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Standardized Phenotyping in Black Raspberry

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Abstract

Black raspberry (*Rubus occidentalis* L.), is one of a group of economically important members of the genus *Rubus*. In this paper, we describe a multi-state project to phenotype 43 traits in black raspberry. Two mapping populations that had parental material from multiple sources, including wild germplasm from North Carolina, Ontario, and Maine were used to assess phenotypes in 11 geographically distinct locations. A summary of the means, sample size, and range of traits including important phenological stages, flowering, plant and fruit characteristics, and fruit chemistry traits are provided in this paper. Variation in traits across populations, locations, and years was observed but was trait dependent. This phenotypic data will be included in the Genome Database for Rosaceae (GDR) (http:/www.rosaceae.org/).

Black raspberries (*Rubus occidentalis* L., *2n=2x=14*) are a high-value crop ($16.9 million farm gate value – US, 2014) showing increasing demand with health-conscious consumers. Despite their long history of production dating back to the 1900s in North America (Dossett, 2007; Jennings, 1988), the black raspberry industry has stagnated over the past 75 years in the US due to a lack of adapted and disease-resistant cultivars (Halgren et al., 2007). Traditionally, black raspberries have been grown almost exclusively for food processing as whole fruit, puree, or juice, in addition to usage as a natural food dye (Bassil et al., 2014). However, in recent times, this production ratio has split approximately 50:50 as interest in fresh berry production has grown (NASS, 2015). Domestically, black raspberries are grown on approximately 1,700 acres (688 hectares), centered in the Pacific Northwest (NASS, 2015). Although black raspberry has been in cultivation over a century, this crop has not been widely grown outside of the Pacific Northwest and fresh fruit is scarce in local and commercial markets.

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Lack of genetic diversity has hindered traditional breeding efforts in black raspberry for many decades. Additionally, very few molecular genetic resources are available for all *Rubus* cultivated crops, including red raspberry (*R. idaeus*), blackberry (*R. spp*), and black raspberry (*R. occidentalis*). Such resources could be used to rapidly develop cultivars for production in diverse climates. Genetic tools have been used for years in row crops such as corn (Stuber and Edwards, 1986), soybean (Cregan et al., 1999), and rice (Chen et al., 2000), and allow germplasm screening at the seedling stage, saving time and space in the field while also saving resources needed to phenotype large populations of plants. 'Developing the Genomic Infrastructure for Black Raspberries Breeding Improvement', a multi-state US Department of Agriculture National Institute of Food and Agriculture (USDA-NIFA; award number 2011-51181-30676) Specialty Crop Research Initiative project, began in 2012 to improve black raspberry breeding resources. From this study, a linkage map (Bushakra et al., 2015), the draft genome (VanBuren et al., 2016), and now a list of phenotypic traits of black raspberry have been developed.

Traditional breeding efforts are time-consuming, therefore the development of molecular tools allowing breeders to screen germplasm at the seedling stage can expedite cultivar development (Bernardo 2008). However, one of the first steps to developing modern tools is based on building reliable phenotypic data sets to calculate the relationship between genotype and phenotype across environments (Bassil and Volk, 2010). Reliable phenotyping is essential for accurate statistical analyses that are used in the development of quantitative trait loci and marker-trait associations for marker assisted breeding (MAB). Standardized phenotyping protocols have been the backbone in the success of other crops in the RosBREED project (Iezzoni et al., 2010), including apple (Evans et al., 2012; Schmitz et al., 2013), peach (Frett et al., 2012), tart cherry (Stegmeir et al., 2014), sweet cherry (Chavoshi et al., 2014), and strawberry (Mathey et al., 2013). In this paper, we present the first standardized phenotyping protocol developed in the genus *Rubus* for 43 plant and fruit traits of black raspberry. Phenotyping was based on the evaluation of two populations planted in multiple locations across the United States.

Materials and Methods

Plant material. Two half-sib mapping populations, that included a wide range of parental germplasm with the aim of incorporating wild germplasm, were planted at research and grower sites in four states in 2012. The populations were established in 2009 in Oregon. The common parent between the black raspberry populations is ORUS 3021-2, which resulted from a cross of NC 84-10-3 × 'Jewel' (Dossett, 2007; Dossett et al., 2008). The cross of ORUS 4158-2 × ORUS 3021-2 resulted in population ORUS 4304 that contained 192 plants. The cross of ORUS 3021-2 × ORUS 4153-1 resulted in population ORUS 4305 that contained 115 plants. The parental sources of germplasm were chosen due to disease and insect resistance, as well as their geographically distinct native sources (Dossett et al., 2008; Dossett and Finn, 2010). The populations were created with the intention of germplasm improvement and diversification.

The black raspberry plant, like many *Rubus* species, has a biennial above-ground growth habit with a perennial root system. Primocanes usually remain vegetative in the first season, although some genotypes of raspberry, blackberry, and a few black raspberry will produce fruit on the primocanes in the first year. In the fall and winter, canes transition from primocanes to floricanes. In the second year, the floricanes produce fruit and after the fruit ripens, the canes senesce. Standard production practices were followed for cultural, fertility and water management of the field and plants based on local recommendations (Barney and Miles, 2012; Bushway et al., 2008; Fernandez et al., 2016; Funt

et al., 1999). Plants were trained to a trellis and spent floricanes were pruned in the late summer. Additional pruning to a standardized cane number of five canes occurred in the fall for cane numbers and diameter and in late winter/early spring for winter injury.

For each of the three growing seasons, plants at research locations (OH, OR, NY, and NC) were most closely evaluated (>1x weekly), while commercial sites were visited as often as possible (≥once per season). For the purposes of statistical analyses in this publication, only research location datasets were included. Plant populations were shipped to each research site, and commercial cultivars Mac Black, Jewel, and Bristol were evaluated alongside the mapping populations as standard controls. Separate research suggested that 'Jewel' and 'Bristol' were synonymous with 'Black Hawk' and 'Munger', respectively (Dossett et al., 2012). Since the populations were planted in fall 2012 and spring 2013, some data were missing or inconsistent in the first season, as plantings were being established. Individual plants that died in the winter of 2012 or spring of 2013 were re-planted in fall of 2013.

Phenotyping. Tissue-culture propagated genotypes from populations ORUS 4304 and ORUS 4305 were shipped to 11 research and grower sites in Ohio (OH), Oregon (OR), New York (NY), and North Carolina (NC) in 2012. At least 168 of 192 progeny from 4304 and 89 of 115 progeny 4305 plants were shipped to each site and planted in non-replicated trial plots. Phenotyping procedures are described below and detailed in Table 1. The frequency of observations depended on variability of trait (i.e., some traits such as prickle shape do not change from year-to-year). Dates for this data set were reported as days from 1 Jan. to 31 Dec. each year (1-365).

Phenology and flowering .Phenological data were recorded at least once weekly from budbreak through fruit ripening. Vegetative budbreak was recorded when approximately 3 mm of green leaf tips were seen protruding from buds in the top 1/3 of the canes. Bloom date and fruit ripening date were recorded when the first fully open flower and first fully ripened fruit, respectively, were observed in the top 1/3 of the canes. For these observations, a date was not recorded unless the rest of the plant was not more than 1-2 days behind, in order not to count 'outlier' canes that may have been diseased or damaged. The number of flowers per lateral shoot and fruit per lateral shoot was counted during or soon after full bloom, or at the time of fruit formation, on three fruiting shoots from the same floricane: one each from the top, the middle and bottom portion of the cane. In addition, subterminal fruiting nodes were counted at the same time, and each node under the terminal fruiting node was included in this count. Fruit set was scored in the interval between bloom and fruit ripening, scored as a percentage of fruit set (observations of drupelet formation overall and in individual flowers), and ranged from no drupelet set to full drupelet set on a scale of 1 – 9.

Plant morphological traits. Plant traits were observed at several points throughout the season. Each plant was scored individually for each trait, and plant traits for each genotype were evaluated using typical evaluation methods for raspberry in the field, by qualitative scoring methods of a 1 – 5 or 1 – 9 scale. Unless otherwise noted, a score of 1 being the least desirable and 5 or 9 being the highest level of the trait (Table 1). Prickle density, shape and length, cane color, glaucousness, and biomass were scored at the time of winter pruning. Prickle density in the mid-portion of the cane (45 – 76 cm from crown) ranged from dense (1) to prickle-free (9), and prickle shape was scored as straight (1) or recurved (2). Prickle length was a visual observation of prickle size, also from the mid-portion of the cane, ranging from small (1) to large (3). Cane color was scored using Royal Horticultural Society (RHS) color chart cards (Royal Horticultural Society, 1966). Black raspberry canes are often identified based on their 'waxy' appearance, and this cane glaucousness ranged from no

wax (1) to very waxy (5). Cane number was estimated on each plant (before winter pruning) and cane diameters were measured in millimeters (mm) by calipers on eight canes per plant. A biomass estimate was calculated as [cane number * average (cane diameter)] at the base of each plant. Cane diameter was measured at approximately 30 cm above the ground and cane numbers were counted in a 30 cm square at the base of the plant.

Winter injury to canes was scored based on presence of necrotic tissue of the cane starting from the apex and moving downward. Winter injury to canes was scored once in late winter or early spring before bloom, and again after bloom. Winter injury scores range from 1: entire cane was necrotic (dead) to 9: all live tissue (no damage). Due to atypical warm temperatures during bloom (32 °C), flower bud injury was scored at sites in NC in 2014, ranging from all buds (receptacles) dead (1) to no damaged buds (receptacles) (9) (State Climate Office of North Carolina, 2015). Floricane vigor was scored at bloom, and primocane vigor was scored in early fall after summer pruning; both ranging from dead canes (1) to extremely vigorous cane growth (9). Total number of fruiting lateral shoots was counted on one representative floricane on each plant. Similarly, lateral length of three fruiting shoots from one floricane of approximately equal size was measured around bloom time at the top, middle and bottom of the cane. Final numbers reported in Table 2 are an average of these three measurements. Primocane emergence was the first date when primocanes could be seen emerging from the crown on each plant, and primocane leaflet number was recorded on fully opened primocane leaflets on new primocanes at least 1/3 m tall. Basal bud density, or the number of buds emerging from the crown area, was scored during the harvest period and ranged from none (1) to many (9).

Fruit characteristics. Fruit traits were observed at the time of harvest and each trait was scored based on observations of five to ten berries, picked at random from each plant (i.e., berries were not all harvested from the same cluster, and not only "king" berries were picked). Fruit traits for each genotype were recorded using typical evaluation methods for black raspberry in the field, by assigning qualitative scores of 1 – 5 or 1 – 9 (Table 1). Fruit traits measured included cluster tightness, fruit load, adherence to torus, drupelet cohesion, fruit color, gloss, pubescence, firmness, shape, size, and flavor. Cluster tightness was scored on a scale of 1 – 5, with 1 being a cluster with ripe fruit touching one another to 5 being a cluster with fruit not touching and spaced widely apart. Fruit load was scored on a scale of 1 – 5 based on a visual observation at peak ripening for each plant, 1 was no fruit, 3 indicated a moderate crop and 5 was a heavy yield. Adherence was scored based on how easily the fruit pulled away from the torus when picked by hand and was scored from very difficult (1) to very easy (9). Cohesion of ripe berries upon harvest was defined by maintenance of fruit shape and crumbliness of drupelets and scored from very crumbly (1) to intact (9). Fruit color was scored from red (1) to black (9), and gloss was scored from dull (1) to very shiny (9). Fruit pubescence in black raspberry arises from small trichomes on the surface of and in between drupelets (Robbins et al., 1988). Fruit pubescence was scored from very high (1) to very low (5). Relative firmness was tested by lightly squeezing fruit between fingertips scoring from very soft (1) to very firm (9). Fruit shape was scored from round (1) to conical (5). A qualitative score of fruit size of small (1) to large (5) was taken at harvest (see fruit size below). Flavor was scored 1 – 9, based on presence or absence of off-flavors and aromas and a good balance of sweetness and tartness, 1 = soapy, not sweet, dry, unpleasant flavors, unpalatable, 9 = sweet, juicy, pleasant flavors. Torus shape of five fruit from each plant was measured with a digital caliper at four locations (one vertical and three horizontal at the calyx, midline, and tip) and then assigned a shape based on these measurements. The following criteria

Table 1. Name, categorical description, and criteria used to evaluate 44 phenotypic traits in two black raspberry populations and standard cultivars. These traits were measured in 2013 – 2015 in four research locations (OH = The Ohio State University, Wooster, OH; OR = Lewis Brown Farm, USDA-ARS NCGR, Corvallis, OR; NY = Cornell University, Geneva, NY; NC = Sandhills Research Station, Jackson Springs, NC).

Trait	Evaluation criteria	2013 sites	2014 sites	2015 sites
Phenology and flowering traits				
Bloom date	Date when first fully open flower in the upper 30% of the canopy was observed	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY; NC
Budbreak	Date when ~3 mm of green tips were seen protruding from vegetative buds on the upper 30% of the canes	OR; NC	OR; NC	OH; OR; NY; NC
Fruit ripening	Date when first fully ripened fruit in the upper 30% of the canopy was observed	OH; NY; NC	OH; NY;	OH; OR; NY
Flowers per lateral shoot	Measured during full bloom. Count of flowers per fruiting lateral shoot. Three fruiting lateral shoots from the same floricane were sampled: one each from the top, middle and bottom portion of the cane	OR; NY; NC	OH; NY; NC	NY
Fruit set	Recorded between full bloom and fruit ripening. Scored 1 – 9; 1 = no fruit set, 5 = 50% fruit set or half of drupelets, 9 = full drupelet set	OH; OR; NY; NC	OH; NC	OH; OR
Basal bud density	Recorded between fruit ripening and summer pruning. Scored 1 – 5; 1 = no basal buds, 5 = many basal buds	NC	NC	-
Plant characteristics				
Floricane vigor	Cane health/growth measured at bloom, scored 1 – 9; 1 = dead, 9 = extremely vigorous	OH; OR; NY; NC	OH; OR; NC	OH; NC
Primocane vigor	Cane health/growth measured in early fall, after summer pruning. Scored 1 – 9; 1 = dead, 9 = extremely vigorous	OH; OR; NY; NC	OH; NY; NC	OH; NY; NC
Prickle density	Recorded during winter pruning. Prickle density was observed in the mid-portion of the cane (approx. 45 – 76 cm height range). Scored 1 – 5; 1 = dense prickles, 5 = prickle-free	OH; NY; NC	-	-

Prickle length	Visual observation taken during winter pruning on prickles. Scored from 1 (small) to 3 (large)	OH; NY; NC	-	-
Prickle shape	Observed during winter pruning. Scored as 'recurved' or 'straight'	OH; NY; NC	-	-
Cane color	Recorded during winter pruning. Scored based on Royal Horticultural Society (RHS) color chart	NC	-	-
Cane glaucousness	Recorded during winter pruning. Scored 1 – 5; 1 = no wax, 5 = extremely waxy	OH; NY; NC	-	-
Cane number	Counted prior to winter pruning. Number of live primocanes growing from the crown	OH; NY; NC	OH; OR; NY; NC	OH; OR; NY
Cane diameter	Measured prior to or during winter pruning. Using calipers, width of maximum of eight primocanes measured in mm at 15 cm from crown	NC	OR; NC	OR
Biomass estimate	Calculation of [(cane number) * average(cane diameter)]	NC	NC	
Winter injury	Recorded in late winter or early spring, before bloom. Scored 1 – 9; 1 = entire cane dead from cold injury, 5 = 50% of cane dead, 9 = no cold injury	NC	NY; NC	NY; NC
Flower bud injury	Recorded after full bloom, during fruit development. Number of receptacles blackened. Scored 1-9; 1 = all receptacles dead, 5 = 50% dead, 9 = all alive		NC	
Primocane emergence	Date when primocanes were first seen emerging from the crown	OH; NC	OH; OR; NC	OH; OR; NC
Primocane leaflet no.	Number of leaflets per leaf on a fully opened primocane leaf; measured on new primocanes at least 1/3 m tall	OH; NY; NC		

Subterminal fruiting nodes	Number of nodes per lateral fruiting shoot below the terminal fruit cluster. Counts were recorded from three fruiting shoots on the same floricane, one each from the top, mid, and bottom portion of the cane	OR; NY; NC	OH; NY; NC	OH; NY
Fruiting lateral length	Measured during or soon after full bloom. Length of three lateral fruiting shoots on the same floricane, sampled from the top, mid, and bottom portion of the cane	OR; NY; NC;	OH; NY; NC	OH; NY
Fruiting laterals	Recorded between bloom and fruit harvest. Count of total fruiting lateral shoots from one floricane	NY; NC	OH; NC	OH; OR
Fruit Characteristics				
Cluster tightness	Visual observation taken when fruit were ripe. Scored 1 – 5; 1 = ripe fruit within the same cluster were touching/rubbing together, 5 = ripe fruit within the same cluster were spaced widely apart	OH; OR; NC	OH; OR; NY; NC	OH; NY
Adherence	Recorded in the field at fruit harvest. Scored 1 – 5; 1 = ripe fruit was difficult to pick, tears and/or remains stuck to torus, 5 = ripe fruit was easily picked	OH; OR; NC	OH; OR; NC	OH
Color	Recorded in the field at fruit harvest. Scored 1 – 9; 1 = red, 5 = purple, 9 = black	NC; OH	NC	-
Firmness	Recorded in the field at fruit harvest by picking several ripe fruit and squeezing lightly between fingertips. Scored 1 – 9; 1 = very soft, fruit collapsed under pressure, 5 = very firm, fruit maintained shape under pressure	OH; OR; NC	OH; OR; NC	OH
Cohesion	Recorded in the field at fruit harvest. Scored 1 – 5; 1 = fruit falls apart when picked, drupelets are ‘crumbly’, 5 = fruit maintains shape when picked	OH; OR; NC	OH; OR; NC	OH

Winter injury	Recorded in late winter or early spring, before bloom. Scored 1 – 9; 1 = entire cane dead from cold injury, 5 = 50% of cane dead, 9 = no cold injury	NC	NY; NC	NY; NC
Flower bud injury	Recorded after full bloom, during fruit development. Number of receptacles blackened. Scored 1-9; 1 = all receptacles dead, 5 = 50% dead, 9 = all alive		NC	
Primocane emergence	Date when primocanes were first seen emerging from the crown	OH; NC	OH; OR; NC	OH; OR; NC
Primocane leaflet no.	Number of leaflets per leaf on a fully opened primocane leaf; measured on new primocanes at least 1/3 m tall	OH; NY; NC		
Shape	Visual observation recorded at fruit harvest. Scored 1 – 5; 1 = round, 5 = conical	NC	NC	-
Flavor	Recorded in the field at fruit harvest. Scored 1 – 9; 1 = soapy, not sweet, dry, unpleasant flavors, unpalatable, 9 = sweet, juicy, pleasant flavors	NC	NC	-
Fruit per lateral shoot	Recorded when fruit had developed. Count of fruit per fruiting shoot. Three fruiting lateral shoots total from the same floricane were sampled: one each from the top, middle and bottom portion of the cane	NY	NY; NC	NY
Fruit load	Visual observation recorded at peak ripeness. Scored 1 – 5; 1 = no fruit, 3 = moderate crop, 5 = heavy crop	NC	NC	-
Fruit size	Visual observation taken at peak ripeness. Scored 1 – 5; 1 = no fruit, 3 = moderate crop, 5 = heavy crop	OH; OR; NC	OH; OR; NC	OH
Fruit weight	Calculation of average [25 berry weight (g)]	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY
Drupelet count	Calculation of [seed number / fruit weight]	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY

Seediness	Calculation of [seed weight (mg) / fruit weight (g)]	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY
Fruit Chemistry				
pH	Fruit previously frozen, thawed to room temperature and pureed. Measured by pH meter	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY
Titratable acidity (TA)	Measured by automated titrator to a pH endpoint of 8.1, reported as percent citric acid	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY
Soluble solids (SSC)	Measured by digital refractometer using 0.5 ml fruit puree	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY
Total anthocyanins	Measured by pH differential method (Lee et al., 2002)[1], reported as mg cyanidin-3-glucoside equivalents / 100 g fresh weight fruit	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY
Total phenolics	Measured by Folin-Ciocalteu method (Singleton et al., 1999)[2] and reported as mg gallic acid/100 g fresh weight	OH; OR; NY; NC	OH; OR; NY; NC	OH; OR; NY

[*] Lee, J., R.W. Durst, and R.E. Wrolstad. 2002. Impact of juice processing on blueberry anthocyanins and polyphenols: Comparison of two pretreatments. J. Food Sci. 67:1660–1667.

[†] Singleton, V.L., R. Orthofer, and R.M. Lamuela-Raventós. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299:152–178.

were established: conic: a gradually tapering conical shape (greater than 0.2 mm wider at the calyx than the midline or tip); oval: the same calyx and midline widths (within 0.2 mm); sphere: a greater midline than calyx width (greater than 0.2 mm).

Yield and fruit size estimation. At each research site, a 25-fruit sample was harvested once a season from each plant (in 2015, no fruit samples were taken in NC because of plant decline). Mean fruit weight was calculated from the 25-fruit sample from each plant. After fruit was weighed, seeds were extracted from the 25-fruit sample, and seed number per fruit was determined. Drupelet count was calculated as the number of seeds per fruit, and seediness was calculated as the proportion of mean seed weight/mean fruit weight.

Fruit chemistry. Approximately 50 g of fruit was harvested, packed on ice or dry ice and transported to the Plants for Human Health Institute in Kannapolis, NC for fruit chemistry analysis. Frozen fruit was held at -20 °C until analyzed. Fruit were thawed, pureed, and analyzed for pH, percent soluble solids concentration (SSC), titratable acidity (TA), total anthocyanin and total phenolic concentration as described by Perkins-Veazie et al. (2016). Extracts for total anthocyanin and phenolics were re-extracted and supernatants were combined for analysis. Details of the fruit chemistry units reported are summarized in Table 1.

Statistical analysis. Phenological traits based on date of occurrence were initially recorded as dates each year, then converted to days from 1 Jan. to 31 Dec. (1-365). These

are the measure of days reported in Table 2. The R statistical software and JMP Pro 14.1 was used to calculate means, minimums, and maximums for each set of traits by year and location, as well as to calculate Spearman's correlation coefficients and between yield and other traits in NC 2013 dataset. The package agricolae in R was used for analysis of variance (ANOVA) and mean separation based on Tukey's Honest Significant Difference test. A full analysis of the data will be included in the online data set housed at the GDR.

Results and Discussion

Individual traits measured at each research location in years 2013 – 2015 are presented in Table 1. The parents of these two populations were chosen and used in our crosses with the intention of diversifying the gene pool of black raspberry, and therefore variation of traits in the populations was expected. Significant range in the recorded scores of traits across populations, locations, and years was observed. This is demonstrated by the wide range of measurements for each trait (Table 2), averaged over populations and locations by year. In general, phenological traits varied the most between locations, most likely due to climatic differences in temperature, light intensity, and day lengths between OH, OR, NY, and NC. However, the mean and range of these traits did not vary by year, indicating that they are likely genetically controlled and heritable. Additionally, fruit traits varied little between locations and years but displayed some differences among the populations, adding to the list of traits that could be improved by breeding.

Floral fecundity was negatively impacted by high temperatures during flowering in NC. In 2014, atypically warm temperatures in Jackson Springs, NC during the first week of May ($\geq$ 32 °C) caused browning of the flower stamens, resulting in subsequent poor drupelet set presumably due to a lack of pollinization and ultimately reduced yield. Stamen injury has been documented in eastern blackberry (*Rubus* L. subgenus *Rubus* Watson) when flowers were suddenly exposed to 35 °C in growth chamber studies, while there was no injury at 29.4 °C (Stanton et al., 2007). Black raspberry flower bud injury was scored at both sites in NC to compare the effect of temperature at these locations. We observed that plants flowering during this week were most affected, whereby plants that flowered earlier and later were not affected (data not shown).

Budbreak occurred earliest in OR, regardless of year, and on average ~ 20 days prior to vegetative budbreak in NC and 30 days prior to OH or NY. This could be due to earlier fulfilllment of chilling units in the winter or early accumulation of heat units in the spring. Bloom date (date when first fully open flower in the upper 30% of the canopy was observed) occurred on average in four consecutive weeks starting in NC, then OR, OH, and NY. Fruit ripening date (date when first fully ripened fruit in the upper 30% of the canopy) was observed in NC and OR was an average of 30 days before NY or OH. The primocane emergence date was similar to budbreak, where OR was approximately 20 days ahead of NC and 30 days ahead of OH each year. Primocane leaflet number was recorded in 2013 in OH, NY, and NC, and determined to be a static trait, with three leaves per leaflet on primocanes and five leaves per leaflet on floricanes. The number of flowers per lateral shoot was highest in 2014 and lowest in 2013, regardless of location. Fruit set was highest in OR regardless of year, and fruit set / fruit per lateral shoot were lowest in NC in 2014, following the flower bud injury that occurred earlier that year.

Primocane vigor was highest in 2013 (OR, OH) or 2014 (NC, NY) depending on location. In the following year (2014), floricane vigor was highest at all locations, and declined in the last year. Since the primocanes of one year are the floricanes in the following year, these high scores in subsequent years on the essentially the same canes were not surprising. Winter injury to floricanes was

Table 2. Mean values and range of observations for 42 phenotypic traits (variables) in black raspberry measured in four locations (OH, OR, NY and NC) and reported by year. Cane color and primocane leaflet number scores are presented in the results section.

	2013			2014			2015		
Variable	n	Mean	Range	n	Mean	Range	n	Mean	Range
Bloom date	871	138.2	116 – 168	901	134.5	121 – 155	962	132.3	115 – 153
Budbreak	596	87.8	60 – 122	564	88.8	60 – 121	1011	95.2	61 – 122
Fruit ripening	674	174.5	152 – 200	439	183.0	179 – 191	740	170.1	155 – 189
Flowers per lateral shoot	596	9.3	3 – 24	612	12.7	3 – 26.7	208	14.4	3 – 28.3
Fruit set	843	7.2	1 – 9	523	5.8	1 – 9	524	7.2	1 – 9
Basal bud density	216	2.4	1 – 5	283	3.4	1 – 5	-	-	-
Floricane vigor	988	5.6	1 – 9	878	6.5	1 – 9	537	3.9	1 – 8
Primocane vigor	1171	6.4	1 – 9	809	6.2	1 – 9	766	5.2	1 – 9
Prickle density	701	2.6	1 – 5	-	-	-	-	-	-
Prickle length	700	2.0	1 – 3	-	-	-	-	-	-
Prickle shape	707	1.6	1 – 2	-	-	-	-	-	-
Cane glaucousness	701	2.5	1 – 5	-	-	-	-	-	-
Cane number	709	5.6	0 – 52	821	6.5	0 – 24	475	6.9	1 - 21
Cane diameter	215	6.7	3.2 – 9.5	532	10.7	2.6 – 13.6	245	10.7	4.0 – 16.0
Biomass estimate	215	33.2	4.4 – 77.7	287	49.9	4.0 – 144	-	-	-
Winter injury	215	6.3	1 – 8	385	7.2	1 – 9	516	4.2	1 – 9
Floral injury	-	-	-	296	4.9	1 – 9	-	-	-
Subterminal fruiting nodes	598	1.2	0 – 6	615	3.0	0 – 8	452	3.3	1 – 10
Lateral length	595	24.8	3 – 75	612	20.5	3.7 – 91.2	451	24.8	6.3 – 48.3
Fruiting laterals	427	17.8	0 – 50	433	34.7	0 – 100	478	17.1	4 – 64

Cluster tightness	619	2.7	1 – 5	815	2.6	1 – 8	294	2.6	1 – 7
Adherence	610	4.2	1 – 5	733	3.9	1 – 5	247	4.9	2 – 5
Color	450	7.9	3 – 9	258	7.4	4 – 9	-	-	-
Firmness	607	4.7	1 – 9	503	6.3	1 – 9	247	5.2	1 – 9
Cohesion	608	4.4	1 – 9	734	4.1	1 – 9	247	5.4	1 – 9
Torus shape	564	1.1	0 – 3	490	2.4	1 – 5	247	2.2	1 – 4
Gloss	450	5.4	1 – 9	596	5.8	1 – 9	328	5.5	1 – 9
Pubescence	200	3.5	1 – 5	258	4.0	1 – 5	-	-	-
Shape	201	2.1	1 – 4	258	1.8	1 – 3	-	-	-
Flavor	194	6.0	3 – 9	258	5.9	3 – 8	-	-	-
Fruit per lateral shoot	204	7.4	2.7 – 15.3	361	10.7	0.3 – 150	205	12.9	3 – 25
Fruit load	213	3.6	1 – 5	276	2.2	1 – 5	-	-	-
Fruit size	622	2.0	1 – 5	745	2.1	1 – 5	247	1.9	1 – 4
Fruit weight g	559	1.92	0.17 – 8.75	924	1.52	0.24 – 3.46	701	1.65	0.39 – 2.93
Drupelet count	561	67.0	5.5 – 129.7	1156	64.9	18.2 - 126	706	76.1	33.0 - 139
Seediness	551	0.95	0.07 – 4.16	924	0.69	0.14 – 1.40	701	0.77	0.15 – 1.38
pH	661	3.8	3.2 – 4.9	916	3.9	2.9 – 4.9	678	3.8	3.0 – 5.0
Titratable acidity	660	1.1	0.4 – 1.9	916	1.1	0. 4 – 1. 9	678	1.0	0.4 – 1.8
Soluble solids	663	10.1	5.2 – 18.7	915	13.1	7.7 – 21.4	697	12.9	6.3 – 23.9
Anthocyanins mg/100g	663	408	157 - 789	914	346	123 - 695	698	354	93.0 – 778
Phenolics mg/100g	663	334	199 - 472	916	383	261 - 521	697	460	253 - 728

measured in NC and NY, and in both locations canes suffered the least damage in 2014 and the most damage in 2015. This decrease in winter hardiness was correlated with a decrease in floricane and primocane vigor seen overall in the populations. Floricane number was highest in NY and lowest in OH, and floricane diameter was larger in OR than NC regardless of year, also indicative of differences in plant stature in different regions.

Fruiting lateral length was longest in OR and NY, and shortest in NC; however, the number of fruiting laterals per floricane were higher in 2014 than 2013 or 2015. Subterminal fruiting nodes on laterals were most abundant in 2014 and least abundant in 2013, regardless of location. Basal bud density was scored on plants only in NC, and number of buds counted was higher in 2014 than 2013. Basal buds may not be an issue in some locations or populations, but fruit from these crown shoots typically produce larger fruit late in the season, and may affect yield, therefore must be taken into consideration and either excluded or included when looking at yield datasets. Also, in NC a decline in vigor was observed over time, thus a decrease in basal buds could be a result of that decline. This decline in vigor has been observed in red raspberry in NC and has been attributed to heat stress (Ballington and Fernandez 2008).

Because prickle density, prickle length, prickle shape, and cane glaucousness were static over locations and years, these traits were only measured in one year in OH, NY and NC. The differences among these clonal populations could be due to environmental differences, but are most likely a result of experimental error caused by subjective judgements of the different individuals phenotyping in different locations. As a protocol such as this one evolves, a clear understanding of the trait range can be better defined; however, as to be expected, our initial observations at different locations were sometimes scored differently. Cane color was measured in NC in 2013 using RHS (Royal Horticultural Society) color charts, and all floricanes fell in the range of RP59A, RP59A/B, RP60A (dark red), or RP77A (purple) (need description of color for these, see if Gina can send me a pick of these).

Fruit traits were scored from 2013 to 2015 (Table 1). However, no fruit traits were scored in 2015 in NC, due to the decline in the population over time. Cluster tightness was scored similarly in OR, NC, and OH, and higher in NY. Berry adherence to the receptacle upon picking was scored moderately easy in OR and NC, and easy in OH. Cohesion of berry fruit upon picking was scored similarly to berry adherence, as there is likely a relationship between the two traits and also the tendency to score higher or lower by that individual in each of those locations. Torus shape measured in OR and OH was more spherical in 2013 and more oval in 2014. The related fruit shape measured in NC contained more spherical shaped fruit in 2013 and 2014. A visual estimate of fruit size ranked OR with the largest fruit in 2013 and 2014, and NC with the smallest fruit in 2013. Fruit firmness was highest in NC berries in 2013 and 2014, and lowest in OR and OH in 2013, possibly due to drier weather conditions and smaller fruit in NC. Fruit color was fully black in OH, and averaged darker purple in NC. Fruit gloss (OH, NC, and NY) and flavor (NC only) was consistently moderate over sites and years, and in NC, the fruit pubescence was less apparent in 2014 than in 2013.

In 2013, total fruit yield was harvested from each plant in both populations in NC. In 2014, yield loss was so substantial due to weather that harvest data could not be used. In 2013, actual yields per plant ranged from 2 – 4,369 g per plant, with average fruit yield of 1,381 g per plant. Spearman's correlation coefficients between yield and 10 other phenotypic traits for 2013 in NC is found in Table 3. Floricane vigor ($\rho = 0.64$) was most highly related to actual yield, followed by biomass ($\rho = 0.57$), fall primocane vigor ($\rho = 0.55$), fruit load ($\rho = 0.44$), and subterminal nodes ($\rho = 0.44$). These traits may be the

Table 3. Phenotypic correlations among 11 black raspberry plant and fruit traits for 2013 at the Sandhills Research Station, Jackson Springs, NC analysed for yield estimates.[1]

Trait	Fruit Weight	Yield	Fruit Set	Fruit Load	Floricane Vigor	Winter Damage	No. Fruting Laterals	Lateral Length	Primocane Vigor 2012	Primocane Vigor 2013
Biomass	NS	0.57***	NS	0.19**	0.48***	NS	NS	0.25**	0.60***	0.27***
Fruit Weight		0.18**	0.37***	-0.16*	NS	-0.18**	NS	0.19**	0.15*	0.21**
Yield			0.29***	0.44***	0.64***	-0.30***	0.37***	0.39***	0.65***	0.44***
Fruit Set				NS	0.33***	-0.44***	0.22**	0.21**	0.23**	0.35***
Fruit Load					0.25**	NS	0.25**	NS	NS	NS
Floricane Vigor						-0.41***	0.41***	0.21**	0.56***	0.39***
Winter Damage							-0.28***	-0.15*	-0.25***	-0.34***
No. Fruiting Laterals								NS	0.28***	0.23***
Lateral Length									0.34***	0.28***
Primocane Vigor 2012										0.48***

[*] Spearman's ρ is reported. NS= no significance, * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$

most useful for yield estimation in the future, however it would be necessary to take data on a larger plot over multiple years and locations to confirm this and develop a more precise yield estimation protocol. Other phenotypic traits that were less well correlated to yield in NC (which may be different and more telling in a different climate) included lateral length (ρ = 0.39), fruiting lateral no. (ρ = 0.37), fruit set (ρ = 0.29), and average fruit weight (ρ = 0.18). Most of the negative correlations were between winter damage and yield traits, this was expected.

Fruit weight and seediness was highest in NY and OH in 2013, and lowest in NY and NC in 2014, and likely reflects wetter and drier weather conditions in the Eastern US during those years (State Climate Office of North Carolina, 2015), i.e., in the wetter year, fruit size was larger. NC fruit had low drupelet counts (53.6 drupelets per fruit) in comparison to fruit from all other locations (72.6 drupelets per fruit). Drupelet count, in conjunction with seediness, are indicative components of fruit size, and explain the discrepancy between NC and other locations.

Fruit chemistry traits were analyzed in the laboratory and varied by production site and year. Soluble solids concentration (SSC) was lowest in 2013 and highest in 2014, with the exception of samples from OR in 2015, which were higher than all other years and sites. Titratable acidity (TA) was higher in samples from OH and NC overall compared to NY and OR. Fruit pH ranged from 3.7 – 4.1 for all samples. Anthocyanins and phenolics concentrations were highest in berry fruit from OR compared to fruit from the other states. Previous studies with red raspberry showed that flavonoid composition varied with location, where at warmer temperatures levels of specific anthocyanins increased, although there was also a significant effect due to genotype (Bradish et al., 2012).

This data set provides the first comprehensive description of traits of black raspberry. This dataset has 43 plant and fruit traits, and was a collective effort of multiple scientists, students, and technicians over three seasons, but required years of preparation before and after. This standardized phenotyping set is the first for the genus *Rubus*, and could be transferable to red raspberry, blackberry, and other crops in the Rosaceae family. The data from this study and the protocol will become a part of the resources for Rosaceae stored

in the Genome Database for the Rosaceae (GDR) (http://www.rosaceae.org) (Jung et al., 2018). The majority of the traits included in this data set are qualitative. Previous phenotyping studies in RosBREED projects have struggled with the usefulness of qualitative of data in genotyping assessments (C. Finn, pers. comm.). However, the wide range in values of the traits, especially in those that responded to temperatures (e.g Bloom Date, Budbreak), points to the value of conducting this type of trial in a wide range of climates. The utility of a standardized phenotyping set is essential for breeders to capture important data on their crops. Ultimately, standardized phenotyping allows breeders the ability to more consistently score traits in multiple locations. This precision over space and time is necessary to develop genetic resources for black raspberry and other fruit crops and puts us on track for developing more efficient breeding programs.

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