ANNUAL REPORT FOR 2019

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Photo by Kim Hummer

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Nahla Bassil, Geneticist-Plants
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April Nyberg, Bio. Science Tech-Genetics
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Ozge Yalcin, GRA OSU, Hort.
Craig Hardner, Australia
Linlin Chang, China
Priscilla Marchi, Brazil

Stakeholder/Service Accomplishments

- 13,007 active accessions, 73 genera and 799 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.
- Shipped 6,605 items.
- Collaborated with NGRPL, Ft. Collins, CO, on backup seedlot preservation, pollen 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.
- Trained/Collaborated with visiting scientists from China, Australia, Brazil, and the US.

Research Accomplishments

- Determined a *Rubus* phylogeny using target capture sequencing
- Determined that the most recent common ancestor for *Rubus* is from North America and that it dispersed over land bridges to Asia, Europe, and South America during the early Miocene.
- Determined that *Rubus* diversified greatly on many continents (particularly China) during the middle of the Miocene.
- 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 chloroplast DNA sequence data to differentiate pear species groups, and to identify genetic relationships between pears and other related crops in collaboration with NCGRP, Fort Collins.
- Used interstem grafts to evaluate pear germplasm for dwarfing potential. Correlated pear mother tree architecture traits with dwarfing potential.
- Developed a high-density SNP array for large-scale genotyping of pear germplasm for marker assisted breeding and germplasm collection diversity analysis in collaboration with UC Davis.
- Analyzed genetic diversity and population structure of American wild southeastern blueberry germplasm in the NCGR collection- Identified true-to-type Florida-4B using parentage analysis and provided evidence of its hybrid status (*V. darrowii* and *V. fuscatum*).
- Demonstrated the diagnostic potential of a current marker for *Phytophthora* crown rot in the University of Florida breeding program but not in other diverse germplasm preserved at the NCGR.
- Demonstrated the usefulness of a bioinformatics pipeline in identifying subgenomes of the octoploid strawberry.
- Identified *Vaccinium* germplasm that is slow to become infected with, and potentially resistant to Blueberry shock virus.

Administrative Overview

Kim Hummer, Research Leader, Specialty Crop Curator

Staffing Changes

Over the past several years, we have seen the beginning of the changing of the germplasm guard. The year 2019 was no exception with several great changes in our staffing. Joseph Postman (photo right), our quintessential pear curator and employee of 38 years, resigned in August 2019. He began working at the NCGR in a technical position in 1980. He began his career testing our plants for viruses and diseases and managing the pathogen elimination program. Over the years, he developed an appetite for pear and pome fruit germplasm. He was selected as our tree curator. He worked very hard for the tree fruit and nut research community. He participated on many international plant collecting expeditions for the National Plant Germplasm System. He forged great friendships with the North American Fruit Explorers, Home Orchard Society, Seed Saver's Exchange, and Northern Nut Growers. He worked with the Animal and Plant Health Inspection Service on the Tier 1 committee to determine the development and course of the National Clean Plant Network. For the next several months, Joseph will be finishing a few of his grant projects on a volunteer basis, so we at the NCGR-Corvallis see him from time to time. We have begun the recruitment for the vice-Postman replacement. This vacancy recruitment process is very slow for permanent positions in the federal government now so we will send out a notice when our action becomes active. In the meantime, Dr. Barbara Gilmore (left) has been our acting pome fruit curator. She is busy managing the tree collections for disease infections and coordinating the order process for dormant scionwood distribution.

This past summer, Jack Brennan returned to Corvallis. We were able to hire him in a temporary Bio. Sci. Technician position for six months. He has greatly helped with farm management operations.

Memoriam

Dr. Chad Finn, Geneticist, at USDA ARS Horticultural Crops Research Unit, died unexpectedly on 17 December 2019. Although Chad Finn was not our employee, he was an integral part of our team. He was an advisor on our Small Fruit Crop Germplasm Committee and chaired that committee for a decade or more. He collaborated so closely with our scientists that we would see him almost daily. His friendly imposing personality and booming laughter brought the scientific world of berry genetics and breeding together for positive collaboration. We still hear his commanding voice echoing down our halls. We will work to complete our cooperative projects as a tribute to his great ability to bring people together for plants, science, and fun.

EEO/CR/Outreach

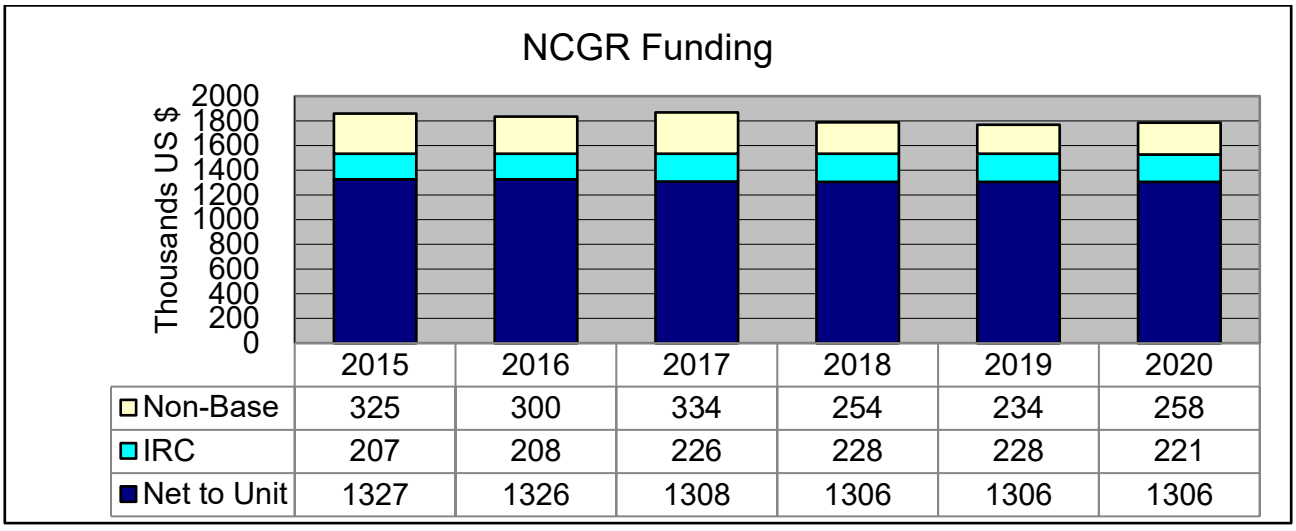
- At least 4 physically-challenged individuals were trained in horticultural plant management and label preparation.

- 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.
- 15 mentally or physically challenged individuals from a local private organization (Work Unlimited) were trained in strawberry greenhouse activities.
- NCGR staff attended Fascination of Plants day; 2 job fairs, and mentored high school students to improve job recruitment skills; Periwinkle School Science Night with staff from HCRU
- NCGR staff gave approximately 45 tours and presentations to industry practitioners, representatives and producers, and three presentations to schools on genetic resource conservation, fruit tree grafting and demonstrating determining botanical nomenclature.
- NCGR staff provided site tours and visits to approximately 243 students from local high schools and community colleges, Oregon State University, Oregon School of the Deaf, Willamette University, Western Oregon State University, and Washington State University.
- Awarded PWA-DIA funds for Oregon School of the Deaf tour.
- Provided extra Ribes plants to Philomath High School for annual plant sale; provided extra Ribes and pear plants to Urban Farm for use in student training.

Budget

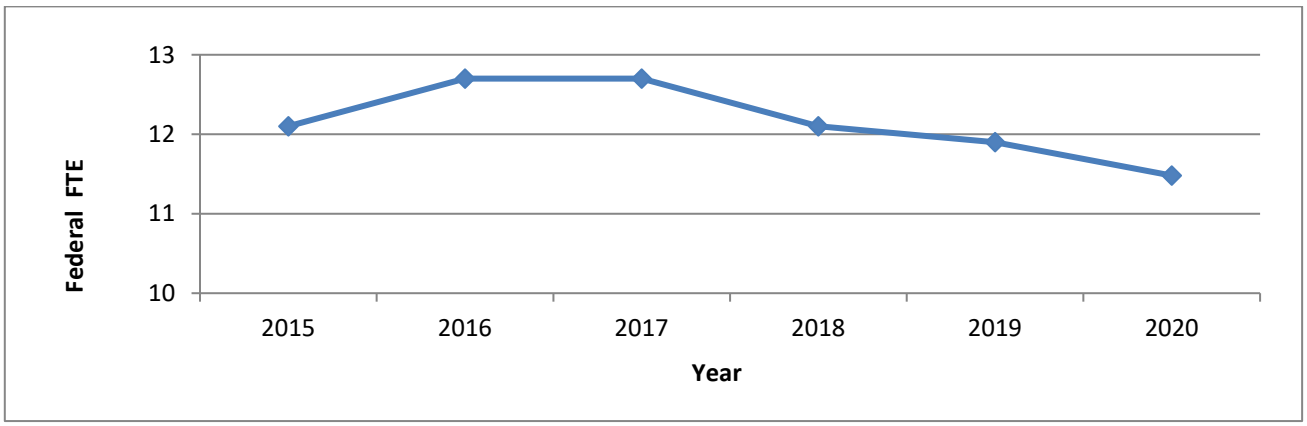
Our total federal budget for 2019 was $1.534 million. The FY 2019 net to unit budget remained level at about $1.3 million since 2005. Our federally supported staff had a peak of 17 FTE in 2005 but has been declining since then to 11.48 FTE for FY 2020. Each year our scientific staff obtains extramural funding of $200 to $300 K from a wide variety of research granting opportunities to supplement our base federal funds. 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
Non-Base	325	300	334	254	234	258
IRC	207	208	226	228	228	221
Net to Unit	1327	1326	1308	1306	1306	1306

Employee Summary



Facilities

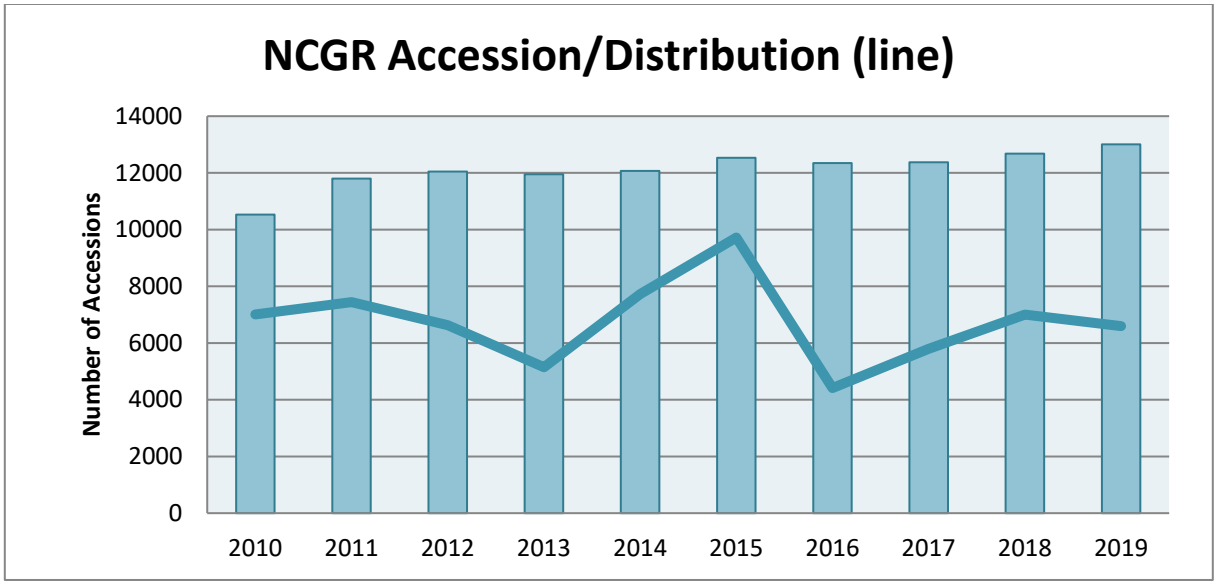
Our boiler sprung leaks in the summer and was replaced in November. Electrical circuits throughout the building complex, particularly in the greenhouses and screenhouses, were improved with fault protection. The potting area was moved from the headhouse to outdoors under a roof. This reduces the particulates that could potentially be airborne in the headhouse. Algae built up in our greenhouses and on the outsides of the headhouse and on the concrete screenhouse walkway. Pressure washing of our facilities was arranged through a local contractor. A temporary growing area, SH-11, remains under construction next to SH-07. This will provide space for *Vaccinium* which are presently over-running the greenhouses.

BIG NEWS:

The NCGR-Corvallis appeared on the President's budget for architectural planning funding to replace 4 greenhouses and 6 screenhouses. This facilities repair was passed by US Congress! This year, NCGR-Corvallis received $13.5 M facilities funding for wholesale repair and replacement of our 6 screenhouses and 4 greenhouses, and the attached headhouse. We are working with ARS administrators and facilities managers to develop the plans and repair/replace these growing structures. This is GREAT! We are looking forward to FY 2020. We'll keep you posted on the progress of the upgrade of our facilities. These are the "before" images of house 5.

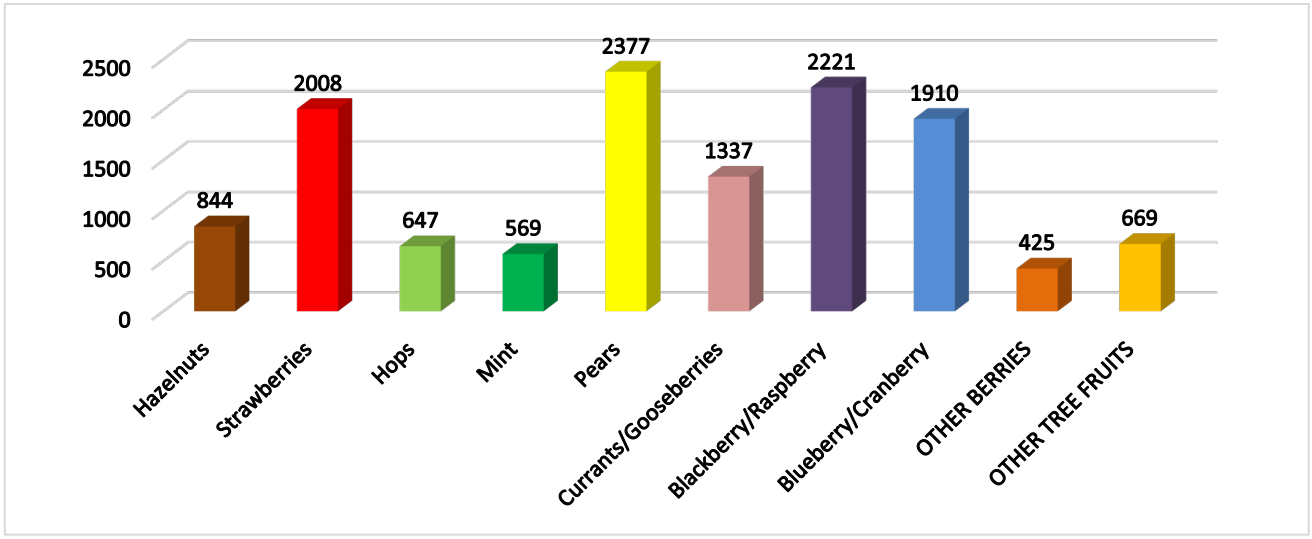
Germplasm Collections

Corvallis Germplasm Collections 2010 -2019



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

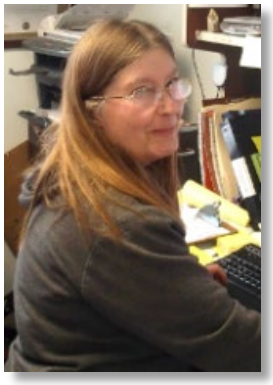
Corvallis Germplasm Collections – Accession counts by crop – January 2020



***In Vitro* collection. Jeanine DeNoma**

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 remainder 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.

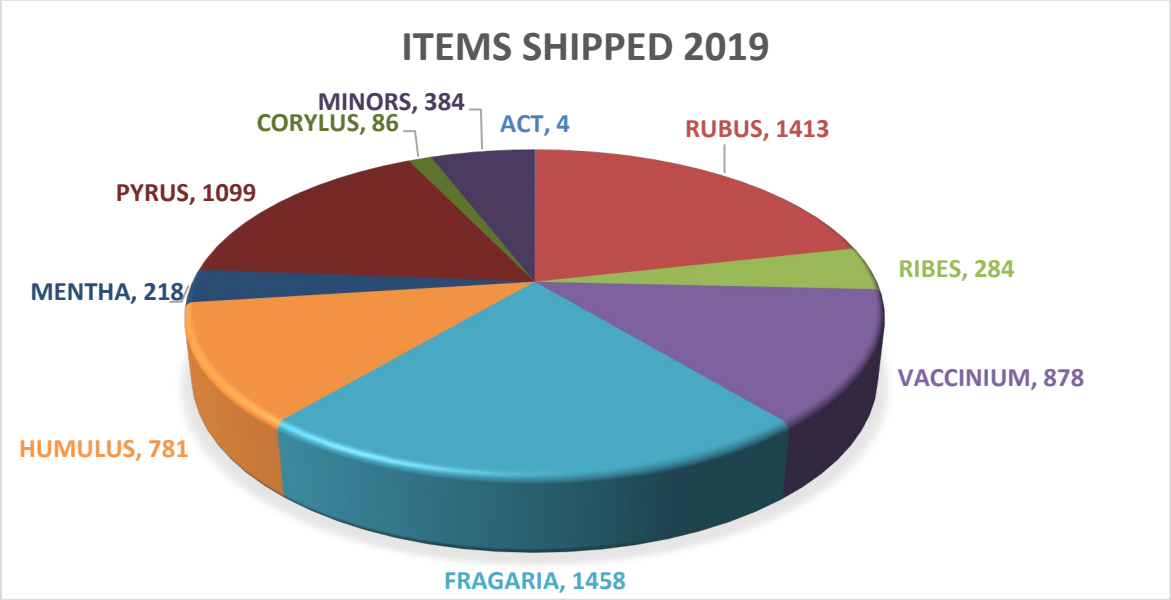
Distribution **Missy Fix**

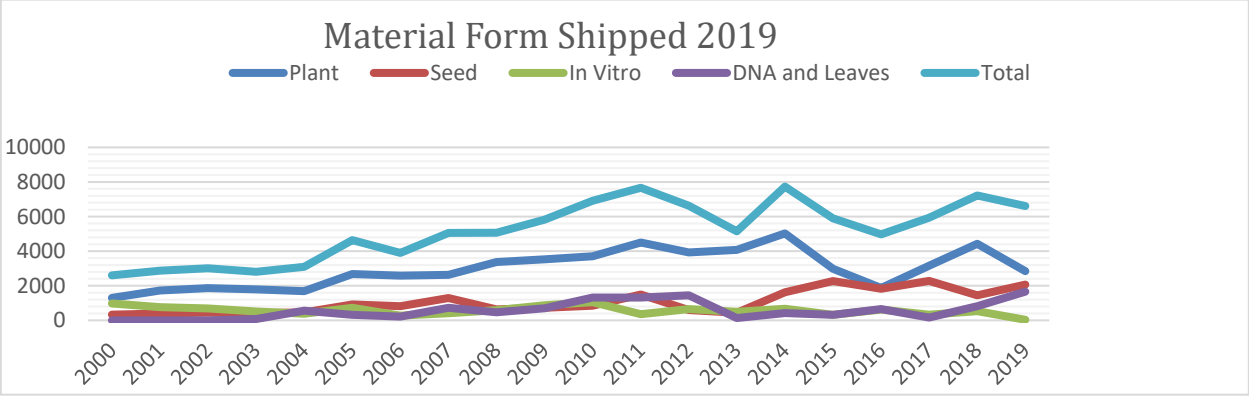


During CY 2019, 1899 seed accessions were shipped. 1672 were from the small fruit genera and 227 from the tree genera. The most requested Genus was the *Fragaria* with 934 requests. *Humulus* was the next popular with 817 requests. We received and or collected 92 seed accessions –70 *Fragaria*, 26 *Humulus*, 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, Ribes* have been selected thus far, in increments of 250, 500, or 1000 (depending on the seed amount totals per accession). These seeds being sent for backup preservation at Ft. Collins. For those accessions with more than 3000 seeds on hand will also be backed up at Svalbard in 1000 seed increments.

- In CY 2019, NCGR staff shipped 6,605 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.
- The *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.





Rubus/Ribes/Sambucus Collections

By Jill Bushakra

The *Rubus* and *Ribes* collections are undergoing regeneration efforts. These two genera have been under managed due to limited staff hours in previous years. This year was an effort to repot, cut back, fertilize, and regenerate these prickly collections. The *Rubus* in the screenhouse was of great interest for genetics work. Much time was spent to collaborate with Pair-wise, a group of berry crop geneticists. NCGR staff worked with their staff to propagate the entire living collection for genetic evaluation project in combination with their research and Rubus breeder research throughout the country and the world.

The *Ribes* collection was repropagated. Species collections were moved to open spaces to consolidate the *Ribes* field plot. Trellising and drip irrigation was installed. Mulch was added to the plants and weeds removed.

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

By Jim Oliphant

The greenhouse plants have been grouped by climate needs. Humid subtropical conditions were established in GH1 to maintain tender accessions. A montane- like environment is under construction for subtropical high elevation crop relatives. Temperate and Alpine plants are located in GH 2. Many Vaccinium are difficult to identify when collected without any inflorescenses. Several *Vaccinium* species bloomed this year and were identified to *V. triflorum* and *V. globosum*.

Molecular Genetics Laboratory
By Nahla V. Bassil

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

Completed Projects

Developed a handbook for strawberry DNA tests: Since its beginning in 2009, the USDA-NIFA-SCRI-funded RosBREED project has developed many genomic resources, including diagnostic tests, to facilitate DNA-informed breeding for horticultural quality and disease resistance traits. Diagnostic tests have also been developed by international partner organizations during this same time. DNA testing is an important tool that can help breeders select potential cultivars without the need to maintain plants long term or perform expensive phenotypic trials. DNA tests allow breeders to make decisions sooner in the selection process, prioritize offspring with higher potential, and be more efficient with available resources. It also allows curators to develop specific collections known to have alleles for desirable traits. To assist breeders and laboratories interested in diagnostic testing, we developed a handbook of DNA tests available at this time for strawberry. This handbook has detailed information about each test and the names of positive and negative controls available at the NCGR for each test. The handbook will continue to be updated as new tests are developed in its online location at the Genome Database for the Rosaceae (GDR).

Mapped the Black Spot Resistance Locus *Rdr3* in the Shrub Rose 'George Vancouver' and developed Diagnostic Markers for DNA-Informed Breeding: *Diplocarpon rosae, the cause of rose black spot,* is one of the most devastating foliar pathogens of cultivated roses (*Rosa* spp.). The primary method of disease control is fungicides and they are viewed unfavorably by home gardeners due to potential environmental and health impacts. Planting rose cultivars with genetic resistance to black spot can reduce many of the fungicide applications needed in an integrated pest management system. To date, four resistance genes have been identified in roses (*Rdr1, Rdr2, Rdr3,*

and *Rdr4*). *Rdr3* was never mapped and is thought to be unique from *Rdr1* and *Rdr2.* It is unknown if it is an allele of *Rdr4*. To assess the novelty of *Rdr3*, a mapping population was created by crossing the *Rdr3* containing cultivar George Vancouver with the susceptible cultivar Morden Blush. The mapping population was genotyped with the rose 68K Axiom array and mapped using the 'polymapR' package. *Rdr3* was mapped to a chromosome 6 homolog confirming it is different from *Rdr1* and *Rdr2,* found on chromosome 1, and from *Rdr4*, found on chromosome 5. The mapping information was used in conjunction with the *Rosa chinensis* genome assembly to develop new tightly-linked SSRs for marker assisted breeding. Three markers were able to predict the presence of *Rdr3* in a 63-cultivar validation set. Additionally, 12 cultivars appear to have resistance genes other than *Rdr3.* The improved diagnostic markers will be a great asset to the rose breeding community toward developing new black spot resistant cultivars.

Used Blackberry fingerprinting set to confirm parentage in new cultivars and identity in the NCPN collection: An 8-SSR fingerprinting set has already been developed to fingerprint and validate parentage in blackberries. We used this fingerprinting set to confirm parentage of two new releases from Chad Finn's breeding program, 'Eclipse' and 'Galaxy'. Comparison of the fingerprints of 51 blackberry accessions from the NCPN to that from Chad Finn's and/or that from the NCGR identified a single cultivar representative, 'Black Pearl' with two genotypes. 'Black Pearl' from the NCGR (CRUB 2232.001) is very closely related to that from Chad's field and from NCPN. Parentage analysis is in progress to identify the genotype that could have resulted from the reported cross.

Confirmed 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 5-SSR fingerprinting set of tri-nucleotide-containing SSRs in blueberry. The objectives of this study were to use this 5-SSR blueberry test to compare fingerprints of all plants representing the most requested blueberry cultivars in the screenhouse and field collections of the NCGR; conduct parentage analysis to confirm identity; establish reference fingerprints for these cultivars; and expand the fingerprinting set with additional long core repeats, if needed. The SSR-set distinguished all but 'Lateblue' and 'Berkeley' and was supplemented with five additional SSRs with long core repeats to generate a 10-SSR fingerprinting set. Genotyping 367 samples with one or both of these SSR sets and conducting parentage analysis when possible detected 54 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. A public database of these reference genetic profiles is available on GRIN-Global. We plan to continue adding to this database and eliminate redundant genotypes for more efficient management of blueberry diversity. Confirmed blueberry genotypes will benefit the germplasm community for use in continued breeding and genetic studies.

Contributed to a new reference genome for pear: Developed a high density genetic map of 'Bartlett'. This map was used as an anchor to the improved assembly of the double haploid European pear (*Pyrus communis* L.) genome (referred to as BartlettDHv2.0), obtained using a combination of Pacific Biosciences RSII Long read sequencing (PacBio), Bionano optical mapping, chromatin interaction capture (Hi-C), and genetic mapping. A total of 496.9 million bases (Mb) corresponding to 97% of the estimated genome size were assembled into 494 scaffolds. Hi-C data and a high-density genetic map allowed us to anchor and orient 87% of the sequence on the 17 chromosomes of the pear genome. About 50% (247 Mb) of the genome consists of repetitive

sequences. Comparison with previous assemblies of *Pyrus communis* and *Pyrus x bretschneideri* confirmed the presence of 37,445 protein-coding genes, which is 13% fewer than previously predicted.

Mapped fire blight resistance in pear: Three pear families were phenotyped for fire blight resistance at the USDA-ARS-AFRS in Kearneysville during 2017 and 2018. These populations were also genotyped with the new 70K pear array. The percentage of current season's shoot that was blighted was calculated. The phenotypic distributions for each population were severely skewed toward resistance and the number of genes mediating resistance could not be easily discerned. Chromosome scale linkage groups were established for each population using a cross-pollinating mapping approach with JoinMap 5. Each high-quality map had approximately 30,000 markers distributed across the whole genome. An integrated two-way pseudo-testcross approach was used to map QTLs using MapQTL 6. A significant QTL ($\alpha = 0.05$) mediating resistance was identified for each population in a similar region on chromosome 2. Fire blight resistance QTLs have been previously reported in this region for 'Harrow Sweet' and 'Moonglow' (a parent of 'Potomac'). The presence of the chromosome 2 QTL in NJA2R59T69 is interesting as the resistance originated from a non-*P. communis* source like 'Potomac' and 'Old Home'.

Projects in progress

Using synteny and candidate genes to identify loci controlling fruit sweetness in blackberry: Increased sugar content is one of the most important traits desired by blackberry consumers. A synteny-based approach was used to identify candidate genes responsible for sugar production in blackberry (*Rubus* L.). Three sugar quantitative trait loci (QTL) were identified from the GDR QTL database that are conserved among apple, peach, and alpine diploid strawberry. The physical regions for these QTLs were identified in the *F. vesca* v1.1 assembly and 26 genes with functions associated with sugar production were extracted. Additionally, 789 sugar-associated genes were extracted from the *M. domestica* v3.0.a1 assembly. The strawberry and apple genes were used to conduct a BLAST search in the GDR *Rubus* reference transcriptome. Of 279 *Rubus* candidate transcripts identified, predicted exons were used to design 9,355 Hyb-Seq baits. The baits covered 99.6% of the targeted regions. These baits were used in conjunction with PacBio sequencing to genotype 40 cultivars with high and low sugar content from the University of Arkansas and USDA blackberry breeding programs. A total of 430,167 high quality circular consensus sequences (CCS) were generated. Alignment to the 'Hillquist' blackberry and *Rubus occidentalis* genomes, followed by variant identification resulted in 929,430 and 1,324,854 markers, respectively. Welch's t-test and a Benjamini-Hochberg correction identified 467 and 312 significant loci from the 'Hillquist' and the *R. occidentalis* genotype tables, respectively. Population structure modeling identified a total of 173 loci that were significantly ($\alpha = 0.05$) associated with sugar production regardless of population structure. We are in the process of validating these loci using KASP genotyping.

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 of a 1,000 locus RhAMPSeq assay that would allow for a single multiplexed reaction. The assay will be used to genotype of our red raspberry collection. 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: 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. The Knapp group has 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 and ~40,000 SNPs well distributed across the new 'Camarosa' genome assembly. Curation of the genotypic data is currently in progress.

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.

Improving sampling and detection protocols to survey *Ribes* germplasm for black currant reversion virus: Reversion disease, caused by the eriophyid mite transmitted black currant reversion virus (BRV) is one of the most economically important diseases of black currants (*Ribes nigrum*) worldwide. As such, a national quarantine program has existed in the United States to prevent the entrance of both the mite and BRV. The enforcement of the BRV quarantine can be difficult as the virus exists at a low titer and can be unevenly distributed within the plant. In October 2016, BRV was detected in several black currant accessions at the USDA NCGR in Corvallis, OR using deep sequencing and reverse transcription PCR (RT-PCR). To better determine how widespread BRV is within the collection, we are conducting a survey of the *R. nigrum* germplasm using RT-PCR and are evaluating droplet digital PCR (ddPCR) as a method to improve detection. Samples have been collected during three time points (May, July, and October) to try and identify ideal sampling times given the virus's low titer in planta. Preliminary work has shown ddPCR is better at overcoming PCR inhibitors naturally present in the *Ribes* leaves. No positive samples were observed during the July collection, including positive controls. The virus may not have built up a high enough titer by July for detection. A subsequent test of the samples from October identified a large number of false positives. We are currently assessing new markers to develop an improved test.

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 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. The DNA tests and fingerprinting information will be made available to service providers.

Tree Fruit

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 each year, but it remains a perennial pest.
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* 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 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 results in branch death. This will require manual inspection of each tree in future years. The pustules on healthy appearing branches caused us to decide to increase our spray schedule to six times per spring instead of the 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.

Plant Pathology

By Jason Zurn (and Joseph Postman)

Reversion in black currants, a devastating disease of black currants in Europe, is caused by Blackcurrant reversion virus (BRV). This disease is spread by the black currant gall mite, not present in North America. A quarantine has been in place for many years to prevent both BRV and the eriophyid mite (*Cecidophyopsis ribis* J. C. Fischer), from entering the United States. In 2016, BRV was detected in the US for the first time using high throughput DNA sequencing. It was found in the black currant cultivar *Ribes nigrum* 'Burga' growing in the NCGR *Ribes* collection in Corvallis, Oregon. A second test using reverse-transcription polymerase chain reaction (RT-PCR) was conducted on 'Burga' as well as 11 other black currants which exhibited suspicious leaf symptoms to confirm the presence of the virus. Four of the 11 black currants tested positive for the disease and subsequent sequencing of the amplicons confirmed the presence of BRV. Three of the four black

currants which tested positive were growing in the U.S. for more than 20 years. These plants were tested when they entered the U.S. using a graft assay and were determined to be BRV-free. At the time, this was the only tool available for BRV detection. In early 2019, deep sequencing data generated by Ruhui Li (Research Plant Virologist, USDA-ARS NGRL, Beltsville, MD) was analyzed confirming the presence of BRV. A note reporting the presence of BRV in the U.S. was published in Plant Disease (Zurn et al., 2019).

Fire blight, caused by the bacterial pathogen, *Erwinia amylovora*, is a constant challenge for pear growers in the U.S. In 2019, work was conducted to map fire blight resistance loci in three segregating populations using resistance data collected by Jay Norelli (USDA-ARS AFRL, Kearneysville, WV) during the 2017 and 2018 growing season in Kearneysville. In each population a significant loci were detected on chromosome 2 for each growing season. The loci in two of populations are identical and correspond to a previously identified resistance locus. The locus in the third population was found in a slightly different location and appears to be unique. Within the two regions there are a total of 30 genes that have functions typically associated with disease resistance. Continued research efforts are being focused on identifying the genes responsible for conferring fire blight resistance. In order to preserve these populations for future research efforts, bud wood was sent from Kearneysville to the USDA-ARS NCGR in Corvallis, OR. Bud grafting was conducted on OHxF 333 rootstocks and the germplasm is being maintained are part of the collection.

Over the past 40 years, clonal germplasm known to be infected with viruses, viroids, and phytoplasmas were acquired by the staff of the USDA-ARS NCGR. These plants were obtained to be positive controls for pathogen testing of the NCGR collection and are maintained in quarantine greenhouse. We have now added these virus positive standards to the NCGR accession and inventory data on GRIN-Global. Virologists and plant protection agencies will find these accessions interesting as positive controls or as unique isolates for study within their research programs. As such, a focus was placed on curating plants containing virus and phytoplasma isolates in 2019, for distribution to plant virologists and plant protection agencies. Individual accession numbers were assigned for these pathogen isolates that separate them distinctly from non-infected clones. In total 172 new accession numbers were added for these pathogen isolates that commonly afflict 7 genera maintained at the USDA-ARS NCGR in Corvallis, OR. These isolates may be an important resource for plant virologists and can be distributed on a case-by-case basis. Orders can be placed on GRIN-Global by searching for accession group name = virus/species through the “Advanced Search Criteria” option.

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