FINAL PROJECT REPORT

YEAR: 3 of 3

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Co-PI: Organization: Telephone: Email:	Richard Bell USDA-ARS 304-725-3451 ext. 353 richard.bell@ars.usda.gov		
Cooperators:	Stefano Musacchi		
Budget:	Year 1: \$37,492 Year 2	: \$26,640*	Year 3 : \$39,430*

Project Title: Cold hardy quince: Propagation, rapid multiplication and field trials

*We were approved for \$26,640 for year 2 (2013); however, due to delays in developing and providing plant material (see report) several of the objectives were unachievable in 2013. As a result only \$8,900 of the requested Year 2 budget was spent in 2013. This results in a surplus of \$17,740. Of this amount, only \$8,400 will be shifted to the Year 3 budget (2014); the remaining \$9,340 will not be requested.

Other funding sources: None

Dudget I Dui bara Recu & Sobeph	1 ostinun				
Organization Name: USDA-ARS	Contract Administrator: Chuck Myers				
Telephone: 510-559-5769	Email address: c	ail address: chuck.myers@ars.usda.gov			
Item	2012	2012 2013			
Wages	\$29,400				
Benefits	\$2,352				
Equipment					
Supplies ¹	\$5,500	\$1000			
Travel					
Miscellaneous					
Total	\$37,252	\$1000	\$0		

Budget 1 – Barbara Reed & Joseph Postman

Footnotes: ¹ rootstocks and greenhouse supplies to produce additional trees to fill gaps in Helios stool beds and Kearneysville plot and package and ship trees to Kearneysville for fire blight study.

Budget 2 - Richard Bell Organization Name: USDA-ARS Telephone: 304-725-3451 ext 332

Contract Administrator: Stephanie Kreger

Item 2012 2013* 2014				
2012	2013*	2014		
		\$ 8095		
		\$ 648		
	\$ 7,908			
	\$ 632			
	\$ 800	\$ 800		
		\$200		
\$ 0	\$ 9340*	\$ 9743		
	2012	2012 2013* \$ 7,908 \$ 632 \$ 800		

Footnotes: ¹ supplies to produce *Erwinia amylovora* inoculum and maintain quince field plot ²plot fees of \$200 were added to 2014.

*2013 budget was not spent and will not be requested in 2014

Budget 3 – Todd Erickson

Organization: Helios Nursery (owner; Tye Fleming)Contract Administrator: Todd EricksonTelephone: 971-241-8116Email address: toddaericksonsr@hotmail.com

Item	2012 2013* 2014*			
Wages ¹	0	8,400	16,800	
Benefits				
Supplies				
Travel				
Plot Fees				
Total	\$0	\$8,400*	\$16,800	

Footnotes: ¹2013-2014 costs are to bud 3,500 rootstock liners (including ½ with interstems), and raise for one-year in nursery (\$16,800). Costs are distributed over 2013-2014. *2013 budget of \$8,400 was not spent. These funds will be shifted to 2014.

Budget 4 – Yongjian Chang

Organization: North American Plan Telephone: 503-474-1852		Contract Administrator: Yongjian Cha Email address: ychang@naplants.		
Item	2012	2013	2014	
Wages	0	\$7900	0	
Benefits				
Supplies				
Travel				
Plot Fees				
Total	\$0	\$7900	\$0	

Footnotes: ¹2013 costs are to produce 3,500 rootstock liners in vitro (250 plants for each of 12 quince selections plus smaller number of 6 additional), to be supplied to Helios Nursery for grafting.

Budget 5 – Kate Evans

Organization: WSU-TFRECContract Administrator: Carrie Johnston & Kevin LarsonTelephone: 509.335.4564, 509.663.8181Email address: carriej@wsu.edu ; kevin_larson@wsu.eduItem201220132014

Wages			\$ 1,000
Benefits			\$ 173
Supplies ¹			\$ 2,750
Travel			
Plot Fees			\$2,000
Total	\$0	\$0	\$5,923

Footnotes: ¹ to cover field preparation, fumigation and irrigation costs

Budget 6 – Todd Einhorn			
Organization Name: OSU-MCA	REC Cont	ract Administrator: I	L.J. Koong
Telephone: 541 737-4866	Email address: 1.j.koong@oregonstate.edu		
Item	2012	2013	2014
Wages			\$ 1,000
Benefits			\$110
Supplies ¹			\$ 2,750
Travel			
Plot Fees			\$ 3,104
Total	\$0	\$0	\$ 6,964

Footnotes: ¹to cover field preparation, fumigation and irrigation costs

Three Year Project Objectives:

- 1) Determine effective propagation methods for quince with commercial nursery partners.
- 2) Test graft compatibility of cold hardy quince rootstocks and commercial pear cultivars.
- 3) Determine fire-blight resistance/sensitivity of cold-hardy quince rootstocks.
- 4) Deliver 10-12 rootstock clones grafted to Bartlett and Anjou for field trials in Wenatchee and Hood River.

Significant Findings:

 Cutting Propagation/Stoolbed Establishment – Rooting of cuttings (hardwood and softwood) ranged from 0% to 62%. In general, propagation by softwood cuttings was more successful than hardwood. Hormone dips improved rooting. Many of the quince accessions were observed to root more efficiently than OH x F clones (success rates between 0% and 7%). Rooted hardwood cuttings of 15 of the 22 cold-hardy accessions were successfully stablished in a stool bed at Helios Nursery.

2. In vitro multiplication-

- a. Many of the clones grew well in vitro on improved Pear medium. Eight quince clones had multiplication rates >10 and twenty one had multiplication rates ≥ 6. A multiplication rate of 6 is considered good. Only 8 of the accessions had low multiplication rates.
- b. After a thrips outbreak in 2012, 70% of the cold-hardy quince clones were successfully cultured *in vitro* at N.A.Plants in 2013. All accessions were transplanted to media and successfully rooted. In most cases, the numbers of transplants per accession exceeded the number required for field trials.
- c. The 7 remaining clones are presently being cultured at North American Plants for multiplication.
- d. Cultures have been maintained for all accessions.
- **3.** Production of quince trees for fire blight field trial 15 of the 20 accessions were evaluated for fire blight susceptibility in 2013 and 2014 field trials. The level of susceptibility was much less than anticipated. Few infections spread into older wood, and no trees of any of the quince clones were lost to fire blight. Many of the accessions could apparently have a level of resistance sufficient for commercial use as rootstocks. It is recommended that these clones be inoculated in the coming year to verify the level of resistance.
- **4.** Liner production for field trials In 2014, a sufficient number of rootstock liners of ~15 accessions, received by Helios nursery as explants from N. A. Plants, were successfully grown to budding/grafting size. Half of each accession was budded/grafted in late summer 2014 with 'Comice' (selected as the interstem). The other half was left un-grafted. Plants will be grown out for the 2015 season. In late summer of 2015, the total number of plants per accession (50% with interstems and 50% without) will be divided into equal groups—one group will be budded to 'd'Anjou', the other to 'Bartlett'. Trees will either be established in field plots in spring 2016 as sleeping eyes or spring 2017 as 1-year-old scions. Initial compatibility between 'Comice' and quince accessions will be evaluated spring of 2015; compatibility between quince and 'Bartlett' and 'd'Anjou' will be evaluated fall of 2015.

Results and Discussion:

In our previous efforts (2009-2011) we identified 22 quince taxa that showed 50% or less browning following exposure to -22 °F. These accessions had equal or greater cold hardiness than our currently used Pyrus rootstocks (OH \times F 87, OH \times F 97). Our main objectives for this phase of the project are to develop propagation knowledge/protocols for these accessions, and to produce an adequate volume of trees for field evaluations. The three propagation techniques under evaluation are in-vitro (tissue culture and rooting of explants), cutting (hard- and soft-wood), and stooling.

Propagation- In vitro initiation was successful on newly developed pear medium (Reed, USDA-ARS NCGR). Eight quince clones had multiplication rates >10 and 21 had multiplication rates \geq 6 during in vitro establishment (Table 3). A multiplication rate of 6 or higher is considered good. Despite being delayed from our initial timeframe due to loss of these cultures to a thrips infestation (October, 2012), N.A. Plants rapidly multiplied a sufficient number of transplants for liner production. Fifteen of the 22 clones were cultured, transplanted, and rooted in 2013 at N. A. Plants (Table 1 and photos). Slight alterations to tissue culture media were made to optimize in-vitro production of the different genotypes. In 2014, plants were delivered to Helios Nursery and transplant to a liner bed. Acceptable growth accrued during 2014 and half the plants of each accession were budded to 'Comice' (selected as the interstem). Plants will be grown out in 2014 (half with interstems and half without). In late summer all plants will be T-budded to the scions ('d'Anjou' and 'Bartlett'). All clones are maintained in culture permitting rapid production of additional plants should the need arise.

In general, propagation by softwood cuttings was more successful than hardwood cuttings (Table 2), though some clones had superior rooting from softwood and others from hardwood cuttings. Rooting hormone improved levels of rooting. Eight accessions had \geq 19% rooting success from hardwood cuttings with hormone, and only two rooted at this rate with no hormone. Twelve accessions had > 25% rooting with hormone from softwood cuttings. Only one top selection did not root (Table 2). Softwood results were scored at 6 weeks to identify the most easily rooted clones. Although the proportion of cuttings that rooted was relatively low, many of the quince accessions were observed to root more efficiently than OHxF clones (Table 2). Only one clone (the hardiest of the population) did not root easily from either soft or hardwood cuttings.

Fire blight- 1). 2013 Planting. Fifteen of the 20 cold-hardy quince clones were evaluated for fire blight susceptibility (Table 3). Several additional quince accessions were evaluated; the cold hardy clones in Table 3 are highlighted in grey. For the entire population evaluated, mean shoot length varied from 60 mm for V-7 o. p. seedling3 to 301 mm for 'Avia from Gebeseud'. Mean lesion length varied from 0 mm for OHF 87 to 255 mm for IV-36 o. p. Lesion lengths and percent lesion lengths for the first inoculation were generally more severe. The mean percent lesion length varied from 0 for OHF 87 to 149 for V-46 o. p., indicating spread of infection into 1-year-old wood. Due to considerable variability among trees, there were many overlapping significance classes for all traits, but especially for mean percent lesion length. Based on lesion length and percent lesion length, the most resistant quince rootstocks were V-46 o.p. seedling 3, V-7 o. p. seedling 3, and ×Pyronia vetchii IRP 82-1. However, these values were only based on 2, 1 and 1 replicate trees. There were six additional clones which had percent lesion lengths of 20 mm or less. There was a great difference between V-46 o.p. and it's offspring V-46 o. p. seedling 3, exhibiting the most susceptible and most resistant disease reactions.

2). 2014 Planting. The 2014 planting included 10 of the 20 cold hardy quince accessions. As in the 2013 planting, additional quince were evaluated for fire blight susceptibility. Of the entire population evaluated, mean shoot length varied from 121 mm for W-4 to 300 for 'Avia' from Gebeseud. The mean lesion length varied from 0.1 mm for OHF 97 to 162 mm for 'Bartlett' seedling. The mean percent lesion length varied from 0.1 for OHF 97, indicating only one infected leaf, to 116 for 'Bartlett' seedling, indicating infection into 1-year old wood. The results for these resistant and susceptible standards are similar to previous results. Based on the percent lesion length, the most resistant quince clone was 'Avia' from Gebeseud, the second most hardy of the 20 hardy clones. However, there was

only one replicate tree of this clone. Quince C7/1, 'Bereczki', 'Megri' and Pigwa S-1 were the next least susceptible clones.

Overall, the level of susceptibility was much lower than expected, especially given the high inoculum concentration. However, the level of susceptibility of 'Bartlett' seedling rootstock was similar to many previous studies. The Spearman's correlations between plantings for shoot length, lesion length and percent lesion length were all non-significant.

Objectives not yet completed:

An evaluation of initial graft incompatibility (with the interstem, 'Comice') will take place this spring. An evaluation of T-budding success with the scions 'Bartlett' and 'd'Anjou' will be performed in fall of 2015.

Methods:

Fire Blight-

Thirty-five quince clones propagated and sent from the National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon were planted in the spring of 2013 in a field plot at the Appalachian Fruit Research Station in Kearneysville, WV. The plot design was a randomized incomplete block design. The number of replicate trees of each clone varied primarily from one to 14. Because many trees were small and the trees varied considerably in size and number of shoots available for inoculation, the trees were grown for one year (2013) before fire blight assays were commenced in the spring of 2014. Trees of Quince A were considered the susceptible control and 10 trees of 'Old Home × Farmingdale 87' were the resistance controls.

In 2014 a second set of 13 quince clones was received from NCGR, and were planted in a separate plot, again in a single-row randomized incomplete block design with 10 blocks. Bartlett seedling rootstocks were planted as the susceptible controls and trees of 'Old Home × Farmingdale 97' were planted as the resistant controls. The number of replicate trees for each clone varied from 1 to 10.

A suspension of fire blight inoculum was prepared using the isolate Ea273, with the concentration adjusted to 1×109 cfu•ml-1. One inoculation was performed on 23 May 2014 in which one shoot per tree was inoculated, but because the characteristic necrotic symptoms were slow to develop after 4 days, a second set of inoculations was performed on 30 May 2014. On this date one to three shoots on each plant were inoculated, where available. Shoot growth was vigorous on both inoculation dates, but the current year's extension growth was relatively short, compared with that usually seen on established pear seedlings. Inoculations were performed by dipping a pair of scissors in the inoculum suspension and cutting the top two expanding leaves on a selected shoot of each clone. The length of current season's growth of each shoot was measured. Inoculated shoots which only exhibited necrosis in the leaf midrib, with no infection in the shoot were scored as "1 mm" to indicate infection. For the first inoculation, the trees were inspected for infection 10 days post infection, but no data was taken, due to limited symptom development. Data on the length of the resulting necrotic lesions for both inoculations were measured beginning on day 17 post infection, and repeated on days 24, 31, and 38, when progression of the lesions ceased for most inoculated shoots. End of season data were collected at 122 days postinfection.

Table 1. Fifteen of the of twenty-two clones previously determined to possess adequate cold hardiness for the PNW have been successfully tissue cultured by Dr. Yongjian Chang at N.A. Plants. Explants were planted in three stages (October, 2013; January, 2014; and, February, 2014).

Variaty	Accession	Number of tissue-cultured explants transplanted				
Variety	Accession	Planted in October	Planted in January	Ready for planting in Feb		
CYD 118.001	C. oblonga - Seghani	563				
CYD 65.001	Quince C7/1	520				
CYD 23.001	WF-17	466				
CYD 22.001	W-4	430				
CYD 57.001	Quince S	400				
CYD 99.002	Kashenko no. 8	307				
CYD 67.001	Akhtubinskaya O.P. seedling 1		500			
CYD 67.004	Akhtubinskaya O.P. seedling 4		500			
CYD 68.002	Krukovskaya O.P. seedling 2		500			
CYD 70.001			500			
CYD 128.001	C. oblonga - Babaneuri			500		
CYD 29.001	Quince W			400		
CYD 123.001	Trentholm			300		
CYD 32.004	Tashkent AR-232 seedling 4			300		
9.001	Pyronia Veitchii			200		



Photos: Quince accessions in tissue culture at NA Plants (above images); rooted explants planted into media in October, 2013 (lower). All photos taken Oct. 24, 2013.

Table 2. Summary of percent rooting success following three separate cutting propagation trials. Dates signify when cuttings were produced. Results were evaluated after 6 weeks and 6 months for soft-wood and hard-wood cuttings, respectively.

					-	
Hardiness Rank	Accession	Browning Score (1-6)	hardwood cutting 01,11/2012	softwood cuttings 06/2012	in vitro multiplic.	Helios Layer Bed
1	C. oblonga - Arakseni, Armenia	1.50	-	0.0	6.8	-
2	Aiva from Gebeseud	2.39	0.0	33.3	3.7	X
3	Akhtubinskaya O.P. seedling 4	2.42	0.0	33.3	6.9	X
4	Tashkent AR-232 seedling 4	2.75	4.8	4.2	7.0	Х
5	Skorospelka O.P. seedling 1	2.86	-	12.5	19.0	Х
6	Quince S	3.00	23.8	8.3	12.5	X
7	Quince W	3.00	42.9	29.2	8.6	Х
8	C. oblonga - Megri, Armenia	3.03	4.8	12.5	5.1	Х
9	C. oblonga - Seghani, Armenia	3.08	9.5	25.0	11.4	X
10	Tashkent AR-232 seedling 2	3.14	0.0	37.5	6.0	X
12	C. oblonga - Babaneuri, Georgia	3.61	14.3	8.3	10.5	X
13	Krukovskaya O.P. seedling 2	3.64	-	45.8	2.0	X
14	W-4	3.69	33.3	12.5	5.7	-
15	Trentholm	3.75	0.0	12.5	14.8	-
16	WF-17	3.75	28.6	25.0	4.6	X
17	Bereczki [Beretskiquitte]	3.78	-	29.2	6.0	X
18	Kashenko No. 8	3.81	-	12.5	15.1	X
19	Quince C7/1	3.86	57.1	8.3	8.7	X
20	Pyronia veitchii	3.89	23.8	-	-	-
Standard	OHxF 97	3.86	7.1	0.0	-	-
Standard	OHxF 87	3.83	-	4.2	- -	-

% rooting w/ hormone

Base of freshly made cuttings dipped in rooting hormone (0.8% indole-3-butyric acid; Hormex No. 8 powder).



		Mean	Mean	Mean
		shoot	lesion	percent
	No.	length	length	lesion
Clone	trees	(mm)	(mm)	length
V-46 o.p.	2	149 cd	112 bcd	149 a
IV-36 o.p.	1	295 abcd	255 a	89 abcde
9) Seghani o.p.	6	139 bcd	99 bcd	85 abcde
4) Tashkent AR-232 Sdlg 4	4	280 ab	231 a	82 ab
17) Bereczki	2	274 abcd	168 abcd	68 abcde
VI-7 o.p. Sdlg 3	2	179 abcd	81 bcd	68 abcde
10) Tashkent AR-232 Sdlg 2	12	213 bcd	129 b	66 abc
14) W-4	5	205 bcd	74 bcd	65 abcd
13) Krukovskaya o.p. Sdlg 2	4	265 abc	161 ab	58 abcde
8) Megri	1	164 abcd	86 bcd	53 abcde
IV-40 o.p. Sdlg 3	2	188 abcd	113 bcd	48 abcde
Akhtubinskaya o.p. Sdlg 1	2	173 abcd	80 bcd	44 abcde
18) Kashenko No. 8	3	174 bcd	48 cd	39 bcde
7) Quince W	10	152 cd	50 cd	36 bcde
Texas Scarlett	5	239 abcd	81 bc	33 bcde
19) Quince C7/1	9	222 abced	71 cd	33 bcde
Pillnitz 2	3	238 abcd	65 bcd	32 abcde
I-83 o.p. Sdlg 1	3	199 abcd	76 bcd	32 bcde
Pigwa S-1	14	173 d	53 cd	29 cde
2) Avia from Gebesud	5	301 a	71 bcd	25 bcde
1) Arakseni	5	129 bcd	29 cd	24 bcde
Pillnitz 3	11	166 de	39 cd	24 bcde
Pillnitz 5	18	145 d	31 cd	24 cde
Quince A	10	233 abcd	54 cd	23 cde
Pillnitz 1	6	185 bcd	42 cd	22 bcde
5) Skorospelka o.p Sdlg	3	190 abcd	36 cd	20 bcde
BA-29C	10	199 bcd	40 cd	20 de
Quince E	6	179 bcd	34 cd	19 de
Babaneuri	5	239 abcd	47 cd	19 cde
3) Akhtubinskaya o.p. Sdlg 4	11	246 abc	45 cd	17 de
Pigwa S-2	6	195 bcd	30 cd	16 cde
20) xPyronia vetchii IPR 82-1	2	140 bcd	11 d	11 de
V-7 o.p. Sdlg 3	1	60 e	1 d	2 e
V-46 o.p. Sdlg 3	1	65 e	1 d	2 e
OHF 87	9	242 abcd	0 d	0 e
Significance of effects			0.000	
Clone		< 0.0001	< 0.0001	< 0.0001
Inoculation		< 0.0001	0.0007	0.0002
Clone x Inoculation		0.6424	0.0909	0.0004

Table 3. Fire blight infection in 2013 planting of prospective quince rootstock germplasm (2014 data, 122 days post-infection). Accessions highlighted in grey represent cold hardy clones; the number preceding the name represents the cold hardy rank

Table 4. Fire blight infection in 2014 planting of prospective quince rootstock germplasm (2014 data, 122 days post-infection). Accessions highlighted in grey represent cold hardy clones; the number preceding the name represents the cold hardy rank.

Clone	No. trees	Mean shoot length (mm)	Mean lesion length (mm)	Mean percent lesion length
Bartlett seedling	7	140 c	162 a	116 a
18) Kashenko No. 8	7	153 c	68 b	78 b
Babaneuri	4	183 bc	77 b	48 c
9) Seghani	1	175 bc	75 b	43 c
4) Tashkent AR-232 Sdlg 4	7	172 bc	66 b	40 c
14) W-4	6	121 d	31 c	39 c
Pigwa S-2	10	159 c	59 b	36 c
5) Skoropelka o.p. Sdlg	8	149 c	51 b	35 c
16) WF-17	4	173 bc	44 b	30 c
19) Quince C7/1	7	177 bc	40 bc	26 cd
17) Bereczki	2	173 bc	20 c	17 cd
8) Megri	5	167 c	26 c	15 d
Pigwa S-1	3	228 b	12 cd	7 d
2) Avia from Gebeseud	1	300 a	1 d	0.3 e
OHF 97	9	162 c	0.1 d	0.1 e
Significance of effects				
Clone		0.1218	< 0.0001	0.0016
Inoculation		0.8733	0.7075	0.8433
Clone x Inoculation		0.1197	0.7866	0.9364

Executive Summary:

In the project's initial phase (2009-2011) we characterized 22 quince accessions as cold hardy; defined by visual observation of \leq 50% oxidative browning of vascular tissues following exposure to -22 °F (predetermined critical temperature). These accessions had equal or greater cold hardiness than the commonly utilized commercial Pyrus rootstocks (OH × F 87, OH × F 97). Our main objectives for the second phase of the project were to develop propagation knowledge/protocols for these accessions and to produce an adequate volume of trees for field evaluations.

In general, propagation by softwood cuttings was more successful than hardwood cuttings. Rooting hormone improved levels of rooting. Eight accessions had \geq 19% rooting success from hardwood cuttings; 12 accessions had > 25% rooting from softwood cuttings. Although the proportion of cuttings that rooted was relatively low, many of the quince accessions were observed to root more efficiently than OH x F clones (rooting success between 0% and 4%). Fifteen of the 22 cold-hardy accessions were successfully stablished in a stool bed at Helios Nursery (from hardwood cuttings).

In vitro initiation was successful on newly developed pear medium (Reed, USDA-ARS NCGR). Eight quince clones had multiplication rates >10 and 21 had multiplication rates \geq 6 during in vitro establishment. A multiplication rate of 6 or higher is considered good. Despite being delayed from our initial timeframe due to loss of these cultures to a thrips infestation (October, 2012), N.A. Plants rapidly multiplied a sufficient number of transplants for liner production. Seventy percent of the cold-hardy quince clones were successfully cultured *in vitro* at N.A.Plants. All accessions were transplanted to media and successfully rooted. In most cases, the numbers of transplants per accession exceeded the number required for field trials. Cultures have been maintained for all accessions.

In 2014, sufficient numbers of rootstock liners of ~15 accessions, received by Helios nursery as explants from N. A. Plants, were successfully grown to budding/grafting size. Half of each accession was budded/grafted in late summer 2014 with 'Comice' (selected as the interstem). The other half was left un-grafted. Plants will be grown for the 2015 season. In late summer of 2015, the total number of plants per accession (half with interstems and half without) will be divided into equal groups—one group will be budded to 'd'Anjou', the other to 'Bartlett'. An evaluation of initial graft compatibility between the quince accessions and pear scions will be performed in the fall of 2015.

Fifteen of the 20 accessions were evaluated for fire blight susceptibility in 2013 and 2014 field trials. The level of susceptibility was much less than anticipated. Few infections spread into older wood, and no trees of any of the quince clones were lost to fire blight. Many of the accessions could apparently have a level of resistance sufficient for commercial use as rootstocks. It is recommended that these clones be inoculated in the coming year to verify the level of resistance.