherry (*Prunus avium* L.) to Facilitate an Understanding of Trait Inheritance

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Abstract

The USDA Specialty Crop Research Initiative-funded RosBREED project has the objective of enabling marker-assisted breeding (MAB) in the economically important agricultural family of Rosaceae. To standardize and increase the accuracy of MAB in Rosaceae, it is necessary to characterize many horticultural and fruit quality traits in representative germplasm. We describe a very comprehensive phenotyping protocol for productivity traits, fruit quality traits and undesirable horticultural traits, developed for use in the Washington State University (WSU) Sweet Cherry Breeding Program. In addition, selected correlations among traits are presented. The protocol facilitates standardization of data among researchers working with sweet cherry across various environments and institutions. Data collected from a sweet cherry 'Crop Reference set' and a 'Breeding Pedigree set' between 2010 and 2012 were evaluated for trait correlations and the results are presented. Selected results indicate significant moderate correlations for harvest date with fruit weight and fruit firmness ($r = 0.26$, $P < 0.0001$ and $r = 0.39$, $P < 0.0001$, respectively), with later-maturing cultivars tending towards larger and firmer cherries, in general. Also, fruit weight had a positive significant association with pedicel-fruit retention force ($r = 0.43$, $P < 0.0001$). However, soluble solids concentration showed a negative relationship with fruit weight and firmness ($r = -0.34$ and $r = -0.20$, respectively). Progress in breeding for multiple traits simultaneously will be faster with the significant positive correlations obtained in this study.

Sweet cherry (*Prunus avium* L.) is important to the United States (US) horticulture industry. In 2012, the US produced approximately 424,000 t of sweet cherry (NASS, 2013). The majority (80%) of the sweet cherries were sold fresh and had a total worth of $778.0 million. Washington was the leading producer in the US with 264,000 t of sweet cherries, followed by California (92,300 t), Oregon (56,000 t), Michigan (4,250 t), and Idaho (3,600 t). Due to the economic importance of sweet cherries in the US, there is the expectation that breeding programs will develop new cultivars to meet the ever changing market needs. New cherry cultivars with superior traits, performance and market acceptance will benefit all players in the supply chain through improved quality, availability, affordability, health benefits, and safety. However, breeding programs require significant financial, human and time resources to develop, evaluate and commercialize new cultivars, especially for a crop with a long juvenile period such as sweet cherry (Fuglie and Walker, 2001). Often, it can take over 20 years from the initial cross to when the cultivar is commercially available. Thus, any strategy that hastens this process and improves the efficiency of cherry breeding programs has high potential economic impact for producers and processors.

MAB may improve the efficiency and cost effectiveness of breeding programs. MAB involves the use of DNA information in breeding decisions taking advantage of the association between DNA markers and

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phenotypic traits. The technology is particularly useful in cases where trait phenotyping is time-consuming, difficult, or has low heritability. High-quality phenotypic data are needed for understanding trait inheritance and integration with genotypic data for marker-locus trait association studies. Standardizing the phenotyping protocol will be necessary to maximize genetic determination and comparison of studies between different programs.

Standardized phenotyping protocols have been developed by RosBREED (a US community initiative to enable marker assisted breeding for Rosaceous crops) for other Rosaceae fruit crops, including apple (Evans et al., 2012), peach (Frett et al., 2012), strawberry (Mathey et al., 2013), and sour cherry (Stegmeir et al., 2014). This paper describes the phenotyping protocol for productivity, fruit quality and undesirable horticultural traits, developed by the Washington State University (WSU) sweet cherry breeding program as well as selected correlations among traits.

Materials and Methods

Sweet Cherry Crop Reference Set and Breeding Pedigree Set

Approximately 600 pedigree-linked individuals including the US Sweet Cherry Crop Reference (CR) set and the Pacific Northwest Sweet Cherry Breeding Pedigree (BP) set were used in this study. The CR set consists of 268 pedigree-linked cultivars, ancestors, selections and seedling populations, efficiently representing the genomes of 25 important breeding parents and their 15 known ancestors (Peace et al., in press). A total of 333 seedlings were chosen as the BP set, which includes mainly breeding parents and their progenies specific to the WSU sweet cherry breeding program. All trees were located at the WSU Roza Farm, Prosser, WA (46˚29'N and 119˚73'W). The trees were irrigated weekly from bloom to leaf senescence with microjet sprinklers and grown using standard orchard management practices. A total of 83, 155 and 203 F_1 seedlings were planted in 2006, 2007 and 2008, respectively. A majority of these seedlings flowered when the trees were five years old.

Phenotyping Protocol

The protocols published in Stegmeir et al. (2014) were adapted to phenotype the accessions, particularly for the common traits between sweet and tart cherries. A different protocol was used for traits unique to sweet cherry. Both protocols are described below for each category of traits.

Productivity Traits. Trees were observed daily for bloom status beginning in February. Bloom date was recorded as the date when 50% of the flowers on a tree had opened. Days from 1 Jan. and growing degree days (GDD) were used to quantitatively describe bloom date. GDD (a measure of heat accumulation) was calculated as the cumulative heat units from 1 Jan. to the date of 50% bloom using a base temperature of 4.4°C (40°F) (Stegmeir et al. 2014) (data from http://weather.wsu.edu/awn.php). Similarly, harvest date was recorded as the date the fruit were harvested and also expressed in days from 1 Jan. and GDD. Fruit maturation was observed regularly beginning in May, with periods between evaluations decreasing from weekly to daily as ripening progressed. Fruit were harvested at optimal ripeness as determined by skin color, firmness and taste. A sample of at least 25 fruit per tree (when possible) was collected randomly around the tree, avoiding fruit from the highest and lowest points of the canopy. Fruit were sampled at least twice on separate dates spanning at least one week, with optimum maturity date based on evaluation of fruit quality data obtained in the laboratory (see Table 1).

Fruit Quality Traits. A sample of 25 fruit per tree (when possible) was assayed for quantitative traits such as fruit size including fruit weight and fruit equatorial diameters, firmness, pedicel-fruit retention force, percent soluble solids concentration, titratable acidity, pH, stem length, fruit and pit length

Table 1. Phenotypic traits, trait code, unit of measure and description used to categorize sweet cherry accessions.

Trait[1]	Trait code	Unit of measure	Description
		Productivity traits	
Bloom Date*	BD	Julian date	The number of days since January 1 to date of 50% bloom
Bloom Time	BT	Growing Degree Days	GDD accumulated from January 1 to date of 50% bloom calculated with a base temperature of 4.4°C
Harvest Date*	HD	Julian date	The number of days from January 1 to date of harvest
Harvest Time	HT	Growing Degree Days	GDD accumulated from January 1 to date of harvest calculated with a base temperature of 4.4°C
		Fruit quality traits	
Fruit Firmness*	Fir	g·mm	Measured by compression (cheek to cheek) on a Firmtech (FirmTech II BioWorks Firmtech II, Kansas, USA) using 5 TF[2]
Bulked Fruit Firmness	FirB	g·mm	Measured by compression (cheek to cheek) on a Firmtech (FirmTech II BioWorks Firmtech II, Kansas, USA) using 20 BF[3]
Pedicel-Fruit Retention Force*	PFRF	g	This is the force required to pull the pedicel off the fruit measured with a pull force gauge (Imada DS2-4 digital force gauge, Northbrook, IL, USA). Five TF samples are used.
Soluble Solids Concentration*	SSC	Brix%	Juice from 5 TF samples pulled together is used and Brix% is recorded on a refractometer (Atago PAL-1, Atago Co, Ltd., Bellevue, WA, USA).
Bulked Fruit SSC	SSCB	Brix%	Juice from BF samples is used.
Titratable Acidity*	TA	%	Measured with a 10 mL strained juice from BF on an autotitrator (Metrohm, Florida, USA) titrated to pH endpoint 8.2
pH	pH	pH	Same as above
Fruit Weight*	FWt	g	Average weight of 5 TF
Bulked Fruit Weight	FWtB	g	Average weight of 20 BF
Stem Length	SL	mm	Average of 5 TF
Fruit Length*	FL	mm	Measured from stem to blossom scar, average of 5 TF
Fruit Width 1*	FW1	mm	Measured from the suture to opposite cheek, average of 5 TF
Fruit Width 2*	FW2	mm	Measured from cheek to cheek, average of 5 TF
Mahogany Skin Color	CMS	#	Using the CTFIL color chart and recording 1 to 6

Blush Skin Color	CBS	#	1 = 0-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100%
Fruit Shape*	ShF	#	Ratio of Fruit Length and Width1; 1 = kidney, 2 = flat-round, 3 = round, 4 = elongate, 5 = cordate (heart-shaped)
Flesh Color*	CFl	#	Visual Flesh Color Card rating 1-5; 1 = cream, 2 = pink, 3 = red, 4 = red purple and 5 = mahogany
Free Stone*	FS	#	Pit cling: 1 = no cling, 2 = slight cling, 3 = semi cling, 4 = cling, 5 = total cling, average of 5 TF
Pit Weight*	PWt	g	Average pit weight of 5 TF
Pit Length*	PL	mm	Pit length from point to point, average of 5 TF
Pit Width 1*	PW1	mm	Pit width from suture to back, average of 5 TF
Pit Width 2*	PW2	mm	Pit width perpendicular to width 1, average of 5 TF
		Undesirable fruit traits	
Pitting incidence	Pi	%	Calculated as the percentage of fruit with pitting out of a total of 25 fruit sampled
Rain cracking incidence	Cr	%	Calculated as the percentage of fruit with splits out of a total of 25 fruit sampled
Powdery mildew incidence in field	PM	Scored	0 = no visible symptoms, 1 = very slight infection on leaves, 2 = slight infection on leaves, occasionally on shoots, 3 = moderate infection on leaves and/or shoots, 4 = heavy infection on leaves and/or shoots and 5 = very high infection on leaves, most shoots damaged
Bacterial Canker incidence based on a detached leaf assay	BC	Scored	0 = no necrosis, 1 = line of infection, 2 = ≤ 50% of the leaf, 3 = > 50% of the leaf and 4 = total necrosis

[1] Traits that are common to this study and Stegmier et al. (2014) are in asterisk.
[2] Tracked Fruit (TF): five carefully chosen fruit out of a total of 25 harvested
[3] Bulked Fruit (BF): Usually 20 out of 25 harvested fruit samples pulled together for measurements

and width, pit weight, skin and flesh color, fruit shape and freestone tendency (Table 1). Fruit were collected into paper bags in the field and transported to the fruit quality laboratory at least twice a day during harvest. Barcoded sample labels were scanned on entry into the laboratory into an Excel (Microsoft, Redmond, WA) spreadsheet "pick list" as a sample management procedure, and samples were held at 0°C (32°F) in a cold room for at least 12 h before fruit evaluation. After cooling, samples were removed from the cold room and fruit allowed to equilibrate to room temperature prior to evaluation. Twenty-five fruit were selected from each sample, discarding any obvious outliers such as over- or under-ripe fruit, mechanically damaged fruit, etc. These fruit were placed into a sample tray and five optimal fruit from the entire sample (as perceived by the analyst; usually the largest and firmest fruit) were numbered with a permanent marker to be "tracked" through the evaluation process as a quality control measure. Incidence of pitting (Pi,

proportion (%) of fruit displaying pitting) and cracking (Cr, proportion (%) of fruit displaying splits) was assayed and recorded as the number of fruit affected with each defect. Weight of the sample was measured on a top-loading digital balance with readability to two decimal places. Average weight per fruit (FWtB, g) was determined by dividing the *net* total weight by the number of fruit weighed. Skin color distribution of the entire sample was observed and, for red/mahogany fruit (CMS), recorded as number of fruit per color designation according to the Center Technique Interprofessionnel des Fruits et Légumes (CTIFL) color chart (see: www.ctifl.fr, Table 1). For blush, number of fruit per category delineation was also recorded, with category numbers referring to percent area of individual fruit overlaid with red tint (CBS - see description, Table 1). For example, a recording of 3 = 14 would indicate that 14 fruit were observed to have red tint over 51-75% of their skin. Individual color of the five tracked fruit was also recorded.

Sample fruit were next loaded onto turntable of a BioWorks Firmtech II (BioWorks, Inc., Wamego, KS, USA) for instrumental firmness measurement. Cherries were placed on the rotating table with stems attached, each fruit oriented perpendicular to its suture with respect to the table. Firmness of the bulk sample was calculated as the average of the entire sample in g·mm (FirB). The five tracked fruit occupied the first five spots and individual firmness was recorded for each (Fir, g·mm).

Individual traits recorded for tracked fruit included shape by visual observation (ShF), pedicel-fruit retention force (PFRF - Imada DS2-4 digital force gauge with a custom fitted polyvinyl chloride attachment for fruit detachment, Northbrook, IL, USA), flesh color (CFl - Washington State University Flesh Color index that can be found at www.rosbreed.org/resources/fruit evaluations), adherence of flesh to pit (Free Stone, FS) and pit weight (PWt, g, after scrubbing the flesh off). Other individual measurements taken were: pit length (PL, mm), fruit and stem length (FL, SL, mm), and fruit and pit width in two orientations (FW1, FW2, PW1, PW2, mm). Fruit and pit length and width measurements were taken with a digital caliper (Fisher Scientific, Pittsburgh, PA, USA). (See descriptions of all measurements in Table 1). In addition, SSC was measured on each tracked fruit (% Brix) by piercing the fruit and squeezing a drop or two of juice onto the sample window of a hand-held digital refractometer (Atago PAL-1, Atago Co, Ltd., Bellevue, WA, USA).

Juice from the bulked fruit sample was collected and filtered through three layers of disposable laboratory wipes, then used for determination of % Brix and titratable acidity (TA, reported as % malic acid). TA was assayed via potentiometric titration of 10 mL juice (diluted to 50 mL with 18 megohm water) against 0.1 M NaOH to pH 8.1 with a Metrohm 848 autotitrator (Metrohm, FL, USA). Juice pH was recorded prior to titration.

Undesirable fruit traits

Powdery mildew and bacterial canker incidence. Susceptibility to powdery mildew (*Podosphaera clandestine* Wallr.) infection was assayed in the field in late August or early September to allow for full expression of disease pressure. Preventive spray was withheld from surveyed trees. Personnel were sent into the field to record observations of individual trees according to a 6-point scale (Table 1). Bacterial canker incidence was assessed in the laboratory on a 5-point scale (Mgbechi-Ezeri et al., in press) based on a detached leaf assay following inoculation with the pathogen, *Pseudomonas syringae* var *syringae* Van Hall 1904. Symptom expression was recorded from 2 to 8 d following inoculation.

Data analyses

The data were adjusted to remove outliers. Means were calculated for representative traits including fruit weight, firmness, soluble solids concentration, titratable acidity, pedicel-fruit retention force, harvest date

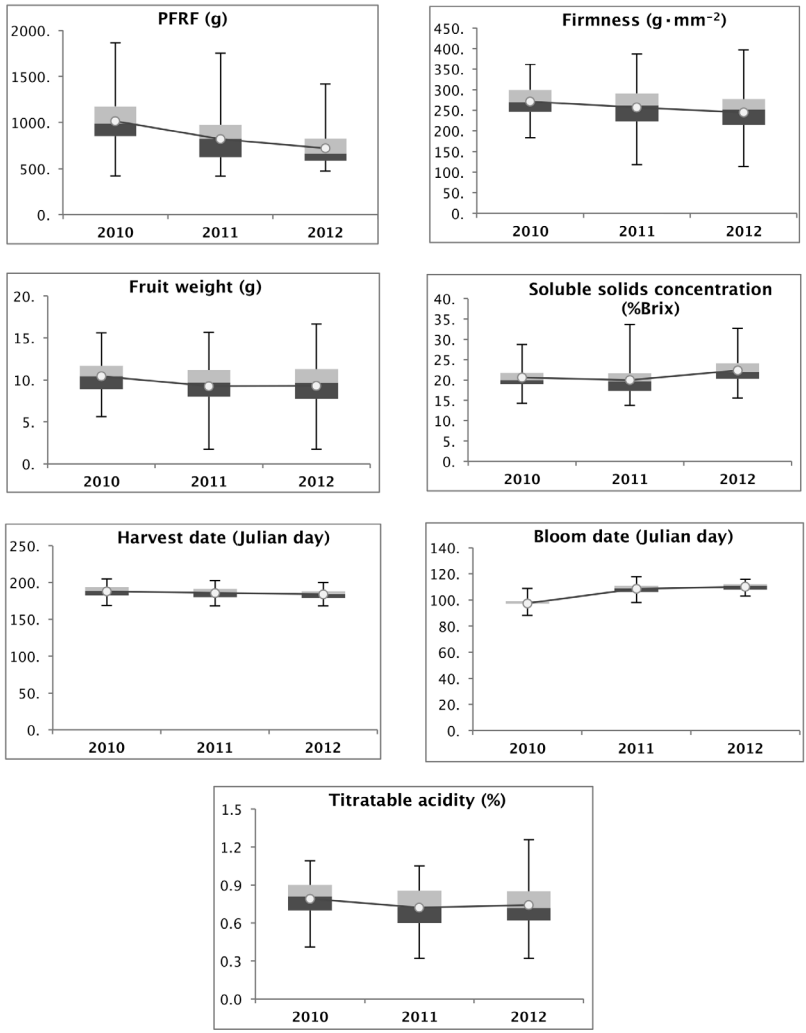


Fig. 1. Box plots of pedicel-fruit retention force, firmness, fruit weight, soluble solids content, harvest date, bloom date and titratable acidity in sweet cherry based on three years data. Dark shading represents the median value minus the 25 percent quartile while light shading represents the median value minus the 75 percent quartile.

and bloom date. Correlations between pairs of traits were calculated for all traits with three years data using Proc Corr in SAS 9.2 version (2008).

Results and Discussion

There were significant differences in PFRF among years ($P \leq 0.001$) with PFRF declining from 1041 g in 2010 to 821 g in 2011 and 719 g in 2012 (Fig. 1). Similar results were obtained by Zhao et al. (2013a) showing significant genotype × year interaction that was attributed to climate variability between years. We believe that inaccurate determination of harvest maturity may also contribute to PFRF differences between years. PFRF

was significantly associated with all the fruit size measurements (FL, FW2, FWt) indicating that accessions with larger fruit tended to have higher values of PFRF. The correlations between PFRF and pit dimension traits (PL, PW2, PWt) were also positive and significant (Table 2). Similarly, the association of PFRF with firmness (Fir) was significant although low (r = 0.16) indicating that firmer fruit in general have higher PFRF values. The mean values of SSC were similar between 2010 and 2011 (20.6% vs 20.0%) but these were significantly different ($P \leq 0.05$) from the 2012 data (22.4%). TA values were similar in 2011 and 2012 (0.72 vs 0.74%) but significantly different ($P \leq 0.05$) from the 2010 data (0.79%). The significant correlation between SSC and TA (r = 0.33) suggests that individuals with higher TA values also had fruit with high sugar concentrations. This association concurs with Zhao (2013) who observed co-location of quantitative trait loci (QTL) for SSC and TA on linkage group (LG) 4 in sweet cherry. Co-location of QTL for the two traits has also been reported by Cantín et al. (2010), Dirlewanger (2006a, 2006b) and Quilot et al. (2004) in peach. The genetic linkage between genes controlling both traits could have contributed to the significant positive phenotypic correlation. Both traits may be improved simultaneously by taking advantage of this genetic correlation. It may be possible to identify accessions that have favorable values of SSC and TA as parents to develop new cultivars with superior taste attributes. SSC, in contrast, had a negative association with all fruit and pit dimension traits (FL, FW2, FWt, PL, PW, PWt) suggesting that breeding for high SSC and large fruit size may be slow.

The mean fruit weights were consistent between 2011 and 2012 (9.26 g vs 9.31 g) but higher in 2010 (10.43 g) ($P \leq 0.05$). There were strong positive associations among all fruit dimension measurements (FL, FW2, FWt) ranging from 0.89 to 0.95 (Table 2). This relationship was also observed by Zhang et al. (2010) and Zhao et al. (2013b). In sweet cherry, QTLs associated with fruit size were originally identified in 'EF' x 'NY 54' populations (Zhang et al., 2010). The QTLs were mapped on LG 2 and LG 6, with the LG 2 QTL postulated to affect fruit size by controlling mesocarp cell number. This is consistent with recent findings that QTL effect on fruit size is highly associated with flesh rather than pit size (De Franceschi et al., 2013). This could explain the high correlation between fruit weight and equatorial diameters. The correlations among all the pit dimension measurements (PL, PW2, PWt) were also positive and highly significant. Moreover, fruit size measurements, fruit firmness and harvest date were positively correlated with each other. Late season accessions tended to have large fruit and firm texture (Table 2).

The incidence of freestone attribute positively correlated with fruit size measurements (FL, FW2, FWt), indicating that pits in larger fruit detached easier from the mesocarp than pits in smaller fruit. Freestone had a negative association with SSC showing higher attachment between pit and mesocarp in cherries with higher values of SSC. However, the correlation between freestone and SSC was low (r = -0.18).

Firmness was highest in 2010 (271 g□mm) and lowest in 2012 (245 g□mm) with significant differences between years at $P \leq 0.001$. Fruit firmness (Fir) and bulked fruit firmness (FirB) had a strong correlation (0.77), confirming that five fruit may suffice to assess overall firmness. Firmness had a positive correlation with all fruit and pit dimension traits suggesting that selection for high firmness would indirectly select for bigger cherries. Significant associations also existed between fruit firmness and PFRF, fruit shape (FS) and harvest date (HD). The negative correlation between firmness and SSC would hinder progress in simultaneously selecting for sweeter fruit.

Harvest was three and five days later in 2010 (189 d from 1 Jan.) than in 2011 (186 d from 1 Jan.) and 2012 (184 d from 1 Jan.), respectively. The latest bloom time was ob-

Table 2. Phenotypic correlations[1] among 19 sweet cherry fruit traits[2] based on three years combined data. Traits that had less than three years data or where data have been collected in different years were excluded from analysis.

	SSC	TA	FL	FW2	FWt	PL	PW2	PWt	FS	CMS	CBS	CFl	ShF	Fir	FirB	HD	HT	BD	BT
PFRF	ns	ns	0.38	0.41	0.43	0.24	0.19	0.27	ns	ns	ns	ns	ns	0.31	0.20	*0.16*	*0.16*	ns	ns
SSC		0.33	-0.41	-0.38	-0.34	-0.25	-0.15	-0.15	*-0.18*	0.29	ns	ns	ns	-0.20	-0.13	*-0.18*	*-0.17*	ns	ns
TA			-0.14	ns	ns	-0.17	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.23	0.23	ns	ns
FL				0.90	0.89	0.71	0.38	0.49	0.24	-0.15	ns	0.14	0.25	0.41	0.30	0.24	0.23	ns	ns
FW2					0.95	0.57	0.45	0.50	0.23	ns	-0.29	ns	*0.18*	0.43	0.31	0.24	0.23	ns	ns
FWt						0.61	0.44	0.53	0.15	ns	-0.31	ns	*0.19*	0.39	0.21	0.26	0.25	ns	ns
PL							0.29	0.60	0.13	ns	ns	0.14	0.21	0.28	0.20	0.20	0.20	ns	ns
PW2								0.43	ns	ns	ns	ns	ns	*0.18*	ns	0.16	0.15	ns	ns
PWt									ns	ns	ns	ns	ns	0.27	0.18	0.26	0.26	ns	ns
FS										ns	ns	0.15	ns	ns	ns	ns	ns	ns	ns
CMS											.	0.66	ns	-0.16	ns	*-0.19*	*0.19*	ns	ns
CBS												ns	ns	ns	ns	ns	ns	ns	ns
CFl													ns	ns	ns	-0.26	0.26	ns	ns
ShF														0.19	0.21	ns	ns	ns	ns
Fir															0.77	0.39	0.40	ns	ns
FirB																0.20	0.20	ns	ns
HD																	0.99	0.26	0.24
HT																		0.24	0.23
BD																			0.95

[1] Correlation values significantly different at $P < 0.0001$ level are underlined, $P < 0.001$ level in italics, and all remaining values are at < 0.01 level. Values not significant at any of these levels are identified as non-significant (NS). Correlations were calculated with the Proc Corr procedure in SAS version 9.2 (2008).

[2] PFRF = pedicel-fruit retention force, SSC = soluble solids concentration, TA = titratable acidity, FL = fruit length, FW2 - fruit width, FWt = fruit weight, PL = pit length, PW2 = pit width, PWt = pit weight, FS = freestone, CMS = mahogany skin color, CBS = blush skin color, CFl = flesh color, ShF = fruit shape, Fir = Firmness, FirB = bulked fruit firmness, HD = harvest data, HT = harvest time, BD = bloom date, BT = bloom time.

served in 2012 (110 d from 1 Jan.) and the earliest in 2010 (98 d from 1 Jan.). It is quite common for some cultivars (such as 'Kiona') to bloom late and yet be harvested mid-season, while others that bloom early to mid-season ripen late (e.g., 'Sweetheart'). This explains the generally low (r = 0.24) but positive correlation between bloom and harvest times. The associations between harvest date (HD) and harvest time (HT), 0.99, and BD and BT, 0.95, were very high. For breeding it will be faster and more cost effective to phenotype either BD or BT, and likewise HD or HT. HD was the most highly correlated with fruit firmness (Fir). Harvest date had positive associations with all fruit and pit dimension traits and clearly showed later maturing individuals tending to accumulate more fruit biomass.

Conclusions

The phenotyping protocol for sweet cherry included measurements of fruit quality attributes that determined marketability and/or consumer acceptability of fresh sweet cherry products. These include fruit weight, firmness, soluble solids concentration, titratable acidity and skin color. Also included was pedicel-fruit retention force which gives an indication of harvestibility with and/or without stem and hence, amenability to mechanization. Measuring either bulked fruit firmness or firmness based on five fruit only, and bloom and harvest time in lieu of either days from 1 Jan. or growing degree days will streamline the phenotyping protocol and improve cost effectiveness. The significant and positive correlations between soluble solids concentration and titratable acidity as well as between harvest date, fruit size and firmness suggests a genetic linkage of the genes controlling these traits. Use of this standardized phenotyping protocol will enable comparisons of data between different years and programs.

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Literature Cited

Cantín, C.M., C.H. Crisosto, E.A. Ogundiwin, T. Gradziel, J. Torrents, M.A. Moreno, and Y. Gogorcena. 2010. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. Postharvest Biology and Technology 58(2):79-87.

De Franceschi, P., T. Stegmeir, A. Cabrera, E. van der Knaap, U.R. Rosyara, A.M. Sebolt, L. Dondini, E. Dirlewanger, J. Quero-Garcia, J.A. Campoy, and A.F. Iezzoni. 2013. Cell number regulator genes in *Prunus* provide candidate genes for the control of fruit size in sweet and sour cherry. Molecular Breeding 32(2):311-326.

Dirlewanger, E., P. Cosson, K. Boudehri, C. Renaud, G. Capdeville, Y. Tauzin, F. Laigret, and A. Moing. 2006a. Development of a second-generation genetic linkage map for peach [*Prunus persica* (L.) Batsch] and characterization of morphological traits affecting flower and fruit. Tree Genetics and Genomes 3(1):1-13.

Dirlewanger, E., P. Cosson, C. Renaud, R. Monet, J. Poëssel, and A. Moing. 2006b. New detection of QTLs controlling major fruit quality components in peach. Acta Hort. 713:65-72.

Evans, K., J. Luby, S. Brown, M. Clark, Y. Guan, B. Orcheski, C. Schmitz, C. Peace, E. van de Weg, and A. Iezzoni. 2012. Large-scale standardized phenotyping of apple in RosBREED. Acta Hort. 945:233-238.

Frett, T.J., K. Gasic, J.R. Clark, D. Byrne, T. Gradziel, and C. Crisosto. 2012. Standardized phenotyping for fruit quality in peach [*Prunus persica* (L.) Batsch]. Journal of the American Pomological Society 66(4):214-219.

Fuglie, K.O. and T.S. Walker. 2001. Economic incentives and resource allocation in US public and private plant breeding. Journal of Agricultural and Applied Economics 33(3):459-474.

Mathey, M.M., S. Mookerjee, K. Gündüz, J.F. Hancock, A.F. Iezzoni, L.L. Mahoney, T.M. Davis, N.V. Bassil, K.E. Hummer, P.J. Stewart, V.M. Whitaker, D.J. Sargent, B. Denoyes, I. Amaya, E. van de Weg, and C.E. Finn. 2013. Large scale standardized phenotyping of strawberry in RosBREED. Journal of the American Pomological Society 67(4):205-216.

Mgbechi-Ezeri, J., K. Johnson, and N. Oraguzie. 2014. Effect of inoculum concentration, isolates and leaf

age on bacteria canker disease development in sweet cherry (*Prunus avium* L.) cultivars. 7th International Cherry Symposium. Acta Hort. (in press).

National Agricultural Statistical Service (NASS). 2013. Non-citrus Fruits and Nuts, 2012 Preliminary Summary. http://usda01.library.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-01-25-2013.pdf.

Peace, C.P., J.J. Luby, W.E. van de Weg, M.C.A.M. Bink, and A.F. Iezzoni. 2014. A strategy for developing representative germplasm sets for systematic QTL validation, demonstrated for apple, peach and sweet cherry. Tree Genetics and Genomes (in press).

Quilot, B., B.H. Wu, J. Kervella, M. Génard, M. Foulongne, and K. Moreau. 2004. QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*. Theoretical and Applied Genetics 109(4):884-897.

SAS. 2008. Statistical Software Version 9.2. SAS Institute, Inc., Cary, NC, USA.

Stegmeir, T., A. Sebolt, and A. Iezzoni. 2014. Phenotyping protocol for sour cherry (*Prunus cerasus* L.) to enable a better understanding of trait inheritance. Journal of the American Pomological Society 68(1):40-47.

Zhang, G., A.M. Sebolt, S.S. Sooriyapathirana, D. Wang, M. Bink, J.W. Olmstead, and A.F. Iezzoni. 2010. Fruit size QTL analysis of an F_1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. Tree Genetics and Genomes 6(1):25–36.

Zhao, Y., B. Athanson, M. Whiting, and N. Oraguzie. 2013a. Pedicel-fruit retention force in sweet cherry (*Prunus avium* L.) varies with genotype and year. Scientia Horticulturae 150(4):135-141.

Zhao, Y., H.P. Collins, N.R. Knowles, and N. Oraguzie. 2013b. Respiratory activity of 'Chelan', 'Bing' and 'Selah' sweet cherries in relation to fruit traits at green, white-pink, red and mahogany ripening stages. Scientia Horticulturae 161(24):239-248.

Zhao, Y. 2013. Understanding the genetics of pedicel-fruit retention force in sweet cherry (*Prunus avium* L.). Washington State University. PhD Diss.

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In *vitro* micrografting of cherry (*Prunus avium* L. 'Regina') onto 'Piku® 1' rootstock [*P. avium* × (*P. canescens* × *P. tomentosa*)]

An early diagnosis for compatibility between cherry varieties and rootstocks is required to launch new plant material on the market. An *in vitro*, slit-micrografting technique for *Prunus avium* L. 'Regina' on 'Piku® 1' rootstock [*P. avium* × (*P. canescens* × *P. tomentosa*)] is presented as a model for cherry species. *In vitro* shoot tips, cut in a V-shape, were used as scions, while shoots induced for *in vitro* rooting were used as rootstocks. The success of various parameters was examined: micro-scion size, carbon source, number of *in vitro* scion multiplication cycles, composition of the medium, and grafted tissue treatment. Micrografts were cultured on a modified Murashige and Skoog (MS) medium without added plant growth regulators (PGRs). The highest percentages of successful grafts (80% at 4 weeks after grafting and 58% at 3 months after acclimatisation) were obtained using glucose as the sole carbon source, vermiculite in the agar medium, and 3 – 5 mm-long shoot tips formed *in vitro* as scions. Additional chemicals such as kinetin, ascorbic acid, citric acid, polyvinylpyrrolidone (PVP), or 6-benzyladenine (BA) did not improve grafting, but a rapid micrografting process was most important. The number of *in vitro* scion multiplication cycles was shown to have no effect. Using *in vitro* cultures of both scion and rootstock, aseptic conditions were maintained throughout the micrografting process and no problems of contamination were encountered. Abstract from: Laurence Bourrain and Gerard Charlot, 2014. The Journal of Horticultural Science & Biotechnology 89(1):47-52.