



Use of an Artificial Inoculation Technique to Identify American Beech Trees with Resistance to the Beech Scale Insect



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Objective

A small percentage of American beech (*Fagus grandifolia*) trees remain disease free in stands long-affected by beech bark disease and challenge trials have shown that they are resistant to the scale insect (Houston 1982). Increasing the number of resistant beech trees while reducing the proportion of susceptible trees is currently thought to be the best management approach to minimize the overall impact of beech bark disease (Mielke et al., 1986). Even in heavily infested areas, trees that remain clear of scale may be "escapes" and not truly resistant. Previous work by David Houston (1982) reported an artificial inoculation technique that confirmed the resistance of older, scale-free trees and successfully infested one-year old seedlings. We are currently setting up experiments designed to determine if this technique will be an effective tool in distinguishing resistant from susceptible American beech trees. To directly compare resistant and susceptible individuals we are using three different tree sources: 1) root sprouts, whose clonal relationships will be confirmed using DNA analysis, 2) softwood cuttings, and 3) seedlings, both from open-pollinated sources (half-sibs) and from controlled cross-pollinations (full-sibs). The artificial inoculation technique will allow cross-progeny to be "screened" for their resistance phenotype, giving insight into the genetic basis and mechanism(s) for resistance. Eventually, superior resistant progeny will be selected and used to develop seed orchards to provide an enriched source of resistant beech for plantings. Resistant trees are needed for planting ahead of the disease front to minimize the impact, and in aftermath forests to restore healthy, productive American beech trees to the forest landscape.

Houston, DR (1982) USDA Forest Serv. Res. Pap. NE-507. 8p.
Mielke ME, Houston DR, Bullard AT (1986) In: Proceedings, Integrated Pest Management symposium for northern forests; 1986 March 24-27; Madison, WI: University of Wisconsin, Cooperative Extension Service; 272-280.

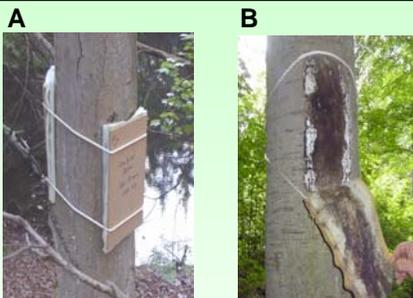


Figure 1. Insect traps. A. Insect traps, patterned after those used by Houston, 1982, were set in 2002 in Ludington State Park, MI, the Allegheny National Forest, PA, and Holden Arboretum, OH. B. The foam pad is pulled back revealing the concentration of scale colonies underneath in comparison to the rest of the tree (July 2003). Eggs for challenge experiments were easily collected from traps like this.

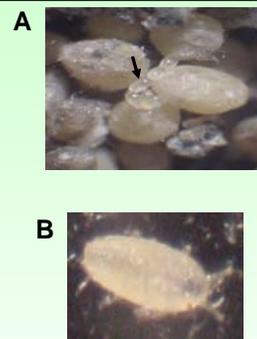


Figure 2. A. Scale eggs. Swollen, egg-laden adult scale insects (0.5 mm) can be seen in the background with a chain of five smaller eggs in the foreground (black arrow). B. Newly hatched "crawler".



Figure 3. Field challenge experiments. A cluster of 20 resistant trees (background) was challenged with insect eggs, along with a cluster of 8 susceptible trees (one can be seen in the foreground) in Ludington State Park. A similar trial was set up in the Allegheny National Forest. DNA analysis will confirm the clonal relationships of the individuals within the clusters. This experiment was designed to determine the level of variability between clonal replicates of varying size & age when the artificial inoculation technique is used to test for resistance in a forest setting.

Treatment	% rooted	% new growth
IBA	22	10
IAA	53	9

N=8



Figure 4. Softwood cuttings of American beech. A. Summary of success rates of treatments given to softwood cuttings taken at bud break. B. Root formation of an individual cutting. C. Six weeks after cutting were taken. Softwood cuttings will be another approach to testing clonal replicates with the artificial inoculation technique.

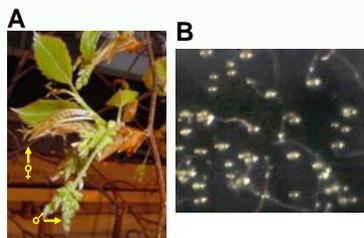


Figure 5. A. American beech flowers. To collect pollen for use in controlled cross-pollinations dormant branches were brought into the greenhouse to force pollen production prior to natural pollen release. B. Pollen viability assay. Prior to use in controlled crosses, all pollen was tested for viability on germination media. The formation of a pollen tube confirms viability of an individual pollen grain.

Pollen Donor	Maternal Parent	Percent Viability 5/19/02	Percent Viability 6/24/02	No. flowers brushed	No. of nuts per flower
1501 (I)	1504 (R)	50	4	69	0.51
1506 (S)	1504 (R)	30	16	249	0.32
1504 (R)	1506 (S)	50	41	98	0.97
1504 (R)	1505 (R)	40	28	115	0.53
1506 (R) @ 4° C	none	51	50	N/A	N/A
1506 (R) @ 20° C	none	54	50	N/A	N/A

Figure 6. Pollen viability in American beech. The viability of pollen used in the controlled crosses ranged from 30 to 50 percent. The percent viability of pollen correlated somewhat with the number of nuts produced per flower pollinated. Viability assays were performed on the pollen samples after they were used for pollination and all pollen retained at least some viability after being brought out into the field for several hours then returned to cold storage for a month. The drop in viability for pollen samples not used in pollination experiments and simply kept in cold storage for a month was very small. R = resistant, S = susceptible, I = intermediate.

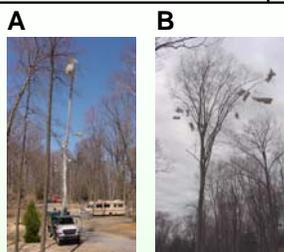


Figure 7. Initiation of controlled cross-pollinations. With the help of a bucket truck (A) from Consumers Energy, pollination bags were placed over branches prior to flower emergence to prevent pollination from occurring with any contaminating pollen. Once the female flowers had emerged and were receptive, the bags were removed only long enough to brush the desired pollen directly on the individual flowers. The pollination bags (B) were kept in place for an additional three weeks post-pollination.

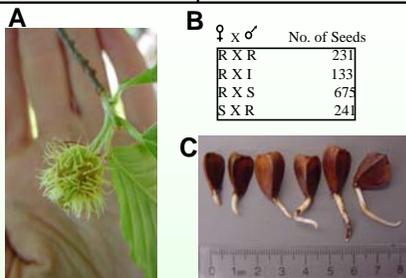


Figure 8. A. Developing beech seed after removal of pollination bag. B. Table showing the number of seeds harvested from each combination of parents used in controlled crosses. R = resistant, S = susceptible, and I = intermediate. C. After harvest, beech seeds were removed from the outer burr and subjected to cold-stratification. Germination began after 100 days of cold treatment. Seeds shown are from a single cross.

♀ x ♂	No. of Seeds
R X R	231
R X I	133
R X S	675
S X R	241

Open-Pollinated Seedlings

Parent Tree	No. of Seeds	% Full	Germinative Capacity	No. of Plants
1506 (S)	802	35	60 %	168
1504 (R)	2081	28	8.5 %	49
1511 (S)	478	24	2.6 %	3
ME (R)	283	75	53 %	149

Figure 9. Open-pollinated seedlings derived from seeds of American beech trees. Between 24 and 35 percent of the seeds collected from Ludington State Park were full, which is only slightly higher than the 13 to 29 percent sound seed collected in a previous study in East Lansing, MI (Gysel, 1971). The percentage of sound seed collected from the tree in Maine (ME) was much higher, at 75%. This value is comparable to the 75 to 88 percent sound seed collected from beech in the White Mountain National Forest by Leak & Graber (1993). The significantly lower number of full seed collected in Michigan is likely attributable to several environmental factors, among which is the fact that Ludington State Park is at the northern-most border of the natural range of American beech.

Gysel, LW. 1971. J. of Wildlife Mgmt. 35(3): 516-519.
Leak, W.B., Graber, R.E. 1993. USDA Forest Service Res. Pap. NE-677. 6p.

Cross-Pollinated Seedlings

Cross ♀ x ♂	Seeds					% Full	Germinative Capacity	Total No. Plants
	Full	Germinated	Rotten	Empty	Total			
1506 (S) x 1504 (R)	11	84	0	146	241	39	81 %	77
1504 (R) x 1506 (S)	49	31	10	585	675	13	12 %	11
1504 (R) x 1501 (I)	35	0	0	98	133	26	37 %	13
1505 (R) x 1504 (R)	28	33	0	170	231	26	84 %	51

Figure 10. Controlled cross-pollinated seed from American beech trees. The germinative capacity observed for cross-pollinated seed was similar to that for open-pollinated seed (Fig. 8), indicating that the pollination bagging process did not negatively affect seed development. The variation in germinative capacity between different parent combinations indicates some mating incompatibilities. For example, when 1504 was used as a pollen donor with either 1506 or 1505 the resulting seeds had a high germinative capacity (81 and 84 percent, respectively). However, when 1504 was used as the maternal parent with either 1506 or 1501 as the pollen donor, the seed produced had much lower germinative capacities (12 and 37 percent, respectively).

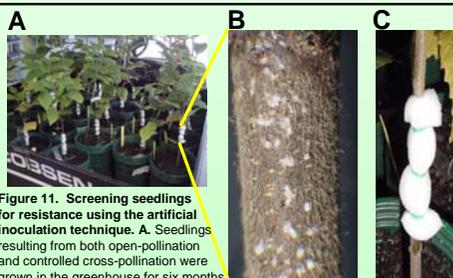


Figure 11. Screening seedlings for resistance using the artificial inoculation technique. A. Seedlings resulting from both open-pollination and controlled cross-pollination were grown in the greenhouse for six months before being transferred to a lathe house at Holden Arboretum, Kirtland, OH. B. Seedlings were challenged with either 30 egg-laden adult scale insects (8-12 eggs) or 300 individual eggs. C. Six weeks after the challenge was initiated, peeling back the bark and began secreting the white wooly wax substance characteristic of the beech scale insect. The seedling in this photo was derived from seed collected from an open-pollinated, susceptible tree.

Summary

Field experiments to test the effectiveness of the artificial inoculation technique on clonal replicates of varying age and size using naturally occurring clusters of both resistant and susceptible trees have been initiated. DNA analysis will be used to confirm the relatedness of individual trees within the clusters. In the summer of 2004, the initial data on the number of reproductive colonies per challenged tree will be collected. A technique to make softwood cuttings has been established and will be optimized over the next year. Softwood cuttings will be used in future experiments to test the insect challenge technique on clonal replicates of the same age. Data collected from both the field experiments and experiments using softwood cutting-generated replicates will be used to develop guidelines for using the artificial challenge technique as a management tool in identifying and retaining beech bark disease-resistant American beech.

Both full- and half-sib families have been generated for use in genetic studies. Currently, the seedlings are being challenged using the artificial inoculation technique to determine their resistance phenotype. By summer of 2004, we should be able to determine if the artificial challenge technique is sensitive enough to "screen" for resistant seedlings. The ability to screen for a desired trait is a prerequisite to a successful breeding program. Information on how resistance may be inherited will also be obtained.

A longer term goal of this work is to preserve resistant germplasm and maintain regional adaptive genetic variability. The softwood cutting technique for vegetative propagation can be applied to this goal by using it to preserve mature, superior resistant trees found throughout the United States. These superior trees can subsequently be used as parents in a breeding program.

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