FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Cold hardiness of quince

PI:	Todd Einhorn	**Co-PI(2)**:	Joseph Postman
Organization:	OSU-MCAREC	**Organization**:	USDA/ARS
Telephone:	(541) 386-2030 x13	**Telephone**:	(541) 738-4220
Email:	Todd.einhorn@oregonstate.edu	**Email**:	joseph.postman@ars.usda.gov
Address:	3005 Experiment Station Drive	**Address**:	33447 Peoria Road
City:	Hood River	**City**:	Corvallis
State/Zip:	Oregon 97031	**State/Zip**:	Oregon 97333

Cooperators: Amit Dhingra, Kate Evans

Total Project Request: **Year 1:** $41,196 **Year 2:** $42,898 **Year 3:** $41,369

Other funding sources

Agency Name: National Plant Germplasm System
Amt. awarded: Requested amount of $12,192, Awarded amount, $9,750
Notes: USDA Plant Germplasm Evaluation Program approved funding March 19, 2010 to develop a precise system for determining the lowest survival temperatures of quince and pear accessions tested herein. We have successfully developed and implemented differential thermal analysis (DTA) techniques for measuring plant hardiness, and will be correlating those data with results from this project.

Budget 1 Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: Cynthia Cox**
Telephone: 541 737-3228 **Email address: cynthia.cox@oregonstate.edu**

Item	2009	2010	2011
Salaries	$18,000	$18,720	$19,469
Benefits	$10,942	$11,380	$11,835
Wages	$1,000	$1,040	$1,080
Benefits	$ 88	$ 92	$ 95
Equipment			
Supplies	$1,000	$1,500	$1,500
Travel	$500	$500	$500
Miscellaneous			
Total	$31,530	$33,232	$34,479

Footnotes: [1] **Salaries include ~ 50 % of a full-time Technician (salary and OPE) for project management, data collection, and equipment maintenance. Increases in years two and three reflect a 4 % rate increase.** [2] **Wages include approximately 90 hours of hourly labor @ $11/hr.** [3] **Travel is for one trip to the Plant Clonal Germplasm Repository, Corvallis, OR per year.**

Budget 2 Joseph Postman
Organization Name: USDA/ARS **Contract Administrator: Cynthia Cox**
Telephone: 541 737-3228 **Email address: cynthia.cox@oregonstate.edu**

Item	2009	2010	2011
Salaries			
Benefits			
Wages	$7,000	$7,000	$5,000
Benefits	$616	$616	$440
Equipment			
Supplies	$1,800	$1,800	$1,200
Travel	$250	$250	$250
Miscellaneous			
Total	$9,666	$9,666	$6,890

Footnotes: [1] **Salaries include 0.25 of a temporary part-time employee (8.8 % benefit rate) for sampling procedures Sept-April, and assistance in propagation of germplasm.** [2] **Travel is for one trip to the MCAREC, Hood River, OR per year.**

Objectives

1) Determine the depth of cold hardiness within the representative quince germplasm and identify changes in hardiness throughout dormancy and early and late season non-acclimated tissue in each of three years (Einhorn: lab analyses, Postman: sampling management).

2) Root quince cuttings in year one and transfer to containers for de-acclimation studies in years two and three (Postman: rooting and transplanting, Einhorn: de-acclimation studies).

3) Determine the tissue zone most sensitive to freeze injury (Einhorn).

4) Determine the value of electrolyte membrane leakage chambers for high-throughput cold hardiness screening (Einhorn).

Significant Findings Sep 2009-Feb 2012

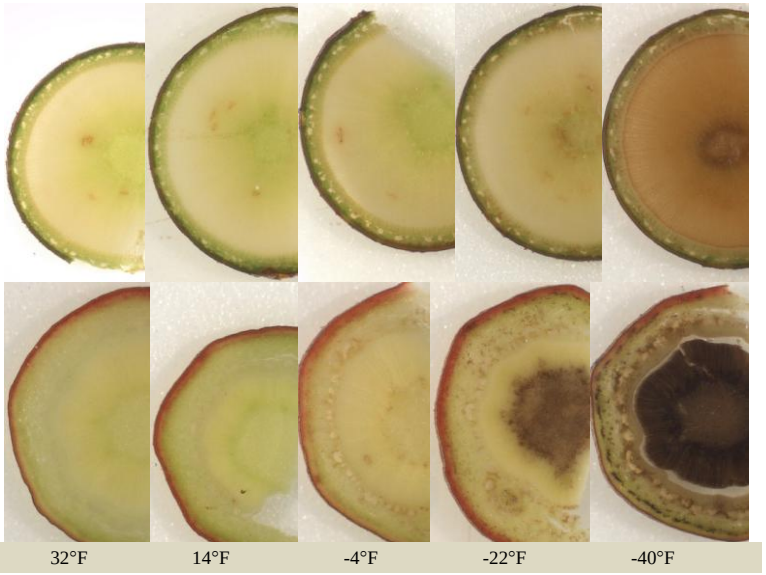
- Following cold acclimation 22 of the 56 quince accessions tested withstood -30 °C [-22° F] with less than 50% browning of tissues(i.e., data are means of three years).
- Two intergeneric hybrids (Quince x Pear, and Pear x Sorbus [i.e. Mt. Ash]) were hardy to -22 F.
- Three Amelanchier (Serviceberry) rootstock clones were hardy to -40 F, without observable tissue injury at peak hardiness. These clones have been budded to commercial pear cultivars and will be planted in trials spring of 2012.
- None of the pear accessions tested, including OHxF 87 and OHxF 97, and the scion clones 'd'Anjou', 'Bosc' and 'Bartlett' were hardy to temperatures below -22 F.
- In all years plants reached peak hardiness levels in December/January. Beginning in mid-late January, de-acclimation was evident as tissue injury was observed at higher temperatures.
- The cambial zone was observed to be more sensitive to cold injury throughout the measurement period, especially in early fall, and late spring, followed by xylem. Phloem developed the greatest hardiness in mid-winter. Differential thermal analysis (DTA) data confirmed these observations. We did not detect a low-temperature freeze event in phloem tissue within the measurement range (32 F to -50 F).
- The cold acclimation period from September through November varied among years. Frosts occurred earliest (October 6) in 2009. All years provided adequate acclimation conditions, though 2010 was fairly mild through mid-November. Irrespective, maximum cold hardiness levels were fairly consistent during December and January among test years (within 1 point on a six point tissue browning scale). No significant low temperature episodes were observed in any year, and it is likely that greater hardiness would be attainable in colder climates.
- Variation in rootability of different quince clones was high. Initial rootability tests were done using soft-wood cuttings. Many of the rootstock accessions (Pillnitz series, and Pigwa S series) were observed to root easily, while other fruiting clones did not.
- We have developed a DTA system to precisely identify low temperature freeze points of quince and pear tissue. Isolated tissue zones (phloem; xylem; pith) from stems showed that exotherms (i.e., freeze events) observed in the whole stem were related to freeze events in the xylem and pith, indicating these tissue zones as weak links to survivability.
- We have developed a novel grafting system to test survivability and regrowth of accessions following our controlled rate freeze tests. Evaluations will begin spring 2012.

Methods

Objectives 1 and 3: Mature, current season shoots from ten Pyrus (Pear) clones and 56 quince clones, were collected from trees located in the NCGR orchards (Corvallis, OR) and shipped next-day to

MCAREC. Tissue was sampled at ~three-four week intervals, beginning in late September, through bud-break (March-April), in each of three years (2009-2011). The protocol is briefly outlined below:

- Shoots were harvested from trees and shipped next-day to MCAREC. Upon receipt, samples were placed in 42° F storage, and sectioned into one-inch pieces. Samples were weighed, and their fresh weights recorded. Four replicate stem pieces per accession per treatment (i.e., temperature) were made. These replications also accounted for possible biological differences occurring within a shoot (i.e., rep 1 was always taken from the thicker, earlier growth at the basal portion of the one-year-old shoot, rep 2 with increasing distance toward the tip, rep 3 further, and rep 4 comprised the apical region, not including the terminal two inches of the shoot).
- Stem pieces were loaded into a programmable Tenney T2C Freeze Chamber, and subjected to freezing at a rate of 4° C per hour. Samples were removed following a one hour 'soak' at each of five treatment test temperatures (0, -10, -20, -30, and -40°C [32, 14, -4, -22, -40°F]), with the exception of the first sample period [Sep 2009], when samples were subjected to 0, -10, -25, and -40° C to account for a shortage of shoot material. Each of the four replicates was run on a separate date.



- Upon removal from the freeze chamber, stem samples were placed in sealed plastic bags with moistened paper towel, and allowed to incubate at room temperature for one week prior to microscopic evaluation.
- Transverse sections of stems were made midway into the one-inch sample, placed under a stereomicroscope, and individual tissue zones (phloem, cambium, and xylem) were rated according to the degree of oxidative browning observed using a six point scale, where 1, no damage [white]; 2, no damage [off-white]; 3, ~ 25% area lightly browned; 4, ~ 50% area browned; 5, >75% area browned; 6, 100% completely oxidized [black]. Visual assessment of freeze injury was performed by one technician, and all samples were prepared and rated in a double blind manner. The lowest exposure temperature which resulted in the absence of any observable levels of injury (i.e., a rating < 4) was termed the temperature prior to incipient damage. Photo shows examples of microtome sections under magnification of one cold-hardy quince accession (above) and OHxF 87 (below), following freezing and incubation. Browning within different tissue zones occurs with decreasing temperatures.
- Following analyses, sample pieces were dried in an oven at 70°C and weighed until a constant weight was attained (i.e., dry weight). Relative water content was derived from fresh and dry weights as, [(Fresh Weight - Dry Weight)/Fresh Weight] *100

Objective 2: In late May and early June, 2009, softwood cuttings were taken from 53 quince clones and one clone each of *Pyronia veitchii* (Pyrus x Cydonia) and *Sorbopyrus auricularis* (Sorbus x Pyrus), with the goal of generating 10 self-rooted trees of each. Sixteen cuttings were initially made for each genotype. Each cutting contained at least 3 nodes (~ 6 cm), and the base was dipped in a powdered rooting product containing 0.8% IBA before sticking in Oasis® Rootcubes and rooted

under mist with bottom heat to keep media temperature at about 24° C. For genotypes that failed to thrive or produce any roots after 4-6 weeks, a second set of cuttings was made in July, 2009.

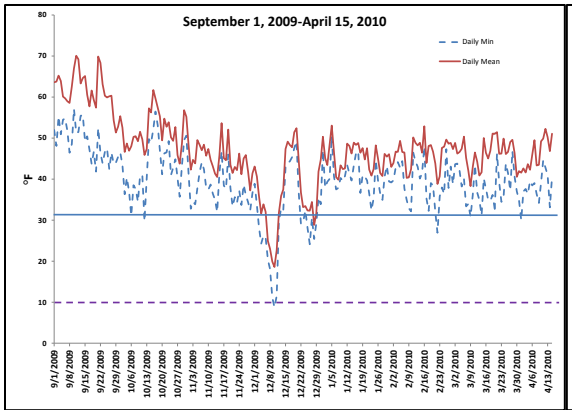
We have developed a novel system to test survivability and regrowth following our controlled rate freeze tests. Four-node shoot pieces of several accessions were subjected to test temperatures of 32, 14, -4, -22, and -40 F (as described above). Upon removal from the freeze chamber, shoot pieces were cut in half; one half was incubated and evaluated for tissue browning (according to methods described above), the other half was stored at 38 F and will be grafted in late February 2012. Quince accessions will be grafted to Quince A rootstock (to avoid incompatibility). OHxF 87 was used as a control and will be grafted to OHxF 87 rootstocks. All grafts will be potted and placed in a greenhouse for evaluation of spring budbreak (date) and weekly measurement of shoot growth, throughout 2012. Shoot initiation and growth of buds from intact two-node shoot sections will provide a direct assessment of freeze injury to correlate with oxidative browning and DTA data.

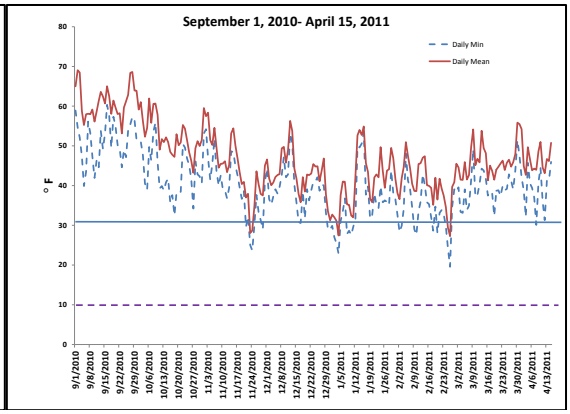
Objective 4: Due to the previously determined precision of differential thermal analysis (DTA) we have substituted this technique for the electrolyte leakage studies that we originally proposed to develop. We leveraged funding awarded from the USDA to design a DTA system for detection of plant kill points (acknowledgements to Dr. Markus Keller's lab group at WSU-Prosser for sharing their experience and insights on DTA system design). The system was modified (Dr. David Gibeaut, OSU-MCAREC) from Mills et al. (2006). Briefly, shoots were harvested and shipped as described above. Samples were cut, wrapped first in a piece of damp paper towel then aluminum-foil, placed on thermo-electric modules (TEMs) and subjected to a 4 °C per hour freeze rate. The TEMs were wired into a data acquisition system (Keithley 2700-DAQ-40; Keithley Instruments, Cleveland, OH, USA) and data were recorded at 30 second intervals. The exotherm (heat release) associated with the phase change of water produces a temperature gradient across the TEM resulting in a voltage output (y-axis) that was plotted against temperature (x-axis). Temperature was measured via a thermocouple placed on a reference TEM without tissue. The height of the low temperature exotherm peak was then used to identify the temperature associated with the freeze event.

Results and Discussion

Objectives 1 and 3: Early fall 2010 temperatures recorded near the NCGR field site did not decline as quickly, nor did they result in frosts as observed in either 2009 or 2011 (Fig 1). In fact, no frost events were recorded prior to November 21, 2010, as compared to the light frosts observed on October 6 and 12, 2009, and October 30, 2011. A seasonal, gradual progression of declining minimum and mean temperatures followed by hard freeze events as observed in 2009 and 2011 are conducive to cold acclimation, a process by which plants acquire hardiness through exposure to increasingly lower temperatures, albeit, in the Willamette Valley (NCGR) this process occurs later than in most pear growing regions of the PNW. Mid-winter temperatures varied substantially among years (Fig 1). The only significant low temperature (<10F) episode was observed in December of 2009. Despite the differences among years and overall lack of cold, no consistent patterns emerged in the hardiness of the accessions tested among years. It is plausible that when established in colder environments, significant gains in hardiness would be observed.

For each sample date, we have determined the warmest temperature at which injury was observed (temperature of incipient damage), and report minimum hardiness level as that temperature which immediately preceded the temperature of incipient damage [i.e., lowest exposure temperature resulting in < 50% browning]; a point that is highly debatable since anecdotal evidence from cutting wood following winter freeze events suggests that pear re-growth the following spring is not impaired at such light browning levels. Subsequently, our estimates of maximum hardiness are extremely conservative.





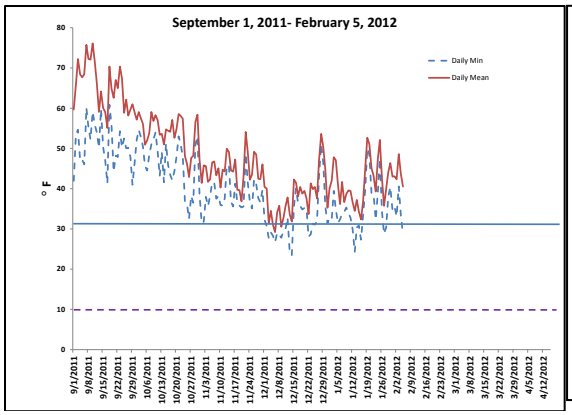


Figure 1. Daily mean and min temperatures (°F) from Sep 1, 2009 - April 15, 2010 (top left), Sep 1, 2010 - April 15, 2011 (top right), and Sep 1, 2011 - February 5, 2012 recorded at the Hyslop farm located ~ 6 miles N.E. of the NCGR quince site. Horizontal solid line denotes 32°F. Horizontal hashed line represents previously published threshold temperature necessary for woody plants to attain maximum hardiness levels, when exposed for period lasting several weeks.

We have identified a group of cold hardy taxa which have consistently performed well (<50% browning) following freeze tests. This group consists of 22 quince selections, 2 intergeneric hybrids, and 4 Amelanchier clones that were equal to, or hardier than the cold-hardy pear genotypes tested as controls (Tables 1-4). Data are maximum hardiness levels from December/January samples, and are means of three years of data. We intend to continue work with this subset of cold hardy selections, should funding be provided in 2012.

Following December sampling, maximum hardiness values decline, until hardiness is completely lost in April (not shown). As the season progressed, cambial tissue (meristematic tissue responsible for cellular division, lateral trunk growth and ultimately new xylem and phloem tissue) appeared to be consistently more sensitive to sub-freezing temperatures than either of the vascular tissues [i.e., phloem or xylem] (data not shown). At the maximum hardiness level [December] phloem tissue was hardier for most accessions, but differences between oxidative browning ratings for xylem and phloem rarely exceeded 1. Interestingly, DTA tests have shown that freeze events in the samples are occurring in the xylem and pith, and not in the phloem (Fig 2). This freeze resistance strategy has been documented in other Rosaceae species plants. It appears that phloem tissues avoid cellular freezing by a mechanism which facilitates the migration of water out of cells to extracellular ice. Accordingly, ice crystals in extracellular spaces will 'pull' water from cells, since the vapor pressure of water is higher over liquid than ice at the same temperature (free energy theory dictates that water will move down the energy gradient from a higher (liquid) to lower (ice) energy status. Further, the osmotic concentration of the cells is increased. This process, in turn, lowers the freeze point. In these cases, damage can be incurred by dehydration. Xylem, on the other hand, tolerates subfreezing temperatures by supercooling. However, supercooled liquids eventually freeze, as was observed near -38 F (Fig 2).

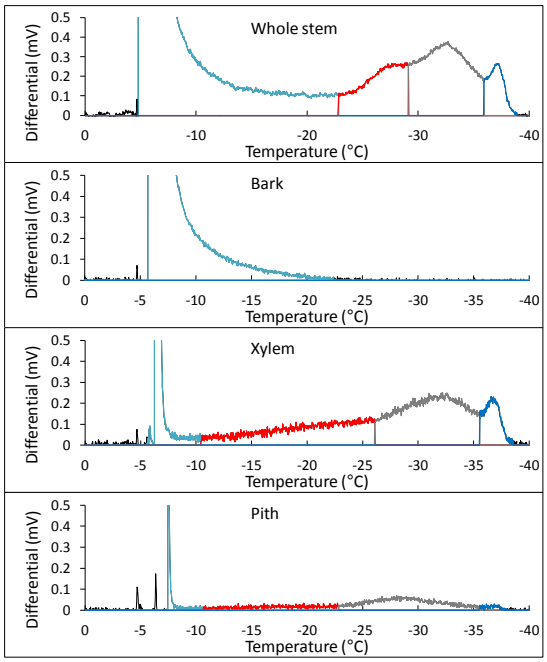


Figure 2. Differential thermal analysis (DTA) of quince cultivar "WF-17". Analyses were performed on first year stems cut from trees during their period of greatest cold hardiness. Bark samples included all tissues from the cambium outward. Xylem was stripped of all cambium then split lengthwise avoiding all pith. Pith was dissected by splitting stems lengthwise in four planes leaving a small amount of xylem.

Because the increment between measurement points is 10°, and in several cases the first temperature at which injury is detected results in quite significant browning (i.e., much higher levels of injury [score of 4-6]), the data reveal little about the qualitative nature of the temperature of incipient damage. Representative data collected from the quince accession 'Aiva from Gebeseud' is provided in Figure 3 to illustrate this point, and shows how hardiness is gained and lost (Fig 3). For example, in December oxidative browning was barely evident at -30 °C, and completely black (fully oxidized) at -40 °C (Fig 3). Once tissue damage is observed, we are unable to define whether the actual injurious event, or kill point, occurs following a 1° or a 9° lowering of the temperature from the previous test temperature.

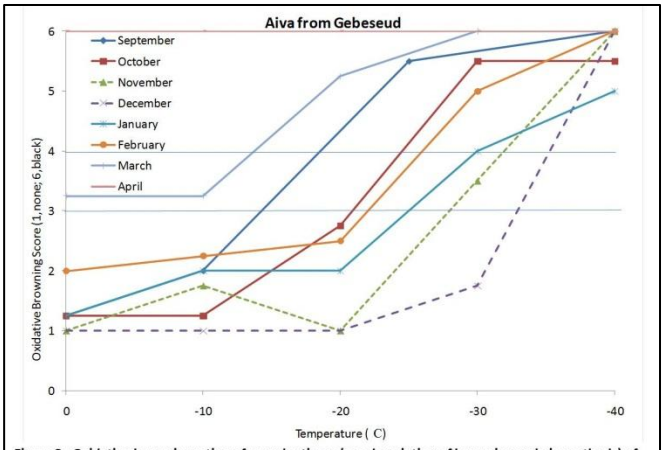
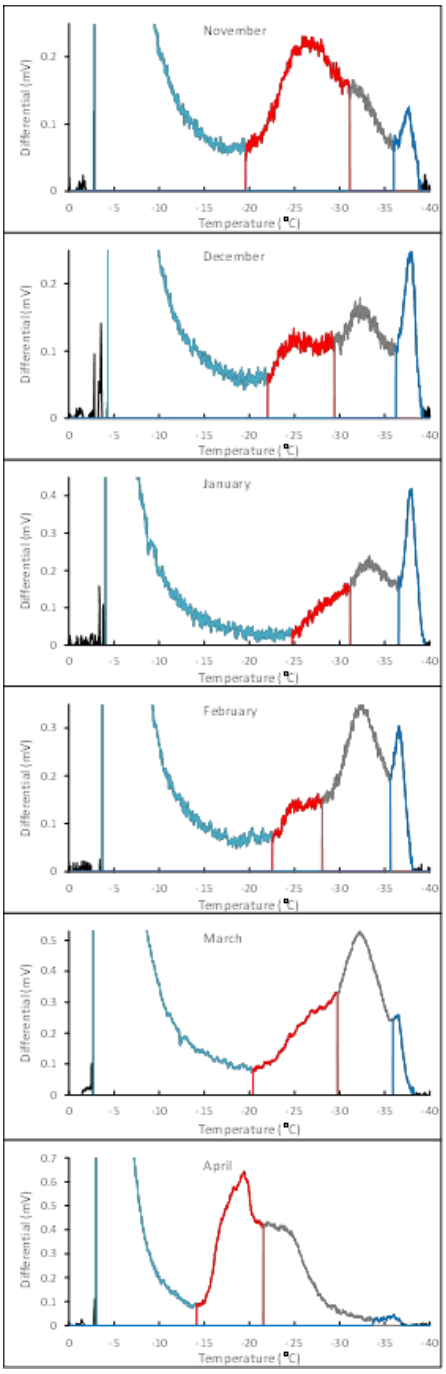


Figure 3. Oxidative browning rating of vascular tissue (see description of browning scale in methods) of the quince accession 'Aiva from Gebeseud' from Sep 2009-April 2010. Horizontal line on y-axis at 4, signifies the threshold for injury (i.e., ,50% tissue area lightly browned).

Overall, results are very encouraging. A large group of quince taxa exist with the apparent capacity to acclimate and attain sufficient levels of cold-hardiness for many regions of the PNW, and these data are supported from three years of sampling. Additionally, previous reports have suggested that full expression of hardiness is associated with exposure to temperatures below 10° F for several weeks (hashed line in Fig 1). Temperatures at the test orchards did not attain these values for any extended period of time, indicating that greater cold tolerance is entirely possible when planted in colder climates.

Objective 2: Roughly 300 rooted cuttings have been established from 31 quince genotypes (~half of the sample population) having an average of 8 rooted cuttings per clone. Plants were budded with one bud of 'd'Anjou' and one of 'Bartlett' for preliminary information regarding compatibility. However, symptoms expressed in shoots of 'Bartlett' suggested that the 'Bartlett' budwood was virus-infected Consequently, results are not reliable. Rooting of soft-wood cuttings varied substantially (data not shown). Further work will be required to develop effective hardwood, softwood and tissue-culture protocols to determine ease of propagation of these taxa.

Objective 4: DTA data show changes in hardiness behavior of an open pollinated quince seedling, Akhtubinskaya- Volgograd, Russia (Fig 4) from December through April. The first peak (occurring at



~ -5 °C) signifies the freezing of extracellular water in the tissue, and is termed the high temperature exotherm. It is a non-lethal, non-injurious event. The next series of peaks represent cellular freezing (low temperature exotherms), and these are highly associated with injury of tissue and, in the case of the low temperature exotherm at -38 °C, likely tissue death. Preliminary anatomical observations indicate that these separate freeze events appear to be unrelated to specific tissue zone injury, but further work is required to understand these events. Across the population of taxa evaluated, the low temperature exotherm consistently occurs near -40C, however intermediate peaks vary with cultivar. The changes as taxa acclimate in the fall, attain maximum hardiness in mid-winter, and de-acclimate in the spring agree with our oxidative browning results (data not shown in final report, but reported in earlier continuing reports).

We intend to align DTA data with results from regrowth assays this spring to gain a better understanding as to the nature of these freeze events.

Figure 4. Differential thermal analysis (DTA) of quince cultivar "Akhtubinskaya O.P. seedling - Volgograd, Russia". Analyses were performed on first year stems cut from trees approximately 30 days apart during the critical periods of dormancy, cold hardiness and break of dormancy.

Tables 1-4. The lowest temperature sustained resulting in oxidative browning levels less than 50% for all accessions tested, at their maximum hardiness level. Maximum hardiness levels were observed between December and January sampling periods in all years evaluated. Accessions highlighted in grey are those suggested to withstand most PNW production region climates.

Amelanchier (Serviceberry) accessions	Hardiness Temperature (°F)
A-2	-40
A-7	-40
A-10	-40

Intergeneric hybrid accessions	Hardiness Temperature (°F)
Pyronia veitchii (= IGC 9)	-22
Sorbopyrus 'Smokvarka'	-22

Pyrus (Pear) accessions	Hardiness Temperature (°F)
Harbin (P. ussuriensis) (cold hardy)	-22
Krylov (cold hardy)	-22
Anjou	-22
Bosc	-22
OHxF 97	-22
OHxF 87	-22
Bartlett (Hood River)	-22
Lesnaia Krasavitza (cold hardy)	-4
Bartlett (Corvallis)	-4
Pyrus pashia	14
Pyrus koehnei	14

Cydonia (Quince) accessions	Hardiness Temperature (°F)
Tashkent AR-232 seedling 2 (A)	-22
Tashkent AR-232 seedling 4 (B)	-22
C. oblonga - Arakseni, Armenia	-22
C. oblonga - Megri, Armenia	-22
Skorospelka O.P. seedling	-22
Aiva from Gebeseud	-22
C. oblonga - Seghani, Armenia	-22
Akhtubinskaya O.P. seedling (B)	-22
Akhtubinskaya O.P. seedling (A)	-22
Bereczki [Beretskiquitte]	-22
C. oblonga - Babaneuri, Georgia	-22
Kashenko No. 8	-22
Krukovskaya O.P. seedling	-22
Quince A	-22
Quince C7/1	-22
Quince S	-22
Quince W	-22
Teplovskaya O.P. seedling	-22
Trentholm	-22
Van Deman	-22
W-4	-22
WF-17	-22
Aiva from Kara-Kala No.9	-4
C. oblonga - Alema, Armenia	-4
C. oblonga - Dusheti, Georgia	-4
Cooke's Jumbo	-4
Ekmek	-4
Fontenay	-4
Karp's Sweet Quince - Majes Valley, Ara	-4
Kaunching	-4
Kichikara Dede 88-1 (virus?)	-4
Kichikara Dede 88-2	-4
Krimskaya	-4
Kuganskaya	-4
Le Borgeot	-4
Limon	-4
Maslenka Rannaya O.P. seedling	-4
Meech's Prolific	-4
Pigwa S-1 - Poland	-4
Pigwa S-2 - Poland	-4
Pigwa S-3 - Poland	-4
Pillnitz 1	-4
Pillnitz 2	-4
Pillnitz 3	-4
Pillnitz 5	-4
Pineapple	-4
Portugiesische Birnquitta	-4
Provence (BA 29-C)	-4
Quince E	-4
Quince Evalina	-4
Seker Gevrek	-4
TE-2-73	-4
Tekes	-4
Tencara Pink	-4
Yuz-Begi 89-1	-4
Zeakli 89-1	-4

Executive Summary
Over a three-year period, shoots of fifty-seven quince, two intergeneric hybrids, and ten pear accessions were tested monthly in a programmable freeze chamber to characterize freeze resistance and hardiness. Shoots were sampled from *in-situ,* own-rooted trees located at the USDA NCGR in Corvallis, Oregon between September and April of each year. In addition, we tested three Amelanchier rootstock clones developed in Germany, and maintained in containers at the OSU Mid-Columbia Agricultural Research and Extension Center in Hood River, Oregon.

Oxidative browning assays and differential thermal analysis (DTA) were used to quantify freeze events and injury sustained following controlled rate freezing experiments. All accessions acclimated and reached maximum hardiness levels in mid-December to early January, though the range of hardiness varied markedly among the sample population. Twenty-two of the quince accessions and both intergeneric hybrids tolerated -22°F without significant tissue browning (<50% browning) during the maximum hardiness period. The three Amelanchier clones tolerated -40°F with <50% tissue browning. Importantly, these results were consistently observed in all years. No pear accessions were capable of attaining greater hardiness levels, based on degree of browning or DTA. Commercial pear rootstocks, OHxF 87 and OHxF 97, tested hardy to -22°F.

Cambium tissue was observed to be slightly more sensitive than xylem or phloem throughout the test period, but DTA showed that freeze events in the stem were related to freezing in the xylem.

We will evaluate survivability and regrowth of shoots subjected to a range of sub-freezing temperatures using a novel grafting assay. Budbreak and growth rate analyses of grafts will be related to oxidative browning and DTA.