FINAL PROJECT REPORT
WTFRC Project Number: PR-03-339

Project Title: Introduction and propagation of pear rootstocks

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Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	14,126	NA	NA
Benefits	9,182		
Wages	5,000		
Benefits	37		
Equipment	0		
Supplies	500		
Travel	0		
Miscellaneous	0		
Total	28,845		

Objectives: The overall objectives of this project were: 1) help the flow of clonal rootstocks, from research programs towards commercial propagation, and 2) improve propagation of these clones.

Significant Findings:
1) Propagated 2000-2500 liners each of Horner 4, Horner 10 and OHF87 for rootstock trials. These will be shipped to Van Well Nursery in May, 2007.
2) Demonstrated the feasibility of auxin-treated tie-off layering (ATTOL) for propagating pear rootstocks.
3) Three rootstocks from Kazakhstan are difficult to propagate.

Methods:

Layering. Stock plants of rootstock clones OHF 40, 87, 90 and 708-2, 12 and 36, were pruned near ground level in spring, 2006. The resulting shoots were layered during the second week of July. Treatments are described in the Results and Discussion. All layers were covered with clean sawdust contained by roofing felt.

Layers were harvested the second week of December, 2006. Rooting response was scored using the following system: 3 = well-rooted; 2 = acceptable, sufficient rooting to support growth and development; 1 = rooted, but unacceptable; 0 = unrooted.

Micropropagation. Cultures were established using vigorous shoot tips collected during active growth.
These shoots were surface sterilized in 10% bleach solution and planted in individual tubes containing DKW medium consisting of 0.8% agar, 3% sucrose plus DKW salts and vitamins. Shoots which were sterile and still actively growing were transferred to a multiplication medium consisting of DKW medium plus 1 ppm benzylaminopurine (BAP). Every 4-6 weeks, shoot clumps were divided into single shoots and re-cultured on multiplication medium.

When liquid medium is used in double-phase culture, enough liquid is added, about 25 ml, to nearly cover shoots that had just been divided and transferred (Figure 1).

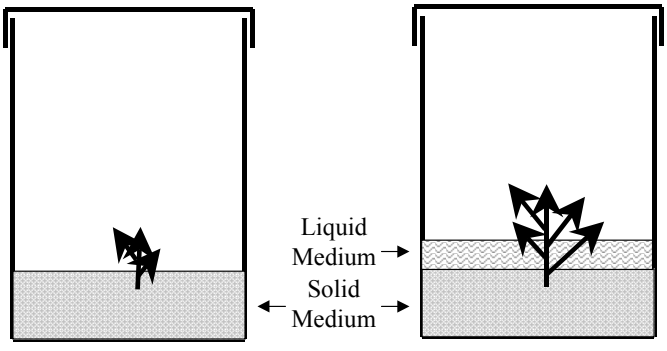

Figure 1. Single-phase vs. double-phase tissue culture.

When a sufficient number of shoots are available, the surplus is treated with indolebutyric acid (IBA) to stimulate rooting. Rooted shoots are transplanted into clean potting medium, grown under intermittent mist for two weeks and then transferred to the greenhouse. In the greenhouse, the shoots are grown to liner size and transferred to other research programs.

For transfer to commercial micropropagators, shoot cultures are sealed in sterile, plastic pouches containing a small amount of DKW solid medium and mailed to the nursery.

Table 1. Pear rootstock clones in tissue culture at OSU, December 2006.	
517-9	OHxF 87
708-13	OHxF 97
96FI11	Pyronia
96FI12	Q29857
Fox 11	Q29858
Fox 16	Q29859
96FI15	Horner 4
OH11	Horner 10
OHxF 40	

Results and Discussion.

1) **Horner rootstocks.** A small trial in the 1990's of the so-called Horner collection of pear seedlings found at least two rootstocks, Horner 4 and 10, worthy of more study. This is a remarkable result from such a small sample, suggesting that the entire collection should be screened as quickly and as systematically as possible. Delaying more complete studies of the population's potential could cost a decade or more.

Starting in 2001, we began to clonally propagate all of the Horner seedlings to produce liners for small trials of the entire population. These trees are now at Hood River and will be evaluated in coming years.

- We initiated shoots of Horner clones 4 and 10 into tissue culture and in 2006 produced about 2000-2500 liners of each of these clones plus OHF87 for orchard trials. These liners are growing through the winter and will be transported to Van Well Nursery in May, 2007 for budding and tree development.

- At the 2006 Pear Research Review, we also proposed to initiate tissue cultures of additional Horner clones based on early evaluations of the field trial and grower consultation. The rationale was that as the trials progress and more information becomes available, cultures will either be culled or continued. When interest warrants larger trials of a given clone, the cultures will already be established, enabling more rapid liner production for the next round of trials and a source of cultures for interested nurseries, thus keeping the process moving.

This early propagation strategy has proven very useful for the Oregon hazelnut industry. As Eastern Filbert Blight-resistant cultivars are developed at OSU, promising clones are initiated in culture and are ready for release to nurseries in advance of the variety release.

In 2006, several clones appeared to have promise, although the trees are still young. Discussion among the pear research committee felt it was too early to initiate 10 or 20 clones. Luigi and I would respond that this is the point of our program. Initiating and maintaining a few shoots of several clones is relatively simple compared to initiating clones at a later date when demand may soar. The complication now, however is that the OSU propagation program will end by January 1, 2008. Other tissue culture labs may differ with us on when to initiate prospective clones.

2) **Propagating rootstocks by layering.** Clonal propagation of pear rootstocks is not a trivial problem. Pear does not respond to standard mound layering used for apple. Hardwood cuttings are difficult to propagate, yielding a low percentage of rooting and poor root quality. Softwood cuttings root well, but require significant skill and attention. Most clones respond to tissue culture, but availability is probably doubtful early in a rootstock's development period.

Another facet of the problem is that sales of pear trees in recent years have not been particularly attractive to the nursery industry. As a result, one nursery is the main source of pear liners and these are propagated by **hardwood cuttings**.

Our view is that hardwood cuttings of pear are an inherently limited option for which breakthroughs are unlikely to occur. Tissue culture alone or in combination with softwood cuttings or modified forms of layering offer better options.

Tissue culture of pear is well-established and most genotypes respond, with some notable exceptions. [OHxF 51is difficult, as is the Brossier series.] However, the OHxF series are generally easy to culture, as are Horner 4 and 10. Thus, besides rootstock potential, a criterion for rootstock selection, especially in the Horner series, should be propagation potential.

Until there is a "killer" rootstock that creates a significant market for trees, nurseries will be reluctant to commit major resources to propagating pear liners. In the meantime, tissue culture will probably be the method of choice, if a lab can be persuaded to cooperate. In 2006, pear growers have reportedly teamed-up to ensure larger-scale micropropagation of pear rootstocks.

Softwood cuttings. A useful property of tissue culture is that liners have higher propagation potential for a few seasons afterwards. We have documented that such plants used as a source of softwood cuttings root at higher percentages with high quality root systems. Unfortunately, softwood cuttings are a specialized technique that many tree fruit nurseries are uncomfortable with. Even with the higher propagation potential of cuttings from tissue cultured stock plants, pears demand a highly-regulated mist environment. This presents a challenge that nurseries so far have been unwilling to deal with- given the state of the market.

Mound layering, mechanized to produce millions of liners, revolutionized the apple industry. Pears don't respond well to this technique. Other species share this problem. These species do respond, however, to layering when shoots are girdled and treated with auxin before being layered. This technique, designated auxin-treated, tie-off layering (**ATTOL**), is commonly used by the Oregon hazelnut industry. My hope is that ATTOL can fill the gap between the level of OSU production and the point at which large-scale propagation begins. Perhaps it may even compete with tissue culture at higher levels of production.

As noted above, this effort is based on the fact that until recently, the nursery industry showed little interest in producing pear rootstocks. Adding ATTOL as an option along with tissue culture and softwood cuttings may help more nurseries, or even orchardists, see themselves as pear propagators thereby increasing competition, as well as total liner output.

• In 2006, we tested ATTOL on pear. Own-rooted stock plants established for cutting production were cut off near ground level to stimulate shoot production. We tested: 1) the effects of girdling and IBA application on rooting and 2) the effect of IBA concentration.

Table 2. Effects of indolebutyric acid (IBA) and girdling on root formation on layered pear shoots. All shoots on a stock plant received the same treatment. Treatments were randomized across stock plants. IBA concentration was 20,000 ppm.

		Clone		
Treatment	Response	OHF 40	OHF 87	OHF 97
Untreated	Total Rooting (%)	31.2	0	1.2
	Acceptable Rooting (%)	1.6	0	0
IBA	Total Rooting (%)	37.0	0	4.2
	Acceptable Rooting (%)	9.0	0	0
Girdle	Total Rooting (%)	74.5	41.2	39.2
	Acceptable Rooting (%)	43.4	17.6	17.2
IBA + Girdle	Total Rooting (%)	91.3	61.7	73.7
	Acceptable Rooting (%)	63.3	33.3	44.6

This experiment confirmed that pear responds poorly to simple mound layering. The key factor for stimulating rooting is girdling, as IBA alone had no effect. Based on our observations and the experience of David Smith of the OSU hazelnut breeding program, the quality and location of the girdle are important. Metal rings are most effective, as non-metal materials, such as nylon-ties, expand as shoots grow and fail to girdle the stem.. Care must be taken to ensure a tight fit when the ring is applied. Placement of the ring as close to the base of the shoot as possible may improve rooting and enables shoots to be easily snapped off at harvest.

Girdling and IBA combined were the most effective treatment. Even with this combination of treatments, 30-50% of the rooted shoots were graded unacceptable. An important question is whether

these culled liners can be used? Are we grading too stringently? Are layered pears equivalent to pear hardwood cuttings, which often have poor growth and high mortality, or are they like some layered apple rootstocks which develop few roots during layering, but grow and develop well subsequently?

• Response to IBA concentration varied somewhat (Tables 3-5). Overall, we would recommend use of 20,000 ppm IBA, however 708-36 consistently produced better rooting in response to 5,000 ppm. Clone 708-36 will not be a commercial rootstock, but clones under development should be screened for IBA response.

• The stock plants we used for this study were mature. As a result, our trials provide a conservative assessment of ATTOL. We compared the response of similar-aged stock plants originally propagated by either softwood cuttings or micropropagation. Even after ten years, the micropropagated stock plants were more productive (Tables 3, 4). Young, micropropagated stock plants will respond at least as well, possibly much better. We will conduct a small trial on two year-old micropropagated stock plants in 2007.

Regulating shoot growth will be an important management question for ATTOL. In 2006, we fertilized the stock plants, which proved unnecessary, as many of the shoots were too large for use as liners. In the future, however, years of girdling may reduce vigor. Experience will teach appropriate management.

Table 3. Effect of IBA concentration on rooting of shoots of pear clone 708-12. Stock plants were originally propagated in 1997 from cuttings or tissue culture. All shoots were girdled.

		IBA (ppm)			
Stock Plant	Response	0	5,000	10,000	20,000
Cutting	Total Rooting (%)	33.4	48.9	58.8	53.1
	Acceptable Rooting (%)	11.3	28.8	29.3	36.9
Micropropagated	Total Rooting (%)	44.4	26.2	74.5	85.0
	Acceptable Rooting (%)	12.5	19.0	40.7	68.7

Table 4. Effect of IBA concentration on rooting of shoots of pear clone 708-36. Stock plants were originally propagated in 1997 from cuttings or tissue culture. All shoots were girdled.

		IBA (ppm)			
Stock Plant	Response	0	5,000	10,000	20,000
Cutting	Total Rooting (%)	23.3	60.0	54.3	52.3
	Acceptable Rooting (%)	13.3	43.3	41.0	29.5
Micropropagated	Total Rooting (%)	80.0	100	89.5	82.9
	Acceptable Rooting (%)	50.0	77.8	65.5	75.2

Table 5. Effect of IBA concentration on rooting of shoots of pear clone 708-2. All shoots were girdled.

		IBA (ppm)			
Stock Plant	Response	0	5,000	10,000	20,000
Cutting	Total Rooting (%)	26.4	17.7	50.0	81.7
	Acceptable Rooting (%)	3.8	8.3	30.8	53.3

3)**Kazakhstan clones**.

• In February, 2002, we received budwood from three clonal rootstocks, Q29857, Q29858, Q29859, from Kazakhstan. These were initiated into tissue culture. APHIS released these clones spring, 2005.

Our experience to date is that these clones are generally difficult to micropropagate.

Clone	Approx. Shoot Multiplication Rate	Rooting (%)
Q29857	1x (poor)	NA
Q29858	2x (slow)	ca. 50%
Q29859	4x (good)	ca. 50%

Propagation of Q29859 is feasible, but at this point, the other two have doubtful propagation potential. Repeated application of liquid medium has only limited effect on shoot growth. Rooting of both 858 and 859 has improved from 17% in initial trials to 50% in recent work with the addition of 1% Activated Charcoal in the rooting medium.