

ANNUAL REPORT FOR CALENDAR YEAR 2017
USDA ARS

National Clonal Germplasm Repository
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National Clonal Germplasm Repository Staff



NCGR Summer Staff July 27, 2017 Corvallis, Oregon

Permanent & Term Federal Staff

Kim Hummer, Research Leader/Curator
Joseph Postman, Plant Pathologist/Curator
Nahla Bassil, Geneticist-Plants
Jim Oliphant, Bio. Sci Tech/Greenhouse
Jeanine DeNoma, Tissue Culture Mgr.
Missy Fix, Bio. Science Tech/Distribution
April Nyberg, Bio. Science Tech-Genetics
Jill Bushakra, Rubus/Ribes Mgr.
Barb Gilmore, Ag Science Tech/Field Mgr.
Jason Zurn, Research Associate, Genetics
Jack Brennan, Ag Science Research Tech
Kyle Nemes, Program Support Assistant

Temporary Staff & Students

Debra Hawkes, GH Bio. Science Tech
Jane Olson, GH Bio. Science Tech
Sunny Green, GH Bio. Science Tech

Garren Laroux, GH Bio. Science Tech
Gwen Moulton, GH Bio. Science Tech
Cory Paterson, Bio. Science Tech
Tyler Young, BENCO
Spencer Gilbride, IT Pathways Student
Lilian Gharib, Work Study, OSU
Melanie Reese, Work Study, OSU
John Derry, Wallace-Carver Student
Harrison Schreiber, ASE HS Student
Christopher Cebra, ASE HS Student
Ashley Winters, Office Automation Intern

Graduate Students & Visiting Scientists

Katie Armes Carter, GRA, OSU, Hort.
Christina Mulch, GRA, OSU, Hort.
Brooke Getty, GRA, OSU
Weijian Cai, China
Ruth Meiers, Netherlands

Stakeholder/Service Accomplishments

- 12,443 accessions, 73 genera and 774 taxa of 677 species of temperate fruit, nut, and specialty crops were conserved.
- Managed > 3,600 accessions of fruit tree and nut crops on 22 acres of orchard.
- Obtained a total of 80 new accessions and 290 new inventory items in CY 2017.
- Received 1,202 order requests and shipped 5,789 items.
- Collaborated with NGRPL, Ft. Collins, CO, on 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 visiting scientists from the Netherlands, China, and the US.
- Participated on Governing Board for USDA National Clean Plant Network.
- Participated as Science Editor of the ISHS Proceedings and Journals.
- Provided tissue culture assistance to hop breeders for a hop mutation breeding project.
- Collaborated with Forest Service scientists and USDA botanist and geneticist on in situ conservation of cranberry within National Forests of eastern US.
- Implemented dormant bud cryopreservation as one of several backup strategies for woody clonal germplasm accessions.
- 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.
- Trained two high school student recruited through the Apprenticeship in Science and Engineering Program in field and molecular evaluation of blueberry, blackberry, red and black raspberry plants.
- Trained visiting graduate student in evaluating sequence variation possibly associated with powdery mildew resistance in red raspberry.
- Provided DNA testing expertise in blueberry to the Federal Bureau of Investigation to assist in solving a murder mystery.
- Enabled marker assisted seedling selection for black raspberry aphid resistance, perennial flowering in strawberry, and aromatics profile in the USDA-ARS-Corvallis, the University of Florida and University of New Hampshire small fruit breeding programs.

Research Accomplishments

- Determined and published on susceptibility/resistance to egg laying of spotted wing drosophila in fruit of *Vaccinium* species.
- Screened strawberry crop wild relatives for perennial blooming in screenhouses in Corvallis, Oregon, and published list of accessions with photoperiod-insensitive flowers.
- Determined phylogenetic relationships in *Rubus* species based on target enrichment DNA sequencing.
- Detected Black currant reversion virus infection in several 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.
- Discovered a potentially novel gene for black spot resistance in rose.
- Identified *Vaccinium* germplasm that is slow to become infected with, and potentially resistant to Blueberry shock virus.

Administrative Overview

Staffing Changes

We have had many staffing changes at the NCGR. Our field technician, Jack Brennan, resigned and took another horticultural job outside of Corvallis. Cory Paterson was selected to fill the position on a temporary basis. In May Kyle Nemes, our Program Support Assistant, resigned to take an administrative position with the state. We were able to recruit Ashley Winters as an Office Automation Trainee. Replacement of our permanent vacant positions are pending because of the regulations requiring a federal hiring freeze within our agency.

Debra Hawkes and Jane Olson were promoted to GS-7 Biological Science Research Technicians.

EEO/CR/Outreach

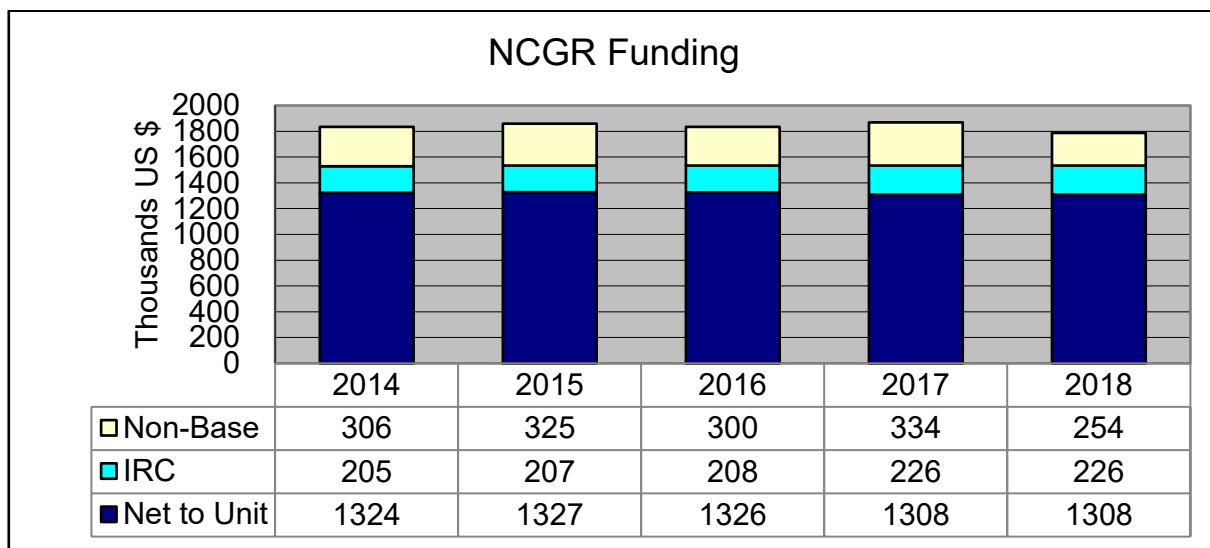
- At least 4 physically-challenged individuals were trained in horticultural plant management and label preparation.
- Through a Research Support Agreement with Oregon State University 1 female graduate students was trained and two undergraduate students were trained.
- During the winter, 3 physically 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
- 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.

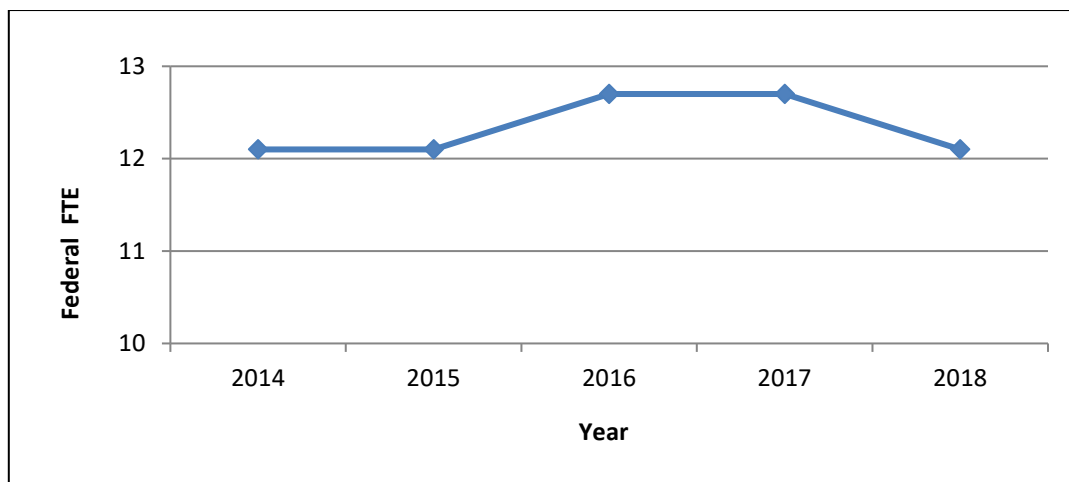
Budget

The FY 2017 year remained level because of continuing resolution most of the year. Our total federal budget is about \$1.54 million. Every year our scientific staff is encouraged to obtain soft funding from a wide variety of research granting opportunities to supplement our base federal funds. Our scientists have been successful with obtaining grants from commodity commissions and research consortium funding. Our SY FTE decreased from 4 scientists positions to 3 in 2016. This enable us to hire more technical help. As salary costs have increased through promotions and Corvallis becoming part of the Portland Pay Locality, our total FTE numbers were decreased in 2018 to keep salary costs relatively level. Administrative costs are at 14.6 %.

Budget History



Employee Summary

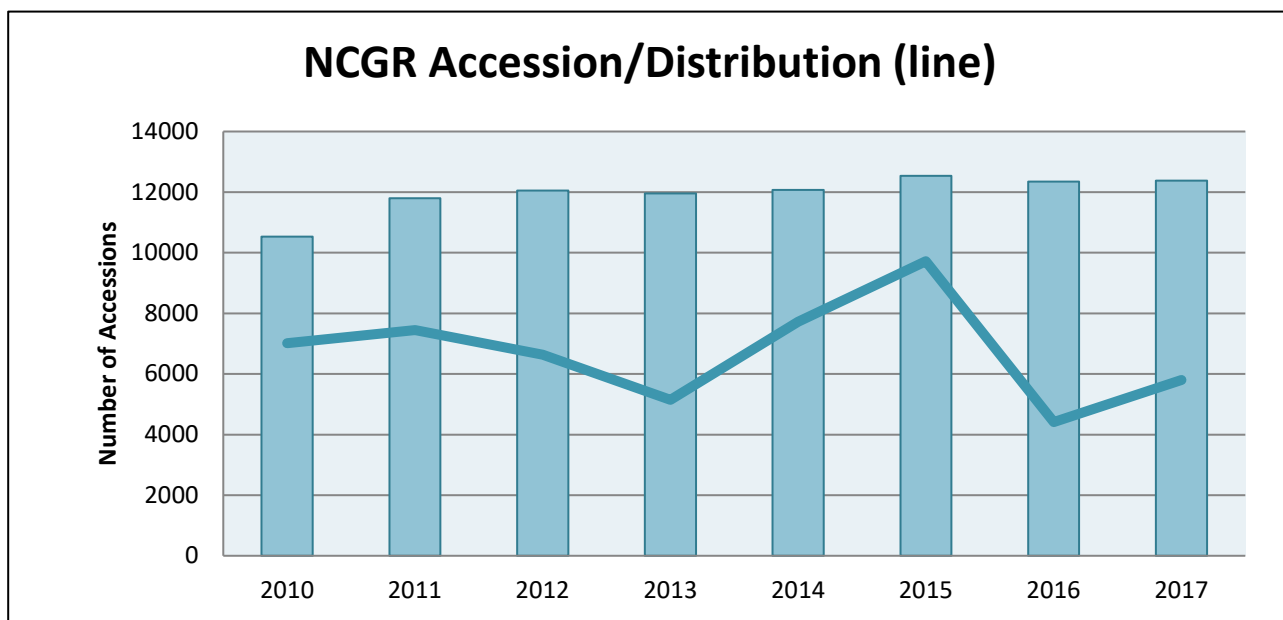


Facilities

Our screenhouses received new “skin” in summer 2017. In addition rotted wood structures were repaired, replaced, and repainted. We are grateful to area and headquarters repair funding so that these repairs could be completed.

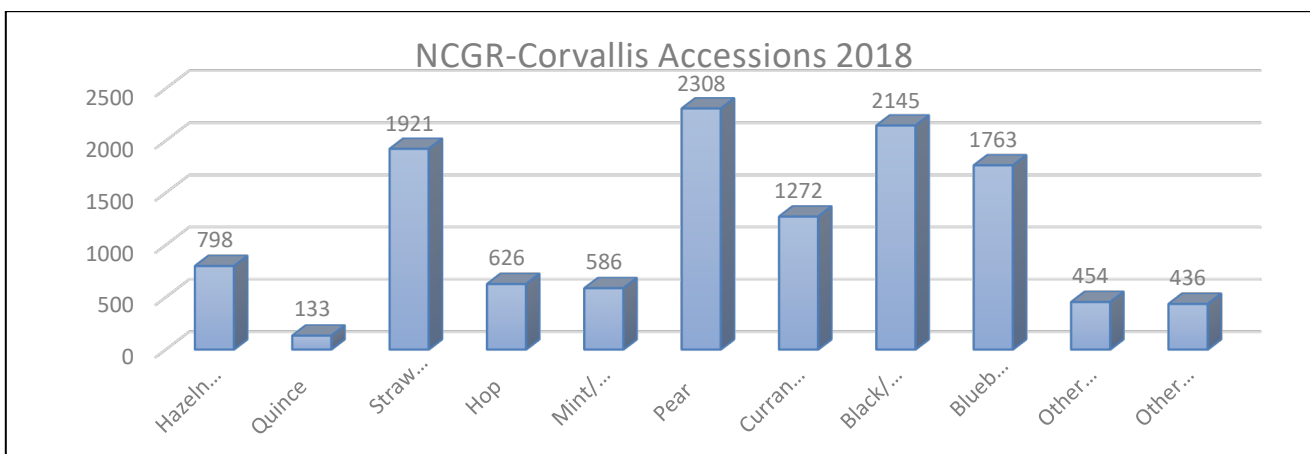
Germplasm Collections

Corvallis Germplasm Collections 2010 -2017



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

Corvallis Germplasm Collections – Accession counts by crop – January 2018



Corvallis Germplasm Collections – Total accessions (12,443) by genus, January 2018

Genus	Common Name	Accessions	Genus	Common Name	Accessions
<i>Actinidia</i>	Hardy Kiwifruit	4	<i>Kalmia</i>	Mountain Laurel	1
<i>Agapetes</i>	Blueberry relative	20	<i>Lonicera</i>	Blue honeysuckle	82
<i>Amelanchier</i>	Serviceberry	48	<i>Lycium</i>	Wolfberry	14
<i>Amelasorbus</i>	Inter-generic hybrid	1	<i>Macleania</i>	Blueberry relative	5
<i>Arbutus</i>	Strawberry Tree	3	<i>Malus</i>	Apple	10
<i>Aronia</i>	Aroniberry	9	<i>Mentha</i>	Mint	461
<i>Asimina</i>	Pawpaw	8	<i>Mespilus</i>	Medlar	61
<i>Buxus</i>	Boxwood	1	<i>Micromeria</i>	Blueberry relative	1
<i>Camellia</i>	Tea Camelia	1	<i>Peraphyllum</i>	Crab apple	7
<i>Castanea</i>	Chestnut	3	<i>Pernettya</i>	Blueberry relative	1
<i>Cavendishia</i>	Blueberry relative	5	<i>Physocarpus</i>	Ninebark	1
<i>Ceanothus</i>	Ceanothus	42	<i>Potentilla</i>	Quincefoil	8
<i>Celtis</i>	Hackberry	1	<i>Psammisia</i>	Blueberry relative	1
<i>Chaenomeles</i>	Asian quince	48	<i>Pseudocydonia</i>	Asian quince	4
<i>Cornus</i>	Cornelian Cherry	1	<i>Pycnanthemum</i>	Mountain mint	125
<i>Corylus</i>	Hazelnut	798	<i>Pyracomeles</i>	Inter-generic hybrid	1
<i>Crataegomespilus</i>	Inter-generic hybrid	3	<i>Pyronia</i>	Pear-Quince hybrid	7
<i>Crataegosorbus</i>	Inter-generic hybrid	1	<i>Pyrus</i>	Pear	2308
<i>Crataegus</i>	Hawthorn	28	<i>Rhododendron</i>	Rhododendron	6
<i>Crataemespilus</i>	Inter-generic hybrid	1	<i>Rhodomyrtus</i>	Rose Myrtle	1
<i>Cydonia</i>	Quince	133	<i>Ribes</i>	Currant/Gooseberry	1272
<i>Dimorphanthera</i>	Blueberry relative	3	<i>Rubus</i>	Black/Raspberry	2145
<i>Docynia</i>	Asian quince	1	<i>Sambucus</i>	Elderberry	173
<i>Empetrum</i>	Crow berry	17	<i>Schisandra</i>	Magnoliavine	10
<i>Epigaea</i>	Blueberry relative	1	<i>Sibbaldia</i>	Strawberry relative	2
<i>Fagus</i>	Beech	2	<i>Sorbaria</i>	False spiraea	1
<i>Fragaria</i>	Strawberry	1921	<i>Sorbaronia</i>	Inter-generic hybrid	7
<i>Gaultheria</i>	Blueberry relative	34	<i>Sorbocotoneaster</i>	Inter-generic hybrid	3
<i>Gaylussacia</i>	Huckleberry	15	<i>Sorbopyrus</i>	Sorbus-Pyrus hybrid	11
<i>Hippophae</i>	Sea buckthorn	1	<i>Sorbus</i>	Mountain ash	136
<i>Holodiscus</i>	Beauty Bush	3	<i>Symphysia</i>	Blueberry relative	2
<i>Humulus</i>	Hop	626	<i>Vaccinium</i>	Blueberry	1763
<i>Juglans</i>	Butternut	28	<i>Zelkova</i>	Zelkova	1

Corvallis Tree Collections 2017

Joseph Postman

Ten most requested USDA *Pyrus* accessions from 1980 to 2017

accession	taxon	name	shipped
PI 541262	<i>Pyrus communis</i>	Seckel	191
PI 541287	<i>Pyrus communis</i>	Yellow Huffcap (perry)	154
PI 300693	<i>Pyrus communis</i>	Bartlett	152
PI 541317	<i>Pyrus communis</i>	Red Pear (perry)	151
PI 541273	<i>Pyrus communis</i>	Thorn (perry)	147
PI 541271	<i>Pyrus communis</i>	Taynton Squash (perry)	145
PI 541123	<i>Pyrus communis</i>	Barland (perry)	144
PI 541195	<i>Pyrus communis</i>	Gin (perry)	137
PI 541156	<i>Pyrus communis</i>	Butt (perry)	133
PI 312507	<i>Pyrus communis</i>	Summer Blood Birne	127

Pears. The most genetically diverse collection of world pear germplasm is very likely the NPGS pear collection at NCGR-Corvallis. The Repository maintains approximately 2,200 clonal accessions of pear as well as 400 seedlots, which represent 36 *Pyrus* taxa that originated from 55 countries. About 20% of the clonal collection is backed up onsite, either as *in vitro* shoot cultures or as small potted greenhouse trees. Accessions at higher risk of loss due to lack of cold hardiness or susceptibility to disease are prioritized for backup. The field collections are grown on 10 hectares of orchard plots with a single tree per accession. As of early 2018, the NCGR orchards include 850 wild relative species trees and 1350 cultivars. Over 1000 European cultivars, 185 Asian cultivars and 125 hybrid cultivars are represented.

A much larger number of accessions represent the species from which large-fruited European and Asian cultivars were developed. Cultivar genetic diversity for *Pyrus* is well represented. Future growth of the *Pyrus* collection is anticipated to target crop wild relatives from a few under-represented geographic regions including east-Asia, north-Africa, Scandinavia, and several countries in the Middle East. Crop wild relatives are more efficiently maintained as seed, sometimes supplemented by a small population of seedlings. Finite field space, staff, and budget limit the number of seedlings that can be established long-term as living trees, therefore 3 to 5 seedlings are often used to represent a seedlot in the orchard. A larger number of seedlings might be grown out for rare taxa, or to represent germplasm likely to possess valuable genetic traits or from under-represented region.

Taxonomic gaps in the collection include species native to north Africa (*P. gharbiana* and *P. mamorensis*) and species native to central and western Asia (*P. armeniacifolia*, *P. korshinskyi*, *P. syriaca*, *P. xerophila*) as indicated by the accession counts in the table above. There are also geographic gaps in the collection for species that may be represented from elsewhere in their native range. For example Greece, the Balkan region, several countries in the Middle East, Central and Southeast Asia are under-represented.

Quince, Hazelnuts, Medlars, etc. A few unique clones of European quince and medlar were added to genebank collections in 2017 (table below), including some Russian and Ukrainian cultivars released from USDA-APHIS quarantine. An effort was begun in 2016 to expand the diversity of fruit-producing (as opposed to flowering ornamental) Asian quinces available in the United States. Cuttings and seed samples were obtained from several commercial nurseries as well as arboreta and botanic gardens, particularly from the extensive *Chaenomeles* holdings at the Arnold Arboretum in Boston. Seedling populations will be evaluated with the goal of introducing enhanced germplasm that can be used to develop cultivars with increased fruit production, combined with more upright habit, lack of thorns, and resistance to diseases. The fermented cider industry is thirsty for delicious new fruit sources, and fragrant Asian quinces may have much potential in that market. Combined with the widely appreciated ornamental traits of “flowering” quinces, there should be edible landscape potential as well.

Corvallis Field Collections

Our efforts focused on fighting Fire Blight in the *Cydonia* and Eastern Filbert Blight in the *Corylus*. In the *Cydonia* we used antibiotic spray applications and a compound that stimulates the plant’s systemic activated resistance (SaR) response. Pruning of infected branches on a weekly basis also helped slow the spread of the disease. Our plan is to use aggressive tactics to decelerate the spread of this pathogen to neighboring collections.

The *Corylus* received four antifungal sprays in two week intervals starting at the end of March, 2017. The trees are being pruned to lower heights so that the air blast sprayer can be more effective in the fungicide applications. Also infected branches were aggressively removed in the later days of September to remove any in field inoculum reserves.

Most of our time during the year was spent watering, fertilizing, pruning, and weed control of the collections. Keeping the orchards and fields healthy was time consuming, but very rewarding.

Greenhouse/Screenhouse Fragaria, Vaccinium, Mentha, Humulus Collections

By Jim Oliphant

- Coordinated the movement of plants, benches, and irrigation out of and back into all 6 screenhouses in preparation for the replacement of the screening and repair of the wooden structures of the screenhouses.
- Improved irrigation in each screenhouse. Repaired damaged
- Rearranged plants in 3 screen houses to give *Fragaria* cultivars the house with the lowest average temperature for improved plant growth.
- Established humid tropical conditions in GH1 to maintain tender accessions
- Re-propagated numerous *Vaccinium* crop wild relative accessions including the entire cranberry collection

Rubus/Ribes/Sambucus Collections

By Jill Bushakra

Rubus

- Replaced cinder block and Trex benches with plastic benches in house 5; hung plastic for winterizing; reinstalled trellis system in house 6
- Participated in plant moving during greenhouse renovations

- Pruning and fertilizing entire collection
- Propagation of tip-layering genotypes as needed
- Field collection in Southwestern Oregon with Katie Carter; prepared voucher specimens and extracted seed from fruit collected
- Provided material to Ioannis Tzanetakis to experiment with mite rearing; provided material to Patrick Di Bello (Bob Martin) for RNA analysis
- Contributed to the Rubus and Ribes chapter of Crop Wild Relatives book
- Presented at Northwest Center Caneberry Day; attended the NCCC212 and Small Fruits CGC meeting; presented posters at NARBA meeting
- Wrote SOP for soil cart pasteurization, boiler operation, pumice handling, soil and pumice cart maintenance, spraying using the 50 gallon rig
- Repotted, labeled and updated inventory for plants in house 1

Ribes field

- Assisted Forest Service personnel in the collection of Ribes for white pine blister rust study
- Propagated black currants from softwood cuttings
- Pruned, weeded (hand and chemical), and fertilized field collection
- Mating disruption and monitoring of Currant Cane Borer moth with lure traps
- Worked with Inga Zasada on detecting dagger nematode responsible for the transmission of Tomato Ringspot Virus (TRV) in red currants
- Worked with Joseph to test red currants for TRV and black currants for Black currant Reversion Virus
- Inventoried collection and updated GRIN records
- Field collection in Southwest Oregon with Katie Carter; prepared voucher specimens and extracted seed from fruit collected
- Harvested dormant wood for cryopreservation
- Learned to drive tractor with air blast sprayer to spray dormant oil

Ribes house

- Rejuvenated quarantine plants and moved to house 5
- Moved non-hardy plants to house 2 to make room in house 3 for Joseph
- Fertilized all plants
- Potted new accessions from Beltsville
- Updated GRIN records as necessary

Sambucus

- Planted propagated plants in new field site
- Updated GRIN records
- Sprayed with dormant oil; fertilized

Corvallis Seed Lab

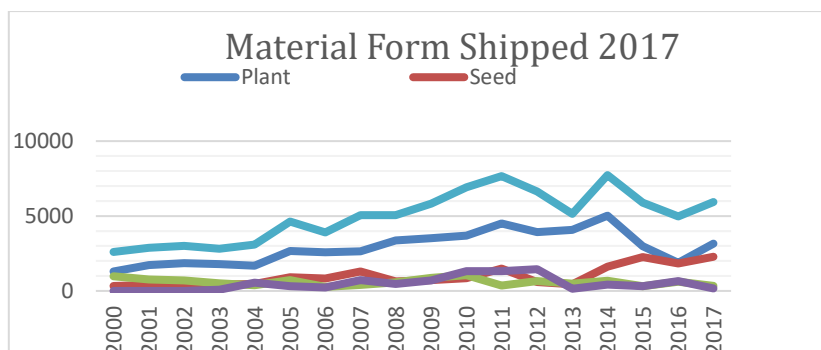
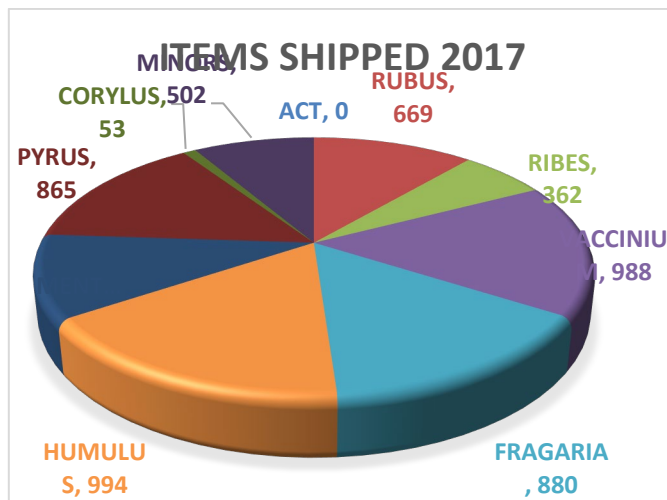
By Missy Fix

During CY 2017, 2281 seed items were shipped. 2171 were from the small fruit genera and 110 from the tree genera. The most requested Genus was the Vaccinium with 845 requests. Humulus was the next popular with 817 requests. We received 40 new seed accessions –13 Rubus, 01 Fragaria, 26 Sambucus. 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. Each genus has been a welcomed offering among the various communities.

Distribution

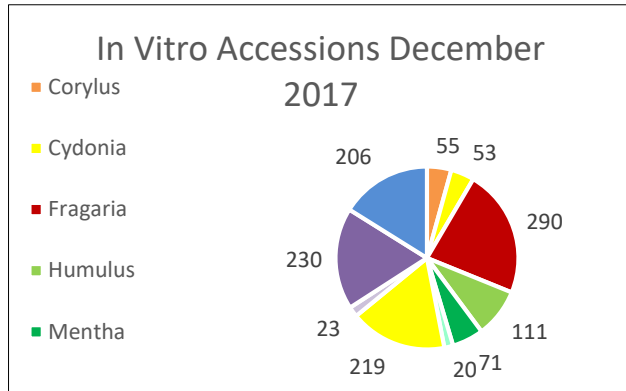
- In CY 2017, NCGR staff shipped 5798 items as seeds, cuttings, runners, scionwood, rooted plants, tissue cultures and DNA and leaf samples and informational material.
- In CY 2017, 1194 new orders were received. More than 817 orders were shipped. 784 of these were domestic orders and 34 international. 761 were completed orders.
- The pears and strawberries topped the list of crops distributed again this year.
- Domestic individuals, state agencies and universities, and ARS researchers received the most germplasm from Corvallis in 2017.
- 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 757 educational seed packets and plant cuttings were distributed.



Tissue Culture

By Jeanine DeNoma

***In Vitro* collection.** The *in vitro* collection contains a subset of the collection held at the Repository, with emphasis on core accessions, those at risk outside the lab and accessions frequently requested. In December 2017, there were 1,265 accessions in tissue culture, most held in StarPac bags in cold storage at 4°C. Accessions in storage are evaluated every three to four months. Accessions are repropagated and returned to the growth room when they decline, then after being refreshed, they are put back into StarPac bags and returned to cold storage.



Accessions initiated into culture. Initiation of new cultures into tissue culture was limited in 2017 while our screenhouses were being rebuilt. We collected 18 accessions of *Humulus* and 5 accessions of *Pyrus* for future virus elimination work.

Service work. We propagated and rooted the strawberries Marshall (FRA 511.001) and Marshall Bainbridge clone (FRA 511.002) and sent to the greenhouse for additional studies.

Also rooting a number of *Mentha* and *Vaccinium* to replace plants lost in greenhouse. Rebecca Reeves maintained a number of accessions of *Pyrus* and *Ribes* that had gone through virus elimination in 2016; these are to be rooted and established in the greenhouse to be retested.

Molecular Genetics

By Nahla V. Bassil

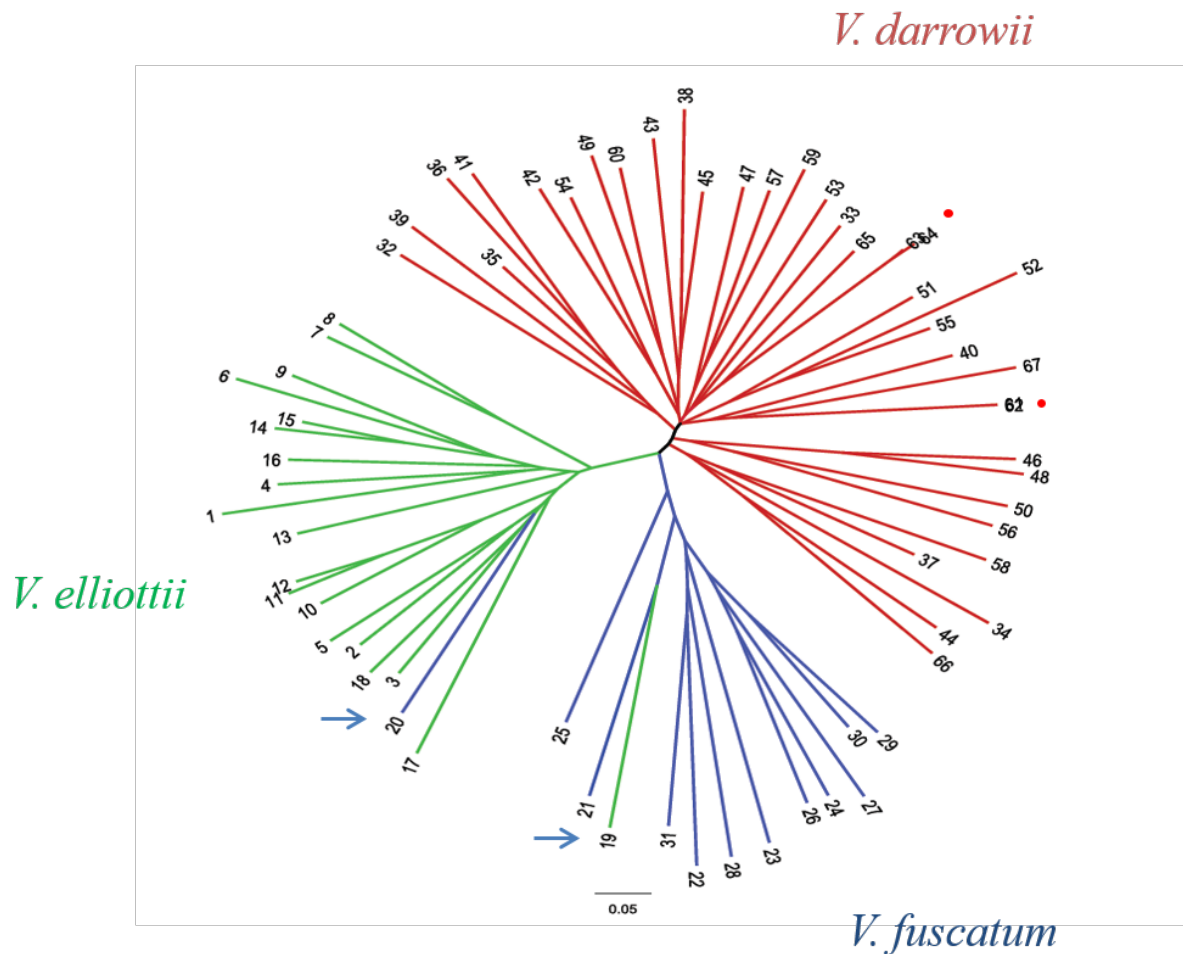
Students

Saturday Academy high-school summer interns Christopher Cebra and Harrison Schreiber worked in the lab on determining hybridity of ‘Nocturne’ x *Vaccinium smallii* progeny and evaluating usefulness of a blackberry fingerprinting set in red and black raspberry, respectively.

Ruth Meiers, a MS student from Wageningen University in the Netherlands spent four months at the NCGR identifying sequence variations in 95 red raspberry accessions at the *Mildew resistance locus O* (*MLO*) genes known to be associated with powdery mildew resistance in other species.

MS student Katie Carter conducted phylogenetic analysis in *Rubus* using targeted capture at ~1,000 genes.

Assessed population structure and diversity of American wild southeastern blueberries. Wild species representatives from northwestern, central, and south Florida and neighboring US states were collected in multiple USDA expeditions and are being preserved at the NCGR-Corvallis. In this study, we used microsatellite markers to evaluate genetic diversity in 67 individuals from three southeastern species. Results from our analyses indicated that the samples from each species could be reliably resolved with these DNA markers. In addition, we were able to detect a mis-labeled cultivar ‘Johnblue’, two mis-classified accessions PI 554933 and PI 613656, and four accessions of previously undescribed hybrid origin; PI 554933, PI 657207, PI 657192, and Florida 4B_1790. Florida 4B_1790, the donor of low chilling for the southern highbush blueberry, was confirmed as the parent of US 74 and a hybrid between *V. darrowii* and *V. fuscatum*.



Cluster analysis of 67 wild diploid blueberry accessions illustrating the three species groups evaluated in addition to mis-labeled (arrows) and undifferentiated (red dots) accessions.

Tested markers associated with *Phytophthora cactorum* in strawberry. We evaluated a Kompetitive Allele Specific PCR (KASP) DNA marker and two high-resolution melting (HRM) markers associated with the *Phytophthora* crown rot resistance gene *FaR_{PC}2* in 38 strawberry accessions with known disease responses based on a literature review. While predictive of resistance in the University of Florida germplasm, there was no clear association between the markers and the reported phenotypes in the diverse 38 accessions from the NCGR.

Clarified subgenomic positions of quantitative trait loci for flowering habit and fruit quality in strawberry. A technique called pedigree-based analysis was used with a high throughput genotypic and phenotypic data to identify quantitative trait loci (QTL) for fruit quality and flowering habit in pedigree-linked populations from Oregon and Michigan. QTLs previously reported for soluble solids content, fruit weight, pH and titratable acidity were confirmed. In addition, a major QTL for flowering was detected on LG 4A, coinciding with the previously reported *FaPFRU* locus. Marker haplotype analysis in this *FaPFRU* region indicated that the homozygous recessive genotype was highly predictive of seasonal flowering.

Resolved ancestral subgenomes in octoploid cultivated strawberry evaluated by genotyping by sequencing. Genotyping-by-sequencing (GBS) was used to survey genome-wide single-nucleotide polymorphisms (SNPs) in three biparental strawberry (*Fragaria ×ananassa*) families. GBS sequence data were aligned to the *F. vesca* ‘Fvb’ reference genome in order to identify these single nucleotide variations, SNPs. Numbers of polymorphic SNPs per population ranged from 1,163 to 3,190. Linkage maps consisting of 30-65 groups were produced from the SNP sets derived from each parent. A phylogenetic analysis performed using the POLiMAPS pipeline revealed linkage groups that were most similar to ancestral species *F. vesca* for each chromosome. Linkage groups that were most similar to a second ancestral species, *F. iinumae*, were only resolved for Fvb 4. While the quantity of missing data and heterogeneity in genome coverage inherent in GBS complicated the analysis, POLiMAPS resolved *F. ×ananassa* chromosomal regions derived from diploid ancestor *F. vesca*.

Projects in Progress

Phylogenetic analysis in *Rubus*.

Understanding the evolution of blackberries, raspberries, and their wild relatives, collectively called the genus *Rubus*, has been challenging because the group exhibits a number of biologically complicating features. Species can hybridize, are commonly polyploid, and often reproduce clonally by seed. We used target enrichment, an innovative sequencing approach, to provide clarity concerning relationships within *Rubus*. Target enrichment involves sequencing specific regions of DNA instead of the entire genome. We sequenced 926 genes from the black raspberry genome and 247 genes that are found in the apple, peach and strawberry genomes. Preliminary analyses resulted in a phylogeny, or a branching tree indicating evolutionary relationships between caneberry species. Members of many subgenera included in this study were spread throughout different groups in the tree, indicating that traditional divisions of *Rubus* should be redefined. This research will inform efforts to breed better blackberries and raspberries for human consumption.

Black raspberry genomic resource development. Two new techniques consisting of long read sequencing and assembly using proximity-guided assembly generated a chromosome scale assembly of the black raspberry genome. Three new populations segregating for each of the three sources of aphid resistance were generated. We plan to fine map the aphid resistance loci in these populations and generate markers that can predict each source of resistance.

Development of blackberry, red raspberry, pear, strawberry and blueberry fingerprinting sets. A reliable and fast method for confirming identity and paternity in each of these crops is needed. Microsatellite or simple sequence repeat (SSR) markers are ideal for cultivar fingerprinting, paternity testing and identity certification. SSRs with higher core repeats (3-6 nucleotide motifs) have become available in each of these crops and are more reproducible across labs and easier to score than the previously used dinucleotide-containing SSRs. Our objective is to develop a multiplexed fingerprinting set composed of trinucleotide-containing SSRs that can distinguish each accession and use them to confirm identity in each of these crops. At this time, we have developed an 8-SSR blackberry, 6-SSR red raspberry, 10-SSR pear, 6-SSR strawberry, and 10-SSR blueberry fingerprinting sets and they are being analyzed for their distinguishing ability in a germplasm panel consisting of closely related as well as diverse individuals. The 10-SSR pear set contains three dinucleotide-containing SSRs from the 12-SSR set developed by the European Cooperative Program for Plant Genetic Resources (ECPGR). The distinguishing power of this 10-SSR set and the 12-SSR

ECPGR set is being evaluated on a panel of 103 accessions that contain multiple sets of undifferentiated individuals.

RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars. DNA tests that can predict traits in strawberry are available for perpetual flowering (PF), gamma decalactone, and mesifurane were used to predict these traits in three strawberry breeding programs: University of Florida, USDA-ARS-HCRL and University of New Hampshire. DNA tests for these traits are described and routinely updated on the RosBREED website at <https://www.rosbreed.org/breeding/strawberry>. Additional DNA tests are being developed by the scientific community and will be validated in appropriate germplasm sets from our collection to determine their usefulness in predicting the corresponding trait.

Four populations expected to segregate for crown and root rot resistance were created and will be phenotyped and genotyped to identify loci controlling these devastating diseases and develop markers that allow incorporation of durable resistance in strawberry cultivars.

Three pear families were phenotyped for fire blight resistance at the USDA-ARS-AFRS. These populations were also genotyped with the pear array. Awaiting a second year of phenotypic data collection for data analyses.

Identifying blackberry loci associated with higher berry sugar content is currently underway using a synteny based approach. 279 genes thought to be associated with sugar production were identified in blackberry using genes and regions identified in apple and strawberry. A target capture approach will be used to sequence these genes and identify associations with previously collected phenotypic data.

Two rose families were genotyped with the rose arrays. Resistance to one of the most devastating diseases of rose, black spot, segregated 1:1 in a tetraploid ‘Morden Blush’ x Brite Eyes™ population suggesting resistance is mediated by a single locus. Preliminary linkage mapping positioned the resistance locus on a single linkage group that corresponds to one of the chromosome 5 homeologs. To date no resistance loci have been reported on chromosome 5 possibly indicating a previously unknown resistance gene.

Plant Pathology

By Joseph Postman

Periodic monitoring of the NCGR Vaccinium field collection identified accessions that have remained free of Blueberry shock virus after more than 10 years of exposure to field spread. This may indicate accessions that are slow to become infected through pollen transmission, or may indicate genotypes that have genetic resistant to natural spread of the virus. Preliminary results were published in *Acta Horticulturae* in 2017 in collaboration with Chad Finn and Bob Martin (USDA-ARS, Corvallis). More than two dozen clones remain free of the virus, and additional screening will identify candidates to consider as parents for breeding resistant cultivars, or for attempting to elucidate mechanisms that may confer resistance.

Reversion in black currants is a devastating disease caused by Blackcurrant reversion virus (BRV). A quarantine has been in place for many years to prevent BRV and the eriophyid mite that spreads it from entering the United States. In 2016, BRV was detected for the first time in the black currant cultivar Burga growing in the NCGR Ribes collection using high throughput DNA sequencing. A second test was conducted on 'Burga' as well as 11 other black currants which exhibited suspicious leaf symptoms using reverse-transcription polymerase chain reaction (RT-PCR) to confirm the presence of BRV in Oregon. 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 have been growing in the United States for more than 20 years. These plants had been tested when they entered the US using a graft assay and were thought to be BRV-free. At the time, this was the only tool available for BRV detection. During 2018, a survey of black currants in the NCGR collection will determine whether any additional plants are infected. Laboratory testing of black currant clones for BRV was coordinated by post-doc Jason Zurn.

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