Objective

Increasing the number of resistant beach trees while reducing the proportion of susceptible trees is currently thought to be the best management approach to minimize the overall impact of beach bark disease (Mieke 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 have initiated tests to determine if this technique will be an effective tool in distinguishing resistant from susceptible American beach trees. To directly compare resistant and susceptible individuals of a variety of ages, we tested two-year-old seedlings and clonal root sprouts of varying sizes. The seedlings were members of other full- or half-sib families and were produced from seed that was the result of open-pollination of a resistant tree or controlled cross-pollination of either two resistant parents or a resistant and a susceptible parent. A second goal of our work is to determine if resistance is a heritable trait that can be incorporated into an American beach breeding program.

The artificial inoculation technique is used to “screen” these seedlings for resistance. Field trials of the artificial inoculation technique were also established to test the efficacy of these techniques. Clusters of putatively resistant trees were chosen because of the likelihood of the individuals being clonal root sprouts and therefore genetically identical replicates that could give an indication of the reproducibility or variability that results from this baccula for resistance.

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Results

Figure 1. 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 flowers emerged, pollen was collected and controlled cross-pollinations were performed.

Figure 2. Summary of the results of controlled cross-pollinations. The range of full seed produced as a result of the crosses was similar to what was observed in open-pollinated seed (data not shown).

Figure 3. Insect traps for collection of scale insect eggs. A. Trapped traps, performed after those used by Houston, 1982, were set in 2002 in Ludington State Park, MI, the Allegheny National Forest, PA, and Hidden Arboretum, OH. B. The foam pad is pulled out to reveal the collection of scale insects underneath in comparison to the rest of the tree (July 2003). Eggs for challenge experiments were easily collected from traps like this.

Figure 4. A. Scale eggs. Sedges, egg-laden adult scale insects (5.5 mm) can be seen in the background with a chain of five smaller eggs in the foreground (black arrow). B. Newly hatched “crawlers.”

Figure 5. Screening seedlings for resistance using the artificial inoculation technique. Cross-pollinated trees were set out at Hidden Arboretum, Kirtland, OH. Panel B shows scale insects beginning to establish on the bark of the seedling less than a month after 150 eggs were placed on the stem using the foam trap shown in C.

Figure 6. Results of Artificial Infestation of Seedlings. A year after the eggs were placed on the seedlings, the foam was removed and the number of scale insects were counted. Trees with five or fewer insects were deemed resistant. The chi-square goodness of fit test (P) suggests that resistance is not a single gene trait.

Figure 7. Comparison of the frequency of resistance. The proportion of resistant seedlings resulting from an R x S cross is 1.3, which is what is also observed in open-pollinated seedlings from a resistant tree (1504) and also from a susceptible tree (1505) located within ten feet of a resistant tree. The susceptible tree 1510 produced about 10% resistant seedlings. The proportion of resistant seedlings resulting from an R x R cross is about the same observed for open-pollinated seedlings from a resistant tree in Maine (ME). The ME tree was located in a stand where all susceptible trees had been removed so it is likely all potential pollen donors are also resistant trees. The similarity in the proportions of resistant seedlings observed is an indication that the artificial infestation technique is successfully distinguishing between resistant and susceptible.

Figure 8. Field test of the artificial inoculation technique on “clusters” of resistant trees. Clusters of resistant trees such as this have a high probability of being root sprouts and therefore clonally related. Clusters were chosen for the field test as a way to provide "replicates" of trees of the same genotype but of different ages. Two hundred eggs were placed under foam pads on each tree, and were removed to assess insect colonization after one year.

Figure 9. Results of the artificial infestation of field trees. A. Two clusters were tested at Ludington State Park, one that appeared resistant (little to no scale) and a second cluster that was susceptible (medium to heavy scale infestation). RAPD (randomly amplified polymorphic DNA) analysis has shown that all trees listed in red are clonal (genetically identical, derived from root or stump sprouts) and all trees listed in black are clonal (data not shown). The number of markers used in the assessment of genetic relatedness (N=47) can identify clonal relationships with only a 1 in 5239 chance of being incorrect for this population. For the most part, trees that were visually assessed as being resistant had no more than 32 insects under the foam. However, three of the "susceptible" trees had less than 32 scale insects. Tree number 24 had died and was no longer able to support a scale population, but tree 23 had only 16 scale insects and tree 27 had 20 scale insects. Because the visual scale assessment of these trees indicated moderate to heavy scale infestation, it is likely that these trees are false negatives. We hypothesize that excess moisture in the foam pad contributed to the tree being unable to sustain a scale infestation under the foam. Three other trees (11, 12, and 13) retained moisture to the point of causing the pad to turn black with moisture. B. A second cluster of resistant trees (based on visual assessment) were tested in the Allegheny National Forest. DNA analysis again showed that the trees were clonally related. Three individual scales of susceptible trees were tested as controls, but after a year only one tree number 12 still had the foam trap intact. C. Due to the observation that excess moisture in the foam pad contributed to scale insect colonization, a foam trap with a "gutter" and a backing that allows greater air contact was designed and is currently being tested.

Summary

The artificial inoculation technique was used to screen seedlings for their resistance phenotypes. The results after the first year indicate that this test effectively distinguishes between resistant and susceptible seedlings. Results will be confirmed after a second year of testing, and long-term durability of resistance will be tested as the seedlings are established in field plantings. The higher proportion of resistant seedlings resulting from an R x R cross compared to an R x S cross, is the first proof that resistance is a genetically inheritable trait. Furthermore, a chi-square goodness of fit test of the ratio of resistant to susceptible seedlings resulting from the crosses, indicates that resistance is not a single gene trait.

Field trials in Ludington State Park and the Allegheny National Forest of clonally related “replicates” showed some variability in the scale challenge results between trees that are genetically identical. Because the visual scale assessment did not agree with the challenge test results, we hypothesize that excess moisture that is caused by the foam traps caused an environment that is not favorable to scale insect colonization. To address this, a new trap was designed that uses aluminum flashing to form a gutter for drainage and a backing material that allows maximum air contact with the foam. This trap is currently being tested.

Overall, the challenge techniques appears to successfully distinguish between resistant and susceptible trees of a variety of ages. However, it will be important to assess the reproducibility of these data in a second year of scale challenge experiments.

References
