NCCC-212 Annual Report for 2021: Small Fruit
USDA ARS National Clonal Germplasm Repository
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List of projects

Objective 1 – Develop improved small fruit germplasm through cooperative breeding and evaluation programs.

Blackberry and Raspberry (*Rubus*):

1. *Developing two fingerprinting sets in red raspberry:* Jason Zurn, Mandie Driskill, Kim Hummer, Nahla Bassil, USDA ARS NCGR-Corvallis; Chad Finn, Jana Lee, USDA ARS HCRU-Corvallis, OR; Michael Dossett, BC Berry Cultivar Development Inc. (in partnership with Agriculture and Agri-Food Canada), Agassiz, Canada.
2. *Fine mapping black raspberry aphid resistance to the North American large raspberry aphid:* Christina Mulch, Kelly Vining, Oregon State University, Corvallis, OR; Nahla Bassil, Jill Bushakra, USDA ARS NCGR-Corvallis, OR; Chad Finn, Jana Lee, USDA ARS HCRU-Corvallis, OR; Michael Dossett, BC Berry Cultivar Development Inc. (in partnership with Agriculture and Agri-Food Canada), Agassiz, Canada.
3. *Analysis of a multi-environment trial for black raspberry quality traits*: Nahla Bassil, USDA ARS NCGR-Corvallis, OR.; Michael Dossett, BC Berry Cultivar Development Inc. (in partnership with Agriculture and Agri-Food Canada), Agassiz, Canada; Chad Finn, ARS HCRU-Corvallis, OR.
4. *GWAS study by phenotyping diverse Rubus species and cultivars*: Jill Bushakra, Nahla Bassil, and Kim Hummer, USDA ARS NCGR-Corvallis OR; Pairwise Plants, Watsonville, CA; Courtney Weber, Cornell University, Ithaca, NY; Gina Fernandez, North Carolina State University, Raleigh, NC; Margaret Worthington, University of

Arkansas, Fayetteville, AR; Michael Dossett, BC Berry Cultivar Development Inc. (in partnership with Agriculture and Agri-Food Canada), Agassiz, Canada.

Blueberry (*Vaccinium*):

1. *Confirming identity of blueberry cultivars by DNA Fingerprinting.* Nahla Bassil, Kim Hummer, April Nyberg, USDA ARS NCGR, Corvallis, OR; Ozgecan Yalcin, Department of Horticulture, Oregon State University, Corvallis, OR.
2. *Determining amount of unreduced pollen for diverse Vaccinium species.* Kim Hummer USDA ARS NCGR, Corvallis, OR; Ryan Contreras, Sunny Green, Department of Horticulture, Oregon State University, Corvallis, OR.
3. *Evaluating* Vaccinium *germplasm for heat tolerance, drought tolerance, and cold tolerance.* Todd Anderson, Department of Horticulture, Oregon State University, Corvallis, OR; Nahla Bassil, Kim Hummer USDA ARS NCGR, Corvallis, OR; Scott Orr, Dave Bryla, Claire Luby, Michael Hardigan, USDA ARS HCRU, Corvallis, OR.
4. *Phenotyping blueberry for fruit quality traits.* Nahla Bassil, Kim Hummer, USDA ARS NCGR, Corvallis, OR; Marti Pottorff, Massimo Iorizzo, Penelope Perkins-Veazie, Mary Ann Lila, Plants for Human Health Institute, North Carolina State University, Kannapolis, NC; Ted Mackey, USDA ARS HCRU, Corvallis, OR.
5. *Developing a high throughput genotyping platform for blueberry and cranberry.* Nahla Bassil, Mandie Driskill, USDA ARS NCGR, Corvallis, OR; Massimo Iorizzo, Plants for Human Health Institute, North Carolina State University, Kannapolis, NC; Patrick Edger, Department of Horticulture, Michigan State University, E. Lansing, MI; Patricio Munoz, Horticultural Science Department, University of Florida, Gainesville, FL; David Chagne, Plant & Food Research Limited, Palmerston North, New Zealand.
6. *Assisting Breeding Insight (BI) in enabling genomic selection in blueberry.* Nahla Bassil, USDA ARS NCGR, Corvallis, OR; Dongyan Zhao, Moira Sheehan, Department of Plant Biology, Cornell University; Amanda Hulse-Kemp, USDA ARS GBRU, Raleigh, NC; Jodi Humann, Dorrie Main, Department of Horticulture, Washington State University, Pullman, WA.
7. *Testing Allegro Targeted Genotyping for blueberry genome wide association.* Nahla Bassil, USDA ARS NCGR-Corvallis, OR; Amanda Hulse-Kemp, , USDA ARS GBRU, Raleigh, NC; Lauren Redpath, Rishi Aryal, and Hamid Ashrafi, Horticultural Science Department, North Carolina State University, Raleigh, NC.

Strawberry (*Fragaria*):

1. *Assessing genetic diversity in the cultivated strawberry (*Fragaria ×ananassa*) collection at the National Clonal Germplasm Repository.* Jason Zurn, Nahla Bassil, Kim Hummer, USDA ARS NCGR, Corvallis, OR; Michael Hardigan, UC Davis, CA.
2. *Evaluating genotype x environment interactions for predicting SSC in strawberry*. Jason Zurn, Nahla Bassil, USDA ARS NCGR, Corvallis, OR; Mulusew Ali, Craig Hardner University of Queensland, St. Lucia, QLD, Australia; Vance Whitaker, University of Florida, Wimauma, FL; Chad Finn, USDA ARS HCRU, Corvallis, OR; Jim Hancock, Michigan State University, E. Lansing, MI; Iraida Amaya, IFAPA, Malaga, Spain; Helen Cockerton, Richard Harrison, NIAB-EMR, East Malling, United Kingdom; Lise

Mahoney, Tom Davis, University of New Hampshire, Durham, NH; Jodi Neal, Queensland Department of Agriculture and Fisheries, Nambour, Australia.

3. *Phenotyping diverse strawberry cultivars in Corvallis, Oregon.* Kim Hummer, Nahla Bassil, Jason Zurn, Gabriel Flores, and Ryan King, USDA ARS NCGR, Corvallis, OR; Todd Anderson, Department of Horticulture, Oregon State University, Corvallis, OR.

Currants and Gooseberries (*Ribes*)

1. *Developing a* Ribes *fingerprinting set for germplasm management*. Kim Hummer, Nahla Bassil, Jill Bushakra, USDA ARS NCGR, Corvallis, OR; Anton Alvarez, Department of Horticulture, Oregon State University, Corvallis, OR.

Blue honeysuckle (*Lonicera*)

1. *Expand blue honeysuckle collection.* Jill Bushakra, USDA ARS NCGR, Corvallis, OR; Shinji Kawai, Department of Horticulture, Oregon State University, Corvallis, OR.

Elderberry (*Sambucus*)

1. *Researching pollen incompatibility and developing seed germination protocols in diverse* Sambucus *germplasm.* Jill Bushakra, USDA ARS NCGR, Corvallis, OR; Bruce McClure, Michele Warmund, University of Missouri.

Objective 2 – Develop practices for small fruit production tailored for climatic and market needs of growers.
NONE

Objective 3 – Explore the association between fruit constituents and human health impacts.

1. *Fruit trait comparisons of Ayusep* (Vaccinium myrtoides*), Mortiño (*Vaccinium floribundum*), and southern highbush blueberry (*Vaccinium corymbosum*).* Kim Hummer USDA ARS NCGR, Corvallis, OR; Todd Anderson, Department of Horticulture, Oregon State University; Bob Durst, Scott Leonard, Linus Pauling Institute, OSU.
2. *Anthocyanins in wild relatives of strawberry* (*Fragaria* L.). K.E. Hummer, USDA ARS NCGR, Corvallis, OR; T.T.T. Hoai, Plant Resources Center, Hanoi, Vietnam; R. W. Durst, Linus Pauling Institute, OSU.

Objective 4 – Identify opportunities and collaborate on the development of extension resources for multistate, regional, national, and/or international audiences.

1. *Fingerprinting* Ribes *cultivars shared between the two organizations*. Kim Hummer, Nahla Bassil, Jill Bushakra, USDA ARS NCGR, Corvallis, OR; Anton Alvarez, Oregon State University; Claudio Niggli, ProSpecieRara, Basel, Switzerland.

Impact statements

Objective 1 – Develop improved small fruit germplasm through cooperative breeding and evaluation programs.

Blackberry and Raspberry:

1. *Developing two fingerprinting sets in red raspberry:* DNA sequence data from the public domain and that we have previously generated was mined for structural variants and long core repeat simple sequence repeats after alignment to the black raspberry genome. We identified single copy polymorphic loci to compile a list of genome-wide single copy SSRs and structural variants. A total of 1,000 RhAMPSeq loci was developed and used to genotype our raspberry collections and accessions from the Dossett breeding programs. A subset of 48 loci were designed into KASP markers and are being used to genotype 384 diverse accessions of raspberry from the NCGR collection and the Hardigan Breeding Program. SSRs that were determined to be polymorphic based on the RhAmpSeq platform were identified and are being tested for polymorphism by the Dossett Program.

2. *Fine mapping black raspberry aphid resistance to the North American large raspberry aphid:* Market expansion of black raspberry is currently hindered by aphid-vectored viruses, such as *Black Raspberry Necrosis Virus* (BRNV). Natural, genetic resistance to aphids exists and has been identified from three geographic sources: Maine, Michigan, and Ontario. These sources are being used to breed cultivars with durable aphid resistance. We have developed three new populations (ORUS 5291, ORUS 5296, and ORUS 5306), that are expected to segregate for each of these three sources, to fine map this trait. Segregation of resistance in each of these populations was phenotypically evaluated by aphid inoculation resulting in segregation ratios of 1:1 resistant I to susceptible (S) by Chi-squared analysis. Expression profiles in 10 R and 10 S seedlings were assessed with Full-Length Isoform Sequencing (IsoSeq) for one source (ORUS 5306) resulting in complete Isoform information for this source. In addition, Illumina Sequencing for 5 R and 5 S seedlings from each population before and after aphid inoculation has resulted in a differential expression analysis producing lists of candidate genes associated with aphid resistance in each population. We plan on using these results to suggest potential markers for each source of resistance. Our goals are to use these resources to develop useful genetic markers for each source of resistance, and to allow pyramiding of these resistance loci in new breeding populations.

3. *Analysis of a multi-environment trial for black raspberry quality traits:* Improved understanding of genetic control and stability of pomological traits will inform the development of improved black raspberry germplasm and cultivars. To this end, analysis of a multiple-environment trial of a mapping population derived from a cross of a commercial cultivar with a wild accession has provided insights into genetic variation, genotype-by-environment interactions, quantitative trait loci (QTL), and QTL-by-environment interactions (QEI) of fruit quality traits among diverse field environments. Genetic components and stability of four fruit size traits and six fruit biochemistry traits were characterized in this mapping population following their evaluation over three years

at four distinct locations representative of current U.S. black raspberry production. This revealed relatively stable genetic control of the four fruit size traits across the tested production environments and less stable genetic control of the six fruit biochemistry traits. Ten QTL associated with three fruit morphology traits and five QTL associated with two fruit biochemistry traits were identified. Of the fifteen total QTL, eleven exhibited significant QEI. Closely overlapping QTL revealed linkage of several fruit size traits: fruit mass, drupelet count, and seed fraction. These and related findings are expected to guide further genetic characterization of black raspberry fruit quality, management of breeding germplasm, and development of improved cultivars for U.S. production.

4. *GWAS study by phenotyping diverse Rubus species and cultivars*: Collaboration to phenotype and genotype species and cultivars of *Rubus* from the NCGR collection. Approximately 300 genotypes were grown under tunnels in Watsonville, CA by PSI and assessed for 79 traits including phenology; leaf, flower, fruit and cane characteristics; and fruit quality characteristics. A set of 5 industry standards were grown at NCGR, PSI, NCSU, BCB, UA and Cornell for GxE evaluation. Approximately 500 genotypes were submitted for flow cytometry to determine ploidy and whole genome sequencing. All data will be made available to the public through GRIN-Global and the Genome Database for Rosaceae (GDR) hosted through Washington State University. Publications will be submitted beginning in late 2022. Collected open pollinated fruit and extracted seed for genetic back-up from 142 accessions grown at PSI.

Blueberry:

1. *Confirming identity of blueberry cultivars by DNA Fingerprinting:* The genotypic identity of the blueberry cultivars in the NCGR collections is critical to genebank management and operations. We had previously developed a 10-SSR fingerprinting set. Genotyping 367 samples with this SSR set and parentage analysis where possible detected 96 plants representing 54 cultivars that were true-to-type (TTT) cultivars, 13 sets of homonyms and ten groups of synonyms. Parentage analysis identified five of the TTT cultivars among the homonyms (‘Bluecrop’, FL 4B, ‘Nelson’ and ‘Clara’) and ‘Elizabeth’ among the synonym sets. Identity challenges were detected in 50 plants representing 23 cultivars. We have obtained leaf samples of parents and cultivars that had genotypes that were inconsistent with the reported parentage from up to seven different sources and are in the process of genotyping them to resolve these identity challenges. Confirmed blueberry genotypes will benefit the germplasm community for use in continued breeding and genetic studies.

2. *Determining amount of unreduced pollen for diverse Vaccinium species:* During May through August, pollen was harvested by staff at the USDA in Corvallis working with those at Oregon State University Department of Horticulture, from flowers of 15 samples including *Vaccinium corymbosum* cultivars, *V. meridionale*, *V. floribundum*, *V. virgatum*, *V. darrowii*, and tetraploid *V. ovatum* growing in greenhouses, screenhouses, and field plantings. Pollen was stained and examined by microscope. Slides were assessed for

pollen viability. Percent unreduced pollen was counted for the samples. 'Grover' blueberry had 30% unreduced pollen, the highest of the samples examined; a wild blue huckleberry from Oregon next 23%, with a wild Maine blueberry following at 20%. Occurrence of unreduced pollen in *Vaccinium* has allowed for the introgression of divergent species germplasm into higher ploidy levels via unilateral sexual polyploidization.

3. *Evaluating* Vaccinium *germplasm for heat tolerance, drought tolerance, and cold tolerance:* During 2021, staff of the USDA in Corvallis working with those at Oregon State University Department of Horticulture, visited four blueberry plots containing 15 hybrid blueberry families to assess horticultural and chemical traits. A singular heat-dome effect (111 F) occurred at Corvallis in late June. Blueberry fruits are being evaluated for firmness, color, and sugar acid chemistry, after that event and at four other sites in Oregon and Washington with drought and high temperature conditions. Assessment of heritability of desirable traits will be determined through parentage analysis of the crossing families.

4. *Phenotyping blueberry for fruit quality traits:* In the spring and summer of 2020 and 2021, we harvested ripe blueberry fruit from 196 seedlings for the 'Draper' x 'Jewel' population, 159 accessions from the NCGR Field collection, and 960 northern highbush blueberry accessions (GenStudy) from the 2016 and 2017 USDA-ARS-HCRL breeding program as part of the VacCAP project. We used the Texture Analyzer to simultaneously evaluate blueberry texture (Tx), stem scar diameter (ScD), scar tear (ScT), fruit weight (Wg) and shelf life indicators such as wrinkle/shrivel (Wr/Shr), mold, leakage (Lk) at harvest time and six weeks post-harvest (stored at 4°C). Preliminary analyses indicated a wide range of variation for most of the traits and parameters. Fruits for non-volatile chemistry analysis were frozen and shipped to Co-Pis Perkins-Veazie and Lila. Fruits for volatile analysis are being shipped to The Metabolomics Innovation Center (TMIC, University of Alberta, Canada) for analyses. QTL analyses are planned for these traits in the 'Draper' x 'Jewel' population and genome wide association analyses (GWAS) for these traits in the GenStudy set.

5. *Developing a high throughput genotyping platform for blueberry and cranberry:* We lead the Genotyping Team for the VacCAP with the objective to develop a high throughput genotyping platform for blueberry and cranberry. In 2021, we compiled a blueberry SNP catalog of 5.3 M SNPs and 2.2 M INDELS. The 491,588 Existing SNPs were obtained from 13 studies, nine collaborators/institutions detected in diversity panels (DPs) and mapping populations (MP) and mapped to a single location in the P0 reference blueberry sequence (diploid W85). Of these SNPs, 4,675 SNPs were associated with known QTLs, representing a number of traits including fruit quality traits (TA, SSC, Fruit Size, fruit weight, firmness, color, ...). A total of 1.6 M structural variants (SVs) were identified from transcriptome sequences of 16 cultivars available through NCBI, and 6.4 M SVs were generated De Novo (from a DP pf 47 cultivars). Based on survey outcomes and forum discussions, a target capture-based genotyping technique (Flex-Seq Ex-L) was chosen for developing the VacCAP genotyping platform. A *Vaccinium* Consortium was formed which committed to genotyping 7,104 samples (3,670 samples in this project and 3,744 samples by 10 consortium members) in 2022 to secure a genotyping cost of $49.45 per sample with 20,000 loci for blueberry and 15,000 loci for cranberry. We have just

initiated the Testing Phase in 192 blueberry accessions that included representative samples from four industry partners and 10 academic institutions in the US, Canada and New Zealand.

6. *Assisting Breeding Insight (BI) in enabling genomic selection in blueberry:* We identified 384 diverse blueberry accessions and collected them from the NCGR and the blueberry community to test the DarTag genotyping platform selected. We worked with Ted Mackey and Michael Hardigan on identifying traits to phenotype ~2,700 seedlings from the 2017 USDA-ARS-HCRL seedling field. In collaboration with Amanda Hulse-Kemp (USDA-ARS) and Jodi Humann (GDV, WSU), we compiled a comprehensive list of 155 traits being used to phenotype blueberry, and corresponding phenotyping methods from the blueberry research and breeding community (ARS, university, and private companies) and converted the information into the BI template and initiated the Blueberry BreedBase. Data analysis is in progress.

7. *Testing Allegro Targeted Genotyping for blueberry genome wide association:* In collaboration with Hamid Ashrafi and his team at NCSU, 1.7 million SNPs were selected, and the flanking sequences were extracted. Single primer enrichment technology (SPET) was used to specifically target 59,700 SNPs of interest in a diversity panel (DP) of 252 tetraploid individuals that included 77 accessions from the NCGR. Phenotypic data for phenological traits were collected in 2019 and 2020 from the 77 accessions at the NCGR, and ripe fruit were shipped to NCSU for fruit quality trait and anthocyanin analyses. The pooled paired-end libraries of 184 and 96 individuals of two diversity panels were used to generate 308 GB of data with an average of 900 MB per genotype. The Freebayes pipelines was used for SNP identification. A total of 14 M variant positions including SNPs and MNPs were identified in the DP before filtering. After filtering for missing data points, minor allele frequency, and captured targeted region, 30,000 SNPs were retained. Through association of these SNPs to measured phenotypic traits of the diversity panels, candidate genes for fruit size, weight, and color, as well as soluble solid content, titratable acidity, pH, and different anthocyanins, were identified using the GWASPoly software. Further, comparative analysis of resequencing data of native diploid, tetraploid, and hexaploidy *Vaccinium* species will be used to ascertain the origin of introgressed SNPs.

8. *Evaluating ploidy of a diversity set of* Vaccinium *accessions*: In collaboration with Hamid Ashrafi and his team at NCSU, 358 accessions collected from the North Carolina State University blueberry breeding program and the NCGR were evaluated for nuclear DNA content via flow cytometry. The mean (range) DNA content of diploid, tetraploid, and hexaploidy reference species was 1.20 pg (0.99 – 1.41 pg), 2.37 pg (2.11 – 3.01 pg), and 3.64 pg (3.24 – 3.80), respectively. Of the 358 unique accessions analyzed for ploidy, 225 were tetraploid, 28 were diploid, 2 were triploid, 77 were pentaploid or aneuploid with 2C values between tetraploid and hexaploidy values, and 26 were hexaploidy. Pedigree analysis of hybridization in the tetraploid accessions primarily consisted of interspecific crosses within tetraploid species or between diploid and tetraploid species producing tetraploid offspring. Diploid species that readily hybridized with tetraploids included *V. caesariense*, *V. darrowii*, *V. elliottii*, *V. ovatum*, *V. pallidum*, and *V. tenellum*, indicating that they produce unreduced gametes. Tetraploid hybrid pedigrees, which

involved hexaploidy crosses back within three prior generations, had a 2C value range between 2.22 pg and 2.59 pg. Anticipated pentaploid 2C DNA content is ~3 pg; however, the interspecific pentaploid and aneuploid progeny 2C DNA content ranged from 2.61 pg to 3.15 pg. We speculate some of these progeny to be near tetraploids with extra chromosomes from hexaploidy progenitors. Further karyotyping of these individuals is necessary to ascertain aneuploidy anomalies.

9. *Genotype, environment, year, and harvest effects on fruit quality traits of five blueberry cultivars:* In collaboration with NCSU, the genotypic performance of five blueberry cultivars, including 'Echota', 'O'Neal', 'Reveille', 'Summit', and 'Sunrise' was assessed in two locations: NC State University (NCSU) Sandhills Research Station located in Jackson Springs, NC and the NCGR in Corvallis, OR. The selected cultivars were phenotyped for various fruit quality-related traits over two sequential harvests in two years and two locations. Our results indicated that genotype (G) is a major source of variation for most phenotypic traits. Further, the effect of year (Y) × harvest time (H) as well as G × Y × H, significantly affected the majority of studied phenotypic traits. Within the studied genotypes, 'Reveille' and 'O'Neal' phenotypic stability were consistent across locations and years, with 'Summit' characteristics stable across years, locations, and harvests. Clonal plant replicates within a genotype, harvest, and environment, as well as individual fruit measures were the most significant source of variability.

Strawberry:

1. *Assessing genetic diversity in the cultivated strawberry (*Fragaria ×ananassa*) collection at the National Clonal Germplasm Repository:* The USDA-ARS national collection includes 560 diverse *Fragaria ×ananassa* accessions of modern and historical U.S. and foreign cultivars and breeding selections. An initial core subset of 447 *Fragaria* cultivars (304) and world species (143) was identified in the 1980s by the curator and the Small Fruit Crop Germplasm committee members to represent maximum genetic diversity. Very little has been done to characterize these accessions genotypically. Pedigrees are unknown for many. Since the original core designation, an additional 160 cultivated strawberry cultivars were received by NCGR. The objectives of this study was to genotype the entire *F. ×ananassa* collection, assess genetic structure and diversity, confirm pedigrees within the collection, identify a core collection based on genetic data, and determine the prevalence of known disease resistance haplotypes within the collection. The Knapp group had already genotyped 211 of these accessions with the Istraw35 Axiom strawberry array. We submitted DNA from the remaining 332 accessions for genotyping with a new strawberry array that contains 6,000 markers in common with the Istraw35 Axiom array. Population structure analysis of the *F. ×ananassa* collections revealed eight sub-populations associated with geographical regions or major breeding programs. Fst values were very low confirming the narrow genetic diversity within cultivated strawberry germplasm and may also be related to the germplasm sharing by 1950's era breeding programs. Two 100 individual core collections were developed with maximum diversity within the collection and a uniform distribution of alleles. Haplotypes associated with resistance for *FaRCa1*, *FaRCg1*, *FaRMp1*, and *FaRPc2* were found to be prevalent within the collection and globally

distributed. Finally, pedigree links were established between individuals within the collection. These core collections will help breeding programs streamline characterization of the collection for useful traits.

2. *Evaluating genotype x environment interactions for predicting SSC in strawberry:* To assess the effects of structure on global-scale genomic prediction, we examined models for a large, diverse set of global strawberry (*Fragaria* × *ananassa*) collections comprised of 2,064 accessions genotyped with 12,591 single nucleotide polymorphism (SNP) markers. Trials to assess soluble solids content (SSC) were conducted at seven global locations [U.S. (Florida, Oregon, and Michigan), Europe (Spain and UK) and Australia (Queensland and Victoria)] over the course of two or three consecutive years. Population structure analysis of the 2,064 accessions revealed that populations were clustered into two large groups consisting primarily of subtropical or temperate strawberry accessions. The population structure observed was further confirmed by variations in pairwise allele frequency distributions among the two subpopulations. Models using a factor analytic approach for these subpopulation categories were developed and assessed for their ability to improve prediction accuracy in the presence of population structure. The models investigated include: i) the standard GBLUP model (Gfa), ii) a multi-population GBLUP approach where the subpopulation genomic relationship matrix is fitted in the prediction model (Wfa). Of the approaches investigated, Wfa were found to have the highest prediction accuracy (r = 0.8) for SSC, including individual environmental models and the Gfa approach (r=0.68). Thus, accounting for population structure enhances prediction accuracy for multi-environment genomic prediction. This study demonstrates that methods for accounting for population structure in multi-environment GBLUP models which reduce bias in the estimation of prediction accuracy.

3. *Phenotyping diverse strawberry cultivars in Corvallis:* During 2019 and 2020, the USDA in Corvallis evaluated 288 heritage strawberry cultivars for phenotypic traits including bloom date, harvest date, harvest weight, fruit shape, plant height, number of runners, and fruit chemistry such as pH, titratable acidity, soluble solids, total anthocyanins, and anthocyanin profiles. In addition, images of plants, flowers, and fruit were taken. Data were uploaded to GRIN-Global for public reference.

Currants and Gooseberries

1. *Developing a* Ribes *fingerprinting set for germplasm management*: We identified 13 high core repeat SSRs from the literature that appeared to be polymorphic in different species. We evaluated them in a testing panel of 12 accessions representing *R. aureum, R. nigrum, R. uva-crispa*, *R. spicatum*, *R. petraeum*, and *R.* × *nidigrolaria*. We identified 7 SSRs that appear polymorphic across these species and optimized this 7-SSR *Ribes* fingerprinting set and are testing it for ability to identify 175 accessions in the NCGR collection.

Blue honeysuckle

1. *Expand blue honeysuckle collection:* Obtained 27 new Lonicera selections from the estate of Maxine Thompson in collaboration with Shinji Kawai. Propagations in progress before field planting. Selections are all from Japan thereby nearly doubling the

collection and increasing the geographic and genetic diversity of the NCGR collection. The current NCGR collection of 31 plants are mostly from Russia.

Elderberry

1. *Researching pollen incompatibility and developing seed germination protocols in diverse* Sambucus *germplasm:* Collaborating on researching pollen incompatibility and developing seed germination protocols in diverse *Sambucus* germplasm.

Objective 2 – Develop practices for small fruit production tailored for climatic and market needs of growers.
NONE

Objective 3 – Explore the association between fruit constituents and human health impacts.

1. *Fruit Trait Comparisons of Ayusep* (Vaccinium myrtoides*), Mortiño (*Vaccinium floribundum*), and Southern Highbush Blueberry (*Vaccinium corymbosum*):* In 2020, staff of the USDA in Corvallis and Oregon State University, Department of Horticulture and Linus Pauling Institute, collected berries from Filipino and Ecuadorian blueberry species relatives along with southern highbush blueberries growing in greenhouses. The fruit were analyzed for horticultural fruit traits along with pH, soluble solids, total anthocyanins, and anthocyanin profiles. The HPLC profiles of the Filipino and Ecuadorian species displayed peaks not observed in highbush species. Small diameter fruits with internal pigmentation, such as in *V. myrtoides* had higher total anthocyanins (mg/100g fresh wt) than did the highbush. Analysis is continuing.

2. *Anthocyanins in wild relatives of strawberry (*Fragaria *L.):* Fruit chemistry of 9 strawberry species, 8 subspecies, and one outgroup, *Potentilla indica*, were determined by staff at the USDA in Corvallis, Oregon working with those at Linus Pauling Institute, Oregon State University. Total anthocyanins ranged from 49.27 mg/100 g fresh wt (red-fruited *F. moschata* L. with Cy 3-gluc as the primary peak) to about 1 mg/100 g fresh wt or less for white-fruited types. *Potentilla indica* fruit had 89 – 95% atypical anthocyanins, though Cy 3-gluc, Pg-3-gluc and Pg 3-rut were present in reduced quantities**.** White-fruited types contained the three compounds, though in different ratios than red-fruited cultivars. *Fragaria nilgerrensis* had white fruit with trace amounts of Cy 3-gluc > Pg 3-gluc > Pg 3-rut.

Objective 4 – Identify opportunities and collaborate on the development of extension resources for multistate, regional, national, and/or international audiences.

1. *Fingerprinting* Ribes *cultivars shared between the two organizations*. ProSpecieRara focuses on maintaining endangered breeds of Swiss farm animals and traditional crops for dissemination and preservation. We are in the process of exchanging leaf material for DNA extraction and standardizing DNA marker visualization. ProSpecieRara requested live plant material for phenotyping. We are waiting for import permit approval.

Data, germplasm/cultivar descriptions, research results, for discussion at the meeting. Note that this data is preliminary and not for public dissemination

Genus	Accessions	Field	SH /GH	Total seed accessions
Fragaria (Strawberry)	**2,016**	**0**	**1,844**	**418**
Lonicera (Blue honeysuckle)	**121**	**30**	**1**	**93**
Ribes (Currant, Gooseberry)	**1,101**	**586**	**55**	**469**
Rubus (Blackberry, Raspberry)	**2,226**	**0**	**953**	**1819**
Sambucus (Elderberry)	**202**	**47**	**1**	**170**
Vaccinium (Blueberry, Cranberry)	**1,856**	**311**	**686**	**1072**

Berry collections summary for the National Clonal Germplasm Repository.
GRIN-Global searches for accessions can be obtained by searching:
https://npgsweb.ars-grin.gov/gringlobal/search.aspx

4. Publications

Alger, E.I., Platts, A.E., Deb, S.K., Luo, X., Ou, S., Hummer, K.E., Xiong, Z., Knapp, S.J., Liu, Z., Mckain, M.R., Edger, P.P. 2021. Chromosome-scale genome for a red fruited, perpetual flowering, and runnerless woodland strawberry (*Fragaria vesca*). Frontiers in Genetics. 12. Article 671371. https://doi.org/10.3389/fgene.2021.671371.

Chen, K., Volk, G.M., Hummer, K.E. 2021. Strawberry shoot tip cryopreservation (droplet vitrification). In: Volk, G.M., editor. Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules. Fort Collins, Colorado: Colorado State University. Available: https://colostate.pressbooks.pub/clonalcryopreservation/chapter/strawberrycryopreservation/

Hummer, K.E. and N.V. Bassil (2021). Confirming clonal identity: A case study in blueberries Acta Hortic. in press.

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