RESISTANCE IN AMERICAN BEECH TO CRYPTOCOCCUS FAGISUGA: PRELIMINARY FINDINGS AND THEIR IMPLICATIONS FOR FOREST MANAGEMENT

David R. Houston and Daniel B. Houston[1]

Abstract.--Beech bark disease (BBD) occurs when hark of American or European beech, infested by the beech scale, Cryptococcus fagisuga (C.f.), is infected and killed by fungi of the genus Nectria. Since its introduction from Europe to Halifax, Nova Scotia, about 1890, C.f. (and BBD) has spread through the Maritime Provinces, southern Quebec, New England, New York, and northern Pennsylvania. Isolated outbreaks occur in West Virginia, Ohio, and Ontario. High mortality of larger trees characterizes the initial epidemic, while severe defect of residual and emerging young trees is typical of the endemic aftermath phase. Some trees are completely free of C.f. attack and BBD. Especially evident in long-affected forests, disease-free trees are found in low numbers, and often in groups. In repeated challenge trials, C.f. has failed to become established on these trees that are now considered to he immune.

An understanding of the genetic relationships (origins) of extant resistant trees, and the genetic structure of stands in which they occur, is a prerequisite to developing silvicultural guidelines designed to maintain or enhance future levels of resistance. Preliminary analyses comparing trees on the basis of nine polymorphic isozyme systems from dormant buds indicate that within groups of resistant trees, many individuals are genetically identical (clonal), while others have unique isozyme "genotypes." The slight differences noted between many of the unique trees growing in close proximity within resistant groups suggest that they may represent half- or full-sibs.

Additional keywords: Beech hark disease, Fagus grandifolia, beech scale, isozyme

INTRODUCTION

Beech bark disease is a major diehack-decline disease that causes significant mortality and defect in American beech, Fagus grandifolia (Ehrh.). It results when bark, attacked and altered by the beech scale, Cryptococcus fagisuga Lind., is invaded and killed by fungi, primarily Nectria coccinea var. faginata Lohman, Tatson, and Ayers, and sometimes N. galligena Bres. (Ehrlich 1934; Lohman and Watson 1943; Cotter and Blanchard 1981).

Sometime before 1890, the scale was accidentally brought to Halifax, Nova Scotia on nursery stock of European beech (Fagus sylvatica L.); by 1925, it was present throughout mainland Nova Scotia (Ehrlich 1934; Hawholdt 1944). By 1932,

[1] Principal Plant Pathologist, USDA Forest Service, Hamden, CT, and Associate Professor, Ohio Agricultural Pesearch nevelopment Center, The Ohio State University, Wooster, OH.

the scale and an associated Nectria fungus were killing trees throughout the mature beech stands of the Maritime Provinces and in localized areas of eastern and south-central Maine. The scale insect has continued to spread to the north into Quebec and to the west and south throughout New England, New York, New Jersey, and northern and eastern Pennsylvania (Houston et al. 1979; Lavallee 1976; Magasi and Newell 1983; Lachance 1983; Towers 1983; Houston 1984). In 1981, an isolated outbreak of the disease was discovered in a large area of the Monongahela National Forest in northeastern West Virginia (Mielke et al. 1982; Mielke and Ciesla 1984). More recently, the insect was detected in Virginia (near the W. Va. site) and in Ohio (Mielke et al. 1985).

The pattern of insect spread and the subsequent occurrence of Nectria infection and tree death or defect has led to an arbitrary classification of disease development over time and space (Shigo 1972). In the advancing front, areas recently invaded by the beech scale are characterized by forests with many large, old trees supporting scattered sparse, building populations of the insect. In the killing front, areas are characterized by high populations of beech scale, severe Nectria attacks, and heavy tree mortality. And in the aftermath zone are areas where heavy mortality occurred at some time in the past and which now possess some residual big trees and many stands of small trees. Often, these stands are rendered highly defective through the interactions of established populations of beech scale, Nectria fungi, and another scale insect, Xylococculus betulae (Perg.) Morrison (Houston 1975). At present, the killing front is developing in central and southern New York, northern Pennsylvania, and in the oldest areas of the West Virginia outbreak. The aftermath zone occurs in most of New England, especially in Maine, and in eastern New York and the Maritime Provinces of Canada.

Some trees remain disease-free in aftermath stands. Recent challenge trials have shown them to be resistant to C. fagisuga (Houston 1982, 1983). On some trees, no insects became established; on others, they established and overwintered, but then died. By contrast, C. fagisuga readily completed its life cycle on susceptible trees. After three successive annual challenges, C. fagisuga failed to infest resistant trees, but on susceptible trees, even initially established populations have persisted and increased.

R esistant beech trees are found in relatively low numbers. In two stands in Nova Scotia, approximately 15 of 2,100 and 12 of 1,900 beech stems per hectare were free of insects or disease (Houston 1983). Some of these trees exist as single trees, many others in groups. In one 2.4-ha stand, 30 of 32 disease-free trees were in one 0.04-ha area; in a 4-ha block of forest in Maine, 5 of 8 such trees were located in a 0.04-ha area. Croups of resistant trees have been located in forests of Prince Edward Island, Nova Scotia, Maine, New Hampshire, Massachusetts, New York, and West Virginia. The spatial distributions of the resistant trees in stands in eastern Maine are shown in Figures 1-5. The susceptible trees are not shown.

The basis for the observed resistance to C. fagisuga is not known. The complete Freedom from infestation (immunity?) of a low proportion of the beech trees growing in long-affected forests, and the inability of C. fagisuga to become established on these trees even after repeated challenge trials, suggest

the presence of an inhibitory or toxic chemical, or the absence of one renuired for insect development. Resistance, though not complete, occurs in European beech as well (Lonsdale 1983). The basis for this "partial" resistance, which also seems to occur in American beech (Houston 1982), may lie in the anatomy of the bark. Compared to that of highly susceptible trees, the hark of partially resistant trees has layers of thick-walled cells that are relatively more continuous and closer to the surface (Lonsdale 1983).

Regardless of the underlying basis for the expressed resistance, it is important to understand the genetic relationship, i.e., the origins, of resistant trees if sound silvicultural approaches to enhance or maintain levels of resistance are to be developed. Approaches designed to favor production and establishment of seedling progeny from resistant parents presumably would he quite different from those designed to increase, vegetatively, the numbers of root sprouts of resistant ortets.

This paper reports the results of preliminary studies to determine, using isozyme techniques, the genetic relationship of resistant trees in several forests in Maine.

METHODS AND MATERIALS

Stands and Trees

In studies of beech hark disease during the past 7 years, a number of trees free of insects and disease were located in Maine and subsequently challenged to confirm resistance to C. fagisuga (Houston 1983). These trees, growing near Franklin and Amherst, and several others near Lambert Lake that were located and challenged in the course of a stand regeneration trial (Ostrofsky and McCormack 1986) were sampled in this study.

One hundred dormant buds on 10- to 15-cm-long twigs were collected from each of 42 resistant trees in February 1985, and placed over ice in insulated coolers. Buds were removed within 4 days of collection, deep frozen in liquid N_2, and stored at -85 C until analyzed. In preliminary trials, enzyme activity was maintained for at least 1 year.

Isozyme Analysis

Nine enzyme systems were characterized in dormant bud tissue for this study. They included alcohol dehydrogenase (ADH), a-glycerophosphate dehydrogenase (a-GPDH), cytochrome oxidase (CTO), diaphorase (PIA), peroxidase (PER), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), 6-phosphogluconate dehydrogenase (6-POD), and malate dehydrogenase (MDH).

Enzymes were extracted from dormant bud tissue, after removal of bud scales, by grinding single buds in a 0.75-m1 chilled extraction buffer, pH 6.7, containing P7 PVP (w/v, 7:1 of 40M; 360M), 0.3M sucrose, 0.5mM EDTA, 1mM dithiothreitol, 1mM ascorhate, 0.17 bovine serum albumin, 0.4mM NAD, 0.3mM NADP, and 0.2mM pyridoxal 5'-phosphate (Cheliak and Pitel 1984) in a 1-ml Duall homogenizer. Supernatant of crude extracts was absorbed directly into 2 x 6-mm filter paper wicks for electrophoresis.

Buffers used to separate isozymes by starch gel electrophoresis are listed in Table 1. All systems used 12.4% gels except for PER, which was electrophoresed in 12% gels. Following electrophoresis, enzymes were stained in 1.5-mm gel slices using protocols reported by Cheliak and Pitel (1984) for CTO, MDH, and PCM; Siciliano and Shaw (1976) for ADH, a-GPDH, 6-PGD, and POI; O'Malley et al. (1980) for DIA; and Yen and Sadanaga (1977) for PER.

Table 1.--Electrophoresis and gel buffers used to characterize isozymes of dormant beech bud tissue

DIA, 6-PGD, PGI	Electrode: pH 8.0, 0.5M Tris, 0.016M EDTA, 0.65M Borate Gel: pH 8.0, 1:10 dilution of electrode buffer (Siciliano and Shaw 1976)
PGM, MDH, α-GPDH	Electrode: pH 7.0, 0.125M Tris-citrate Gel: pH 7.0, 0.05M Histidine-HCl, 0.0014M EDTA (Cheliak and Pitel 1984)
ADH	Electrode: pH 7.0, 0.135M Tris, 0.043M Citrate Gel: 0.009M Tris, 0.003M Citrate (Siciliano and Shaw 1976)
CTO	Electrode: pH 8.1, 0.06M Lithium hydroxide, 0.3M Borate Gel: pH 8.5, 0.03M Tris, 0.005M Citrate, 1% electrode buffer (Ridgway et al. 1970)
PER	Electrode: pH 8.7, 0.3M Borate, 0.1M NaOH Gel: pH 7.5, 0.014M Tris, 0.004M Citrate (Kristjansson 1963)

Data Analysis

It was not possible to confirm the patterns of inheritance for these enzyme systems due to the limited number of trees included in the portion of the study reported here, and the lack of control-bred or half-sib progenies. Banding patterns should be regarded as gel phenotypes until this is accomplished, and isozyme "genotypes" assigned to trees considered as putative.

Isozyme banding patterns were characterized by measuring Rf values (i.e., Rf equals the distance of hand leading edge from origin divided by the distance of front from origin) for each isozyme band of each sample. Patterns for each enzyme system were verified by repetitive electrophoresis and by co-electrophoresis of individual tree samples. Each tree was then assigned an isozyme "genotype" for each enzyme system, and the nine isozyme genotypes were pooled to form a composite enzyme genotype for each tree.

In lieu of strict genetic analyses, pairwise comparisons between the isozyme patterns of different trees within stands were made by measuring the number of azygous hands and computing a disagreement count for each comparison (Sakai and Miyazaki 1972). A low disagreement count implies a higher degree of genetic similarity, while a high disagreement count indicates a lower degree of relatedness. Disagreement counts also were computed and compared on a stand basis, even though stands did not have equal numbers of trees.

RESULTS AND DISCUSSION

The isozyme technique is a sensitive assay of variation in beech stands, and has potential for measuring the genetic structure of this species. Because of the "mixed mode" of reproduction in American beech (i.e., sexual by seeding, and vegetative by root sprouting), the genetic architecture of this species may prove to be complex and variable from stand to stand, and dependent on past stand history, especially past cultural practices. Analyses of this type will he particularly helpful in defining genetic mosaics that may have developed over time and space.

The technique has been used in studies of the population structure of several conifers (e.g., Roberds and Conkle 1984; Knowles and Grant 1985; Beckman and Mitton 1984), hut relatively few hardwood species. In work with a related species, variation among sources of European beech was studied using peroxidase and glutamate-oxaloacetate transaminase (Thiebaut et al. 1982; Felber and Thiebaut 1984; Thiebaut 1984). The inheritance patterns of acid phosphatase (AP) and leucine aminopeptidase (LAP), as well as the occurrence of viability selection at an LAP locus, also have been described for this species (Kim 1979, 1985). Isozyme studies with American beech have not been reported.

The range of banding patterns observed for several enzyme systems is shown in Figure 1. Banding patterns suggest that at least two loci can be characterized for several enzyme systems, including ADH and 6-PGD, hut confirmation will require examination of a larger number of samples as well as half-sib progenies.

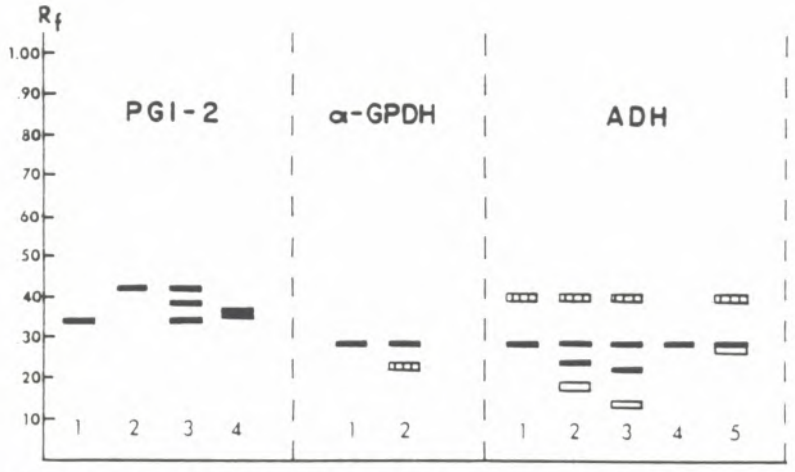

Figure 1(A).--Banding patterns observed in American beech dormant bud tissue for isozymes of phosphoglucose isomerase (PGI-2), α-glycerophosphate dehydrogenase (α-GPDH), and alcohol dehydrogenase (ADH).

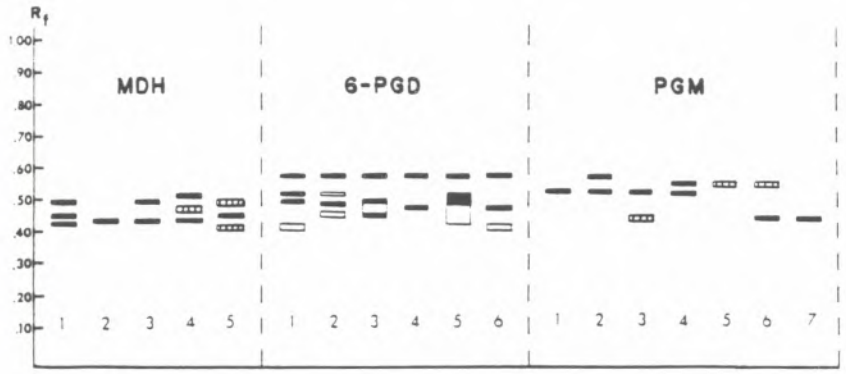

Figure 1(B).--Banding patterns observed in American beech dormant bud tissue for isozymes of malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), and phosphoglucomutase (PGM).

Isozyme analyses indicate that several of the resistant trees in the Maine stands included in this study are ramets of existing clones, or related by descent. Six trees growing in close proximity in Lambert Lake stand 3 have identical isozyme genotypes for all enzyme systems (Fig. 2). This group of trees is presumed to be a clone.

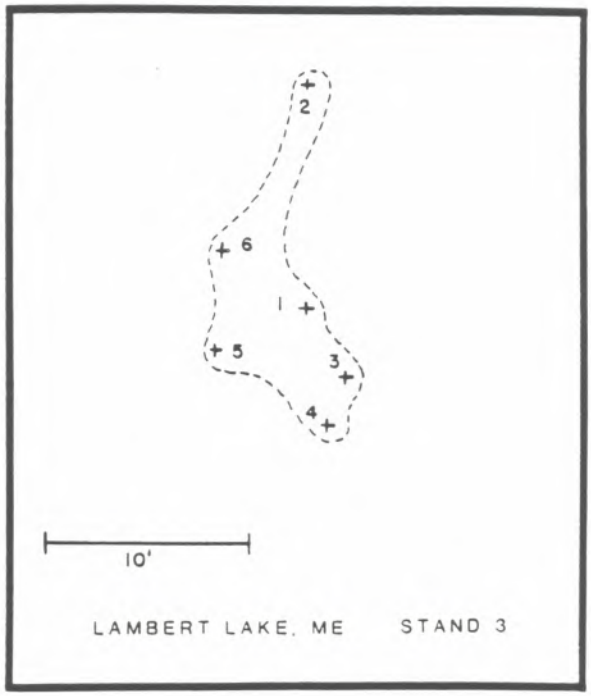

Figure 2.--Distribution of six resistant trees near Lambert Lake, Maine (stand 3). These trees have identical isozyme genotypes. In this and Figures 3-6, resistant trees with identical isozymes are circumscribed by dashed lines; susceptible trees are not shown.

In two other stands in the Lambert Lake area, in addition to some trees that appear to Be ramets of discrete clones, substantial variation was found among resistant trees, with most trees exhibiting unique isozyme genotypes (Figs. 3-4). These trees, and those in the Amherst and Franklin stands (Figs. 5-6), generally were spaced more widely than those in Lambert Lake stand 3, and less likely to be ramets of single clones.

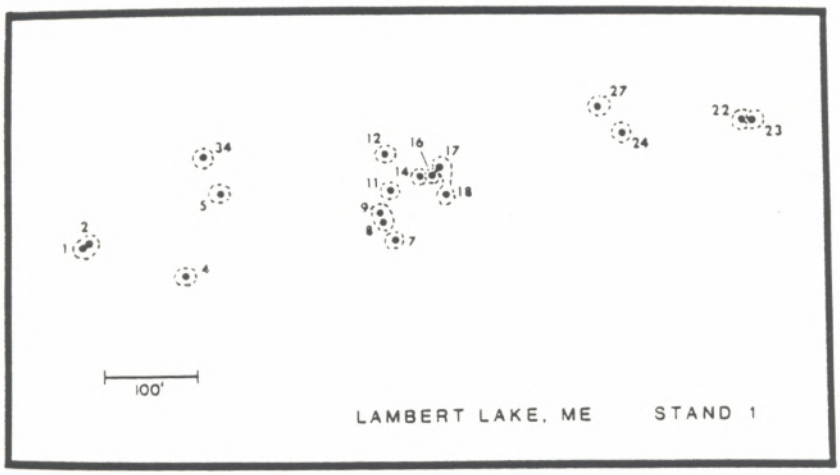

Figure 3.--Distribution of 18 resistant trees in Lambert Lake, stand 1.

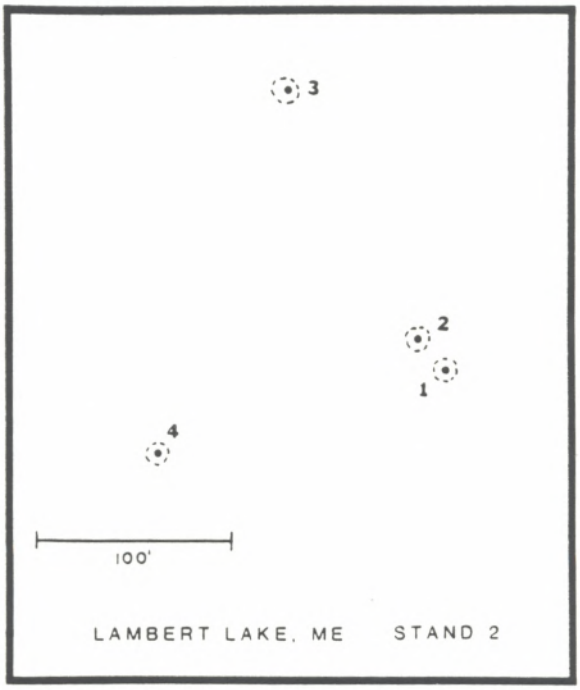

Figure 4.--Distribution of four resistant trees in Lambert Lake, stand 2.

Of the 18 resistant trees in Lambert Lake stand 1 (Fig. 3), three clones were represented by two ramets each, while all other trees had unique genotypes. Two of these, 22 and 23, differ at only two of the nine enzyme loci scored, and because of their close proximity are probably at least half-sibs. The mean disagreement count for trees in this stand was 4.6. If a disagreement count is computed for only the central group of trees in this stand (Nos. 7-18), the mean count is 4.4, slightly less than that for the stand as a whole. All trees in both Lambert Lake stand 2 (Fig. 4) and the Amherst stand (Fig. 5) generally are widely separated and have unique genotypes. The mean disagreement counts for the trees in these stands were 4.8 and 6.3, respectively.

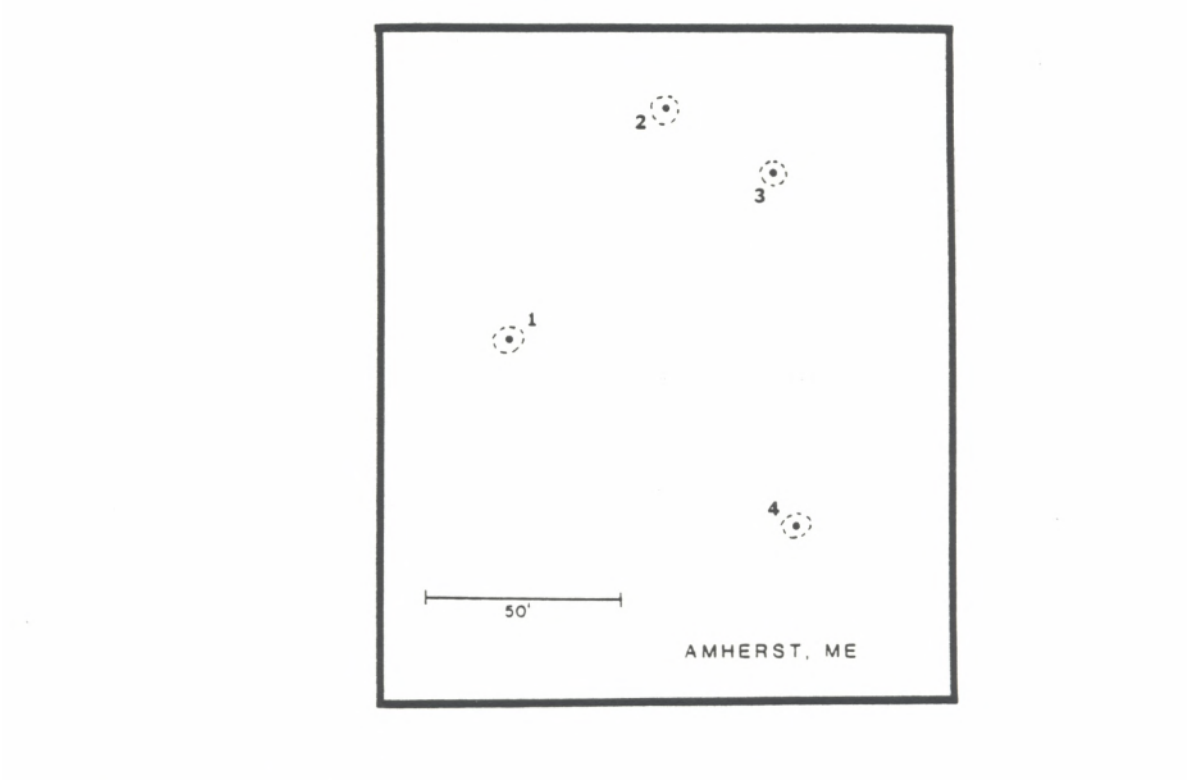

Figure 5.--Distribution of four resistant trees in the Amherst stand.

In the Franklin stand (Fig. 6), no genotypes were identical, hut the degree of similarity of several of these trees generally was higher than that found in stands in which trees were separated by greater distances. Trees in this stand were only slightly more widely separated than those in Lambert Lake stand 1, except for three outliers (Nos. 1-3, not shown in Figure 6) separated from the main group of six trees by approximately 500 feet, and from each other by approximately 400 feet. These three trees also had unique genotypes. several pairwise comparisons (2/4, 2/5, 2/8, 2/10, 4/5, 4/10, 5/10, 6/7 [7], 8/10) differed in only one or two of the nine enzyme loci, and probably represent half-sibs. The mean disagreement count for trees in this stand was 3.5, the lowest value of any of the stands examined.

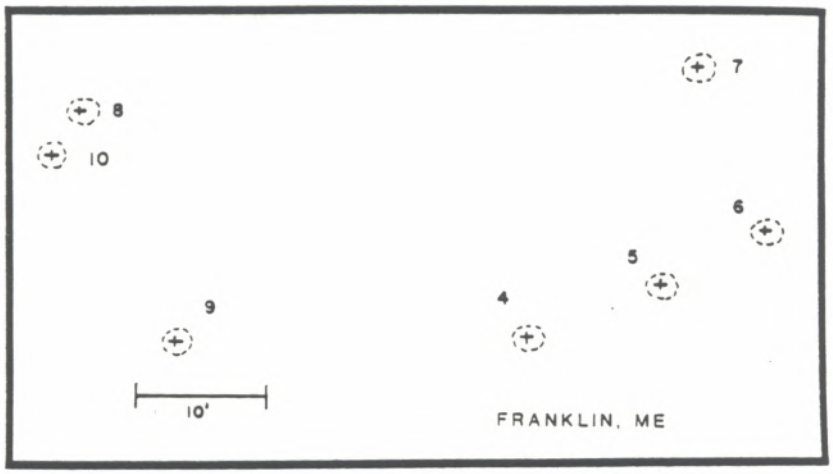

Figure 6.--Distribution of seven resistant trees in the Franklin stand. Three outlying resistant trees are not shown.

In summary, 42 resistant trees were sampled, representing 34 unique isozyme genotypes. Twelve trees were included in four putative clones. There were no distinct isozyme markers for resistance evident in the trees that were surveyed.

IMPLICATIONS FOR FOREST MANAGEMENT

American beech is one of relatively few tree species that reproduce both by *seed* and vegetatively by production of root sprouts (Ward 1961; Bormann and Likens 1979). Root sprouting is an obvious means by which specific genomes are perpetuated and, where conditions are favorable, increased and spread through the forest community. This has advantages when traits are desirable, such as resistance to an insect or disease. It is obviously disadvantageous when the trait is high susceptibility. In the case of beech bark disease, conditions created by the disease, either directly through parent tree (ortet) mortality or indirectly through salvage operations, appear to have encouraged sprouting of affected trees and, therefore, an increase in the number of susceptible stems (Houston 1975).

In the aftermath forests, disease is clearly differentiating between resistant and susceptible trees (Houston 1983). Resistant trees, more frequently than can be attributed to chance, are observed in what appear to be less-than-random groupings, suggesting that individuals within the groupings may be related, either as members of a clone originating as root sprouts from a present (or formerly present) ortet, or as half- or full-sibs, or as a mixture of these.

Knowledge of the genetic structure of forests can provide valuable insights for forest managers as they plan silvicultural treatments for forest stands impacted by beech hark disease. The preliminary data provided by isozyme analysis of resistant trees in the small stands included in this study suggest

that resistant groups of trees are, in fact, familial and/or clonal groups, and that recognition of these groups can assist in the design and timing of stand treatments to favor the propagation of resistant genotypes. It is not possible at this point, given the array of isozyme systems available, to differentiate between resistant and susceptible genotypes in a stand prior to infestation by the beech scale. However, a knowledge of how beech forests are compartmentalized into genetic mosaics will allow a more sensitive evaluation of stand structure with regard to the application of stand treatments.

Resistance in aftermath stands also provides a marker on which isozyme studies can be based to develop information on how beech forests are structured, from both an age and genetic standpoint. This, in turn, may allow the development of models of gene flow in this species that will assist in planning silvicultural strategies for enhancing resistance.

Work is in progress to compare the isozyme genotypes and spatial relationships of resistant trees with those of susceptible neighbors in selected stands in West Virginia and Massachusetts to determine if patterns revealed for resistant cohorts are representative of beech populations in general.

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