ANNUAL REPORT October 2020
National Clonal Germplasm Repository
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National Clonal Germplasm Repository Staff – Socially distanced due to COVID-19

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Kim Hummer, Research Leader/Curator
Nahla Bassil, Geneticist-Plants
Jim Oliphant, *Vaccinium/Fragaria* Mgr.
Jeanine DeNoma, *Humulus/Mentha* Mgr.
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April Nyberg, Bio. Science Tech-Genetics
Jill Bushakra, *Rubus/Ribes* Mgr.
Barb Gilmore, Field Mgr./Curator, Trees
Ashley Winters, Program Support Assist.
Jason Zurn, Research Associate, Genetics

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Ryan King, Bio Science Tech
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Graduate Students & Visiting Scientists

Todd, Anderson, GRA, OSU, Hort.
Christina Mulch, GRA, OSU, Hort.
Ozgecan Yalcin, GRA, OSU, Hort.
Joseph Postman, Plant Path/Curator Ret.

Stakeholder/Service Accomplishments

- 12,802 active accessions, 73 genera and 772 taxa of 683 species of temperate fruit, nut, and specialty crops were conserved.
- Obtained 272 new accessions and 546 new inventory items in CY 2019.
- Received 1,609 domestic and 90 foreign orders and shipped 7,307 items thus far.
- Collaborated with NGRPL, Ft. Collins, CO, on seed preservation and on the cryopreservation protocols of dormant blueberry, hazelnut, pear, currant and gooseberry.
- Collaborated with staff of NCGR-Davis to backup genetic resources of hazelnuts in Parlier, and butternuts and kiwifruit in Corvallis, Oregon.
- Participated on Governing Board for USDA National Clean Plant Network.
- Participated as Editor of *Chronica Horticulturae* for ISHS.
- Participated as President for the American Pomological Association.
- Expanded potted greenhouse backup collections of *Pyrus* and *Cydonia* for accessions represented by a single tree and at risk of loss due to disease susceptibility, lack of hardiness or small tree size.

Research Accomplishments

- Mapped fire blight resistance in three pear populations and identified a similar region on chromosome 2 that controls resistance from the 'Old Home and 'Moonglow' sources.
- Used synteny and candidate genes to identify loci controlling fruit sweetness in blackberry and confirmed association of some of these loci with sweetness in 192 accessions**.**Developed a 10-SSR US Pear Genetic Resources (USPGR) fingerprinting set and demonstrated it is comparable to the pear SNP array and the ECPGR set in determining clonal identity and parentage and more inexpensive to use.
- Detected Black currant reversion virus infection in black currant (*Ribes nigrum*) collection; worked with APHIS to develop a national response plan for this disease.
- Used interstem grafts to evaluate pear germplasm for dwarfing potential. Correlated pear mother tree architecture traits with dwarfing potential.
- Used parentage analysis and our 10-SSR blueberry fingerprinting set to identify 54 blueberry cultivars that matched reported pedigree descriptions and were identified as true-to-typeDetermined phenotypic and phenological measurements for a strawberry field study including 287 cultivars for two years; determined maximum length of runners on one strawberry plant on 7 July 2020: 150 meters; fruit size and chemistry measurements are under analysis.
- Identified *Vaccinium* species that are slow to become infected with, and potentially resistant to Blueberry shock virus.

Administrative Overview

Staffing Changes

Ashley Winters, our Program Support Assistant resigned in September 2020. She took a position working with the National Park Service. We were able to recruit Gabriel Flores, a Hispanic Associated Colleges and Universities (HACU) student for Fall Term, to help with our administrative tasks. Jack Brennan, our field manager, resigned and took a position working with Oregon State University at Hood River, Oregon. We were able to replace his position with Ryan King, in a term capacity. In

addition we hired Stephen Erickson as a seasonal field helper. Jeanine DeNoma, our Biological Science Research Technician who was in charge of our tissue culture program retired after about 20 years of service. Thank you, Jeanine, for all of your years of service to our tissue culture laboratory! Many people throughout the globe appreciate your generosity and knowledge of culture techniques. Please enjoy your retirement years!
Jason Zurn, Post-doctoral candidate supporting Nahla Bassil's program, has moved on to his next position working at Kansas State University. Thank you, Jason, for your service, reason, wit, and good humor. It was great working with you at our unit.

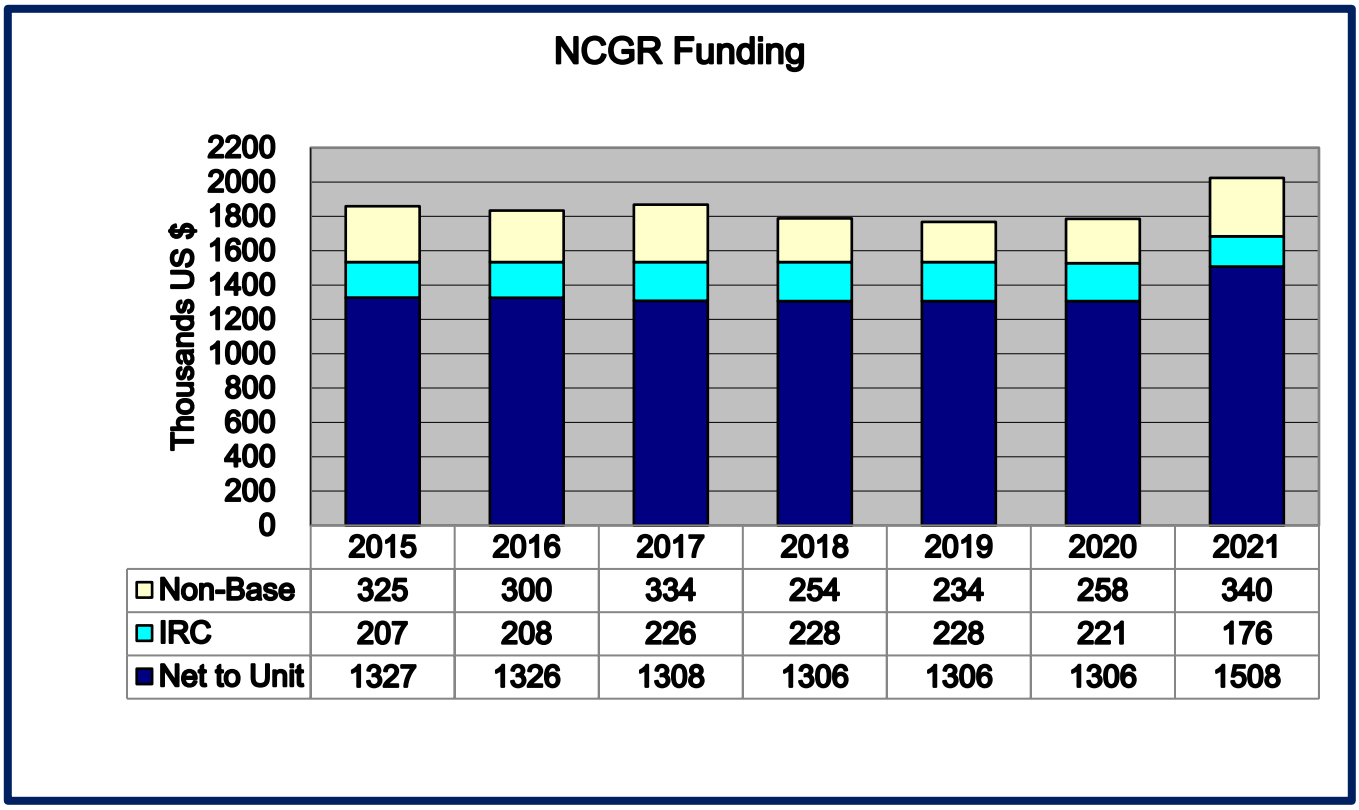
EEO/CR/Outreach

- At least 4 physically-challenged individuals were trained in horticultural plant management and label preparation.
- Through a Research Support Agreement with Oregon State University two female graduate students and two undergraduate students were trained.
- During the winter, 3 physically challenged high school students (program was funded through local school district grants) were trained in greenhouse management activities.
- NCGR staff provided site tours and visits to approximately 75 students from local high schools, community colleges, and Oregon State University.
- Awarded PWA-DIA funds for Oregon School of the Deaf tour.

Budget

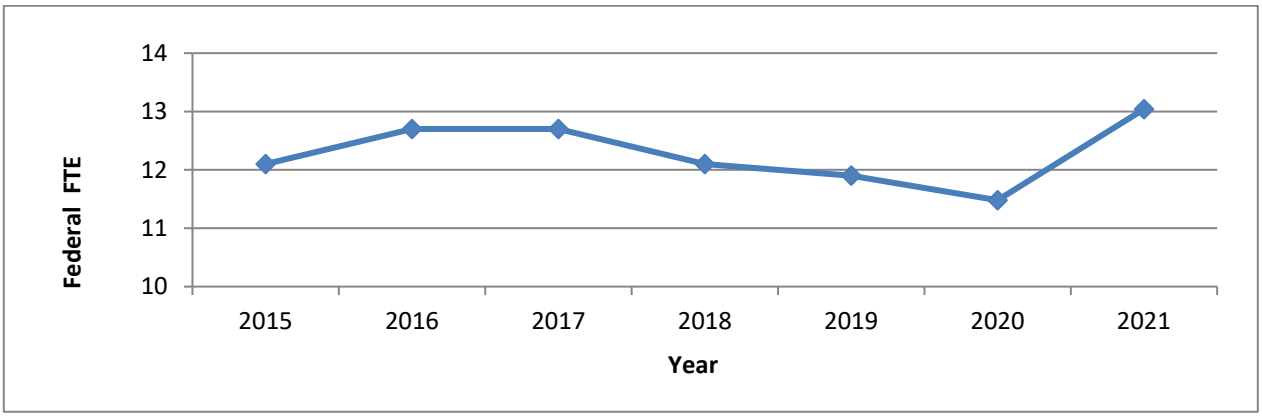
Our total federal budget is $1.684 million. The FY 2020 net to unit budget increased by $150,000 supported in Congress by the blueberry and cranberry genetic resource community. Thank you to those of you who supported the needs of our project! Our scientists have been successful in obtaining federal agriculture grants as well as those from commodity commissions and research consortium funding. Our location administrative costs (IRC) are 14.4 %.

Budget History



	2015	2016	2017	2018	2019	2020	2021
Non-Base	325	300	334	254	234	258	340
IRC	207	208	226	228	228	221	176
Net to Unit	1327	1326	1308	1306	1306	1306	1508

Employee Summary

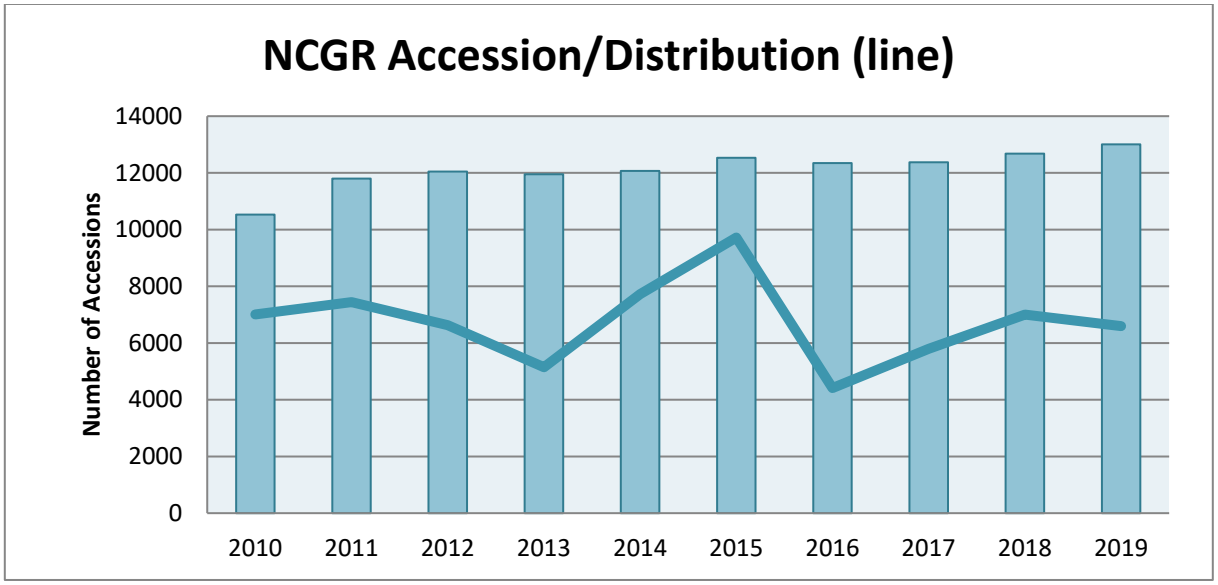


Facilities

The NCGR-Corvallis appeared on the President's budget for architectural planning funding of $13.500 million to replace 6 screenhouses and 4 greenhouses. We are working with USDA headquarters staffing and the Army Corps of Engineers for the design and planning phase of this project at the present time.

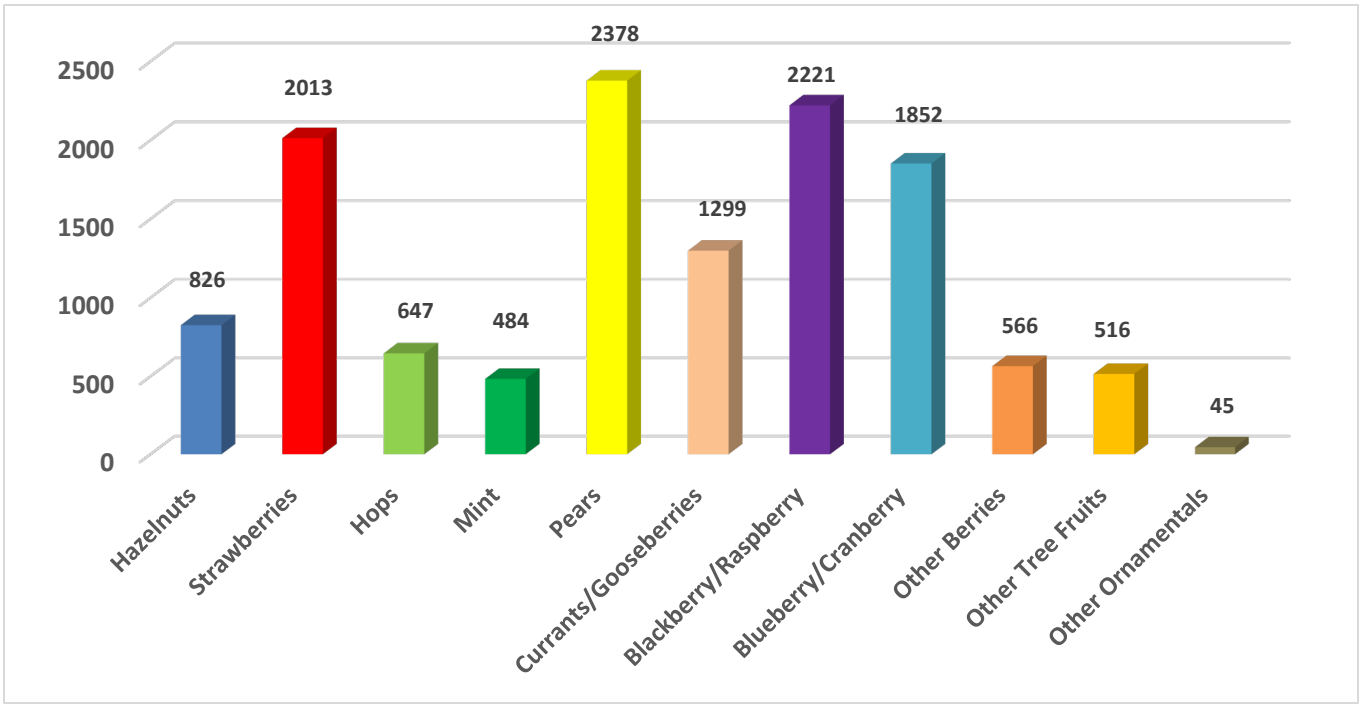
Germplasm Collections

Corvallis Germplasm Collections 2010 -2019



Bars represent number of accessions in the NCGR Collection. Line represents number of accessions distributed.

Corvallis Germplasm Collections – Accession Counts by Crop – October 2020



Corvallis Germplasm Collections – Total accessions (12,802) by genus, January 2020

Major crops

Genus	Common Name	Accessions
Corylus	Hazelnut	826
Fragaria	Strawberry	2013
Humulus	Hop	647
Mentha	Mint	484
Pyrus	Pear	2378
Ribes	Currant/Gooseberry	1299
Rubus	Black/Raspberry	2221
Vaccinium	Blueberry	1852
Total		11720

Other tree relatives

Genus	Common Name	Accessions
Amelanchier	Serviceberry	43
Amelasorbus	Inter-generic hybrid	1
Chaenomeles	Asian quince	48
Crataegomespilus	Inter-generic hybrid	2
Crataegosorbus	Inter-generic hybrid	1
Crataegus	Hawthorn	28
Crataemespilus	Inter-generic hybrid	2
Cydonia	Quince	147
Docynia	Asian quince	2
Juglans	Butternut/ backup for Davis, CA	29
Malus	Apple	28
Mespilus	Medlar	60
Peraphyllum	Crab apple	4
Physocarpus	Ninebark	1
Pseudocydonia	Asian quince	4
Pyracomeles	Inter-generic hybrid	1
Pyronia	Pear-Quince hybrid	9
Sorbaria	False spiraea	1
Sorbaronia	Inter-generic hybrid	7
Sorbocotoneaster	Inter-generic hybrid	3
Sorbopyrus	Sorbus-Pyrus hybrid	9
Sorbus	Mountain ash	86
Total		516

Other berries

Genus	Common Name	Accessions
Actinidia	Hardy Kiwifruit / backup for Davis, CA	3
Agapetes	Blueberry relative	13
Aronia	Aroniaberry	16
Asimina	Pawpaw	22
Cavendishia	Blueberry relative	5
Dimorphanthera	Blueberry relative	1
Empetrum	Crow berry	17
Epigaea	Blueberry relative	1
Gaultheria	Blueberry relative	44
Gaylussacia	Huckleberry	17
Hippophae	Sea buckthorn	1
Lonicera	Blue honeysuckle	82
Lycium	Wolfberry	14
Macleania	Blueberry relative	8
Micromeria	Blueberry relative	1
Pernettya	Blueberry relative	1
Potentilla	Quinquefoil	9
Psammisia	Blueberry relative	1
Pycnanthemum	Mountain mint	95
Sambucus	Elderberry	201
Schisandra	Magnoliavine	10
Sibbaldia	Strawberry relative	2
Symphysia	Blueberry relative	2
Total		566

Other ornamentals

Genus	Common Name	Accessions
Arbutus	Strawberry Tree	2
Camellia	Tea Camelia	1
Ceanothus	Ceanothus	38
Holodiscus	Beauty Bush	3
Kalmia	Mountain Laurel	1
Total		45

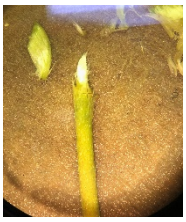
Greenhouse/Screenhouse *Fragaria*, *Vaccinium* and Quarantine Collections

By Jim Oliphant

- Developed a bulk custom soilless medium for use in the containerized collections.
- Hardwood chips have replaced pumice as a topdress on containerized plants in our screenhouses.
- Pesticides were applied to control aphids, scale, and spider mites.
- Pump was installed for the de-ionized water supply for specific challenging-to-grow plants.
- *Vaccinium* and *Rubus* plants remain in quarantine from 2015 collection to Vietnam.
- Established humid tropical conditions in GH1 to maintain tender accessions. A montane-like environment is under construction for subtropical high elevation crop relatives.
- *Vaccinium* crop wild relative including the entire cranberry collection were repropagated for invigoration of the foundation stock.
- Unknown *Vaccinium* species bloomed and were identified to *V. triflorum* and *V. globosum*.

Mentha/Pycnanthemum/Humulus and *In Vitro* Collections

By Jeanine DeNoma



***In Vitro* collection**. Forty-nine accessions of *Fragaria* were identified as having Strawberry Mild Yellow Edge Virus (SMYEV). Two were duplicate accessions; the remainder were scheduled for meristem treatment to eliminate the virus. Five accessions still healthy in StarPacs in cold storage were propagated in tissue culture and used for meristems. The remaining meristems were obtained from runners on screenhouse plants. One accession, CFRA 442.001 Pioneer did not produce runners this year. Meristems were cut from the other 41 accessions. A total of 282 meristems were cut. Of these, 193 meristems survived and were propagated for ELISA testing.

Pycnanthemum: One accession CPYC 44.001 was lost in the greenhouse but recovered from StarPacs in cold storage, propagated and is ready to be rooted for the greenhouse. CPYC 17.001 was highly at risk in the screenhouse. Shoot tips were collected, propagated and rooted in tissue culture and are now ready to replace the screenhouse plant.

Rubus: The accession CRUB 1868.001 Tulameen was recovered from StarPac in cold storage to replace screenhouse material. This particular explant was collected from the screenhouse in 2007 so is of interest for molecular variety identification. Five *Rubus* accessions were received from the berry breeding program; these were propagated, rooted and transferred to the greenhouse for the collection.

Humulus. There are 392 accessions of *Humulus* in the screenhouse. Thirty-four of these accessions are untested heat-treated meristems representing six accessions cut by Dr. Cai in 2016. Ten core accessions were repropagated by rhizome in the screenhouse, following our success propagating 91 accessions by rhizomes in 2017. We up-potted to P86 pots 242 plants representing 113 accessions from softwood cuttings from the virus infected collection in SH7. Another 121 pots representing 54

core accessions are in one gallon pots in the greenhouse, primarily for replacement in the core collection.

Mentha. There are 439 screenhouse and greenhouse accessions in the *Mentha* collection. The entire core and non-hardy *Mentha* collection of 48 accessions were up-potted from rhizomes propagated in 2018 and were replaced with two new pots of each. All 14 of the *Mentha* virus collection accessions were replaced with newly propagated plants from rhizomes. Of the 376 non-core accessions, 229 accessions were repropagated in 2019; the remaining 146 accessions are scheduled for repropagation this coming year.

Pycnanthemum. There are 33 growing accessions in the screenhouse collection. Of these, 21 accessions were repotted to two 5-gallon pots each. Another six accessions were repotted to a single 5-gallon pot and five accessions were repropagated into 1-gallon pots. Two accessions are being recovered in tissue culture and will be moved to the greenhouse in the spring.

Rubus/Ribes/Sambucus Collections

By Jill Bushakra

Rubus

- Pruning and fertilizing entire collection; repotting into new style pots, labelling and updating inventory for plants in houses 1 & 3 completed
- Propagation of tip-layering genotypes as needed
- Provided material to Patrick Di Bello and Melinda Guzman (Bob Martin) for RNA analysis; provided material to Mary Peterson (Chad Finn) for Boysen field trial; worked with staff from Pairwise Plants, North Carolina, to provide approximately 600 accessions of the *Rubus* collection for DNA analysis and phenotyping; provided material for Ava Howard of Western Oregon University for *Rubus* water relations study; collected leaf material for cytology study conducted in The Netherlands
- Attended teleconference for PGOC meeting; presented on Repository resources to growers at Caneberry day and annual Oregon Raspberry and Blackberry Commission meeting
- Wrote SOP for emptying the debris trailer
- Pressure washed aisles and benches in houses 1 & 3; put down weed mat in *Rubus* side of house 3, vacuumed leaves
- Worked with Jun Tanaka on seed evaluation for viability
- Obtained replacement plants from nurseries; obtained new seed lots from Lund University, Sweden; researching identification of NZ *Rubus*
- Obtained virus/pathogen data from Driscoll's on plants sent to them
- Controlled for pests as needed; maintained pesticide applicator license through continuing education credits
- Prepared houses for winter (washing screen, hanging plastic) and for spring (removing plastic, vacuuming leaves)
- Updated GRIN and included patent information; scanning and attaching intake information into GRIN
- Replaced missing labels as needed
- Provided material for fingerprinting *Rubus* cultivars and species

Ribes field

- Propagated species *Ribes* and cultivar *Ribes* in situ layering and cuttings; obtained replacement plants from nursery; obtained new cultivar, 'Oregon Snowflake', developed by Dr. Ryan Contreras, Oregon State University ornamental breeder
- Researching proper name for cultivar 'Redstart'
- Pruned, weeded (hand and chemical), and fertilized field collection
- Worked with Joseph and Jason to test black currants for Black currant reversion virus (BRV); worked with Jeanine to propagate meristems from BRV infected plants
- Inventoried collection and updated GRIN records
- Harvested dormant wood for cryopreservation
- Observed flowers on *Ribes dicanthum* and *R. komorovii* to determine sex of the plants
- Installed out irrigation drip tape in cultivar field. Drove and operated field equipment for flailing, tilling, mowing, and spreading mulch. Mulched entire *Ribes* cultivar field with fir sawdust
- Replaced 15 rows of drip tape with hanging irrigation line
- Incorporated species into cultivar field to reduce redundancy and so that all plants are on drip irrigation. Remapped and relabeled entire field
- Provided material for fingerprinting *Ribes* cultivars

Ribes screenhouse collections

- Fertilized and pruned all plants
- Updated GRIN records as necessary

Sambucus

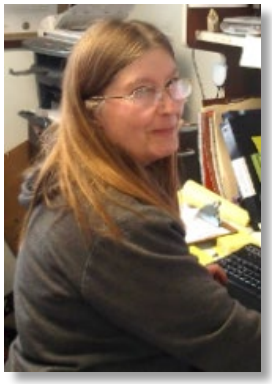
- Inventoried and fertilized
- Received new cuttings from Pat Byers and put in propagation
- Updated GRIN records
- Received new cuttings from Jim Ballington and a private breeder and put in propagation

Lonicera

- Inventoried and fertilized
- Contacted a nursery to look into replacement plants
- Updated GRIN records
- In the process of obtaining material from Maxine Thompson to incorporate into the collection.

Corvallis Seed Lab

By Missy Fix

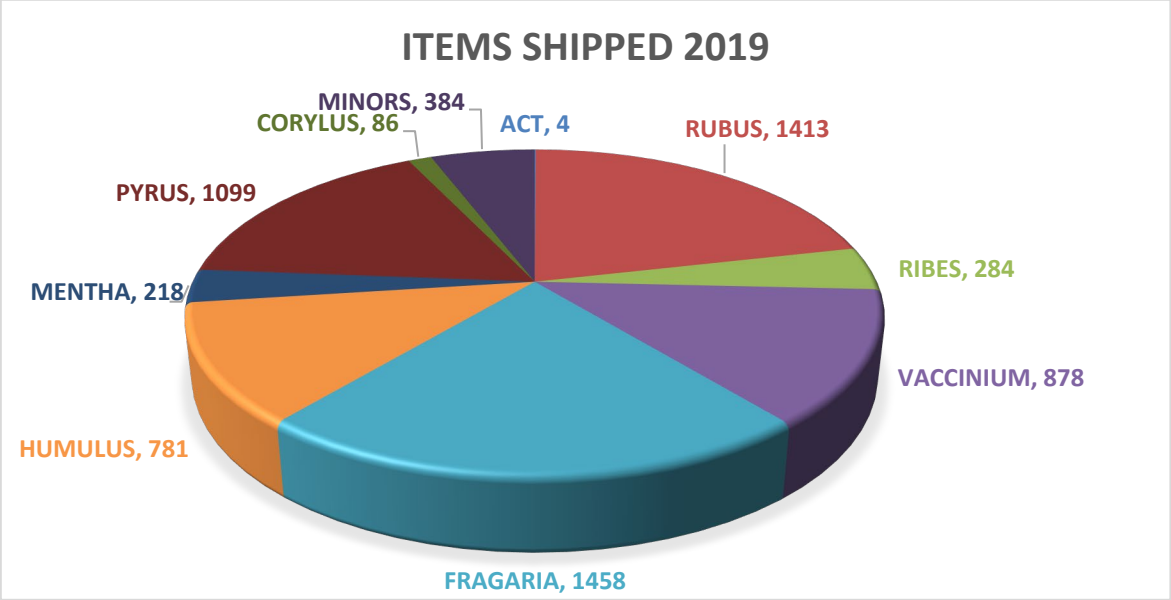


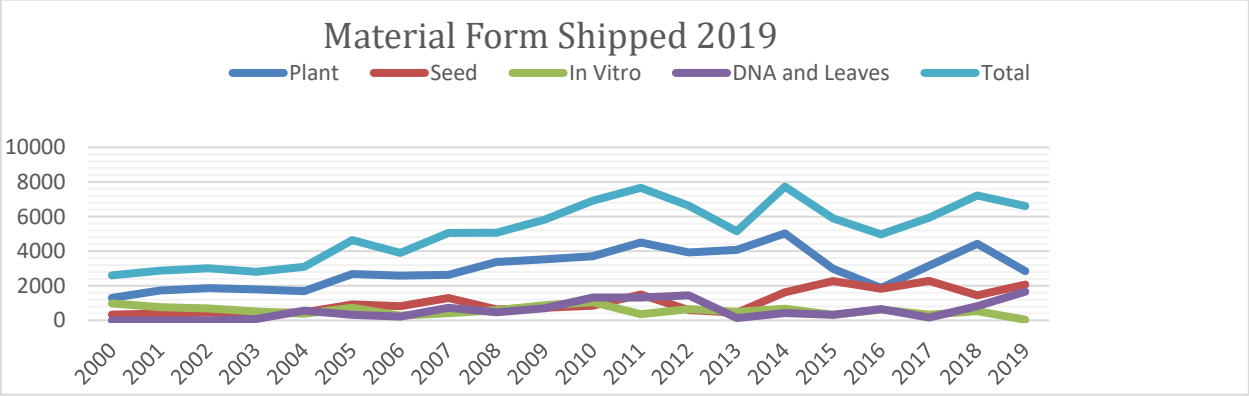
During CY 2019, 1899 seed accessions were shipped: 1672 were from the small fruit genera and 227 from the tree genera. The most requested genus was *Fragaria* with 934 requests. *Humulus* was the next popular with 817 requests. We received and or collected 92 seed accessions –70 *Fragaria*, 26 *Humulus*, 27 *Rubus*, and 18 *Vaccinium*. We continue to support requestors wanting material for educating K-12, home schooling, non-profit, community gardening projects and classes with our educational seed of blackberry, yellow raspberry, red raspberry, blueberry, hops, strawberry, pear seed and mint rhizomes (when available). This service has been for the most part, a welcomed offering among the various communities.

In continuing with germplasm seed preservation, 1349 accessions for *Fragaria*, *Rubus*, and *Ribes* have been selected thus far, in increments of 250, 500, or 1000 (depending on the seed amount totals per accession). These seeds are being sent for backup preservation at Ft. Collins. Those accessions with more than 3,000 seeds on hand will also be backed up at Svalbard in 1,000 seed increments.

Distribution

- In CY 2019, NCGR staff shipped 6605 items as seeds, cuttings, runners, scionwood, rooted plants, tissue cultures and DNA and leaf samples and informational material.
- In CY 2019, 958 new orders were received; 662 orders were completed; 602 of these were domestic orders and 30 international.
- *Fragaria* and *Rubus* topped the list of crops distributed this year – *Fragaria* topped out with 1458 items shipped, *Rubus* with 1413 items shipped. Domestic individuals, state agencies and universities, and ARS researchers received the most germplasm from Corvallis in 2019.
- With the various educational systems such as grade schools, home schooling, and community gardening arenas requesting plant and or seed material, the addition of our educational seed has allowed us to fill orders that otherwise would have been cancelled. In all 205 educational seed packets and plant cuttings were distributed.





Molecular Genetics
By Nahla V. Bassil

Students

Christina Mulch, MS student at OSU, is using expression analysis to fine map aphid resistance in this crop. New MS student, Ozgecan Yalcin, arrived from Turkey and is working on QTL analysis for phenological and fruit quality traits in blueberry and on identifying more true-to-type blueberry cultivars through parentage analysis using SSR markers.

Genetics Lab Team: From Left to Right Jamie Green, Mandie Driskill, Christina Mulch, Nahla Bassil, April Nyberg, and Jason Zurn

Completed Projects

Used synteny and candidate genes to identify loci controlling fruit sweetness in blackberry. We identified blackberries genes that are similar to those know for sugar production in other closely related crops such as apple, peach, and strawberry. We used these genes to conduct targeted sequencing in 40 blackberry cultivars with high and low sugar content from the University of Arkansas (UA) and the USDA-ARS-HCRL breeding programs. Population modeling identified 173 loci that were linked with sugar production in these 40 blackberries. Molecular markers were developed and validated in 192 samples from UA and the USDA-ARS-HCRL breeding programs and some were indeed linked with soluble solids content. The regions identified represent the first sweetness related genomic region in blackberry.

Developed an inexpensive and easy to use US Pear Genetic Resources (USPGR) fingerprinting set. We developed 10-SSR high core repeat fingerprinting set in pear that was used for cultivar paternity testing, and identity certification in pears. The USPGR set was compared to the European set (ECPGR) and had similar performance while being cheaper (one reaction instead of two) and easier to use. The US set was also compared to the new 70K Pear Axiom array and was able to come to the same conclusions when looking at pear diversity or parentage. The US fingerprinting set was quick and inexpensive for determining clonal identity and parentage for a few samples. The US set is being used to manage the US pear genebank collection.

Mapped fire blight resistance in pear. We used DNA markers from the 70K array to construct genetic maps for three pear families. Use of phenotypic data for fire blight resistance for these three families and the genetic maps, allowed us to detect a genomic region mediating resistance in a

similar region on chromosome 2. Fire blight resistance have been previously reported in this region for 'Harrow Sweet' and 'Moonglow'. A genomic analysis of the region using the new pear genome assembly identified 30 genes potentially associated with disease resistance. New tools will be developed to incorporate this resistance region into new pear varieties.

Mapped the Black Spot Resistance Locus in the Shrub Rose 'George Vancouver' and developed Diagnostic Markers for DNA-Informed Breeding. To date, four resistance genes to black spot have been identified in roses. The resistance gene from 'George Vancouver' was never mapped and is thought to be unique and different from two previously identified genes for resistance to black spot. We mapped this gene to one of the four chromosome 6 molecules using genotypic data derived from the 68K Axiom array, confirming it is different from the other identified genes. The mapping information was used in conjunction with the Chinese rose genome assembly to develop new tightly-linked tests for DNA-informed breeding. Three DNA markers in the test were able to predict the presence of this gene in 63 cultivars. The improved diagnostic DNA test will be a great asset to the rose breeding community toward developing new black spot resistant cultivars for the $1 billion North American rose industry.

Identified true-to-type cultivars of blueberry in the USDA National blueberry collection. We used our 10-SSR blueberry fingerprinting set to identify 54 blueberry cultivars that match reported pedigree descriptions in the national USDA collection using parentage analysis. The identity confirmed blueberry genotypes would be the reference foundation USDA genotype for that named blueberry. These results are critical to the resource-efficient germplasm management of the National blueberry collection and special resources will be applied to manage and protect the identity of these correct genotypes. We will continue to add to this database that was made publically available and seek parents or offspring to confirm identity through parentage analysis of the remaining accessions. This DNA test was shared with researchers and service providers to use and provide input about its reproducibility and usefulness in confirming blueberry cultivar identity under their conditions.

Developed a genetic framework to improve the efficiency of bioactive delivery from blueberry. In collaboration with Massimo Iorizzo (NCSU), we applied a novel high-throughput *in vitro* gastrointestinal digestion model to phenotype bioaccessibility of phenolics in 66 diverse blueberry accessions from the NCGR collection in 2017-2019. Results revealed significant ($P<0.05$) differences between accessions, years, and accession by year interaction for relative and absolute bioaccessibility of flavonoids and phenolic acids. Broad sense heritability estimates revealed low to moderate inheritances of relative and absolute bioaccessibility, suggesting that besides environmental variables, genetics factors could control bioaccessibility of phenolics. Acylated anthocyanins had significantly higher relative bioaccessibility than non-acylated anthocyanins. Correlation analysis indicated that relative bioaccessibility did not show significant association with fruit quality or raw concentration of metabolites. The study also identified accessions that have high relative and absolute bioaccessibility values. Overall, combining the bioaccessibility of phenolics with genetic and genomic approaches will enable the identification of genotypes and genetic factors influencing these traits in blueberry. Data from this study will be uploaded to GRIN.

Projects in progress

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 9,717,410 sequence variants and 126,616 putative SSRs. Subsequent filtering identified 1,995 genomic regions for assay design. We submitted these genomic regions to IDT for design of a 1,000 locus RhAMPSeq assay that would allow for a single multiplexed reaction. The assay will be used to genotype 800 samples that include our red and black raspberry collections, and mapping populations from Michael Dossett's program. A second small scale SSR-based fingerprinting assay will be developed using the most informative SSRs from the RhAMPSeq assay.

Converting alleles of all fingerprinting sets from the Beckman CEQ to the CeqStudio capillary electrophoresis platforms. We have compared alleles generated using the Beckman CEQ to alleles for the same accessions separated using our new SeqStudio (ABI) capillary electrophoresis system with our fingerprinting sets (10-SSR Pear USPGR, 10-SSR blueberry, 14-SSR hazelnut, 8-SSR blackberry, 6-SSR strawberry, and 6-di-SSR red raspberry) and were able to create a conversion factor for some SSRs and are scrutinizing challenges encountered in some SSRs.

Developing a mint fingerprinting set. We evaluated 17 high core repeat SSRs in a testing panel of 16 accessions representing *M. aquatica*, *M. canadensis*, *M.* × *gracilis*, *M.* × *piperita*, *M. suaveolens*, *M. spicata*, and *M. longifolia*, and identified 6 SSRs that appear polymorphic across these species. We optimized this 6-SSR mint fingerprinting set and will be testing it for ability to identify true hybrids from interspecific populations and for accession identification in the NCGR collection.

Developing a *Ribes* fingerprinting set. 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 will be testing it for ability to identify 51 accessions in the NCGR collection.

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. Natural, genetic resistance to aphids exits and has been identified from three geographic sources: Maine, Michigan, and Ontario. These sources are being used by Chad Finn 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 (R) to susceptible (S) by Chi-squared analysis. Differential expression in 10 R and 10 S seedlings is being assessed with IsoSeq (Full-Length Isoform Sequencing) for one source (ORUS 5306). In addition, Illumina Sequencing for 5 R and 5 S seedlings from each population before and after aphid inoculation is being evaluated. We plan on performing fine mapping of QTL (Quantitative Trait Loci) for aphid resistance in each of these populations using previously developed microsatellite

markers and new markers identified using IsoSeq. 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.

Assessing genetic diversity in the cultivated strawberry (*Fragaria ×ananassa*) collection at the NCGR. 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 is to genotype the entire *F. ×ananassa* collection, assess genetic structure and diversity, confirm pedigrees within the collection, and identify a core collection based on genetic data. Genotyping was conducted on 534 *F. ×ananassa* samples using either the iStraw35 or the 50K octoploid strawberry Axiom array. These arrays share 5,809 markers that are distributed across the 'Camarosa' genome assembly. Data for the shared markers were curated for call quality, missing data, and minor allele frequency resulting in 4,033 markers. K-means clustering analysis revealed eight sub-populations associated with different geographic breeding centers. Two core collections were determined: one represented a uniform distribution of the gene space, and the other its maximum genetic diversity. Pedigrees are being confirmed for triads and sports in the collection. Genotypic data for this collection will be publically available and could allow identification of genomic regions controlling valuable traits when phenotypic data are obtained.

Evaluating genotype x environment interactions for predicting SSC in strawberry. Strawberry fruit flavor is due to a complex mix of sugars, acids, and aromatic compounds. Consumers tend to prefer sweeter strawberry cultivars. Therefore, sweetness has been an important target trait for breeders. The majority of strawberry soluble solids are sugars, and soluble solid content (SSC) is used as a proxy to determine sweetness. A strong genotype × environment (G × E) interaction has been observed for SSC, causing difficulties when studying the genetics underlying SSC in individual environments. A meta-analysis of multiple environments may provide new insights toward unraveling the genetics underlying SSC. Genotypic and phenotypic data were collected for 3,407 total individuals from seven breeding programs (four in the United States, one from Spain, the United Kingdom, and Australia). Subsets of the individuals were evaluated for SSC in 19 environments. Genotypic information from the 90K and 35K Axiom arrays was reduced to 12,951 high quality single nucleotide polymorphism markers shared by all accessions. Missing data was imputed, linkage disequilibrium was calculated, and a relationship matrix was constructed for all samples. Using this information, multiple G × E models were evaluated for their predictive ability among environments. Results are being analyzed to identify genomic models that can be used to predict strawberry SSC in new environments.

Developing a multiplex fingerprinting set in hops. We are developing, testing, and applying two economically viable sets of DNA-based markers for fingerprinting 328 hop accessions from the USDA ARS National Clonal Germplasm Repository world collection; 223 cultivars and selections from the USDA ARS breeding program; and 26 wild samples from the University of Nebraska-Lincoln. Our objectives are to develop markers that can separate botanical varieties of native hops as well as identify standard hop cultivars. The two DNA tests were developed and are being tested at this time. They consist of a single nucleotide polymorphism (SNP) based fingerprinting set, and a

simple sequence repeat (SSR) based set. The SNP set consists of 28 SNPs from John Henning of the USDA-ARS-NFSPRC hop breeding program that were converted to a Kompetitive allele specific PCR (KASP) KASP assay by the company LGC Ltd. After testing 44 SSR primer pairs in 16 diverse hop accessions, we selected nine highly polymorphic SSR primer pairs to make up a multiplexed DNA test. We are comparing these two tests in 192 samples to ensure they distinguish each unique genotype. Data analysis is in process. The DNA tests and fingerprinting information will be made available to service providers.

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. We surveyed 18 core and non-core *Vaccinium* groups to identify their needs for a high throughput platform that can be of use to the *Vaccinium* research community. We began compiling a SNP catalog by obtaining 47,025 SNPs of interest from linkage maps and QTL studies. We obtained sequence data from collaborators and NCBI and cleaned them up and stored them to use for SNP detection once the pangenome is ready. We also identified four providers that could meet our needs, organized webinars from these companies to provide an overview of their services to the *Vaccinium* community and are in the process of evaluating their offers.

Phenotyping blueberry for fruit quality traits. In the spring and summer of 2020, we harvested ripe blueberry fruit from 196 seedlings for the 'Draper' x 'Jewel' population, 200 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 use 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.

Nahla Bassil meeting with Ted Mackey and Michael Hardigan to coordinate blueberry harvest and collection activities

Blueberry harvest crew preparing to harvest ripe berries from the 960 blueberry plants in the 2016 and 2017 USDA-ARS-HCRL breeding program field

Postdoc Marti Pottorff (NCSU) using the Texture Analyzer to evaluate fruit characteristics and shelf life indicators in harvested ripe blueberry fruit.

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 genotyping platform selected (DArTag), once it is ready. We provided leaf tissue for ~600 samples for two companies to test their blueberry DNA extraction protocols. 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 (GDR, WSU), we compiled a comprehensive list of all traits being used to phenotype blueberry, and phenotyping method from the blueberry research and breeding community (ARS, university, and private companies) and converted the information into the BI template that is interoperable with BreedBase.

Testing Allegro Targeted Genotyping for blueberry genome wide association. In collaboration with Hamid Ashrafi (NCSU), 1.7 million SNPs were selected, and the flanking sequences were extracted. Single primer enrichment technology (SPET) was used to specifically target SNPs of interest in a diversity panel of 252 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. Two bioinformatics pipelines were used for SNP identification. Data analysis is in process. 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, will be investigated. Further, comparative analysis of resequencing data of native diploid, tetraploid, and hexaploid *Vaccinium* species will be used to ascertain the origin of introgressed SNPs.

Accomplishments

- Developed markers for SSC in blackberry using synteny and candidate gene approach from other Rosaceous crops (apple, strawberry and peach).
- Developed a new USPGR fingerprinting set for pear that is comparable to the 70K Array and the ECPGR set in ability to confirm identity of pear accessions and that is easier and cheaper to use.
- Identified true parents of new USDA-ARS blackberry cultivars Eclipse, Galaxy, and Twilight using the blackberry fingerprinting set.
- Mapped the Black Spot Resistance Locus in the Shrub Rose 'George Vancouver' and developed diagnostic markers for DNA-Informed Breeding.
- Applied a novel high-throughput *in vitro* gastrointestinal digestion model to phenotype bioaccessibility of phenolics in 66 diverse blueberry accessions from the NCGR and identify accessions that have high relative and absolute bioaccessibility values.
- Confirmed identity of 54 unique blueberry cultivars using pedigree-based analysis with the blueberry fingerprinting set. We also identified 13 homonym sets, and 10 synonym sets of blueberries in the NCGR national blueberry collection and among those obtained from two nurseries, five breeder collections (OR, MN, MS, NJ, and NC) across the US.

Tree Fruit Curation

By Barb Gilmore, Field Manager and Tree Fruit Crop Manager

This year our efforts have been focused on fighting fire blight in the *Cydonia, Pyrus, Mespilus* and *Sorbus* and continuing the ongoing war with Eastern Filbert Blight (EFB) in the *Corylus* collection. In the *Corylus* we are seeing less and less symptoms each year, but EFB remains a perennial pathogen.

The fire blight invaded the main *Pyrus* collection this year and has required severe pruning of some of the pear trees. We had the pathogen cultured, and this strain is not resistant to streptomycin, the number one preferred antibiotic. The North Farm *Pyrus* trees have been cut down to their main scaffolds, as have some of the *Sorbus* trees. We have also started bringing down the species pears in the main collection; Because of their height we can't harvest scionwood and we can't monitor them for fire blight strikes. This height reduction will allow antibiotic sprays to reach the tree tops and prevent further spread of the pathogen. Also our plan is to spray a dormant copper spray on the trees and then spray with antibiotics during the bloom period for all four orchards. This fire blight pressure made us decide that it is necessary to move the *Sorbus* and the *Cydonia* collections to a different area of the North Farm. Many of the trees in the *Cydonia* collection have

systemic infections of fire blight. This systemic infection resists pruning and sprays. The infection continues moving through the tree throughout the summer with young branches showing flagging and death. Those diseased branches must be pruned out and removed from the field. We hope by starting afresh that we can prevent a systemic infection from reoccurring. The *Sorbus* collection abuts Peoria Road and this location prevents air-blast sprayer use on those trees. We have started *Sorbus* seeds and will use these seedlings for rootstocks. *Sorbus* grafts are most successful when the scionwood is grafted onto the same *Sorbus* species rootstock. Once we have established trees in a new location on the North Farm then the old trees adjoining Peoria Road will be removed.

In the *Corylus* collection all of the trees have been reduced to a more manageable height, about 12 feet high. In past years, we scouted for dead limbs to alert us to EFB strikes, but what we observed this year was EFB pustules on healthy appearing limbs. We had a professor from Oregon State University confirm our diagnosis that this was indeed EFB before it has the chance to girdle the branch, which can result in branch death. This will require manual inspection of each tree in future years. The pustules on healthy appearing branches caused us to increase our spray schedule to six times per spring instead of four. A result of the many species in the collection is a longer leafing-out period, much longer than is seen in a typical Oregon hazelnut orchard. The six times per spring spray schedule will start in Mid-March and continue until mid-May. This extended spray program will better protect the young leaves from infection. There is too much inoculum present in this area to not be ever vigilant with our sprays and scouting.

Another main goal that we have achieved for the North Farm is that many of the collections now have drip irrigation. The drip irrigation will provide a more favorable growing environment for our trees, but even more important is that it will reduce water mist from the water wheel irrigation system that we previously used in past years. The mist that the water wheels produce provides moisture for the fire blight inoculum to spread further. Irrigation rates are highest during warm temperatures which creates the perfect environmental conditions for fire blight to spread.

Weeds are a problem on this farm as on any farm, and glyphosate, Surflan, Rely and Casoron were used on the orchards and fields this year. Another method we used for weed control was the zero-turn Kubota mower. This mower allows us to get close to the trees and knock down living and dead weeds. The Stihl weed wacker was used to remove dead suckers and to knock down weeds in the *Pyrus* and the *Corylus* fields. The pear field was groomed extensively for Joseph Postman's retirement ceremony. Normally, we don't use insecticides on our collections, but a passive yellow jacket control system was implemented in the pear field to prevent yellow jacket injuries.

All collections received fertilizer, and we continued to use the 20-12-8-8 that is purchased from Wilco. The plants respond very favorably to that mix and it can be applied with the tractor pulled applicator. This fertilizer is applied at a low rate, about 10 pounds of nitrogen per acre. At that rate moderate growth is encouraged, but the trees don't demonstrate rampant growth.

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- New seed accessions including 34 Pyrus samples and 2 *Docynia* samples were added to NCGR Corvallis collections. New clonal accessions including 2 *Chaenomeles* trees, 49 *Corylus* trees, 10 *Crataegus* trees, 17 *Cydonia* trees, 6 *Mespilus* trees, 5 *Photinia* plants and 29 *Pyrus* trees were added to NCGR collections.

- Surveyed genetic diversity of mint crop ancestors, *Mentha aquatica* and *Mentha suaveolens*, and determined their ploidy, essential oil composition, and relative Verticillium wilt resistance. This study provided updates of accession descriptions in the GRIN database, and is expected to increase the utility of the *Mentha* collection to the research community.

Publications

Bassil, N.V., Bidani, A., Nyberg, A.M., Hummer, K.E., Rowland, L.J. 2020. Microsatellite markers confirm identity of blueberry plants in the USDA-ARS National Clonal Germplasm Repository collection. Genetic Resources and Crop Evolution. 67:393-409. https://doi.org/10.1007/s10722-019-00873-8.

Bassil, N.V., Zurn, J.D., Hummer, K.E., Hardigan, M.A., Knapp, S.J., Montanari, S., Postman, J.D., Worthington, M., Clark, J.R., Ashrafi, H., Aryal, R., Dossett, M., Finn, C.E., Driskill, M.J., Mulch, C., Vining, K.J., Rapp, R., Ochsenfeld, C., Zhang, X., Poorten, T., Pham, G. 2020. Molecular characterization of caneberries, strawberries, pear, mint and hops at the Corvallis genebank. Abstract for American Society for Horticultural Science Conference, August 9-13, 2020, Orlando, FL

Bradish, C., Bushakra, J., Robbins, L., Karaaoac, E., Sabrina, T., Willard, J.L., Perkins-Veazie, P., Lee, J., Scheerens, J., Weber, C., Dossett, M., Bassil, N.V., Finn, C.E., Fernandez, G. 2020. Standardized phenotyping in black raspberry. Journal of American Pomological Society. 74(1):2-17.

Bushakra, J., Alice, L., Carter, K., Dossett, M., Lee, J.C., Liston, A., Meiers, R., Mulch, C., Nyberg, A.M., Peterson, M.E., Clark, M.C., Vining, K., Worthington, M., Yin, M., Sutherland, B., Zurn, J.D., Clark, J., Finn, C.E., Bassil, N.V., Hummer, K.E. 2020. Status of Rubus germplasm at the US National Clonal Germplasm Repository in Corvallis, Oregon. Acta Horticulturae. 1277:121-128. https://doi.org/10.17660/ActaHortic.2020.1277.17..

Finn, C.E., Strik, B., Yorgey, B.M., Peterson, M.E., Jones, P.A., Lee, J., Bassil, N.V., Martin, R.R. 2020. 'Twilight' thornless semi-erect blackberry. HortScience. 55(7):1148-1152. https://doi.org/10.21273/HORTSCI14992-20.

Finn, C.E., Strik, B.C., Yorgey, B.M., Peterson, M.E., Jones, P.A., Buller, G., Serce, S., Lee, J., Bassil, N.V., Martin, R.R. 2020. 'Eclipse' thornless semi-erect blackberry. HortScience. 55(5):749-754. https://doi.org/10.21273/HORTSCI14891-20.

Finn, C.E., Strik, B., Yorgey, B.M., Peterson, M.E., Jones, P.A., Buller, G., Lee, J., Bassil, N.V., Martin, R.R. 2020. 'Galaxy' thornless semierect blackberry. HortScience. 55(6):967-971. https://doi.org/10.21273/HORTSCI14985-20.

Hummer, K.E., Bassil, N.V., Lange, M.B., Vining, K. 2020. Crop wild relatives as a germplasm resource for cultivar improvement in mint (Mentha L.). Frontiers in Plant Science. 11:1217. https://doi.org/10.3389/fpls.2020.01217.

Hummer, K.E., Bushakra, J. 2020. Recent acquisitions of Rubus L. at the USDA National Clonal Germplasm Repository, Corvallis, Oregon: profiles of four species. Acta Horticulturae. 1277:33-38. https://doi.org/10.17660/ActaHortic.2020.1277.5.

Hummer, K.E., Postman, J. 2020. Guardians of the germplasm: hazelnuts, berries, pears, hops, and mint. Journal of American Pomological Society. 74(2):104-110.

Mengist, M.F., Grace, M.H., Xiong, J., Kay, C.D., Bassil, N.V., Hummer, K.E., Ferruzzi, M.G., Lila, M., Iorizzo, M. 2020. Diversity in metabolites and fruit quality traits in blueberry enables ploidy and species differentiation and establishes a strategy for future genetic studies. Frontiers in Plant Science. 5. https://doi.org/10.3389/fpls.2020.00370.

Montanari, S., Postman, J., Bassil, N.V., Neale, D. 2020. Reconstruction of the largest pedigree network for pear cultivars and evaluation of the genetic diversity of the USDA-ARS national Pyrus collection. G3, Genes/Genomes/Genetics. 10:3285-3297. https://doi.org/10.1534/g3.120.401327.

Staats, E.R., Jenderek, M.M., Hummer, K.E. 2020. Safeguarding strawberry (Fragaria L.) genetic resources. ASHS Centennial Conference.

Whitaker, V.M., Knapp, S.J., Hardigan, M.A., Edger, P.P., Slovin, J.P., Bassil, N.V., Hytonen, T., Mackenzie, K.K. 2020. A roadmap for research in octoploid strawberry. Horticulture Research. https://doi.org/10.1038/s41438-020-0252-1.

Willman, M., Bushakra, J., Bassil, N.V., Finn, C.E., Dossett, M., Fernandez, G., Weber, C., Scheerens, J., Dunlap, L., Fresnedo-Ramirez, J. 2020. Genetic analysis of drupelet count in black raspberry (Rubus occidentalis). Acta Horticulturae. 1277:65-72. https://doi.org/10.17660/ActaHortic.2020.1277.9.

Worthington, M.I., Aryal, R., Bassil, N.V., Mead, D., Fernandez, G.E., Clark, J.R., Fernandez-Fernandez, F., Finn, C.E., Hummer, K.E., Ashrafi, H. 2020. Development of new genomic resources and tools for molecular breeding in blackberry. Acta Horticulturae. 1277:39-46. https://doi.org/10.17660/ActaHortic.2020.1277.6.

Zurn, J.D., Driskill, M.J., Bassil, N.V. 2020. Developing DNA-based tests to breed fire blight resistant pears. Abstract for Plant Health 2020, August 10-14, 2020, Denver, CO

Zurn, J.D., Driskill, M.J., Bassil, N.V. 2020. Creating DNA-based tests for the pear chromosome 2 fire blight resistance region. Abstract for American Society for Horticultural Science Annual Conference, August 9-13, 2020, Orlando, FL

Zurn, J.D., Ivors, K.L., Cole, G.S., Knapp, S.J., Hummer, K.E., Hancock, J.F., Finn, C.E., Bassil, N.V. 2020. Assessing cultivated strawberries and the Fragaria Supercore for resistance to soilborne pathogens. Journal of American Pomological Society. 74(1):18-23.

Zurn, J.D., Meiers, R.C., Ward, J., Finn, C.E., Dossett, M., Bassil, N.V. 2020. Identifying variation in red raspberry MLO genes thought to provide resistance to powdery mildew. Acta Horticulturae. 1277:25-32. https://doi.org/10.17660/ActaHortic.2020.1277.4.

Zurn, J.D., Norelli, J.L., Montanari, S., Bell, R.L., Bassil, N.V. 2020. Dissecting genetic resistance to fire blight in three pear populations. Journal of Phytopathology. 10(7):1305-1311. https://doi.org/10.1094/PHYTO-02-20-0051-R.

Zurn, J.D., Nyberg, A.M., Montanari, S., Postman, J., Neale, D., Bassil, N.V. 2020. A new SSR fingerprinting set and its comparison to existing SSR and SNP based genotyping platforms to manage pyrus germplasm resources. Tree Genetics and Genomes. 16. https://doi.org/10.1007/s11295-020-01467-7.

Zurn, J.D., Zlesak, D.C., Holen, M., Bradeen, J.M., Hokanson, S.C., Bassil, N.V. 2020. Mapping the black spot resistance locus Rdr3 in the shrub rose 'George Vancouver' allows for the development of improved diagnostic markers for DNA-informed breeding. Theoretical and Applied Genetics. 133:2011-2020. https://doi.org/10.1007/s00122-020-