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

Permanent and Term Federal Staff
Kim Hummer, Research Leader/Curator
Nahla Bassil, Geneticist-Plants
Lauri Reinhold, Horticulturist/Curator
Barb Gilmore, Field Mgr., Trees
Jim Oliphant, *Vaccinium/Fragaria* Mgr.
Jill Bushakra, *Rubus/Ribes* Mgr.
Gabriel Flores TC, *Humulus/Mentha* Mgr.
Ryan King, Bio. Sci. Res. Tech., Lab
April Nyberg, Bio. Sci. Res. Tech., Distribution
Vacant, Program Support Assist.

Temporary Staff & Students
Laura Duncan, Bio. Sci. Res. Tech., SH
Sunny Green, Bio. Sci. Res. Tech.
Debra Hawkes, Bio. Sci. Res. Tech.
Jane Olson, Bio. Sci. Res. Tech.
Leonardo Zavala, Ag. Sci. Tech., Field
Sebastian Dibblee, Ag. Sci. Tech., Field
Emma Ciechanowski, Undergrad Tech.

Graduate Students
Todd Anderson, GRA, OSU, Hort.
Christina Mulch, GRA, OSU, Hort.
Ozgecan Yalcin, GRA, OSU, Hort.

Visiting Scientists
Joseph Postman, Curator, Retired

Volunteers
Margaret Kelly, Volunteer /Easter Seals
Chuck Muraz, Volunteer /Easter Seals
Jun Tanaka, Volunteer /Easter Seals
Alex / Work Unlimited

Stakeholder/Service Accomplishments

- Conserved 12,828 active accessions and 759 taxa of temperate fruit, nut, and specialty crops.
- Obtained 63 new accessions and 129 new inventory items in CY 2021.
- Shipped 4,331 items this year.
- Collaborated with NGRPL, Ft. Collins, CO, on backup open pollinated seed preservation for 300 strawberry cultivars.
- 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.
- 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.
- Gave virtual presentation on strawberry anthocyanins to International Strawberry Symposium at Rimini, Italy, in May 2021.
- Gave virtual presentation on blueberry identification to International Blueberry Symposium at Nova Scotia, Canada, in August 2021.
- Presentation on subtropical *Vaccinium* species presented at ASHS annual meeting in Denver, Colorado, August 2021.
- Gave virtual presentation to OSU Hort 511, graduate student seminar at Oregon State University.
- Presented virtual tour of the NCGR to Hort 112 class at Oregon State University.
- Collaborated with Food Innovation Center at Oregon State University on tasting evaluation of pear cultivars.
- Participated as President for the American Pomological Society.
- Participated on Board for the North American Raspberry and Blackberry Association.
- Participated on Board for International Society for Horticultural Science.
- Participated as Editor of *Chronica Horticulturae* for ISHS.

Research Accomplishments

- Established true to type (TTT) system for identification of blueberry cultivars in GRIN.
- Determined unreduced pollen for diverse Vaccinium species.
- Evaluated blueberry breeding lines for heat, drought, and cold tolerance in OR and WA.
- Phenotyped strawberry, blueberries, and Rubus for fruit quality traits.
- Developed high throughput genotyping platform for blueberry and cranberry.
- Advised Breeding Insights (Cornell) in enabling genomic selection in blueberry.
- Tested Allegro Targeted Genotyping for blueberry genome wide association.
- Assessed genetic diversity in cultivated strawberry collection.
- Evaluated G x E interactions for predicting soluble solid content in strawberry.
- Determined anthocyanins in wild relatives of *Fragaria*.

Ozge Yeltsin, OSU Ph. D. candidate, received the ISHS Young Mind Award for Best Poster at the ISHS International *Vaccinium* Symposium in Nova Scotia, August 2021. Her poster was entitled: *Confirming identity of blueberry cultivars with a fingerprinting set*.

Administrative Overview

Staffing Changes

The NCGR has had many staffing change since COVID and the pandemic.
New and reassigned permanent staff members at NCGR.




Left: Dr. Lauri Reinhold, Horticulturist/Curator for tree fruit, nut, hop, and mint collections. She replaces Joseph Postman who retired in July 2018.
Center left: Gabriel Flores, Bio. Sci. Res. Tech, working in tissue culture laboratory and plant maintenance of hop and mint collections. He replaced Jeanine DeNoma who retired in October 2020.
Center right: Ryan King, Bio. Sci. Res. Tech., working in molecular laboratory.
Right: April Nyberg, Bio. Sci. Res. Tech., Plant Distribution Manager. She replaced Missy Fix who retired in April 2021.

Welcome to our new and reassigned staff members!

Ashley Winters, our former 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 2020, to help with our administrative tasks. After his term ended, Gabriel was then selected to be a permanent Bio. Sci. Res. Tech. in our tissue culture laboratory in December 2020. The PSA position has been vacant since then. We hope to hire a replacement PSA soon. In addition, we hired Leonardo Zavala and Sebastian Dibblee (Ag. Sci. Res. Techs) to work in our Field Collections. Their positions will begin in early November 2021.

EEO/CR/Outreach

- We are working with Easterseals, an organization that helps people with disabilities, older workers, and veterans find meaningful employment, to train three individuals.
- We are working with Work Unlimited, an organization that facilities the employment of people experiencing intellectual/developmental disabilities and challenging behavioral issues, to train an individual.
- Through a Research Support Agreement with Oregon State University, four graduate students, (three women and a student of Pacific-Island heritage) and two undergraduates were trained.

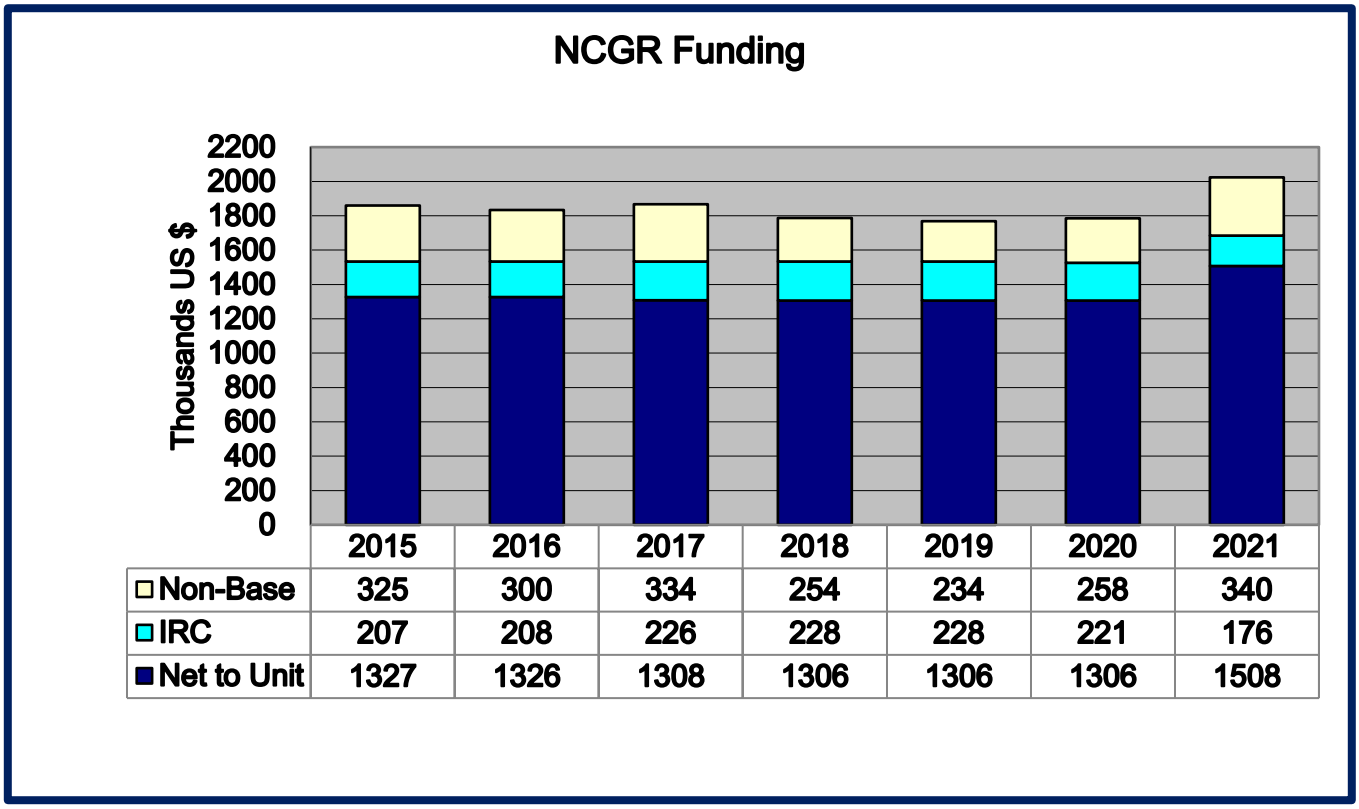


Left: Todd Anderson, Ph. D. candidate
Center: Ozge Yeltsin, Ph. D. candidate
Right: Sunny Green, B.S. student/part time Bio tech

Budget

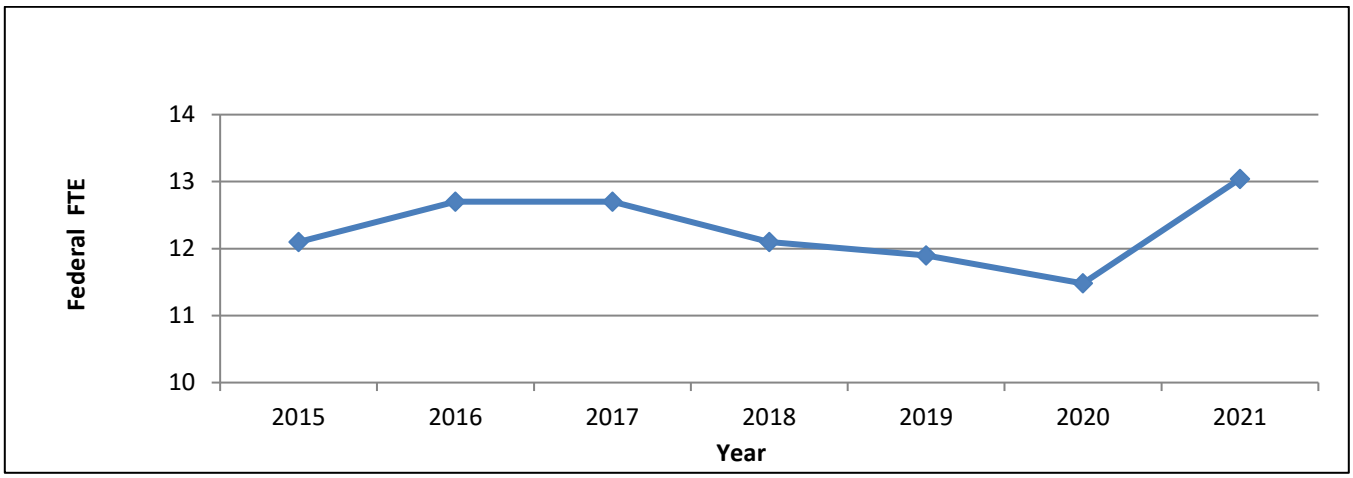
Our total federal budget was $1.684 million for FY 2021. The net to unit budget increased by $150,000 supported in Congress by the blueberry and cranberry genetic resource community. Thank you to those of you who supported the needs of our project! Our scientists have been successful in obtaining federal agriculture grants as well as those from commodity commissions and research consortium funding. Our location administrative costs (IRC) were about 14 % in FY 2021.

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

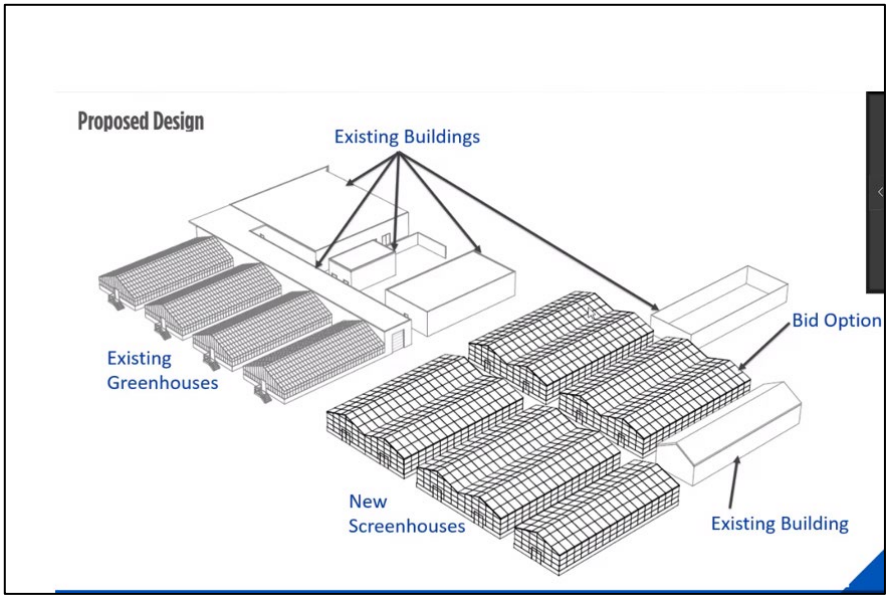
Employee Summary



Facilities

The NCGR-Corvallis appeared on the President's budget in 2019 for architectural planning funding of $13.500 million (no year funding) to replace 6 screenhouses and 4 greenhouses. We are working with USDA headquarters staffing and the Army Corps of Engineers and Burns & McDonnell for the design and planning phase of this project now.

NCGR facilities as of about 2018.



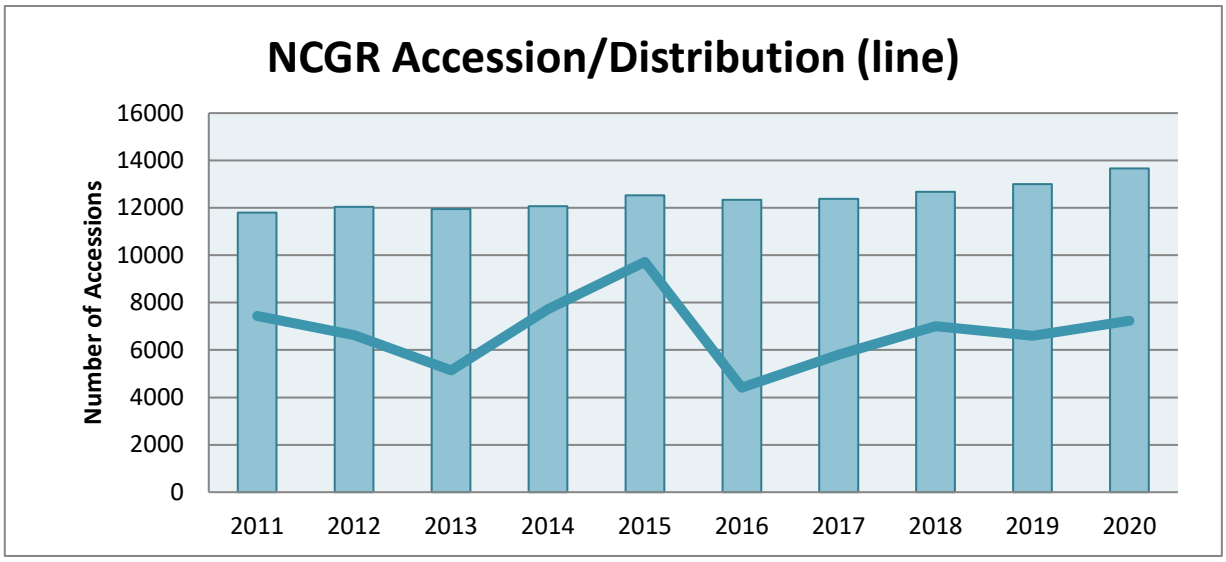
Possible design for the new construction.

Roof damage (photo taken 19 October 2021 due to recent winds. Rocks on roof of SH 7 to hold down the corrugated single-wall polycarbonate roofing (past its lifecycle). The light transmission of the degraded roofing is less than desired.

Inside SH 8 the strawberry house. It now contains blueberries in the center aisle due to lack of space. Carts can no longer go down the aisle. The lack of space makes it difficult to work on plants.

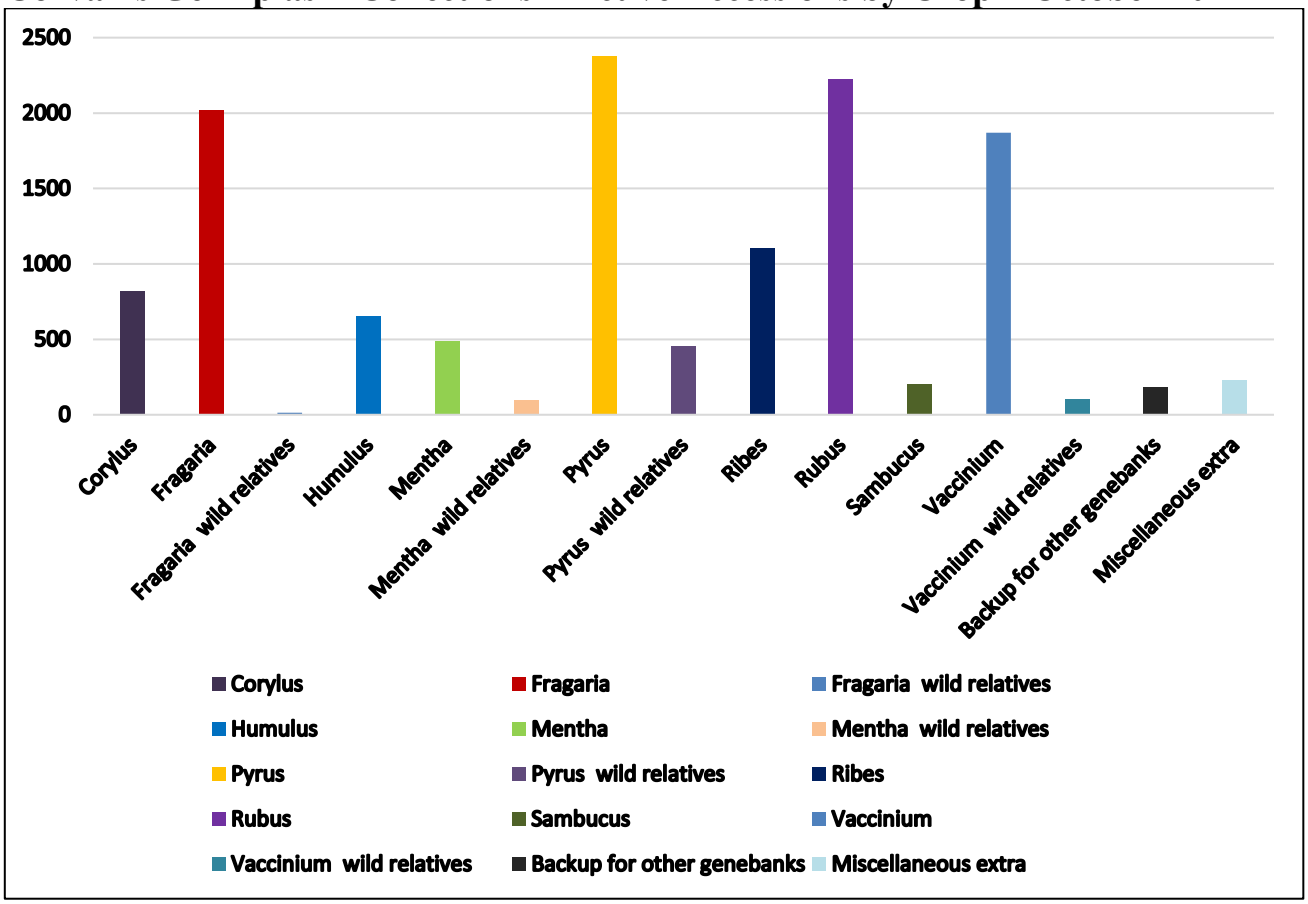
Germplasm Collections

Corvallis Germplasm Collections 2011 -2020



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

Corvallis Germplasm Collections – Active Accessions by Crop – October 2021



Corvallis Germplasm Collections – Total accessions (12,828) by genus, October 2021

Genus	Common Name	Accessions	NCGR Curator/Crop Manager
Corylus	Hazelnut	**822**	Lauri Reinhold
Fragaria	Strawberry	**2016**	Nahla Bassil
***Fragaria* wild relatives**	Tribe: Potentilleae	**13**	Nahla Bassil
Humulus	Hop	**655**	Lauri Reinhold
Mentha	Mint	**484**	Lauri Reinhold
***Mentha* wild relatives**	Mountain Mint	**96**	Lauri Reinhold
Pyrus	Pear	**2378**	Lauri Reinhold
***Pyrus* wild relatives**	Tribe: Maleoideae	**456**	Lauri Reinhold
Ribes	Currant/Gooseberry	**1101**	Jill Bushakra
Rubus	Black/Raspberry	**2226**	Jill Bushakra
Sambucus	Elderberry	**202**	Jill Bushakra
Vaccinium	Blueberry	**1870**	Nahla Bassil
***Vaccinium* wild relatives**	Subfamily: Vaccinioideae	**100**	Nahla Bassil
Backup genera for other genebanks	*Actinidia, Juglans,*	**179**	Lauri Reinhold
Miscellaneous extra		**230**	Nahla Bassil
Total		**12828**	

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 relatives, 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 2020

By Gabriel Flores

Jeanine retired in October 2020. Thank you, Jeanine, for your career of work at our unit!

Mentha: The *Mentha* collection includes 460 accessions located in greenhouse 2, 4, and screenhouse 12. The core and non-hardy collection is located in greenhouse 2. Non-core hardy accessions are in screenhouse 12 and greenhouse 4. About 146 accessions of the non-core collection are in need of repropagation this year.

Pycnanthemum. The mountain mint (*Pycnanthemum*) includes 33 accessions in screenhouse 6. Of these, 21 accessions were repotted to two 5-gallon containers. 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. CPYC 44.001 has been recovered from culture to the greenhouse to replace the missing accession. Three extra cultures of CPYC 44.001 remain in the grow room.

Humulus: The 329 accessions of *Humulus* are conserved in the screenhouses: 170 pathogen (virus and viroid negative accessions) are located in screenhouse 6; 159 known virus positive accessions are in screenhouse 7. Another 121 new propagations representing 54 core accessions are in one-gallon pots in greenhouse 4, primarily for replacement in the core collection. Many accessions remain in P-86 pots in greenhouse 4 awaiting to be assigned a permanent location. Labels are being updated throughout the entire *Humulus* collection to include QR codes.

In Vitro **collection:** After Jeanine DeNoma's retirement, the pace of the tissue culture lab has initially slowed down. Gabriel Flores has taken on the technical responsibilities of the lab. Currently, there are nineteen accessions in the growth room: three accessions from the *Pyrus* collection, eleven accessions from the *Humulus* collection, and five accessions from the *Rubus* collection. The accessions from the *Humulus* collection have yet to sprout any new growth.

As time progresses, cleaning up the *Humulus* collection of Hop latent and Hop stunt viroids will be a priority. This will be accomplished by culturing 1mm cuttings from apical meristems using a procedure developed at the Clean Plant Center in Prosser, Washington. Enzyme-linked immunosorbent assay (ELISA) and rtPCR tests will be used to re-test the collections to determine the success of the pathogen removal. Additionally, these tests will be applied to *Fragaria*, *Rubus*, and *Vaccinium* collections. With limited staffing at NCGR, some testing will be contracted to commercial sources.

Recently, the field staff has discovered that our *Pyrus* collection has been heavily affected by fire blight. Due to concerns of losing accessions, the tissue culture lab will be actively reviving *Pyrus* accessions from the cooler and micro-propagating them until 2 starpacks can be filled. Our goal is to have sufficient germplasm backed up in case of loss of accession in the field. We will focus on backing up the most susceptible accessions first. The NLGRP, Ft. Collins, has sent 16 accessions from their backup inventory that will undergo micro-propagation and backing up as well.

Rubus/Ribes/Sambucus Collections

By Jill Bushakra

Rubus

Pruning and fertilizing entire collection; repotting into new style pots. In the process of repotting everything that was potted prior to 2013

- Propagation of tip-layering genotypes as needed; attempting airlayers of difficult to tip genotypes
- Planted and trellised Rubus standards for Pairwise GxE analysis

- Wrote SOP for label punch
- Put down new weed mat between Rubus houses
- 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), replaced side awning plastic on House 5
- Replaced missing labels as needed
- Organized and attended NARBA virtual meeting; attended APS advisory and business virtual meetings; attended PGOC meeting; attended NCCC-212 and Small Fruits CGC meetings
- Assisted in seed distribution and inventory

Ribes field

- Pruned, weeded (hand and chemical), applied sawdust mulch, and fertilized field collection
- Continuing to monitor Black currant Reversion Virus plants and performed graft inoculation onto indicator stock
- Harvested dormant wood for cryopreservation
- Incorporated species into cultivar field to reduce redundancy and so that all plants are on drip irrigation. Remapped and relabeled entire field; in process of putting in hanging irrigation in rest of field
- Provided material for fingerprinting *Ribes* cultivars and species
- Contacted Brogdale, Royal Horticultural Society and ProSpecieRara to obtain material to confirm fingerprints
- Working with Inga Zasada's lab for nematode project
- Co-author on Ribes cryopreservation manuscript

Ribes screenhouse collections

- Fertilized and pruned all plants; propagated as necessary

Sambucus

- Inventoried and fertilized
- Planted 4 new accessions into field
- Received new material from Patrick Byers and began propagation

Lonicera

- Inventoried and fertilized
- Received cuttings of Kapel from private nursery
- Received cuttings of advanced material from Maxine Thompson's for propagation; received open pollinated seed.

GRIN

- Attending twice monthly GRIN advisory committee meetings; developing protocols for more consistent data entry
- Working with other curators on best practices and new methods for using GRIN
- Conducted inventories on all crops; included patent information when available; scanned and attached supporting documentation and photos

Tree Fruit Collections

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.

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. MS student, 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 (2020).

Completed Projects

Assessed 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 539 *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 100 accession core collections were determined: one represented a uniform distribution of the gene space, and the other its maximum genetic diversity. Pedigree linkages were also confirmed in the collection. Finally, accessions containing disease resistance associated haplotypes for resistance genes *FaRCa1* (anthracnose fruit rot), *FaRCg1* (Colletotrichum crown rot), *FaRMp1* (charcoal rot), and *FaRPc2* (Phytophthora crown rot) were identified. These new core collections will allow for breeders and researchers to more efficiently utilize the *F. ×ananassa* collection. The core collections can be ordered from the USDA-ARS NCGR via the Germplasm Resources Information Network (https://www.ars-grin.gov/). Genotypic data for this collection will be publicly available and could allow identification of genomic regions controlling valuable traits when phenotypic data are obtained.

Evaluated 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 2,064 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. 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 significant variations in pairwise allele frequency distributions among the two subpopulations. Therefore, three 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. Of the approaches investigated, two were found to have the highest prediction accuracy for SSC. Thus, accounting for population structure enhances prediction accuracy for multi-environment genomic prediction. We believe that the approaches investigated in this study will provide new insights about population structure in multi-environment genomic prediction and for the practical implementation of genomic selection in strawberry improvement programs.

Developed two hop fingerprinting sets and used them to genotype the NCGR hop collection. we developed two fingerprinting sets for hop: a 9-SSR fingerprinting set containing high-core repeats that can be ran in a single PCR reaction; and a kompetitive allele specific PCR (KASP) assay of 25 single nucleotide polymorphisms (SNPs). The SSR set contains a sex-linked primer pair, HI-AGA7,

and was used to genotype 629 hop accessions from the US Department of Agriculture (USDA), National Clonal Germplasm Repository (NCGR), the USDA Forage Seed and Cereal Research (FSCR), and University of Nebraska-Lincoln (UNL) collections. It identified unique genotypes except for 89 sets of synonymous samples. These synonyms included: cultivars with different designations, the same cultivars from different sources, heat-treated clones, and clonal variants. Population structure analysis clustered accessions into wild North American (WNA) and cultivated groups. Parentage and sibship analyses were used to identify true-to-type cultivars. HI-AGA7 generated two male- and nine female-specific alleles among the cultivated and WNA samples. The SSR and KASP fingerprinting sets were compared for 190 samples consisting of cultivated and WNA accession for ability to confirm identity, and assess diversity, and population structure. The SSR fingerprinting set distinguished cultivars, selections and WNA accessions while the KASP assays were unable to distinguish the WNA samples and had lower diversity estimates than that of the SSR set. Both fingerprinting sets are valuable tools for identity confirmation and parentage analysis in hop for different purposes. The 9-SSR assay is cost efficient when genotyping a small number of wild and cultivated hop samples (<96) while the KASP assay is easy to interpret and cost efficient for genotyping a large number of cultivated samples (multiples of 96).

Projects in progress

Identifying true to type *Ribes* cultivars using a 7-SSR fingerprinting set. After evaluating 13 reported SSRs 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. This optimized 7-SSR *Ribes* fingerprinting set was used to genotype 51 accessions from the NCGR collection. The results confirmed two synonyms and the identity of 13 cultivated genotypes with the same name from different sources; five cultivars with the same name but with different alleles at one or more SSR loci; two genotypes with different names but the same fingerprint; two unknown accessions; and differences among suspected synonyms. This fingerprinting set accurately separated species and will provide another tool to better manage the botanical and horticultural identity of *Ribes* germplasm collections. In collaboration with Claudio Niggli, from ProSpecieRara (Swiss Foundation for cultural and genetic diversity of plants and animals), we are comparing genotypes of 52 accessions in common between our collection and other European collections to identify true-to-type cultivars. We are also in the process of using this genotyping set to establish base fingerprints of 165 important accessions from our currant and gooseberry collections.

Assessing diversity in the raspberry collection. 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 for use in diversity assessment and development of two fingerprinting sets. The assay was used to genotype 800 samples that include our red and black raspberry collections, and mapping populations from Michael Dossett's program. In collaboration with Dr. Michael Hardigan, the data is being analyzed at this time.

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.

Fine mapping black raspberry aphid resistance to the North American large raspberry aphid. The purpose of this project is to understand the underlying genetic basis of different sources of aphid resistance in black raspberry. 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 the USDA-ARS-HCRU breeding Program to breed cultivars with durable aphid resistance. We developed three new segregating populations (ORUS 5291, ORUS 5296, and ORUS 5306) to fine map this trait for each resistance source. Each of these populations was evaluated by aphid inoculation. Chi-squared tests determined that all three populations fit segregation ratios of 1 resistant (R): 1 susceptible (S). A robust RNA extraction protocol was then developed for Black Raspberry. Presence-absence differential gene expression was assessed for 10 R and 10 S seedlings using long-read IsoSeq (Full-Length Isoform Sequencing) for one source (ORUS 5306). There were 755 transcripts expressed uniquely in the R pool, and 1573 transcripts uniquely expressed in the S pool. In addition, differential gene expression was quantified using Illumina 300 bp paired-end sequencing for 5 R and 5 S seedlings from each population before and after aphid inoculation. This resulted in 45 genes differentially expressed among all comparisons made, and a total of 215 genes identified expressed in only one group. Genomic regions are being cross-referenced to facilitate our goals to use these resources to develop useful genetic markers for each source of resistance and to allow the pyramiding of these resistance loci in new breeding populations.

Converting alleles of all fingerprinting sets from the Beckman CEQ to the SeqStudio 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. When tested, this 6-SSR mint fingerprinting set was not able to easily distinguish hybrids and needed further improvement. We then identified 21 long core repeat-containing SSRs that appeared polymorphic in silico based on sequence alignment between *M. suaveolens* and *M. longifolia* sequences available through our collaborator, Kelly Vining and her team at OSU. After another round of testing in the testing panel, we modified the fingerprinting set to consist of 11 polymorphic SSRs and are optimizing it at this time to use in identifying hybrid seedlings from three bi-parental populations.

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 the needs and uses of a high-throughput genotyping platform for the research community.

We have finished compiling a SNP catalog by obtaining 491,568 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. The *Vaccinium* community selected one out of four providers to use in developing a high throughput genotyping platform of 20,000 loci for blueberry and 15,000 loci for cranberry. We are in the process of conducting SNP variant calling using FreeBayes in 50 whole-genome sequences in blueberry and will be using the same pipeline in cranberry once the cranberry genome reference is completed.

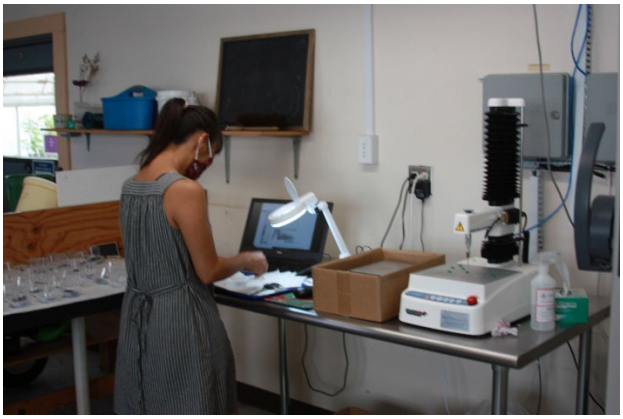
Testing Allegro Targeted Genotyping for blueberry genome wide association. In collaboration with Hamid Ashrafi (NCSU), 1.7 million SNPs were selected, and the flanking sequences were extracted. Single primer enrichment technology (SPET) was used to specifically target SNPs of interest in a diversity 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.

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. We have started the second season of phenotyping.

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. 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. When DNA extraction failed to yield consistent results, we provided lyophilized leaf tissue to two companies to identify a DNA extraction protocol that works well in blueberry and we extracted the DNA for the 384-testing panel and shipped it to DArT for genotyping with the 3,000-DArTag genotyping platform. We are awaiting the results.

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.

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