Background
Beech bark disease complex (BBD) has been attacking American beech in forests since the accidental introduction of the scale insect Cryptococcus faginatus Lind. in Nova Scotia around 1900 (Ehrlich, 1984). BBD begins when bee tissues attacked by the scale insect are rendered susceptible to infection by fungi of the genus Nectria. Since the introduction of the scale, the disease has spread westward and southward through forests of Canada and the United States and is well established in New England, New York, New Jersey, West Virginia, and Pennsylvania. More recently BBD has been discovered in Michigan and the presence of the scale insect has been reported in North Carolina, Tennessee, and Northeastern Ohio. Generally, fungal infection and tree mortality occur 1 to 4 years after heavy build-up of the insect on large trees (Houston, 1997). In aftermath forests, both causal agents establish on small trees of both root sprout and seedling origin. The newly emerging trees and surviving older infected trees are rendered highly defective by the scale-Nectria complex.

Figure 1 Beech Flowers. To obtain pollen to perform controlled crosses, dormant branches from both resistant and susceptible trees were harvested and placed in artificial media in the greenhouse to stimulate flower emergence.

Objective
A small percentage of American beech (Fagus grandifolia) trees remain disease free in stands long-effected 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 infected 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) half-sibs, both naturally occurring and artificially induced through mechanical wounding, 2) grafted material, and 3) seedlings, both from open-pollinated sources (half-sibs) and from controlled crosses (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.

Controlled Cross-Pollinations with Resistant American Beech

Table showing the number of seeds harvested from each combination of parents for controlled crosses. R = resistant, S = susceptible, I = intermediate.

<table>
<thead>
<tr>
<th>Combination</th>
<th>No. of Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>R X S</td>
<td>131</td>
</tr>
<tr>
<td>R X R</td>
<td>223</td>
</tr>
<tr>
<td>S X R</td>
<td>257</td>
</tr>
</tbody>
</table>

Artificial Inoculation Technique to Identify American Beech trees with Resistance to the Beech Scale Insect

Summary
We have initiated work to test D. Houston’s artificial inoculation technique to determine if it will be a reliable method for distinguishing between resistant and susceptible beech. We have set traps for egg collection in OH, MI and PA on susceptible trees to collect eggs for challenge experiments, and on putatively resistant trees to confirm their resistant phenotype.

Figures:
1. Beech Flowers. To obtain pollen to perform controlled crosses, dormant branches from both resistant and susceptible trees were harvested and placed in artificial media in the greenhouse to stimulate flower emergence.
2. Pollen viability. All harvested pollen was tested for viability prior to being used in cross-pollinations. Pollen tube formation on pollen germination media indicates viability. On average, the pollen we used in the controlled crosses had a 50% rate of viability based on these assays.
3. Initiation of Controlled Cross-Pollinations. With the help of a bucket truck 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 desired pollen was brushed directly on the flowers and the pollination bags were kept in place.
4. American Beech Seeds. A. Developing beech seed after removal of pollination bag. B. Beech seeds were harvested and subjected to cold-stratification. Seeds shown are from a single cross, and began germinating after 100 days of cold stratification. Variation in germination rate makes determination of % germination premature at this time. Dissection of a subset of open-pollinated seeds indicated that 40% of the harvested seeds were full. C. Table showing the number of seeds harvested from each combination of parents for controlled crosses. R = resistant, S = susceptible, I = intermediate.
5. Insect Traps. Traps have been placed in OH, MI and PA on susceptible trees to collect eggs for challenge experiments, and on putatively resistant trees to confirm their resistant phenotype.
6. Seeding Inoculation. These photos taken from Houston, DB (1982) USDA Forest Serv. Res. Pap. NE-507, illustrate the technique used to (A) wrap insect eggs around seedling stems, leading to the establishment of scale insect colonies (B) one year later. This technique will be used to test the cross-progeny, using eggs collected from traps like the one shown in Fig. 5.
7. Resistant Clusters. American beech have a propensity for reproducing through the production of root sprouts. Clusters such as this give us the opportunity to test the artificial inoculation technique on clonal replicates and to determine the level of variability between them.