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

Project Title: Field evaluation and propagation of novel cold-hardy quince rootstocks

PI: Todd Einhorn
Organization: MSU
Telephone: 517-353-0430
Email: einhornt@msu.edu
Address:1066 Bogue St
Address 2:Soil Science Building
City/State/Zip: East Lansing/MI/48824

Co-PI (2): Stefano Musacchi
Organization: WSU-Wenatchee
Telephone: 509-663-8181 ext. 236
Email: stefano.musacchi@wsu.edu
Address: TFREC
Address 2: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI(3): Yongjian Chang
Organization: North American Plants, Inc.
Telephone: 503-474-1852
Email: ychang@naplants.com
Address: 9375 SE Warmington Rd.
Address 2:
City/State/Zip: McMinnville/OR/97128

Co-PI (4): Kelsey Galimba
Organization: OSU
Telephone: 541-386-2030 Ext. 38218
Email: kelsey.galimba@oregonstate.edu
Address: MCAREC
Address 2: 3005 Experiment Station Drive
City/State/Zip: Hood River/OR/97031

Cooperators: Sara Serra, Steve Castagnoli, USDA-NCGR curator (tbd), Adam McCarthy, Stemilt

Total Project Request: **Year 1:** $89,508 **Year 2:**$93,636 **Year 3:** $97,684

Other funding sources: **None**

WTFRC Budget: ***None***

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator: Dan Arp**
Telephone: 541-737-4866 **Email address: dan.j.arp@oregonstate.edu**
Station Manager/Supervisor: **Email Address:**

Item	2021	2022	2023
Salaries	8,000	8,400	8,820
Benefits[1]	6,800	7,140	7,497
Wages[2]	2,850	2,993	3,142
Benefits	285	299	314
Equipment			
Supplies	500	500	500
Travel[3]	2,172	2,192	2,213
Cold storage fees[4]	375	386	398
Plot Fees[5]	5,000	5,000	5,000
Total	25,982	26,910	27,884

Footnotes:
[1] Benefits were calculated from actual OPE rates (20% of OSU technician). An annual increase of 5% was applied to years 2 and 3.
[2] Wages are for part-time employee to help with general maintenance during the season; 190 hours at $15/hr. Part-time employee benefits are calculated at 10%.
[3] Travel is to cover mileage to plot for measurements and one trip per year (4 days) for Einhorn (total $1,500) to travel to plots to perform pruning and training tasks and meet with K. Galimba and S. Musacchi and grower

collaborators (airfare was estimated at $750 roundtrip, three nights hotel ($100/night), car rental ($400) and per diem ($60/day).
[4] Cold storage fees are for 3 months at $125 per month with 3% annual increase.
[5] Plot fees are to compensate growers for land, resources and fruit.

Budget 2
Organization Name: WSU **Contract Administrator:** Kathy Roberts, Shelli Tompkins
Telephone: (509) 293-8803 **Email:** katy.roberts@wsu.edu, shelli.tompkins@wsu.edu
Station Manager/Supervisor: **Email Address:**

Item	2021	2022	2023
Salaries	$ 25,133	$ 27,339	$ 29,445
Benefits	$ 9,048	$ 9,842	$ 10,600
Wages	$ 6,000	$ 6,000	$ 6,000
Benefits	$ 1,345	$ 1,345	$ 1,345
Equipment			
Supplies	$ 9,000	$ 9,200	$ 9,410
Travel	$ 3,000	$ 3,000	$ 3,000
Plot Fees			
Miscellaneous			
Total	$ 53,526	$ 56,726	$59,800

Footnotes:
1 Salary for a 6 months of a Research assistant ($4,000/month) (Musacchi)
2 Benefit on salary at 36%
3 One non-student temporary for 10 wks: 40hrs/wk at $15/hr (Musacchi).
4 Benefits on temporary at 22.4%
5 Labware/consumable, fruit sample reimbursement (Musacchi)
6 5,217 miles/year for domestic travel (0.575$/mile) to go to the orchard.

Budget 3
Organization Name: North American Plants, Inc. **Contract Administrator:Yongjian Chang**
Telephone: 503-474-1852 **Email address:** ychang@naplants.com
Station Manager/Supervisor: **Email Address:**

Item	2021	2022	2023
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies[1]	$10,000	$10,000	$10,000
Travel			
Plot Fees			
Miscellaneous			
Total	$10,000	$10,000	$10,000

Footnotes:
[1]Consumables, reagents, nutrients, hormones, storage of cultures, pots, substrate, etc.

Significant Findings:

Objective 1: Continue to evaluate vegetative and fruiting performance of Bartlett and d'Anjou pear trees on nine quince rootstocks in current field performance trials (WA and OR).

- **Based on growth habit, vigor, canopy balance, precocity and production during the first four cropping years (2023 was the 4th crop), roughly half of these rootstocks continued to perform well. Genetic testing of all accessions indicated that several of the 20 accessions (this includes the newly propagated accessions in Obj 2) have a high degree of genetic similarity (via finger printing analysis). These analyses were performed on leaves sampled from suckers at the Entiat, WA site and tissue cultured rootstocks at NAP which were derived from shoot tips collected at the Clonal Germplasm Repository in Corvallis, OR.**
- **Our multi-site trial facilitated a comparison between two very different sites (soil and climate) on tree growth and production. The shorter, relatively cooler growing region of Parkdale with heavier more fertile soils produced trees that were 50-100% larger than their counterparts in WA.**
- **High performing 'D'Anjou' trees on size controlling quince rootstocks in both OR and WA produced between 20 and 40 bins per acre in 2023. This was nearly double the yields of 2022. Production is based on 1210 trees/acre which is the planting density of the trial. Fruit size for these combinations ranged from poor (152 g) to excellent (271 g) and varied considerably between and within sites. Most accessions produced box counts of 80s to 90s in OR and 90s to 100s in WA.**
- **2023 yields of high-performing Bartlett trees on size controlling quince were slightly higher than D'Anjou in WA, resulting in ~ 30 to 50 bins per acre. Tree density was the same as Anjou. In OR, Bartlett yields were lower; trees produced roughly ~50 fruit per tree (on average) equating to 30 bins per acre. Fruit size was generally good (220 g and 190 g on average in OR and WA, respectively).**
- **In the existing trials, Comice serves as the interstem between the quince rootstock accessions and the scions (Anjou or Bartlett). Comice is regarded as having good compatibility with quince rootstocks, in general; however, pear scions do differ in their relative compatibility with quince. Thus, the poor performance of a few rootstocks could be attributed to interstem issues (i.e., incompatible with Comice). This is further supported by their differential behavior when direct-grafted to either Bartlett or Anjou. For example, 99.002 had more vigor in OR for both pear scions without an interstem as compared to trees with Comice interstem.**
- **Trees are maintainable in their 3 ft in-row spacing, even with Amjou in the fertile Parkdale site, with the exception of a few accessions. Pruning of Bartlett trees was mostly by short-pruning. Anjou trees received a combination of short and long pruning techniques. Trees were trained to a spindle architecture with very narrow canopies that were slightly pyramidal in form. All large limbs (~50% of the trunk diameter) were removed with renewal cuts to encourage weak replacement shoots.**

Objective 2: Determine the propagation potential of the remaining 11 cold-hardy quince clones that could not be tissue-cultured and successfully micropropagate them for new field performance trials

- **All cold hardy quince selections that were not previously tissue-cultured were successfully micropropagated from shoot tips in 2022. These represent diverse germplasm of cold hardy and plausibly dwarfing pear rootstocks and include the three hardiest quince taxa of the entire germplasm collection. Rooting of a sufficient number**

of each selection to facilitate new tree production for future field-performance trials is underway.

Results and Discussion:

Objective 1: Continue to evaluate vegetative and fruiting performance of Bartlett and d'Anjou pear trees on nine quince rootstocks in current field performance trials (WA and OR), and successfully micropropagate the remaining 11 cold-hardy quince selections for establishment in new field performance trials.

Confirming the genetic identity of selections (i.e., true to type)

I would like to begin with an accounting of the philosophy and methodology applied to this project which, like any other germplasm exploration, comprises an inherently high degree of risk. The original research that defined the cold hardiness of quince accessions at the National Clonal Germplasm Repository in Corvallis, OR was conducted by PI Einhorn many years ago. From that research, ~20 quince accessions were selected based on their performance over three consecutive years throughout their dormancy transitions (September through April). The NCGR furnished material of these selections to NAP. NAP was successful in micropropagating half of the accessions, initially. These tissue-cultured and subsequently rooted explants were then supplied to Helios nursery. Helios nursery planted these in an OR liner field and grafted them with Comice interstems, later budded Bartlett and Anjou, raised the trees for two years, dug them and sent them to Einhorn in OR. Einhorn divided the trees and sent half to Musacchi in WA. The trees were then planted in their respective sites with appropriate experimental designs. Performance during the first few years of some accessions (dwarfing, growth habit, precocity, yield, and fruit size) showed very good potential. We remained cognizant, however, of the many potential issues facing quince, in addition to cold tenderness, when used as pear rootstocks. Several of these horticultural challenges (decline, incompatibility, fire blight, iron deficiency/chlorosis, etc.) can require many years of field testing (possibly beyond the timeframe of this project) before enough confidence could be gained to advance any promising selections to a subsequent round of testing (i.e., small scale commercial plots). Based on our collective experience with quince and the timeline of this project, we were purposeful not to prematurely 'release' promising selections to commercial entities; an approach intended to avoid scenarios that would cost the industry far more money/resources than the funding already received for the project or the interest we collectively share for identifying a dwarfing, productive pear rootstock. After observing strikingly similar performance and growth of scions on several rootstock selections over several years, we decided to collect leaves from rootstocks suckers in WA as well as from all tissue culture jars at NAP, which originated from the NCGR, to confirm their genetics. Material was sent to an external molecular laboratory specialized in fingerprinting by SSR markers in a blind experimental design that included standard quince rootstocks (i.e., Quince A [from two sources; US and Europe], Quince C, Quince BA29C, Quince Sydo, etc.). The CYD accessions 57.001 and 65.001 were reported to have a high level of genetic similarity (i.e., they are likely identical). 22.001, another promising rootstock, tested as genetically similar to Quince MA, a standard quince. A second year of fingerprinting provided confidence as to individual accession origin, as far as could be traced to the NCGR. All accessions from the project are listed in Table 6 with an explanation of their origin and our recommendation based on fingerprinting and/or performance data to stop future development or continue trialing. Despite issues in traceability back to the NCGR collection in Corvallis, there were no mixups in the field plantings of these trials, despite the many transfers of material from the

inception of tissue culturing to the field trials. This was confirmed by having representation of several replicates of each treatment in the assays.

Mortality

Mortality has been documented in previous reports as the average percent survival for each combination. 68.002 had the highest proportion of dead trees with both scions after approximately 4 years from planting (~50%). The accessions 118.001 and 99.002 also experienced mortality between 35 and 60%. For high-performing combinations, additional mortality after that observed in the establishment year was not observed, at either site. Regarding combinations without an interstem, Anjou/99.002 (direct graft) had the highest incidence of tree failure (83%), while Bartlett/99.002 (direct graft) had 0% mortality in WA. Detailed mortality data from WA is shown in Table 2. These data support a future evaluation of compatibility in order to determine the optimal pear interstem for these rootstocks.

Pruning

Dormant pruning of the Entiat, WA and Parkdale, OR plots was conducted in March and April 2023, respectively. The same methodology as reported in the previous years was executed in each plot. For Anjou, some significant differences emerged when comparing the average pruning weights (as kg per tree) among the 9 combinations in trial with Comice as interstem; Anjou/Comice/99.002 had greater than 2 kg per tree of pruned wood (Table 1), which was significantly greater than all other combinations and agrees with trunk measurements (Table 2) and results from previous years. At the other extreme, Anjou/Comice/68.002 and 67.001 produced $1/6^{th}$ of the pruning weights; these data also aligned with the tree size (as measured by trunks). In OR, pruning weights and trunk size were also the lowest for this combination. For Bartlett, no differences among combinations were observed for average pruning weight in 2023 as shown previously, but clear differences emerged for cumulative pruning weights over the life of the planting (Table 1) and was supported by trunk measures (Table 2). Most combinations had good vigor and produced a similar weight of pruning wood with Bartlett/Comice/118.001 and 68.002 having markedly lower vigor both in pruning weight and trunk size (Tables 1 and 2). At either end of the spectrum, similar observations were seen in OR, suggesting that despite vast differences in climate, very vigorous and very weak genotypes were performing similarly.

In OR, three years of corrective short-pruning facilitated a return of fruiting wood close to the central leader in Bartlett (Photo 1). Despite the characteristic vigor of Anjou, canopies have been maintained in a planar configuration with ample fruiting wood and do not exceed their allotted 3 ft. of in-row space (Photo 1).

Table 1. Pruning wood weight (kg/tree) on March 8th, 2023 and cumulated 6-year pruned wood weight (kg/tree) from 2018 to 2023. All trees are scions of d' Anjou or Bartlett with a Comice interstem and roots belonging to the quince accessions for the present study. Direct graft combinations of scions on quince rootstocks were excluded. The means are averages of 3 replications per combination. Significance, *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. ***The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow in ALL talbes).***

Cultivar	Rootstock	N=	Pruned wood weight (kg/ tree) in 2023		Pruned wood weight (kg/ tree) in the past 6 years (∑2018-2023)	
Anjou	22.001	3	0.93	BC	3.60	BC
Anjou	23.001	3	0.69	BC	3.65	BC
Anjou	57.001	3	0.83	BC	3.95	B
Anjou	65.001	3	0.82	BC	4.02	B
Anjou	67.001	3	0.37	C	2.60	BCD
Anjou	68.002	3	0.42	C	1.43	D
Anjou	70.001	3	1.18	B	3.96	B
Anjou	99.002	3	2.43	**A**	6.35	**A**
Anjou	118.001	3	0.57	BC	1.89	CD
Significance			***		***	
Bartlett	22.001	3	0.69		4.28	AB
Bartlett	23.001	3	0.45		3.56	AB
Bartlett	57.001	3	0.75		4.56	AB
Bartlett	65.001	3	0.84		5.13	**A**
Bartlett	67.001	3	0.40		2.80	AB
Bartlett	68.002	3	0.26		1.54	B
Bartlett	70.001	3	0.97		4.35	AB
Bartlett	99.002	3	0.81		4.06	AB
Bartlett	118.001	3	0.13		1.48	B
Significance			NS		**	

Table 2. Trunk cross section area, TCSA and mortality (%) for d' Anjou and Bartlett in January and October 2023. All trees are scions of Bartlett or Anjou with a Comice interstem and roots belonging to the quince accessions for the present study. The means are averages of N trees per combination (N is varied in the experiment). Significance, *=p<0.05, **=p<0.01, ***=p<0.001. NS, not significant. The mortality, shown as percentage, were performed arcsin() transformation, before performing AOV analysis and post-hoc. Post-doc letters separations are by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. Note, one tree from Comice/99.002 and two from 118.001 were excluded due to the data missing or incorrect TSCA measurements, recorded Oct 2023.

Cultivar	Rootstock	N (for Jan 2023)=	N (for Oct 2023*)=	TCSA (cm^2) on Jan 2023		TCSA (cm^2) on Oct 2023		Tree growth (cm^2) between Nov 2021 to Jan 2023		Tree growth (cm^2) between Jan 2023 to Oct 2023		Mortality (%) on Oct 2023	
Anjou	22.001	22	22	23.47	BC	28.93	BCD	5.00	BC	5.45	AB	0%	B
Anjou	23.001	12	12	22.37	BC	26.09	BCD	4.48	BC	3.73	ABC	0%	B
Anjou	57.001	16	16	26.88	B	32.82	B	5.87	BC	5.94	AB	0%	B
Anjou	65.001	17	17	28.25	B	34.30	B	5.84	BC	6.05	AB	0%	B
Anjou	67.001	12	12	17.99	BC	20.54	CDE	3.03	BC	2.55	BC	0%	B
Anjou	68.002	5	5	12.01	C	12.81	E	1.29	C	0.80	C	64%	**A**
Anjou	70.001	29	29	24.00	BC	30.62	BC	6.84	BC	6.61	AB	26%	**A**
Anjou	99.002	17	17	43.05	**A**	51.11	**A**	13.37	**A**	8.06	**A**	60%	**A**
Anjou	118.001	7	7	16.60	BC	18.81	DE	2.20	BC	2.21	BC	36%	**A**
Significance				**		***		***		***		**	
Bartlett	22.001	24	24	17.50	AB	20.01	AB	1.90		2.51	AB	0%	D
Bartlett	23.001	12	12	19.05	AB	21.97	AB	1.22		2.92	AB	0%	D
Bartlett	57.001	15	15	19.93	AB	22.76	AB	2.09		2.84	AB	0%	D
Bartlett	65.001	17	17	22.07	**A**	25.60	**A**	1.91		3.53	**A**	0%	D
Bartlett	67.001	13	13	14.00	BC	15.59	BC	1.12		1.59	AB	0%	D
Bartlett	68.002	7	7	11.07	C	12.02	C	1.33		0.95	B	56%	**A**
Bartlett	70.001	25	25	18.89	AB	22.06	AB	2.53		3.17	AB	14%	C
Bartlett	99.002	**36**	35	20.19	AB	23.55	AB	2.13		3.02	AB	35%	B
Bartlett	118.001	**25**	23	8.69	C	9.68	C	0.85		0.72	B	34%	B
Significance				**		***		NS		***		***	

Photo 1. *Bartlett (left) and Anjou trees (right) in OR after April 2022 pruning.*

Bloom

The number of Anjou flower clusters per tree counted in spring 2023 was considered excellent, with most combinations having between 200 and 300 clusters per tree in WA (Table 3). Clusters in OR were much lower ranging from 50 to 120 per tree, which was more than half observed in 2022. Bartlett had fewer clusters than Anjou in WA, ranging from 35-80 per tree (Table 3), which was similar to OR (data not shown). No significant differences emerged among the 9 combinations, irrespective of cultivar, for bloom. The phenological status, full bloom (FB) and petal fall (PF) was recorded for both cultivars on May 3rd, 2023. Bartlett showed to be more advanced in its phenological stage, with six combinations of Bartlett in PF stage having 33% or more of the trees than Anjou (Table 3). For Anjou, all combinations were scored as at full bloom (FB) on May 3rd, 2023. There was no significant difference in phenological stage across all nine combinations for either Anjou and Bartlett.

Table 3. Flower clusters number and phenological status for Anjou and Bartlett were recorded on April 17th, and May 3rd 2023, respectively. All trees are scions of d' Anjou or Bartlett with a Comice interstem and roots belonging to the quince accessions for the present study. Direct graft combinations of scions on quince rootstocks were excluded. Significance, *=p<0.05, **=p <0.01, ***=p <0.001. NS, not significant. The count data fit normal distribution, then AOV analysis was applied. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow).

Cultivar	Interstem	Rootstock	N=	Flower cluster counts/tree 2023	Full bloom (% trees in that stage) 2023	Petal fall (% trees in that stage) 2023
Anjou	Comice	22.001	3	307	100	0
Anjou	Comice	23.001	3	297	100	0
Anjou	Comice	57.001	3	366	100	0
Anjou	Comice	65.001	3	267	100	0
Anjou	Comice	67.001	3	277	100	0
Anjou	Comice	68.002	3	322	100	0
Anjou	Comice	70.001	3	186	100	0
Anjou	Comice	99.002	3	250	100	0
Anjou	Comice	118.001	3	283	100	0
Significance				NS	NS	NS
Bartlett	Comice	22.001	3	38	100	0
Bartlett	Comice	23.001	3	55	67	33
Bartlett	Comice	57.001	3	80	100	0
Bartlett	Comice	65.001	3	50	67	33
Bartlett	Comice	67.001	3	66	67	33
Bartlett	Comice	68.002	3	63	33	67
Bartlett	Comice	70.001	3	77	67	33
Bartlett	Comice	99.002	3	35	33	67
Bartlett	Comice	118.001	3	76	100	0
Significance				NS	NS	NS

Productivity

2023 was the fourth cropping year from orchard establishment. Anjou was harvested in WA on 8/29 (roughly 2.5 weeks earlier than 2022) and October 3 in OR. Production of Anjou was higher than the previous year due to frost events and poor pollination conditions of 2022 (discussed in 2022 report). High performing 'D'Anjou' trees on size controlling quince rootstocks in both OR and WA produced between 20 and 40 bins per acre in 2023 (see WA yield data in Table 4; OR data are still being prepared). This was nearly double the yields of 2022. Production is based on 1210 trees/acre which is the planting density of the trial. While yield did not differ significantly among accessions, the mumber of fruit per tree did (Table 4). The largest and smallest fruit number/tree for Anjou was 95 pears for Comice/57.001 and 29 pears for Comice/99.002, respectively. Recall that 99.002 had the largest volume of pruning wood and produces an extremely vigorous tree; thus, even on quince rootstocks precocity and productivty can be compromised by roots imparting too much vigor to the scion. In the case of 57.001 (one of the four similar genotypes) there is a very nice balance of viogor (as seen by pruning weight or trunk size data) and productivity.

For Bartlett, 2023 yields of high-performing quince were slightly higher than D'Anjou in WA, resulting in ~ 30 to 50 bins per acre, based on 1210 trees per acre. In OR, Bartlett yields were lower; trees produced roughly ~50 fruit per tree (on average) equating to 30 bins per acre. Numerical but nonsignificant differences were observed among the accessions in the number of fruit per tree, ranging from Comice/65.001 with 84 pears per tree to Comice/68.002 having 52 pears per tree.

The average fruit weight was affected by the rootstock combination in Anjou showing an expected negative relationship with the number of fruit/tree; trees with higher crop load had smaller pears (Table 4). Anjou/Comice/57.001 and Anjou/Comice/23.001 had the lowest average fruit weights (152 g), while Anjou/Comice/99.002 had the largest fruit weight (246 g). However, no significant difference in average fruit weight in 2023 was found among Bartlett combinations. Fruit sizes in OR tended to be ~1 box size larger than WA (data will be presented at the review).

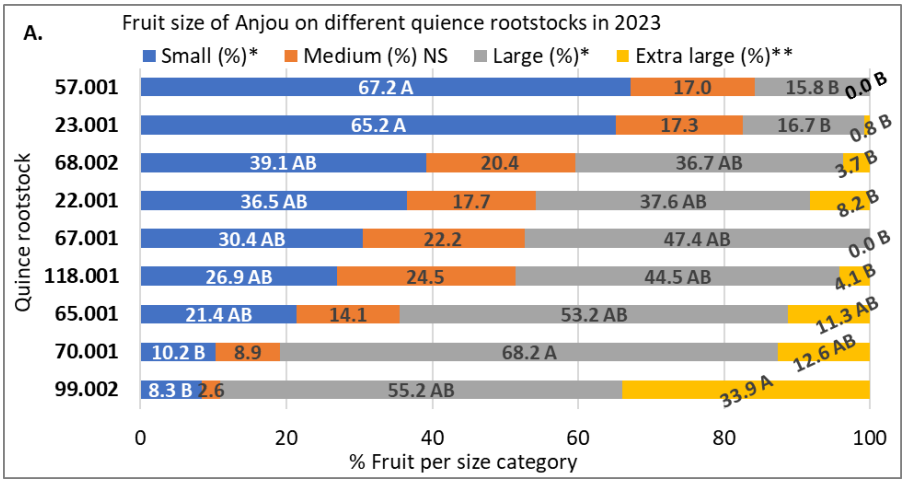
Table 4. Yield parameters for Bartlett and Anjou, with a Comice interstem grafted on quince accessions, on August 24th and August 29th, 2023 (harvest dates for Bartlett and Anjou, respectively). The means are averages of 3 trees per combination (N= 3). Significance, *p=<0.05, **p=<0.01, ***p=<0.001. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each parameter and column. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow).

Cultivar	Rootstock	N=	Fruit number per tree at harvest		Net tree yield (kg per tree)	Fruit weight (g)		Mton per acre	Mton per hectare	Average number of bins per acre
Anjou	22.001	3	57	AB	10.59	190	BC	12.82	30.93	32.11
Anjou	23.001	3	73	AB	10.53	152	C	12.74	30.75	31.92
Anjou	57.001	3	95	A	14.30	152	C	17.30	41.75	43.34
Anjou	65.001	3	44	AB	9.06	203	ABC	10.96	26.46	27.46
Anjou	67.001	3	71	AB	12.86	183	BC	15.56	37.54	38.97
Anjou	68.002	3	79	AB	13.73	181	BC	16.61	40.08	41.61
Anjou	70.001	3	49	AB	10.65	220	AB	12.89	31.10	32.28
Anjou	99.002	3	29	B	7.11	246	A	8.61	20.77	21.56
Anjou	118.001	3	81	AB	15.21	188	BC	18.40	44.41	46.11
Significance			*		NS	**		NS	NS	NS
Bartlett	22.001	3	74		14.14	188		17.11	41.28	42.85
Bartlett	23.001	3	75		13.53	182		16.37	39.51	41.01
Bartlett	57.001	3	70		12.03	191		14.55	35.12	36.46
Bartlett	65.001	3	84		17.35	209		20.99	50.65	52.58
Bartlett	67.001	3	68		12.18	183		14.74	35.57	36.92
Bartlett	68.002	3	52		9.59	201		11.60	27.99	29.06
Bartlett	70.001	3	77		16.40	213		19.85	47.90	49.72
Bartlett	99.002	3	64		11.40	178		13.79	33.28	34.55
Bartlett	118.001	3	57		9.53	176		11.53	27.83	28.89
Significance			NS		NS	NS		NS	NS	NS

Fruit quality

2023 pear grading by size with Aweta sorting line (WA): After harvest in 2023, pears from both varieties were sorted by an Aweta sorting machine, based on fruit weight (Fig. 1). Bartlett and Anjou fruits were both sorted on September 7, 2023 (9-14 days after harvest for Bartlett and Anjou, respectively). The size categories are small (<166 g, ≥ 120 pears/box), medium (166 g to 182 g, 110 pears/box), large (183-260 g, 80-100 pears/box, the optimum size preferred by the market) and extra-large (>260 g, ≤70 pears/box). For Anjou, small size fruit were found in higher proportions in combinations, Anjou/Comice/57.001 (67.2%) and Anjou/Comice/23.001 (65.2%), then followed by Anjou/Comice/68.002 (39.1%). The lowest proportion of small pear size was observed in Anjou/Comice/70.001 (10.2%) and Comice/99.002 (8.3%, Table 8). On the contrary, Anjou/Comice/99.002 had more extra-large size fruit (33.9%), followed by the combination Anjou/Comice/70.001 (12.6%,) and Anjou/Comice/65.001 (11.3 %, Figure 1A). The combination reporting the highest proportion of large fruit size (the optimum size), was Anjou/Comice/70.001 got more large-size fruit (68.2%), then followed by 99.002 (55.2%) and 65.001 (53.2%, Figure 1A). On the contrary, the lowest proportion of large fruit was found in Anjou/Comice/23.001 (16.7%) and

Anjou/Comice/57.001 (15.8%). No significant difference in the proportion of pear in the medium size was found across all nine accessions for Anjou (Figure 1A). In general, at least 80% of fruits for Anjou/Comice/70.001 and Anjou/Comice/99.002 were in the large and extra-large grade. However, this higher ratio of large size and extra-large size fruit in Anjou could be related to the crop load (49 fruits for Comice/70.001 and 29 fruits for Comice/99.001 in 2023 harvest). No specific rootstock effect was found for the proportions of pear fruit in each size category for Bartlett ($p>0.05$, Figure 1B). However, the highest proportions of large size fruit was found in Bartlett/Comice/65.001 (58.1%), followed by Bartlett/Comice/70.001 (48.9%), and Bartlett



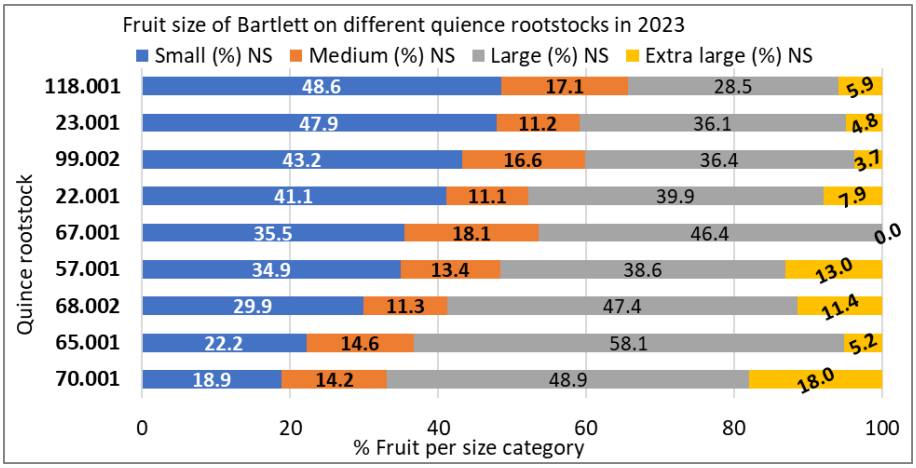


Figure 1. Fruit size distribution for 2023 harvest: **A.** Anjou with Comice interstem grafted on quince accessions, **B.** Bartlett with Comice interstem grafted on quince accessions. Fruit was harvested on Aug 24th, 2023 for Bartlett and Aug 29th, 2023 for Anjou and sorted on September 7th by the use of Aweta sorting machine. 'Small' means fruit weight < 166 g, 'Medium' between 166 g and 182 g, 'Large' between 183 g and 260 g, and 'Extra large' > 260 g. The percentage represents the average of three replications per combination (N= 3). Significance, *=p<0.05, **=p<0.01, ***=p<0.001. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters identify similar means for each 'fruit size' parameter. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity further investigations on 2023 samples are ongoing.

Pear fruit internal quality (2023 harvest)

After seven-day ripening at room temperature, Bartlett IAD was measured then destructive analysis was carried out on October 3rd and October 4th, 2023 (Table 5). Compared with 2022 harvest, 2023

harvest had a lower IAD values on the sorting day and after the ripening process (Table 5). The IAD value after the ripening process was low, ranging from 0.01 to 0.02 for the combinations, Bartlett/Comice/65.001, Comice/23.001, Comice/57.001, and Comice/67.001. The highest IAD mean after ripening was measured as 0.08 in Bartlett/Comice/68.002 (Table 5). Bartlett/Comice/68.001 showed the highest IAD values after the ripening in both years (Table 5). Fruit firmness values were higher in four combinations: Bartlett/Comice/67.001 and Bartlett/Comice/57.001, followed by Bartlett/Comice/118.001. Both Comice/67.001 and Comice/57.001 showed among the largest values for firmness in the two consecutive years in Bartlett (Table 5). SSC did not show a clear discrimination in 2023 for Bartlett (Table 5).

On October 11th and October 12th, 2023, Anjou was measured for all parameters as reported for Bartlett. Unlike 2022 harvest, no significant difference was found in IAD at sorting and IAD after seven days of ripening for 2023 harvest (Table 5). IAD values of some rootstock combinations decreased more after the ripening process, however, the IAD drop did not show differences between combinations, nor did firmness SSC showed significant differences among the Anjou combinations and, in particular, A/Comice/68.002 and A/Comice/118.001 confirmed to produce poor quality fruit in terms of SSC in both years.

Table 5. Internal quality fruit analysis for Anjou and Bartlett, crop 2023, index of absorbance difference (IAD), IAD after seven days, and IAD drop after the ripening process, firmness, and soluble solid content (SSC). All trees are scions of Anjou or Bartlett with a Comice interstem and roots belonging to the quince accessions under evaluation. Direct graft combinations of scions on quince rootstocks were not included in the quality analysis. Significance: *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$. NS, not significant. Post-doc letters separation by SNK for alpha= 0.05. Same letters within each column identify similar means for each parameter. Harvest days: Bartlett 8/24/23 and Anjou 8/29/23, Sorting days: Bartlett and Anjou, 9/26/23-9/27/23. Anjou selected pears of size 65-70 mm with an IAD ranging between 1.81 and 1.94. Bartlett selected pears of size 55-65 mm with an IAD ranging between 0.6 and 1.86. The CYD accessions 22.001, 23.001, 57.001, and 65.001 were reported in 2022 to have some level of genetic similarity, further investigations on 2023 samples are ongoing (yellow shadow).

Cultivar	Interstem	Rootstock	N=	I_{AD} at sorting	I_{AD} after 7-day ripening		I_{AD} drop		Average firmness (kg)		SSC (Brix, %)	
Anjou	Comice	22.001	3	1.80	1.68		0.12	A	7.53		16.35	AB
Anjou	Comice	23.001	3	1.80	1.69		0.11	A	7.47		16.73	AB
Anjou	Comice	57.001	3	1.80	1.67		0.13	A	6.78		17.41	A
Anjou	Comice	65.001	3	1.86	1.74		0.13	A	7.47		16.59	AB
Anjou	Comice	67.001	3	1.86	1.74		0.12	A	6.50		16.10	AB
Anjou	Comice	68.002	3	1.78	1.71		0.07	B	6.32		15.48	B
Anjou	Comice	70.001	3	1.77	1.68		0.09	B	7.07		15.64	B
Anjou	Comice	99.002	3	1.86	1.78		0.08	B	7.92		16.03	AB
Anjou	Comice	118.001	3	1.87	1.78		0.09	B	6.74		15.54	B
significance				NS	NS		***		NS		*	
Bartlett	Comice	22.001	3	1.47	0.04	AB	1.44		0.69	B	14.22	
Bartlett	Comice	23.001	3	1.31	0.01	B	1.29		0.88	AB	15.57	
Bartlett	Comice	57.001	3	1.19	0.02	B	1.17		1.00	A	14.88	
Bartlett	Comice	65.001	3	1.19	0.01	B	1.18		0.87	AB	15.31	
Bartlett	Comice	67.001	3	1.20	0.02	B	1.18		1.00	A	14.65	
Bartlett	Comice	68.002	3	1.33	0.08	A	1.25		0.92	AB	15.50	
Bartlett	Comice	70.001	3	1.27	0.07	AB	1.21		0.73	B	14.89	
Bartlett	Comice	99.002	3	1.55	0.06	AB	1.50		0.73	B	15.62	
Bartlett	Comice	118.001	3	1.24	0.03	AB	1.21		0.96	A	15.36	
significance				NS	**		NS		**		NS	

Objective 2: Determine the propagation potential of previously identified cold-hardy quince clones not included in the field trial described above (a total of 11 accessions).

After several attempts (2021 and 2022) to establish cultures, NAP has successfully cultured all of the missing accessions where material still exists at the NCGR in Corvallis, OR (10 of 11 original accessions) in sufficient numbers to begin generating trees for future rootstock trials. These include the top three cold hardy accessions previously not propagated due to challenges with media/material. Objective 2 is on schedule; tissue cultured accessions have been rooted so that tree generation of ~200 liners per accession can be produced and grafted/budded in a nursery for future trials.

Table 6. Summary Table of all quince rootstocks trialed in the field during the duration of this project or developed in tissue culture for future trialing. We recommend actions based on our findings and informed via genetic finger printing analysis according to 4 classification groups: A, Advance to next trial phase; B, begin new testing trial; C, cease all activities and discard tissue cultures due to unknown genetics as explained in the table and report; and, D, discard material due to poor trial performance, mislabeling or duplication.

ID as reported throughout the project	**Tissue cultures at NAP**	**Field Trialed During this Project**	**Classification Group**	**Grouping Reason/comments** **Recommendations**
57.001/65.001	Yes	Yes	A (Advance to S3 phase, collect data for 6 to 10 years)	Performance interesting; the two accessions are identical
22.001	Y	Y	A	Performance interesting; this can advance but it is not novel, it is equivalent to Quince MA
67.001	Y (reintroduced from root suckers collected from the Entiat trial; no longer at NCGR	Y	A (Advance to S3 phase, collect data for 6 to 10 years)	Performance interesting; Appears to be unique
68.002	No	Y	D (Discard)	Performance poor; High mortality; survivors generally very weak
118.001	N	Y	D (Discard)	Moderate mortality levels, overly vigorous, low yield, *NAP cultures are not genetically similar to source at NCGR and field trees are not the same as either NAP or NCGR material.

70.001	Y	Y	*D (Discard)	Inconsistent performance between sites; low production in WA, high in OR. Genetics from all 3 sites (NAP, NCGR, Entiat) matches.
99.002	Y	Y	D (Discard)	High mortality levels, overly vigorous, low yield, is not genetically similar to source at NCGR
23.001	*Y	Y	C (Cease activities and start over from the source material; retest hardiness)	Performance interesting, but tissue cultures and NCGR material do not match- they are genetically different; potentially same as 29.001. *Therefore, the NAP cultures are not what was trialed in the field.
126.001	Y	N	B (Begin testing in new Phase 2 trial)	Test in Phase 2 small-scale plots 6-10 years then move to Phase 3 test for 6-10 years if warranted, then commercialize if promising
67.004	Y	N	B	
64.001	Y	N	B	
32.002	Y	N	B	
13.001	Y	N	B	
128.001	Not clear	N	B	

29.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
34.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
66.001 and 75.001	Y	N	C	These two are genetically identical and neither of the tissue cultures matches the NCGR material
67.002	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
71.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
104.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different
120.001	Y	N	C	tissue cultures and NCGR material do not match- they are genetically different

Executive Summary

A three-year evaluation comprising three cropping seasons (4th through 6th leaf) evaluated the agronomic performance of 9 cold-hardy quince rootstocks with 'd'Anjou' and 'Bartlett' scions and 'Comice' insterstem at two sites, Parkdale OR and Entiat WA. Four of these rootstocks produced dwarf trees with good vigor to fruit balance and were horticulturally managed within a three foot in-row spacing after 7 years in the field. These accessions produced between 20-50 bins of fruit per year, depending upon year, site, cultivar and rootstock accession, and had relatively low mortality. In general, trees in OR were ~50% larger than in WA for any given accession. Poorly performing rootstocks behaved similarly between sites. In addition to these field trials, previously selected cold hardy quince accessions that were not previously tissue-cultured were successfully micropropagated from shoot tips in 2022 and 2023. An exhaustive, two-year genetic fingerprinting analysis was undertaken to confirm the identity of the rootstock accessions tested and propagated within this proposal to ensure that each accession was true to type and could be traced back to its original progenitor tree at the NCGR. For each of the nine field-tested accessions, material was collected from several replicate trees of a given rootstock at the Entiat WA field trial site (via sucker material), NAP (from tissue culture jars) and NCGR (from the original tree). In some cases, the original tree no longer exists. For the additional 15 accessions tissue-cultured during the project timeline, but not yet field tested, material was selected at NAP (from tissue culture jars) and the NCGR (from the original tree). Analyses revealed two or more accessions to be genetically similar (i.e., duplicates) ***and*** several accessions to have genetic dissimilarity between/among locations (e.g., Entiat, WA, NAP and/or the NCGR), precluding our ability to identify them and link the present material to the original cold hardiness status. Based on these data, we can recommend how each of the 25 rootstock accessions should be handled to avoid severe economic consequences that will inevitably arise if the industry propagates, trials and/or commercializes material of unconfirmed origin. First, the nine accessions field tested for 6 years, including 3 years of cropping: 1) Discard **five** accessions (57.001 ***or*** 65.001 [these are the same], 68.002, 118.001, 99.002, 70.001) due to the duplication, poor vegetative and/or yield performance, high mortality, and/or mislabeling; 2) re-evaluate the cold hardiness of **one** accession (23.001) for which no root suckers could be sampled and material at NAP and NCGR differed from one another (making it impossible to determine if the trees in field trials are the same as the original material evaluated for its cold hardiness); and, 3) advance **three** accessions (57.001 ***or*** 65.001 [these are the same], 22.001 and 67.001) to a Phase 3 trial. Phase 3 trialing should comprise an additional 6-10 years of small-scale commercial plot testing. Rootstocks deemed promising upon completion of phase 3 trialing, based on tree health, cropping, canopy balance, and fruit quality could be candidates for potential commercialization. Between 200 and 600 rooted plants were tissue cultured for each accession during the timeline of the project to facilitate Phase 3 trialing. For the **15** rootstocks accessions previously selected for their cold hardiness, **14** were successfully established in tissue culture. Our recommendation for the handling of these rootstocks is as follows: 1) the genetic identity of **six** accessions was confirmed (13.001, 32.002, 64.001, 67.004, 126.001, 128.001). These were rooted and are ready for grafting (100-500 plants depending on the accession) using interstems and scions to assess their performance in field trials, a minimum of 6 years and preferably 8-10 to include five cropping cycles. Rootstocks that performed well following the completion of these trials could be advanced to a phase 3 trial (as described above). The remaining **eight** accessions (29.001, 34.001, *66.001 and 75.001* [these are the same], 67.002, 71.001, 104.001, and 120.001) were not genetically similar to the mother tree at NCGR. Thus, we recommend discarding the tissue cultures and reanalyzing the cold hardiness of the original NCGR tree to compare with early hardiness data. Those that are hardy can be cultured and rooted for future field trials.