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

Project Title: Interstem grafts to evaluate pear germplasm for dwarfing potential

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Total Project Request: **Year 1**: $18,000 **Year 2**: $9,000 **Year 3**: no cost extension

Budget:

Organization Name: USDA-ARS **Contract Administrator: Richard Kimball**
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Item	**2017**	**2018**	**2019**
Salaries			
Benefits			
Wages	$12,000	$9,000	
Benefits			
Equipment			
Supplies	$6,000		
Travel			
Miscellaneous			
Plot Fees			
Total	**$18,000**	**$9,000**	**$0**

OBJECTIVES

Clonal propagation of pear selections as self-rooted trees for rootstock trials is challenging, and a procedure to pre-screen selections for dwarfing ability will help focus resources. The USDA living pear germplasm collection in Corvallis, OR has numerous potential pear rootstock selections, and also includes a very large and diverse assortment of pear selections and species that have never been evaluated for rootstock potential. The objective of this project was to investigate whether interstem grafts can be used to identify pear selections that have dwarfing potential, and provide a relatively rapid assay for screening pear germplasm to be included in future rootstock trials. Interstem pieces of 30 potential rootstock selections were grafted onto seedling rootstocks, and then each interstem was top-worked with a bud of either ‘Bartlett’ or ‘Bosc’. Trees were grown and evaluated in a greenhouse for the first year, and then in a field planting for two additional years.

SIGNIFICANT FINDINGS 2017 to 2019

- **2017**: The goal of generating interstem trees in one season was accomplished. 194 ‘Bosc’ trees and 204 ‘Bartlett’ trees on 29 different interstem candidates were produced. Following a full season of growth in the greenhouse there was inadequate growth on the potted trees to detect any interstem effect. A single growing season under greenhouse conditions was not adequate for evaluating dwarfing potential (January 2018 Progress Report).
- **2018**: 174 ‘Bosc’ trees and 184 ‘Bartlett’ trees on 28 different interstems were field planted in early July (Figure 1). Rootstock, interstem, and cultivar stem diameters; cultivar stem heights; and number of side branches was recorded at the end of the 2018 growing season to evaluate possible dwarfing. No significant differences were detected between treatments (January 2019 Progress Report).
- **2019**: 154 ‘Bosc’ trees and 192 ‘Bartlett’ trees on 33 different interstems (Table 1) are well established in a field plot (Figure 2). Most grafted trees were generated during early 2017, however 26 trees from a 2016 preliminary greenhouse trial had also been field planted and were evaluated along with the 2017 grafts. The 2016 grafts were not included in the 2018 report, but are included here. Vegetative growth data was recorded in late October 2019:
    - Diameters of the ‘Bartlett’ and ‘Bosc’ scions measured approximately 1 cm above the graft unions did not differ significantly from the controls (‘Bartlett’ and ‘Bosc’ interstems). Trees with ‘**Granatnaya’** and ‘**Passe Crassane’** interstems had mean stem diameters that differed only from the two most vigorous interstem treatments at a very low level of significance (Table 2).
    - Total tree height was not a useful measure of dwarfing. Some trees had single leaders, some had double leaders, and trees differed in number and length of side branches. Tree height was therefore not a reliable measurement of vegetative vigor.
    - ‘Bartlett’ grafts produced twice as many side branches (mean = 15.4) as ‘Bosc’ (mean = 6.9), but when average branch length was calculated, there was no difference (44.8 cm vs. 45.7 cm respectively). Number or length of side branches was not a useful measure of dwarfing, however some side branches appear to have many flower buds and differences in precocity and fruit production may be apparent in 2020 and beyond.
    - When the sum of all branch lengths (main stem and all side branches) was calculated (Table 3), ‘Bartlett’ had nearly twice the vegetative growth (mean = 847 cm) as ‘Bosc’ (482 cm). Two potentially dwarfing interstems produced significantly less vegetative growth than the most vigorous interstems ($p < .05$) although there was no significant difference from the ‘Bartlett’ and ‘Bosc’ interstem controls. The two most dwarfing interstems were ‘**Granatnaya’** and ‘**P. calleryana D6’**.

- A “vegetative growth efficiency” was calculated based on total shoot growth (sum of all stem and side branch lengths) per mm of stem diameter (Table 4). The same interstems as above (‘**Granatnaya’** and ‘**P. calleryana D6’**) differed significantly from the 4 most vigorous interstems ($p < .05$) but not from the control treatments.
- Pear selections known to be either dwarfing or vigorous as rootstocks did not control vigor in a similar fashion when used as interstems during the 3 years of this study. More than 3 years, including the transition from vegetative growth to fruit production, are required to properly evaluate rootstock potential.

METHODS

Interstem Grafts. Scions were collected in January 2017 from interstem candidates and from virus-free mother trees of cultivars Bartlett and Bosc (for top-working) and stored at 4 °C (40 °F). In April 2017 pear seedling rootstocks were planted in 2” x 10” deepots. In May, 15 cm (6 in) long interstems were grafted onto seedling rootstocks in a cool greenhouse. Twenty grafts were made with each of 31 interstem candidates. ‘Bartlett’ and ‘Bosc’ were also used as two of the interstem treatments and can be considered controls. A number of pear selections known to be either dwarfing or vigorous as rootstocks were among the interstem candidates (Table 1). Approximately 2-3 weeks after interstem grafts were made, 10 of each were chip-budded with ‘Bartlett’ and 10 with ‘Bosc’ at the top of the interstem. Grafted trees were maintained in pots and flood irrigated during the growing season. Rootstock and interstem shoots were regularly removed to force the cultivar buds. Interstem graft survival and cultivar top-graft survival was assessed. Length of cultivar bud growth was measured in mid-October. Results from 2017 were reported at the pear research review in February, 2018.

A small number of preliminary interstem grafts were made in late summer 2016 as proof of concept using the dwarfing rootstock ‘Pyrodwarf’ and the genetic dwarf clones ‘Le Nain Vert’ and ‘P. nivalis compact hybrid’. ‘Bartlett’ and ‘Bosc’ interstems were controls. Survival and replication of these preliminary grafts was not sufficient to evaluate statistical significance, but the data may be of interest and is included in the tables below.

Field plot established in 2018. In early July 2018, ‘Bosc’ and ‘Bartlett’ trees on 28 different interstems from 2017 grafts, and 5 interstems from 2016 grafts were field planted in a randomized block design with two blocks per treatment. A narrow irrigation pipe trencher was used to prepare planting furrows for easily lining out the trees on 24 inch centers (Figure 1). Trees were fertilized, regularly irrigated, and weeds were controlled for the remainder of the growing season. The total height of ‘Bartlett’ and ‘Bosc’ shoots was measured from the bud union and the number of side branches was counted after leaf-fall in late October. Stem diameters were measured at three points using a digital caliper: below the interstem graft union (rootstock), at mid-interstem (interstem), and above the interstem (cultivar). Analysis of variance was conducted and Tukey’s HSD was used to compare means from the 2017 grafts; no statistically significant differences were detected. A no-cost extension was requested for this project to assess tree growth for another year in the field.

Field plot growth in 2019. Drip irrigation was installed and weeds were controlled during the 2019 growing season. No tree pruning or training was done, other than removing rootstock and interstem suckers and staking trees that were badly leaning. In mid-October stem diameters were measured for the ‘Bartlett’ and ‘Bosc’ top-grafts approximately 1 cm above the graft union (Table 1), and tree heights were measured at the tallest point (data not shown). In early-November the length of every shoot and side-shoot was measured and the values were added to obtain the number of side branches and the total vegetative growth of each tree (Table 2). As an alternative measure of vigor, total vegetative growth (cm of length) was divided by the scion stem diameter (mm) to obtain a “vegetative growth efficiency” similar to the “yield efficiency” used in fruit productivity studies. The statistical package ASRemi-R was used to account for treatment and replication effects for a mixed linear model, and Tukey’s HSD was used for making all pairwise comparisons.

RESULTS & DISCUSSION

At the end of the 2018 growing season, 358 trees with 28 different interstems had survived in the field. ‘Bosc’ on ‘Bosc’ interstems and ‘Bartlett’ on ‘Bartlett’ interstems were reference controls. While some cultivar/interstem combinations resulted in trees that were generally smaller or larger than controls none of the differences were statistically significant (January 2018 Progress Report).

At the end of the 2019 growing season, 346 interstem trees (154 with ‘Bosc’ scions and 192 with ‘Bartlett’) with 33 different interstems are well established in a field plot (Table 1). A number of very weak trees evaluated in 2018 did not survive, thus reducing some of the variance that impeded pervious year statistical analysis. Both the total length of vegetative growth (Table 3) and the “vegetative efficiency” calculation (Table 4) suggest that the intergeneric hybrid ‘**Granatnaya’** (Crataegus x Sorbus) and the Australian rootstock selection ‘**P. calleryana D6’** deserve further evaluation as size-controlling stocks.

A number of interstem candidates were included that are known to be either dwarfing or non-dwarfing as rootstocks. This should allow the validation of whether the dwarfing effect as an interstem is similar to the expected influence as a self-rooted stock. For example for the two South African rootstock clones, ‘BP-1’ is considered to be semidwarfing and ‘BP-2’ is considered to induce more vigor. Likewise, among the several ‘Old Home x Farmingdale’ clones included, ‘OHxF 97’ is considered to be more vigorous than ‘OHxF 69’ and ‘OHxF 333’. The rootstock clones ‘Pyrodwarf’ and ‘BU 2/33’ (or ‘Pyro II’) should induce dwarfing.

Unfortunately, after 1 year in pots and 2 years in the field, the dwarfing results from this interstem study were not consistent with the expected size control for many of the rootstock clones. While the differences were not statistically significant, ‘BP-2’ was more dwarfing than ‘BP-1’ with ‘Bartlett’ scions, and ‘BP-1’ was more dwarfing when ‘Bosc’ was the scion (Table 3, Table 4). Also, while not statistically significant, ‘OHxF 69’ tended to be more dwarfing than ‘OHxF 97’ as expected, but ‘OHxF 333’ was more vigorous. ‘Pyrodwarf’ had an inadequate number of surviving replicates to evaluate (none for ‘Bartlett’) but the few surviving trees were more dwarf than other interstem treatments. ‘BU 2/33’ had no surviving ‘Bosc’ replicates, and the ‘Bartlett’ grafts did not differ at all from control interstems.

It is not entirely unexpected that cultivars grafted onto size-control rootstocks do not exhibit dwarfing in the nursery or as young orchard trees, and only exhibit dwarfing relative to other rootstocks as a result of precocious fruit production. Shifting tree resources to producing fruit rather than producing shoots and leaves is known to be an important component in vigor control. While 2 or 3 years may be inadequate for assessing the ability of a rootstock candidate to induce dwarfing and precocity, the use of interstems, rather than self-rooted rootstocks, may still have great potential as an efficient method to screen germplasm for rootstock potential. An interstem assay will avoid the expensive and time-consuming effort to generate self-rooted rootstocks using tissue culture or conventional cutting propagation.

2020 and beyond. This interstem field plot is now well established on the USDA germplasm farm. While no flowers or fruit have yet been produced, there is evidence of abundant flower buds on some trees that should produce a fruit yield in 2020. We anticipate much more useful information to result from this planting in future years and hope to continue to monitor this planting and collect production data for several additional seasons.

Acknowledgements: Jaimie Green and Laura Duncan assisted with tree maintenance and data collection. Jason Zurn conducted ASRemi statistical analyses. Jill Bushakra reviewed this report and suggested useful improvements.

Figure 1 – Interstem trees field planted in early July 2018.

Figure 2 – Interstem trees at end of 2019 growing season.

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Table 1. Pear interstem candidates with USDA accession number and National Clonal Germplasm Repository inventory number.

Interstem	Accession	Inventory	Taxon	Name
1	CIGC 5	CIGC 5.001	*×Crataegosorbus miczurinii*	Granatnaya (Crataegus x Sorbus)
2	PI 689459	CIGC 9.001	*×Pyronia veitchii*	Pyrus communis x Cydonia IRP 82-1
3	PI 300693	CPYR 38.001	*Pyrus communis*	Bartlett (2017)
4	PI 300693	CPYR 38.001	*Pyrus communis*	Bartlett (2016)
5	PI 436538	CPYR 101.001	*Pyrus communis*	BP-1 (Bien Donne 1)
6	PI 420810	CPYR 102.001	*Pyrus communis*	BP-2 (Bien Donne 2)
7	PI 322035	CPYR 344.001	*Pyrus communis*	Le Nain Vert (2017)
8	PI 322035	CPYR 344.001	*Pyrus communis*	Le Nain Vert (2016)
9	PI 324134	CPYR 406.001	*Pyrus communis*	Mustafabey
10	PI 131662	CPYR 441.001	*Pyrus communis*	Passe Crassane
11	PI 214185	CPYR 665.001	*Pyrus calleryana*	P. calleryana D6
12	PI 541370	CPYR 726.001	*Pyrus communis*	OHxF 97
13	PI 541745	CPYR 866.001	*Pyrus nivalis hybrid*	P. nivalis compact hybrid (2017)
14	PI 541745	CPYR 866.001	*Pyrus nivalis hybrid*	P. nivalis compact hybrid (2016)
15	PI 541945	CPYR 890.001	*Pyrus regelii*	P. regelii
16	PI 312149	CPYR 920.001	*Pyrus syriaca*	P. syriaca - Armenia No. 1087/62
18	PI 541387	CPYR 1165.001	*Pyrus communis*	Bosc - OP-5 (2017)
19	PI 541387	CPYR 1165.001	*Pyrus communis*	Bosc - OP-5 (2016)
21	PI 541405	CPYR 1329.001	*Pyrus communis*	OHxF 333
22	PI 665738	CPYR 1343.001	*Pyrus communis*	OHxF 69
23	PI 541415	CPYR 1345.001	*Pyrus communis*	OHxF 87
24	PI 541652	CPYR 1496.001	*Pyrus fauriei*	P. fauriei Selection 12-14
26	PI 502179	CPYR 1639.001	*Pyrus elaeagrifolia hybrid*	Sbkta
27	PI 541953	CPYR 1697.001	*Pyrus salicifolia hybrid*	P. salicifolia Russia sdlg. 1
28	PI 541953	CPYR 1697.005	*Pyrus salicifolia hybrid*	P. salicifolia Russia sdlg. 5
29	PI 541007	CPYR 2291.002	*Pyrus betulifolia*	P. betulifolia - Shaanxi
30	PI 617598	CPYR 2522.004	*Pyrus korshinskyi*	P. korshinskyi 94011 - Kyrgyzstan
31	PI 617654	CPYR 2598.002	*Pyrus communis*	Pyrodwarf (2016)
32	PI 617679	CPYR 2699.001	*Pyrus communis*	BU 2/33 - Pyro II
33	CPYR 2704	CPYR 2704.002	*Pyrus communis*	QR 708-12
34	PI 638009	CPYR 2817.001	*Pyrus elaeagrifolia*	Gasparian 38, Kotayk sdlg. 1
35	PI 657923	CPYR 2882.001	*Pyrus sachokiana*	P. sachokiana Georgia-2006-115
36	PI 665763	CPYR 2968.001	*Pyrus spinosa*	P. spinosa Albania-2011-038

Table 2. Mean stem diameter (mm) above interstem/scion graft.
n = number of replicate plants, x = no data)

	Bartlett		Bosc		
interstem	**N**	**Diam (mm)**	**n**	**Diam (mm)**	**p < 0.1**
Pyrodwarf 2016	0	x	2	15.0	
Granatnaya (×Crataegosorbus)	3	14.3	5	16.2	**a**
Le Nain Vert 2017	2	18.0	2	20.0	
BP-2	8	18.4	7	20.0	
QR 708-12	8	18.4	6	19.0	
Passe Crassane	8	18.6	7	13.7	**a**
P. calleryana D6	5	19.0	3	18.7	
P. betulifolia - Shaanxi	6	19.2	4	13.0	
P. sachokiana	6	19.2	5	17.8	
P. regelii	6	19.5	0	x	
Bosc - 2016	7	19.6	0	x	
Pyronia (Pyrus x Cydonia)	7	19.7	8	18.4	
OHxF 87	8	19.8	8	18.1	
Bartlett 2017	8	20.5	5	17.6	
BU 2/33 - Pyro II	7	20.7	0	x	
P. fauriei 12-14	8	20.9	2	15.5	
OHxF 97	7	20.9	2	20.0	
P. syriaca - Armenia	6	21.0	5	15.6	
P. elaeagrifolia Armenia-38	4	21.0	3	17.0	
P. salicifolia (Russia hybrid 5)	8	21.1	6	18.5	
OHxF 69	8	21.3	4	13.8	
Bosc - 2017	7	21.3	8	19.9	
BP-1	6	21.3	7	17.6	
P. nivalis compact 2017	3	21.7	4	19.3	
Mustafabey	7	21.7	7	20.6	**b**
Bartlett 2016	1	22.0	5	16.8	
P. korshinskyi sdlg 4	7	22.1	7	18.0	
P. salicifolia (Russia hybrid 1)	8	22.4	5	20.4	**b**
OHxF 333	8	22.6	7	18.4	
Sbkta (P. elaeagrifolia)	8	22.6	7	18.6	
P. spinosa Albania-38	3	24.0	6	18.3	
P. nivalis compact 2016	2	24.5	4	17.8	
Le Nain Vert 2016	2	25.5	3	16.3	
Mean Stem Diameter		**20.7**		**17.7**	

Table 3. Total vegetative growth: Average sum of all branch lengths (cm).
(n = number of replicate plants, x = no data

	Bartlett		Bosc		
interstem	**n**	**Length (cm)**	**n**	**Length (cm)**	**p < .05**
Pyrodwarf 2016	0	x	2	291.5	
Granatnaya (×Crataegosorbus)	3	399.7	5	346.0	**a**
Bosc - 2016	7	501.3	0	x	
P. calleryana D6	5	599.2	3	457.3	**a**
QR 708-12	8	647.1	6	527.8	
BP-2	8	667.6	7	662.9	
P. nivalis compact 2016	2	674.0	4	382.5	
P. syriaca - Armenia	6	729.7	5	490.6	
Le Nain Vert 2017	2	733.0	2	417.0	
Bartlett 2016	1	750.0	5	379.4	
P. betulifolia - Shaanxi	6	750.3	4	245.5	
BU 2/33 - Pyro II	7	764.0	0	x	
P. regelii	6	769.7	0	x	
Passe Crassane	8	814.3	7	397.4	
Bartlett 2017	8	829.4	5	558.0	
P. fauriei 12-14	8	866.5	2	399.0	
OHxF 69	8	874.1	4	276.5	
Sbkta (P. elaeagrifolia)	8	885.6	7	548.0	
P. spinosa Albania-38	3	891.0	6	468.7	
OHxF 87	8	891.5	8	501.3	
P. salicifolia (Russia hybrid 5)	8	897.8	6	522.2	
P. elaeagrifolia Armenia-38	4	898.0	3	285.3	
BP-1	6	898.7	7	465.9	
Pyronia (Pyrus x Cydonia)	7	919.6	8	489.9	
Bosc - 2017	7	957.1	8	586.0	
Mustafabey	7	998.6	7	699.0	**b**
OHxF 97	7	1006.6	2	547.5	
P. nivalis compact 2017	3	1018.0	4	610.0	
OHxF 333	8	1028.8	7	545.3	
P. sachokiana	6	1057.0	5	496.0	
P. salicifolia (Russia hybrid 1)	8	1114.9	5	720.0	**b**
P. korshinskyi sdlg 4	7	1115.0	7	550.7	**b**
Le Nain Vert 2016	2	1139.5	3	589.3	
Mean Sum of Branch Lengths		**846.5**		**481.9**	

Table 4. "Vegetative Efficiency": Total branch length (cm)/stem diameter (mm).
(n = number of replicate plants, x = no data)

	Bartlett		Bosc		
interstem	**n**	**length/diam (cm/mm)**	**n**	**length/diam (cm/mm)**	**p < .05**
Pyrodwarf 2016	0	x	2	17.6	
Granatnaya (×Crataegosorbus)	3	24.8	5	19.8	**a**
Bosc - 2016	7	25.6	0	x	
P. nivalis compact 2016	2	27.6	4	18.6	
P. calleryana D6	5	30.4	3	24.3	**a**
Bartlett 2016	1	34.1	5	21.9	
P. syriaca - Armenia	6	34.6	5	32.2	
BP-2	8	34.9	7	32.3	
QR 708-12	8	35.3	6	28.0	
BU 2/33 - Pyro II	7	36.3	0	x	
P. spinosa Albania-38	3	37.1	6	25.8	
P. betulifolia - Shaanxi	6	38.0	4	18.3	
P. regelii	6	38.4	0	x	
Sbkta (P. elaeag.)	8	39.2	7	28.5	
Bartlett 2017	8	39.8	5	31.5	
Le Nain Vert 2017	2	40.0	2	20.7	
OHxF 69	8	40.4	4	20.6	
P. elaeagrifolia Armenia-38	4	40.9	3	16.9	
P. salicifolia (Russia hybrid 5)	8	41.1	6	27.2	
BP-1	6	41.3	7	25.4	
P. fauriei 12-14	8	41.4	2	23.9	
Passe Crassane	8	41.8	7	30.7	
OHxF 87	8	43.2	8	27.8	
Le Nain Vert 2016	2	43.7	3	36.5	
Bosc - 2017	7	44.1	8	28.5	
Pyronia	7	45.1	8	25.7	
OHxF 333	8	45.4	7	29.6	
Mustafabey	7	46.0	7	33.8	**b**
OHxF 97	7	46.3	2	26.5	
P. nivalis compact 2017	3	46.8	4	30.6	
P. salicifolia (Russia hybrid 1)	8	49.9	5	35.3	**b**
P. korshinskyi sdlg 4	7	50.6	7	29.1	**b**
P. sachokiana	6	51.8	5	27.5	**b**
Mean Total Length/Diameter		**39.9**		**26.5**	

EXECUTIVE SUMMARY

Project title: Interstem grafts to evaluate pear germplasm for dwarfing potential
Key words: Pear, Pyrus, Rootstock, Dwarfing, Germplasm, Interstem
Abstract: Interstem grafts on seedling rootstocks were evaluated as way to screen pear selections for dwarfing potential with Bosc and Bartlett scions. After 3 years two selections were identified as possibly dwarfing. However known dwarfing or vigorous rootstocks did not perform as expected, indicating 3 years are insufficient to properly evaluate rootstock potential.

The use of interstems as an alternative to grafting onto self-rooted rootstocks was investigated as a more rapid way to screen pear germplasm for dwarfing potential. Interstem pieces of 30 candidate pear selections were grafted onto seedling rootstocks, and then top-worked with buds of either 'Bartlett' or 'Bosc'. Trees were grown and evaluated in a greenhouse for the first year, and in a field planting for two additional years. Based on both total vegetative growth (sum of all stem and side branch lengths), and also on "vegetative growth efficiency" (total shoot growth per mm of stem diameter) following the third growing season, selections 'Granatnaya' and 'P. calleryana D6' were identified as worthy of further evaluation. Several pear selections known to be either dwarfing or vigorous as rootstocks did not control scion vigor as anticipated during the 3 years of this study. Additional years, including the transition from vegetative growth to fruit production, are required to properly evaluate rootstock potential. While a short-term assay may not be adequate for identifying dwarfing, the use of interstems rather than self-rooted rootstocks may still have great potential as an efficient method to screen germplasm for rootstock potential. The use of interstems avoids the expensive and time-consuming effort to generate self-rooted rootstocks by tissue culture or cutting propagation. This interstem field plot is now well established on the USDA germplasm farm, and there is evidence of abundant flower buds on some trees. We anticipate more useful information to result from this planting in future years.