ANNUAL REPORT FOR CALENDAR YEAR 2014
USDA ARS
National Clonal Germplasm Repository
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National Clonal Germplasm Repository Staff

Permanent & Term Federal Staff
Liz Alperin, Bio. Science Tech., Genetics
Bruce Bartlett, Ag. Science Tech., Plant Distribution
Nahla Bassil, Geneticist – Plants
Jill Bushakra, Research Associate, Genetics
Jeanine DeNoma, Bio. Science Tech., TC
Missy Fix, Bio. Science Tech., Plants
Kim Hummer, Research Leader/Curator
April Nyberg, Bio. Science Tech., Genetics
Jim Oliphant, Bio. Science Tech., Greenhouse Manager
Yvonne Pedersen, Program Assistant
Joseph Postman, Plant Pathologist/Curator
Barbara Reed, Research Plant Physiologist
Joe Snead, Ag Science Tech/Field Manager

Pyrus curator Joseph Postman has tentatively identified the 'Hager Grove Pear', a heritage tree growing south of Salem, Oregon, as the lost cultivar 'Dearborn'.

Temporary Staff & Students
Katie Armes, OSU/Mol. Lab Tech
Emily Bouldin, Bio. Science Aid/Field
Jack Brennan, Work Study
Mitchel Elstad, Work Study
Jacob Fought, Work Study
Barb Gilmore, Bio. Science Tech
Brad Greenburg, Work Study
Debra Hawkes, Bio. Science Aid, Greenhouse
Andrew Isaacs, Work Study
Eda Karaagac, Volunteer
Ellen Mering, OSU student worker
Angelina Nachorniy, Bio. Science Aid
Rocco Nguyen, Work Study
Jane Olson, Bio. Science Aid, Greenhouse
Kasey Schaefer, Work Study
Clayton Skillman, Bio. Science Aid/Field
Sabrina Teo, ASE Intern
Kimberly Teo, Volunteer
Frazier Thurman, Work Study
Carly Waddell, Work Study
Tyler Young, BENCO

Graduate Students & Visiting Scientists
Meleksen Akin, GRA, OSU, Hort., Turkey
Larry Alice, Scientist/ Western KY State University
Meera Das, Visiting Scientist/India
Sukalya Poothong, GRA, OSU, Hort. Thailand
Victoria Rivero, Visiting Scientist/Argentina
Natalia Salinas, Fulbright/Ecuador
Sugae Wada, Post Doc. OSU

Stakeholder/Service Accomplishments

- 12,070 accessions, 65 genera and 748 taxa of 653 species of temperate fruit, nut, and specialty crops were conserved.
- Obtained a total of 103 new accessions and 203 new inventory items in CY 2014.
- Received a record number: 1108 order requests and shipped 7,727 items in CY 2014.
- Improved the management and maintenance of 3200 accessions in the pear, hazelnut, quince and related tree field collections.
- Collaborated with NCGRP, Ft. Collins, CO, on cryopreservation protocols of dormant blueberry, hazelnut, pear, and currant.
- Provided tours to high school, undergraduate and graduate school groups
- Trained visiting scientists from Western Kentucky University, Japan, China, and. Kazakhstan.
- Served as advisory panel member for SCRI Research and Ext. Planning Project Seattle.
- Advised *Citrus* and *Malus* community on development of Global Conservation Strategies working with the Global Crop Diversity Trust.
- Participated on Governing Board for USDA National Clean Plant Network.
- Member of the organizing committee and editor of the proceedings for the ISHS IHC symposium on Plant Genetic Resources
- Provided tissue culture assistance to hop breeders for a hop mutation breeding project.
- Mentored an OSU Horticulture Department Intern in tissue culture techniques for 9 months.
- Hosted two hop industry technicians to work with hops tissue culture and cryopreservation.
- Meristemmed heat-treated pear and hazelnut accessions and multiplied them for the virus elimination program.
- Provided tissue culture advice to Oregon and California tissue culture laboratories.

Research Accomplishments

- Published the first high throughput 90K genotyping platform in strawberry an octoploid crop.
- Obtained samples of the threatened *Rubus bartonianus* from Eastern Oregon
- Determined that *Vaccinium myrtillus* has tetraploid and hexaploid cytotypes in Oregon.
- Determined that *Rubus* species in Subgenus *Micranthobatus* have very small sized genomes.
- Reported on improved microsatellite markers for quince and analyzed genetic fingerprints of more than 100 *Cydonia* and *Pseudocydonia* germplasm accessions.
- Reported on successful development of microsatellite markers to assess genetic diversity and phylogenetic relationships of clonal medlar *(Mespilus* sp.) accessions.
- Harmonized SSR profiles of pear cultivars with those from the Brogdale collection in England.
- Determined ploidy levels for *Rubus* species and related genera using flow cytometry.
- Completed pear rootstock *in vitro* rooting study for the pear industry.
- Completed *in vitro* raspberry mineral nutrition studies and produced improved growth media.
- Determined initial *in vitro* nutrient requirements for blueberry.

Administrative Overview

Staffing Changes

Yvonne Pedersen, our Program Support Assistant, retired at the end of February 2015. She worked at her assignment for the past 12 years. She has been the repository soul and our pulse; keeping us in line with regulations, and ensuring that our time cards for everyone (of course the students!) were submitted appropriately. She innovatively developed forms for compilation of scientific manuscripts information. She managed purchase cards users for the unit. She helped guide the use of our budgets; and coordinated the development of research support agreements, foreign visitors, reception desk deliveries, and the use our library-meeting room. We will dearly miss her. We wish her well in her retirement when, no doubt, many salmon and steelhead will have the opportunity to jump to snatch her tempting home-tied flies.

Joe Snead, our Farm Manager (Agricultural Science Research Technician) retired in March 2015. He began working as an Oregon State employed technician at the beginning of the Repository and was converted to federal employment in 1987. Joe has provided oversight for our North Farm property helping to coordinate the cultural maintenance of many research projects at that location. He promoted use of integrated pest management approaches to plant maintenance, including promoting beneficial insects and birds. He assisted in the training of many student helpers over the almost 3 decades of his federal employment.

Dr. Barbara Reed, our Plant Physiologist is retiring from the federal service on 17 July 2015. She received her B.S. from the University of Nebraska-Lincoln, and her M.S. and Ph. D. in Botany from Oklahoma State University, Stillwater. In 1985, she began a Post-Doctoral position and, in 1989, was selected as a Research Plant Physiologist with the US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon. During her career at the NCGR she researched and developed alternative techniques for clonal plant germplasm storage. She improved media for tissue culture of hazelnuts, strawberries, raspberries, pears, and blueberries and their crop wild relatives. She developed protocols for low temperature or other slow growth storage techniques for medium-term storage of these tissue cultures. In addition, she developed protocols for cryopreservation to store meristems in liquid nitrogen. She authored 110 journal publications, two books, 13 book chapters, 19 proceedings papers, two theses, four handbooks of laboratory protocols, and six web-based educational tools. Dr. Reed advised nine M.S. and eight Ph.D. students, and provided short-term training to more than 50 visiting international scientists. Dr. Reed is a Fellow of the Society for *In Vitro* Biology and the Society for Cryobiology. She was an Associate Editor for HortScience for 6 years; and is an Associate Editor for *In Vitro* Cellular and Developmental Biology – Plant, and Plant Cell Tissue and Organ Culture. Dr. Reed was a member-at-large of the Governing Board of the Society for *In Vitro* Biology for 4 years, Secretary for an additional 4 years, and for 8 years the Chair of Publications; she is Treasurer of the Oregon State University Chapter of Sigma Xi and a member of the Board of Governors of the Society for Cryobiology. The staff of the Repository wishes her the best for her upcoming retirement.

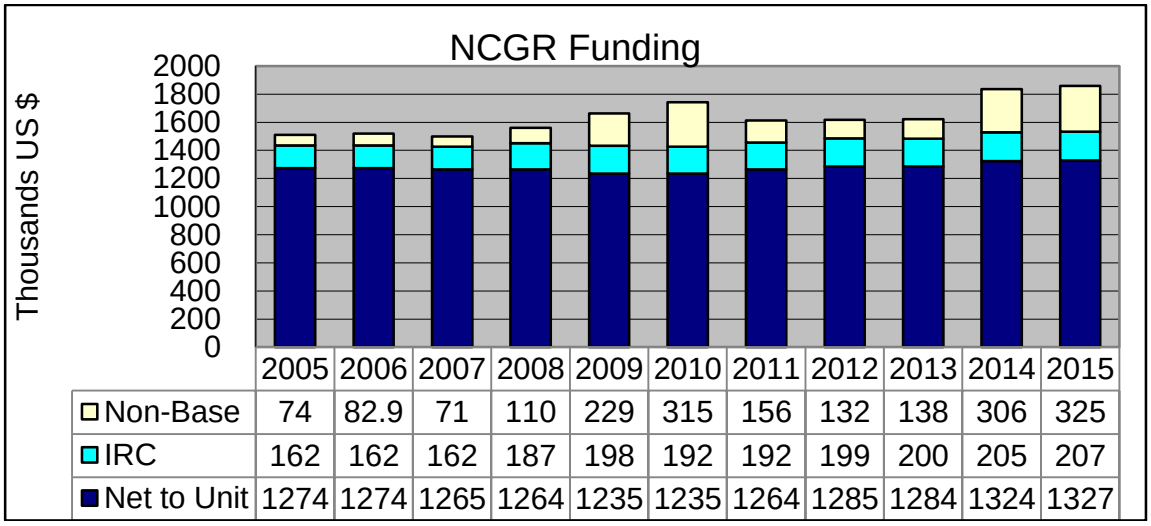
We are proceeding to recruit for a permanent program assistant, a permanent field technician, and a permanent scientist to replace these three critical positions for our unit.

EEO/CR/Outreach

- A male challenged individual was
- A female horticulture student intern was trained for 9 months in tissue culture techniques.
- Through a Research Support Agreement with Oregon State University 3 female graduate students were trained.
- During the winter, 3 disabled high school students (program was funded through local school district grants) were trained in greenhouse management activities.
- A female horticulture student intern was trained for 9 months in tissue culture techniques.
- During the winter an additional 15 mentally or physically individuals from a local private organization (Work Unlimited) were trained in strawberry greenhouse activities.

Budget

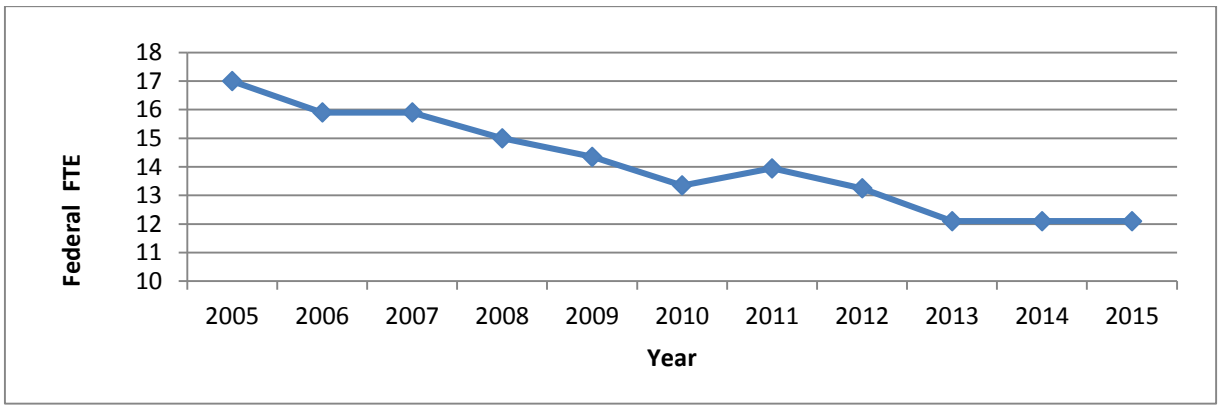
The CY 2014 year was a new reality for our federal budget year for the NCGR. We received a programmatic increase in spring 2014. That increase helped to reduce the effect of the recession and sequestration cuts of FY 2013. Our total federal budget is about $1.54 million. Our scientific staff is encouraged to obtain soft funding from a wide variety of research granting opportunities.



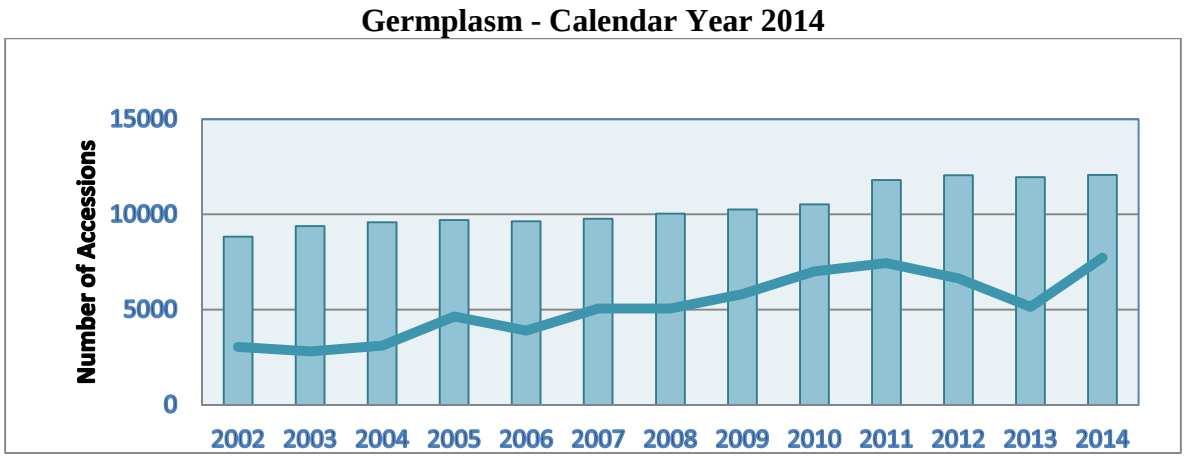
	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Non-Base	74	82.9	71	110	229	315	156	132	138	306	325
IRC	162	162	162	187	198	192	192	199	200	205	207
Net to Unit	1274	1274	1265	1264	1235	1235	1264	1285	1284	1324	1327

Non-base and Extramural Funding for the USDA-ARS NCGR- Corvallis

Research Proposals Funded	**FY 2014**		
California Pear Board	$35,050	Pear rootstock chlorosis	Reed
OR Hazelnut Commission	$12,000	Hazelnut elite selection tissue culture	Reed
ISTC-ARS Kazakhstan	$10,000	Pear and apricot tissue culture	Reed
OAN/ODA Nursery Crops	$24,000	Hazelnut medium development	Reed
SCRI subcontract	$10,000	Hazelnut micropropagation	Reed
Ag Res. Foundation	$12,500	Blueberry medium development	Reed
SCRI GRANT	$127,822	Genomic infrastructure black raspberry	Bassil
Western Kentucky	$12,500	Targetseq in *Rubus*/Cytology	Bassil/Hummer
NWCSFR	$33,905	aphid resist in black raspberry/ Markers	Bassil
Schmidt	$5,000	labeling of *Corylus* with QR code	Postman/Hummer
NARBA	$1,000	labeling of *Rubus* with QR code	Postman/Hummer
WA Tree Fruit Comm.	$2,500	labeling of *Pyrus* with QR code	Postman
PWA Summer Intern	$1,500	labeling of genera	Postman/Hummer
APHIS	$16,500	Survey of Berry germplasm for viruses	Postman
NPGS Evaluation	$1,503	Pear tree architecture	Postman
Total	$305,780		

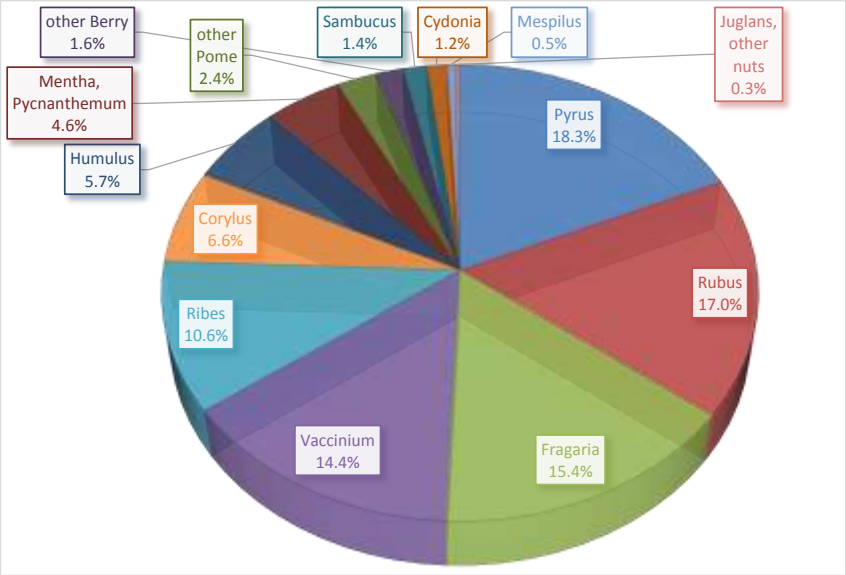


Germplasm Collections



Summary of Corvallis Holdings, May, 2015
12,098 Seed and Plant Accessions

Genus	Accessions
Pyrus	2196
Rubus	2034
Fragaria	1841
Vaccinium	1726
Ribes	1269
Corylus	793
Humulus	684
Mentha, Pycnanthemum	553
other Pome	286
other Berry	196
Sambucus	170
Cydonia	143
Mespilus	60
Juglans, other nuts	34



Top Accessions Requested 01/01/2014 to 1/1/2015 for Site COR

Rank	Accession	Taxon	Plantname	Requests	Shipped
1	PI 557968	Mentha x piperita	Chocolate Mint	34	33
2	PI 557912	Mentha suaveole	Variegata Pineapple Mint	33	32
3	PI 554817	Vaccinium coryml	Rubel	31	30
4	PI 557922	Mentha x gracilis	Double Mint	28	27
5	CFRA 2207	Fragaria x ananas	Strawberry D9-11 educational	25	25
6	PI 617403	Humulus lupulus	Sorachi Ace	25	23
7	CVAC 2184	Vaccinium coryml	Blueberry C 9-11 educational	22	22
8	PI 558681	Humulus lupulus	Cascade	21	17
9	PI 312507	Pyrus communis	Summer Blood Birne	20	20
10	PI 556298	Ribes spicatum	Cherry	20	19
11	PI 541262	Pyrus communis	Seckel	19	19
12	PI 557952	Mentha x piperita	M. x piperita f. lavanduliodora	19	19
13	PI 652482	Lonicera caerulea	Bluebird	19	11
14	PI 541271	Pyrus communis	Taynton Squash	18	18
15	PI 657877	Rubus occidentali	Earlysweet	18	16
16	CRUB 2690	Rubus idaeus sub	Red raspberry D9-11 educatior	17	16
17	PI 285530	Pyrus communis	Johantorp	17	17
18	PI 535808	Humulus lupulus	Mt. Hood	17	16
19	PI 541256	Pyrus communis	Rousselet de Reims	17	17
20	PI 551904	Fragaria x ananas	Ettersburg 121	17	16
21	PI 617584	Pyrus communis	Joey's Red Flesh Pear	17	15
22	PI 541273	Pyrus communis	Thorn	16	16
23	PI 552292	Fragaria x ananas	Variegated Strawberry	16	6
24	PI 553258	Rubus loganobac	Bauer's Thornless	16	13
25	PI 541150	Pyrus communis	Beurre Superfin	15	15
26	PI 551622	Fragaria x ananas	Elista	15	9
27	PI 553543	Rubus idaeus sub	Bababerry	15	4
28	PI 554841	Vaccinium coryml	Razz	15	13
29	PI 557953	Mentha x piperita	White Peppermint	15	15

Facilities

Security. We upgraded the data bridge to our North farm. Our staff has new smart cards for entering locked doors. A new multi-zone system was installed replacing a 30+ year old system. We installed a new stairway to a storage loft to replace a wooden ladder. We are working with Beltsville engineers for potential replacement of our aging screenhouses with a glass or twin-wall polycarbonate growing structure. If approved, construction is anticipated in 2016.

Travel 2014

Compiled by Yvonne Pedersen

Highlighted entries indicate contributed travel, paid by inter/intra agency or outside private funds.

Joseph Postman – Riverside, CA; ARS Citrus Germplasm Repository Review; Jan. 2014

Nahla Bassil – San Diego, CA; Fruit & Nut Workshop and PAG meeting; Jan. 2014

Barbara Reed – Portland, OR; Hop Research Council meeting; Jan. 2014

Joseph Postman – Yakima, WA; pear industry research review; Feb. 2014

Joe Snead – Albany, CA; OSHA 6000 Course; April 2014

Barbara Reed – Watsonville, CA; Global Tissue Culture/Nursery Clean Stock and Plant Health; April 2014.

Kim Hummer – Richmond, CA; attending PWA Leadership Development/Training Conference; April 2014.

Barbara Reed – Kazakhstan; ISTC grant work and International Scientific Conference of Plant Biology and Biotechnology; May, 2014.

Barbara Reed- Savannah, GA; Joint meeting of the Society for *In vitro* Biology and the Society for Cryobiology, May 31-June 5.

Jill Bushakra – Wenatchee and Seattle, WA; Rosaceae Genome Conference; June 2014.

Nahla Bassil – Wenatchee and Seattle, WA; Rosaceae Genome Conference; June 2014.

Joseph Postman – Davis, CA; Western Regional (W-6) Technical Advisory Committee; June 2014.

Kim Hummer – Baker City, OR; plant collecting; June 2014.

Kim Hummer – Seattle, WA; collecting germplasm from Rhododendron Botanical Gardens; June 2014.

Joseph Postman – Riverdale, MD; National Clean Plant Network proposal review; June 2014.

Joseph Postman – Leuven, Belgium; ISHS International Pear Symposium; July 2014.

Jill Bushakra – Orlando, FL; ASHS conference; July 2014.

Kim Hummer – Orlando, FL; ASHS conference; July 2014.

Nahla Bassil – Orlando, FL; ASHS conference; July 2014.

Joseph Postman – Troutdale, OR; co-hosting NAFEX-CRFG-HOS meeting; August 2014.

Kim Hummer – Brisbane, Australia; ISHS planning meeting and International Hort. Congress (IHC); August 2014.

Barbara Reed – Melbourne and Brisbane, Australia; International Association of Plant Biotechnologists and IHC; August 2014

Joseph Postman – Davis, CA; Plant Germplasm Operations Committee; October 2014.

Barbara Reed – Santos, Brazil; Brazilian Congress of Genetic Resources; November 2014.

Visitors in 2014

By Yvonne Pedersen

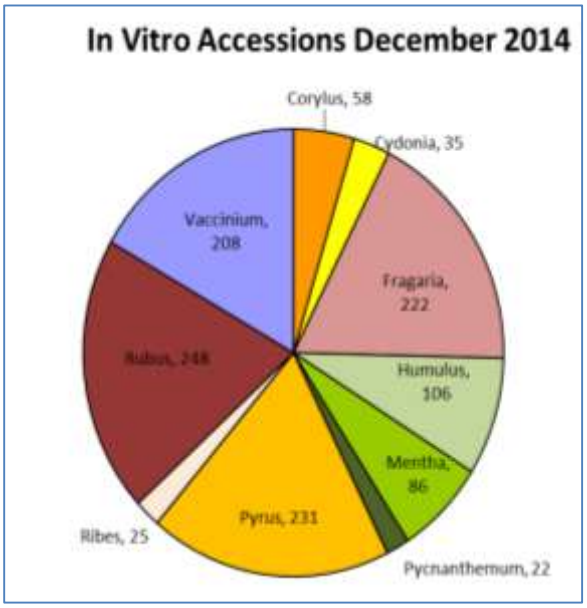
More than 463 visitors came through the Repository's front door during business hours. Guests arrived in large or small groups, as organized class tours or as individuals. About 25-30 people attended a blueberry open house in July. Groups such as the Oregon Processed Vegetable Committee used the Repository conference room for their annual meetings. Educational tours ranging from of 2 to 150 individuals came from Willamette University, Home Orchard Society, Chemeketa and Linn-Benton Community Colleges, Oregon State University, various garden clubs, Eugene Permaculture, Slow Food, and Life-Long Learning Institute. Our ARS-Corvallis Outreach Diversity and Equal Opportunity Committee arranged a tour for 28 summer students to the three ARS Corvallis units. International visitors came from India, China, Japan, New Zealand, Columbia, Spain, Turkey, Thailand, Australia, and Canada. We also hosted graduate students and visiting scholars from Thailand, Ecuador, Turkey, India, Argentina, and Kentucky State University.

Tissue Culture and Cryopreservation

By Barbara Reed and Jeanine DeNoma

In-Vitro Collection

The *in-vitro* collection contains mostly core and highly requested accessions. Jeanine DeNoma and helpers collected new accessions as plants were available during the spring and summer explant season. In December 2014, 1241 accessions were in culture and most were in cold storage (figure right). *In-vitro* accessions are evaluated at four month intervals and re-propagated as needed.



Apricot and Pear Diversity and Germplasm Preservation in Kazakhstan

Our 10 year collaboration with Dr. Irina Kovalchuk, Institute of Plant Biology and Biotechnology for two projects in the ISTC program is nearing completion. Dr. Malli Aradhya of the Davis Repository is also a collaborator. Dormant buds of pears and apricots were cryopreserved using new techniques. Micropropagation medium of apricot was improved by changing mineral nutrients. Apricot seeds were successfully cryopreserved for the first time. DNA was sampled from apricot and pear populations and compared with plant morphological characteristics. Genotyping of 80 apricots and pears was completed. Apricots from the Ketmen metapopulation were added to the US Germplasm System. To date 24 manuscripts on micropropagation, *in vitro* cold storage and cryopreservation have been published from this collaboration.

Medium Optimization for *Pyrus*

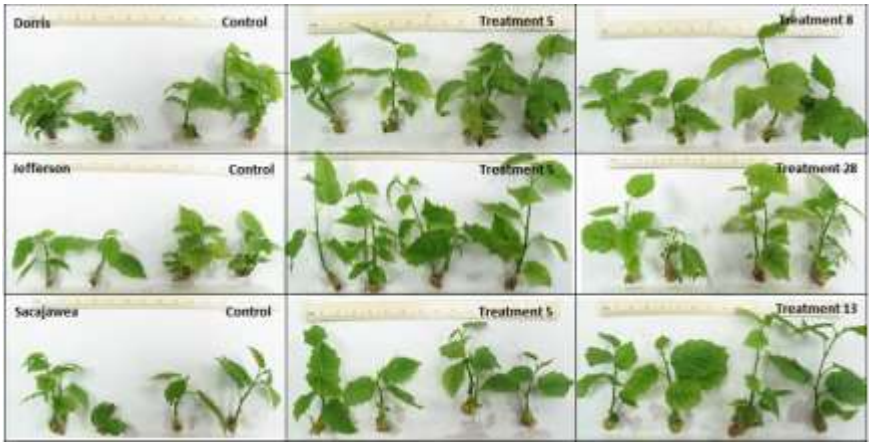
We tested rooting protocols for *in vitro* pear rootstock selections. Dwarfing rootstocks are short and slow growing so they provide some challenges for culture, but the new PRS medium provides good

elongation and multiplication for cultivars tested. PEG and auxins provided excellent rooting for most genotypes (Figure below). The California Pear Board and the Washington Tree Fruit Commission funded Dr. Reed and Dr. Sugae Wada (OSU Horticulture) for this project. We also studied *in vitro* screening for iron chlorosis for the California Pear Board and identified Fe-chlorosis tolerant and susceptible rootstocks. Using 1 mM EDDHA with 10 mM $KHCO_3$ created a sufficient medium pH range (from 5.49 to 9.58 after autoclaving). We found OH×F 87, Fox 11 and Horner 10 were tolerant; OH×F 97 was moderately tolerant; and Pyro 2-33 and OH×F 513 were susceptible to iron chlorosis. This study could help fruit growers to determine which rootstocks should be considered as candidates for field trials, reducing the time and money needed to test all available candidates.

Four pear rootstock selections from in vitro culture 4 weeks after rooting in a soilless mix following treatment with a combination of NAA 5 mM plus IBA 5 mM dissolved in PEG 400. Rooting was 66 to 97% for treated shoots while controls dipped only in PEG rooted at 0 to 25% (Wada and Reed, unpublished)

Corylus Mineral Nutrition

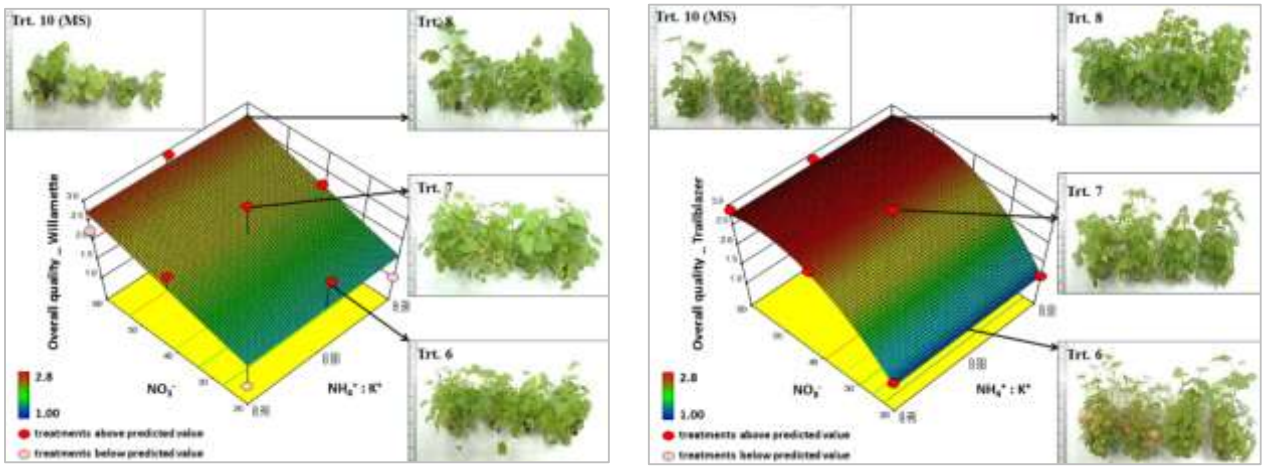
An improved *Corylus* culture medium (Hazelnut 2013 Medium) was developed based on experiments by graduate student Chip Hand. Improved growth and shoot quality in 'Dorris' and 'Jefferson' required greatly increased B, Mo and Zn combined with low Mn and Cu; 'Sacajawea' required increased B, Mn, Zn and Ni with low Mo for the best growth. Ph.D. student Meleksen Akin is continuing this study and hopes to further improve the medium. Several commercial laboratories have adopted the 2013 medium.



Shoots of 'Dorris', 'Jefferson', and 'Sacajawea' hazelnuts grown on control medium and two minor nutrient treatments that produced higher quality ratings. (Hand and Reed, 2014)

Raspberry Mineral Nutrition

Ph.D. candidate Sukalya Poothong successfully defended her study of the response raspberry cultivars to mineral nutrients in October. Dr. Poothong is now a Lecturer in the School of Agriculture and Natural Resources, University of Phayao, Thailand. She used the surface response design to develop improved mineral nutrition for five cultivars. The initial experiments found the mesos stock solution ($CaCl_2$, KH_2PO_4 and $MgSO_4$) was the most significant limiting factor associated with changes in plant quality, multiplication and shoot length in all cultivars followed by the nitrogen components. Follow-up studies found that increasing all three components to 2.5x the MS concentration greatly improved growth; and concentration of NO_3^- (40-60 mM) was a significant factor for improved shoot quality of most cultivars. The five best treatments were also effective for a wider range of *Rubus* germplasm. (Figure below).



Ammonium and potassium proportion (NH4+:K+) and total NO3- effects on quality of 'Willamette' and 'Trailblazer' red raspberries showing the appearance of shoots grown on three levels of total NO3- (right) with 2.5× mesos compared to shoots grown on standard MS (left) (S. Poothong, Ph.D. dissertation 2014).

A metabolomics study to investigate the effects of the mesos components of mineral nutrition on plant metabolism was completed in collaboration with Dr. Claudia Meier, OSU Department of Chemistry. Increased mesos altered mineral uptake in the shoots resulting in increased up take of Ca, S and Mg and slight decreases in Fe, Cu and Zn. This analysis indicated that shoots grown on MS medium were experiencing stress conditions resulting in the production of antioxidant compounds while high mesos medium produced shoots that were actively growing and not stressed.

Vaccinium mineral nutrition

With funding from the OSU Agricultural Research Foundation and collaboration with Fall Creek Nursery, we are evaluating *in vitro* growth medium for blueberry cultivars. The initial study of five stock solutions was completed this year and the follow up experiments will be done in 2015.

Molecular Genetics

By Nahla V. Bassil

Graduate Students

In collaboration with Chad Finn, M.S. student Natalia Salinas continued her work on strawberry, validating markers associated with remontancy and a marker associated with high soluble solids content. She also implemented genotyping by sequencing (GBS) in octoploid strawberry and is analyzing the strengths and weaknesses of this technique.

Natalia Salinas presenting a poster at ASHS annual conference in Palm Desert, CA.

Genetics Lab Team joined by Kim Hummer enjoying a summer break. Left to Right: Kacey Schaefer, April Nyberg, Elisabeth Alperin, Nahla Bassil, Larry Alice, Sabrina Teo, Kim Hummer and Eda Karaagac

Visiting Scientists. Dr. Lawrence Alice, Associate Professor of Plant Systematics and Herbarium Curator at Western Kentucky University, was on sabbatical at the NCGR working with us on implementing a new genomic sequence-based technique for phylogenetic analyses in *Rubus*. Also Dr. Eda Karaagac volunteered to work on molecular and field evaluation of black raspberry germplasm.

Projects Completed in 2014

Compared fingerprints of 61 pear accessions in common between the Brogdale and NCGR collections. We used multiplex PCR to fingerprint the NCGR core collection using a universal fingerprinting set developed by the ECPGR. Comparison of the data from 61 accessions in common between the National Brogdale Collection at East Malling Research (EMR) and the NCGR revealed 41 pairs of individuals with the same name and with identical allele sizes. Twelve accessions from Brogdale were different from those with the same name at NCGR at more than six of the 12 microsatellite loci indicating the possibility of misidentified trees. Six accessions from Brogdale were identical to those with the same name at NCGR at 11 of the 12 microsatellite loci. Detailed

comparisons of electropherograms revealed that three of these were mis-scored and consequently can also be added to the original 41 identical pairs. Two accessions from Brogdale were identical to those with the same name at NCGR at 10 of the 12 microsatellite loci. Further comparison of electropherograms and comparison of phenotypic data is required before these accessions can be confirmed as different. Characterization of germplasm collections at this level should result in more accurate labeling of accessions reducing the possibility of confusion when attempting to compare results between varieties that may be mislabeled. Using this fingerprinting set, we identified 'Patricke' pear as the cultivar 'Leona'.

Used microsatellite markers to evaluate genetic diversity of medlar collection. Twenty-one simple sequence repeat (SSR) markers from apple and two from pear were tested for polymorphism in eleven medlar accessions. Nine apple SSRs were polymorphic in medlar. Five easy- to-score SSRs from apple were used to genotype 41 clones of *M germanica* and one accession of the intergeneric hybrid species *M. canescens* growing at the NCGR. Seven very large fruited clones with five different countries of origin were genetically indistinguishable from 'Monstreuse d'Evreinoff'. Four clones from western Oregon sources with similar phenotypes also produced identical SSR profiles. Two other pairs of synonyms were also documented. One of the five SSRs (NZ05g8) distinguished 'Marron' and 'Macrocarpa' from the ancient English cultivar 'Nottingham'. Flow cytometry confirmed *M. canescens* as triploid and showed all *M. germanica* accessions to be diploid. The relationships determined in this study document the diverse genetic backgrounds represented in the NCGR medlar collection. These results also will permit the removal of unnecessary duplication and improve the efficient management of this collection.

Evaluated 90K strawberry array in 306 cultivated strawberry accessions. A high-throughput genotyping platform is needed to enable marker-assisted breeding in the allo-octoploid cultivated strawberry. Short-read sequences from one diploid and 19 octoploid accessions were aligned to the diploid 'Hawaii 4' reference genome sequence to identify single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) for incorporation into a 90K Affymetrix® Axiom® array.
About 36 million sequence variants were identified in this 19 member germplasm set. Strategies and filtering pipelines were developed to identify and incorporate markers of several types: di-allelic SNPs (66.6%), multi-allelic SNPs (1.8%), indels (10.1%), and ploidy-reducing "haploSNPs" (11.7%). The remaining SNPs included those discovered in the diploid progenitor *F. iinumae* (3.9%), and speculative "codon-based" SNPs (5.9%). In genotyping 306 octoploid accessions, SNPs were assigned to six classes with Affymetrix's "SNPolisher" software. The highest quality classes, *PolyHigh Resolution* (*PHR*), *No Minor Homozygote* (*NMH*), and *Off-Target Variant* (*OTV*) comprised 25%, 38%, and 1% of array markers, respectively, providing markers suitable for genetic studies as demonstrated by generation in the full-sib family 'Holiday' × 'Korona' of a genetic linkage map consisting of 6,593 *PHR* SNPs evenly distributed across 28 chromosomes with an average density of approximately one marker per 0.5 cM, exceeding our goal of one marker per cM.
This array is the first high-throughput genotyping platform for cultivated strawberry and is commercially available to the worldwide scientific community. The array's high success rate is likely driven by the presence of naturally occurring variation in ploidy level within the nominally octoploid genome, and by effectiveness of the employed array design and SNP genotyping strategies. This array enables genetic analyses including generation of high-density linkage maps, identification of quantitative trait loci for economically important traits, and genome-wide association studies, thus providing a basis for marker-assisted breeding in this high value crop.

Developed new microsatellite markers for red and black raspberry using next generation sequencing. In collaboration with Michael Dossett, we demonstrated the efficiency of microsatellite or SSR marker development from short-read sequences using the Illumina® platform in red and black raspberry, compared transferability of markers across species, and tested whether the rate of polymorphism in the recovered markers can be improved upon by how marker sequences are chosen. More than 5,000 markers were identified from sequences in each species, with 144 markers chosen from each species and tested in both. Markers selected based on different percentages (90-97 % as compared to ≥ 98 %) of short read sequence cluster identity did not differ in polymorphism rates from each other or from those originating from singletons. Efficiency of polymorphic locus recovery was nearly twice as high in black raspberry from black raspberry-derived sequences as from red raspberry-derived sequences, while efficiency of polymorphic locus recovery in red raspberry was not affected by the source of the primer sequences. Development of transferable SSR markers for red and black raspberry for evaluation of genome colinearity and to facilitate comparative studies in *Rubus* will be more efficient using SSR markers developed from black raspberry sequences.

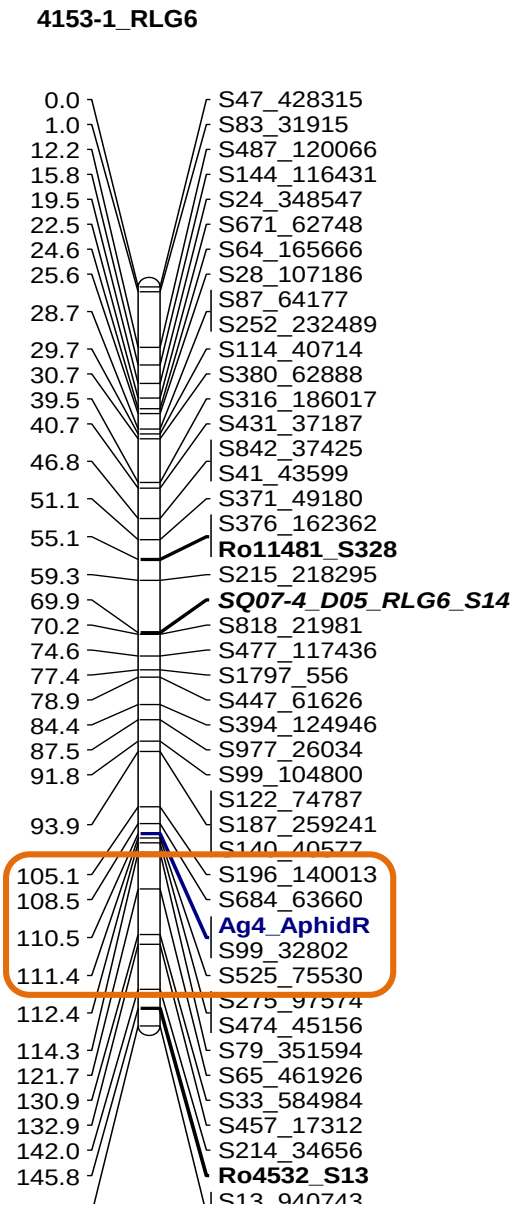
Projects in Progress in 2014

Developing genomic tools for blueberry. Construction of linkage maps in the tetraploid mapping population of 'Draper' x 'Jewel' is continuing. So far, 365 markers were placed across the 12 linkage groups in the tetraploid map. SNP markers were developed for this population using genotyping by sequencing and data analyses is in progress.

Testing markers associated with remontancy in strawberry. In collaboration with Daeil Kim, Jim Hancock, Chad Finn and Beatrice Denoyes, we are evaluating four SSR markers linked to remontancy on LG IV in strawberry in multiple sources of remontancy in the RosBREED-evaluated germplasm. These strawberries were also phenotyped for this trait in at least two geographical locations (Corvallis, OR and East Lansing, MI).

Testing markers associated with high soluble solids content in strawberry. In collaboration with Jim Hancock and Chad Finn we are analyzing the results of genotypic (for one SSR) and phenotypic data in 947 strawberry accessions for association with high soluble solids content. The strawberries were also phenotyped for this trait in at least two geographical locations (Corvallis, OR and East Lansing, MI).

Black raspberry genomic resource development. The USDA-ARS NCGR manages and maintains a collection of over 175 black raspberry germplasm accessions, which includes newly collected wild accessions from 130 locations across 27 US states and two Canadian provinces. Evaluation of this wild germplasm led to the identification of three potential sources of aphid resistance, and potential new sources of resistance to the fungal pathogen *Verticillium dahliae*. We are building the genomic infrastructure for black raspberry by developing, and making available, genomic tools including molecular markers for construction of linkage and physical maps, and a draft genome assembly that will benefit both black and red raspberry breeding programs across the U.S. To date, we have constructed a genetic linkage map consisting of seven linkage groups representing the seven haploid chromosomes of black raspberry which includes a morphological locus for aphid resistance on group 6. A draft genome assembly of 240 megabase pairs (Mbp) was generated from a highly homozygous accession. We are evaluating our populations for tolerance to *Verticillium dahliae* with the intent of placing a locus for this valuable trait on the linkage map. Additional aphid resistant crosses have been generated and are in the process of being evaluated. These genomic resources are essential for building the infrastructure needed for identification of candidate genes or closely linked markers for traits of interest during the development of improved black raspberry cultivars, and will inform decisions regarding germplasm value and usage, crossing, and selection through marker-assisted breeding.



Black raspberry Linkage Group (LG) 6 with the locus for aphid resistance highlighted

NCGR Corvallis Tree Collections

Joseph Postman

Summary of Tree Fruit and Nut Collections, May 2015

Genus	Accessions	Taxa	Genus	Accessions	Taxa
Amelanchier	49	10	*Juglans*	28	3
Amelasorbus	1	1	*Malus*	10	6
Castanea	3	2	*Mespilus*	60	2
Celtis	1	1	*Peraphyllum*	8	1
Chaenomeles	17	4	*Pseudocydonia*	3	1
Corylus	793	21	*Pyronia*	7	1
Crataegomespilus	3	1	*Pyrus*	2196	36
Crataegosorbus	1	1	*Sorbaronia*	7	4
Crataegus	23	9	*Sorbocotoneaster*	3	2
Crataemespilus	1	1	*Sorbopyrus*	11	2
Cydonia	140	1	*Sorbus*	135	46

Pears. As of January 1, 2015, the NCGR *Pyrus* collection included 2232 clonal pear accessions and 335 seedlots representing 36 Pyrus taxa from 59 countries. A collection of 231 clones are backed up *in vitro* as shoot cultures at 40° F, and 320 clones are backed up as small potted screenhouse trees. NCGR houses 950 European Cultivars, 185 Asian Cultivars, 120 Hybrid Cultivars, 170 Rootstock Selections, 28 Perry (cider) Cultivars and 851 trees representing pear wild relatives. Perry pears continue to be some of the most requested accessions, reflecting the surge of interest in hard cider in the U.S. The 8 most requested accessions in 2014 were Summer Blood Birne, Seckel, Taynton Squash, Johantorp, Rousselet de Reims, Joey's Red Flesh Pear, Thorn and Beurre Superfin.

Hazelnuts. The *Corylus* collection consists of 860 living trees including 453cultivars/selections and 407 wild relative species trees. The hazelnut collection represents 20 Corylus taxa from 36 countries. Several Eastern Filbert Blight (*Anisogramma anomala*) were found in early 2015, making annual prophylactic fungicide sprays and pruning to remove infections essential. Propagation efforts continue in support of the establishment of field rows dedicated to the shrubby species *C. americana* and *C. heterophylla*, and to the re-location of various tree-hazel species to a separate plot. We are also in the process of propagating the core collection (177 accessions) as self-rooted trees to replace the remote backup collection at the USDA genebank in Parlier, California. The most requested *Corylus* accessions in 2014 were *C. avellana* Oroban #34, Barcelona, Delta, Zeta, Sacajawea and Jefferson. Delta and Jefferson were also among the most requested in 2013.

Quince. The Corvallis genebank maintains 172 clonal accessions and 25 seedlots of *Cydonia* and the closely related genera *Docynia*, *Pseudocydonia*, *Pyronia*, and *Chaenomeles*. The various species of quince are represented by 9 taxa from more than 21 countries. The collection includes 22 rootstock clones, and 78 selections grown for their edible fruit. SSR fingerprints generated using microsatellite markers developed at NCGR identified a number of identical accessions (either synonyms or misidentified trees). More than a dozen duplicate trees were removed from the *Cydonia* field plot, opening up vacant spaces for new, unique genotypes. The most requested quince accessions in 2014 were Pineapple, Ekmek, Sekergevrek, Champion, Bereczki, and Tashkent AR-232. Pineapple, Champion and Bereczki were also among the most requested in 2013.

Greenhouse/Screenhouse

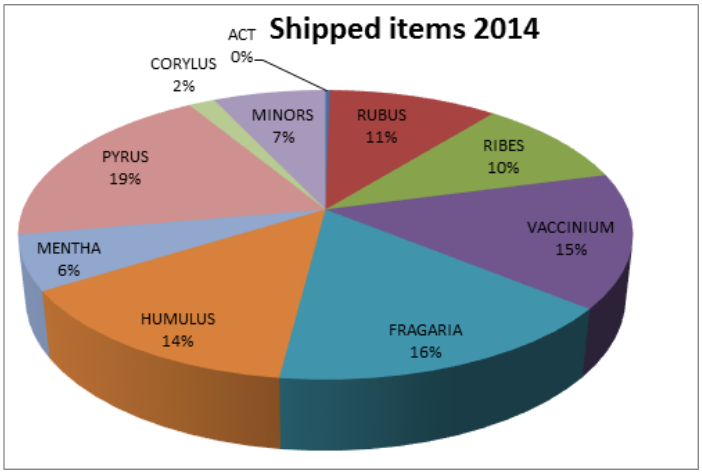
By Jim Oliphant

- Optimized softwood propagation methods greatly improve rooting success
- Cleaned *Vaccinium* house and replaced benches
- Improved GRIN inventory data and developed a working pot label
- Propagated *Mentha* collection
- Continued the rearrangement of the potted *Vaccinium* collection.

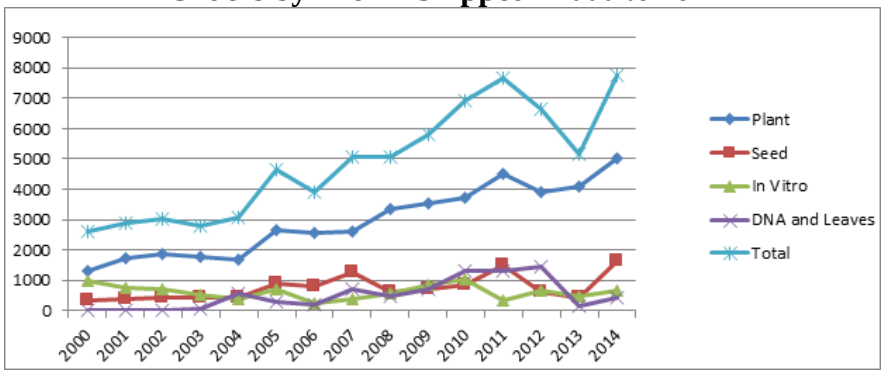
Distribution

by Kim Hummer and Missy Fix

- In CY 2014, NCGR staff shipped 7,739 items as seeds, cuttings, runners, scionwood, rooted plants, tissue cultures and DNA and leaf samples and informational material.
- In CY 2014, 856 new orders were received for small fruit and 179 new orders for scion or budwood. More than 823 orders were shipped.
- The pears and strawberries topped the list of crops distributed.
- Domestic individuals, state agencies and universities, and ARS researchers received the most germplasm from Corvallis in 2014.



Orders by "Form Shipped" 2000 to 2014



Seed Lab

By Missy Fix

During CY 2014 1294 seed items were shipped. 1161 were from the Major genera and 133 from the Minor genera. The most requested Genera was the Humulus with 421 requests. Fragaria was the next popular with 390 requests. Lonicera was the most requested in the minor genera with 44 requests. Sambucus was next with 29 requests.
We received 19 new accessions – 13 Vaccinium, 4 Rubus, 1 Fragaria and 1Pyrus.
To help preserve our seed collection, Kim Hummer donated open pollinated seed of blackberry, yellow raspberry, red raspberry, blueberry and strawberry to be sent out as educational seed for those teaching in elementary through High School or home school environment. These items have also been requested by that conducting community garden education and various non-profit groups.

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