

AMERICAN BEECH VEGETATIVE PROPAGATION AND GENETIC DIVERSITY

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Abstract

Work is underway in two regions of North America to develop the protocols and knowledge required to conserve genetic resources and restore populations of disease resistant beech. The work builds on years of research by Houston and others within the US Forest Service. Two aspects of this work include developing vegetative propagation techniques and understanding patterns of genetic diversity. Although American beech regenerates readily from root suckers under natural conditions, efforts to vegetatively propagate the species have met with limited success. There are no reports in the published literature of successful production of rooted cuttings, micropropagation from buds, or somatic embryogenesis. Grafting is somewhat more successful. Barker and others (1997) and Simpson (2001) were able to develop rooted plantlets through micropropagation of buds but neither were successful in over-wintering them. Present studies focus on reducing incidence of contamination, improving root formation, and maintenance of plantlets after transfer to a soil medium and understanding causes of inability to survive a dormant period. Both Simpson (2001) and Koch (personal comm.) have had success with rooting but none of the rooted cuttings have flushed after over-wintering. Grafting appears promising as a tool for resistance screening and developing seed orchards.

Understanding patterns of genetic diversity within and among populations of resistant and susceptible trees is important for carrying out gene conservation. Genetic parameters including diversity were estimated for populations located throughout the range of beech bark disease (Houston and Houston 1994 2000). They reported that susceptible trees had higher heterozygosity than resistant trees. A study has been initiated to examine the distribution of genetic diversity for 10 populations in a restricted geographical area in New Brunswick, Canada.

Introduction

The American beech component of forests in north-eastern North America has been devastated by beech bark disease caused by the introduced insect-disease complex, *Cryptococcus fagisuga*, a soft-bodied scale, and *Nectria* fungi species (most commonly, *N. coccinea* var. *faginata* and, less commonly, *N. galligena*). In the Maritime provinces of eastern Canada, where the disease was first discovered, beech forests have been reduced to scrubby stands with no commercial value, and much reduced ecological value.

Natural resistance to beech bark disease apparently exists with a low frequency in all areas where the disease occurs and this resistance is believed to have a genetic basis (Houston 1983). The disease is caused by initial infestation by the beech scale and subsequent infection by the *Nectria* fungus. In the absence of beech scale, the disease rarely occurs. Restoration of beech in mixed wood or tolerant hardwood forest ecosystems depends on genetically-based resistance to the disease (or to the scale insect).

Conservation of the resistant gene pool and restoration of disease-free beech requires vegetative propagation techniques and knowledge of genetic structure of populations. Several studies have reported on genetic diversity over the range of the disease. Understanding the resistance mechanism or mechanisms and genetic mode of inheritance (number of genes involved, penetrance, additive or dominant gene action) is essential for developing a program for breeding resistant genotypes.

American beech is notoriously difficult to propagate by vegetative means, in spite of the high frequency of root sprouting commonly observed after disturbance. Cuttings have been successfully rooted and micropropagation has yielded apparently healthy plantlets, both at low success rates, but subsequent development of the plants, especially over-wintering poses the biggest problem.

The purpose of this paper is to provide an overview of current and previous work in vegetative propagation including rooting cuttings, micropropagation, grafting; and in genetic studies including population genetic structure of infected stands and levels of genetic diversity among resistant and susceptible trees.

Vegetative Propagation

Reid (1984) described attempts to produce rooted cuttings from stump sprouts. He used softwood cuttings with a mist enclosure and successfully rooted 10% of the cuttings but they did not over-winter. Simpson (2001) used softwood cuttings derived from root suckers collected from forest stands and from suckers produced in a greenhouse from roots collected in the field and kept in damp peat until suckers were produced. He tried many different culture combinations and found that with a mist enclosure, cuttings that had been dipped in a 5000 ppm IBA solution rooted well (75% of trees tested produced some rooted cuttings), but all died over winter. Simpson reported that his success varied by date of collection, genotype, diameter of the tree from which roots had been collected and the IBA concentration.

Work on rooting cuttings continues in two labs: Koch and Carey (2004a) have achieved a reasonable rate of success with cuttings but the over-wintering problem has not been solved. Ramirez, a Masters student at the University of New Brunswick, has attempted various cultural approaches to rooting cuttings, including a hydroponics system, without success.

Similarly, attempts to produce plantlets by tissue culture of buds or stem segments have met with some success but no plantlets have successfully over-wintered. Barker *et al.* (1997) worked with seedlings, shoot tips from root sprouts, and buds from mature trees with varying success but none survived the transfer to soil. Simpson (2001) worked with buds from mature trees and buds from 47% of trees produced some rooted plantlets; however, none over-wintered. Beech buds are difficult to sterilize and contamination was an on-going problem in both projects. Incomplete rejuvenation may account for the inability of plantlets to survive the transition to soil and break dormancy after winter. Plantlets develop buds and shut down when they are still very small, and their roots are not well-developed.

One of us continues to work on micropropagation using both buds from mature trees and stem segments from root suckers produced in the greenhouse as explants (Fig.1). She has tried culturing epicormic shoots induced in a greenhouse, as well. The contamination is

higher with buds than with stem segments because of the layering of bud scales and the impossibility of eliminating all sources of contaminants that may be stuck between bud scales (Fig. 2a and 3a). Green, non-woody stem segments (1 cm in length) excised from suckers, produced by greenhouse-cultured, meter-long sections of beech roots (ranging from 1 - 3 cm in diameter) in peat moss were easier to sterilize, but no more successful in terms of rejuvenation (Fig 2b and 3b).

Currently, Ramirez is examining the roots from plantlets cultured in standard AC growth medium to determine whether they are structurally different from those of seedlings.

Grafting has proven more successful. In New Brunswick, scions were collected in late February and early March in 2003 from 20 undiseased and 5 diseased trees, and were grafted onto rootstock produced from seed collected from undiseased trees. The success rate was approximately 33% and varied by genotype (Fig. 4a). More significantly, success varied by diameter of the rootstock stem; the smaller the rootstock, the greater the difficulty in matching with a scion of similar size (Fig. 4b).

Genetic Structure and Diversity

Understanding the genetic structure and distribution of genetic diversity is important before embarking on gene conservation and restoration activities. For example, it is important to know whether all resistant trees in a stand are likely to be related or even if they are ramets of the same clone, before deciding if the gene pool of resistant selections will be increased by sampling more than one tree per stand. It is also important to know whether the levels of genetic diversity are consistent among resistant and susceptible genotypes, i.e. whether resistant trees across populations are deficient in genetic diversity. If this is the case, susceptible trees may be needed to retain natural levels of genetic diversity within the resistant gene pool.

If the restoration of beech attains sufficient importance to merit the development and implementation of a breed strategy, it will be important to know whether genetic diversity is distributed evenly across the species range.

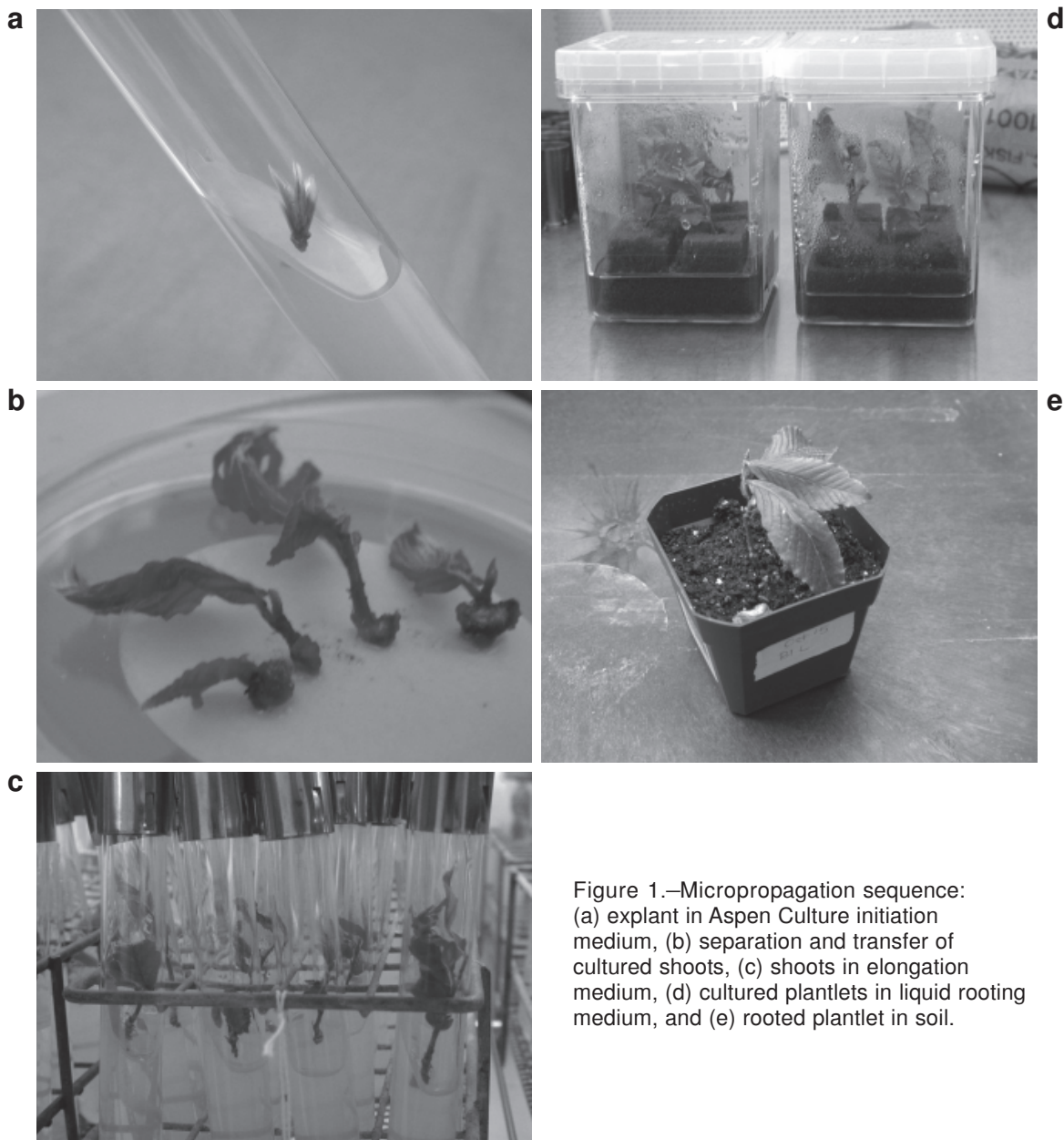


Figure 1.—Micropropagation sequence: (a) explant in Aspen Culture initiation medium, (b) separation and transfer of cultured shoots, (c) shoots in elongation medium, (d) cultured plantlets in liquid rooting medium, and (e) rooted plantlet in soil.

Houston and Houston (1994) reported on allozyme diversity in two mapped stands; one in Massachusetts with 173 trees and the other in West Virginia, looking at 152 trees. They found very little difference in genetic diversity parameters between the two populations. Both appeared to be clonal mosaics. In general they found high expected and observed heterozygosity, although the West Virginia population exhibited a heterozygosity deficiency (observed was lower than expected).

Kitamura and others (2000, 2001) evaluated allozyme diversity in several studies examining the substructuring

of beech populations, particularly comparing populations with and without regeneration by root suckers. In one study, 21 populations were sampled and 12 allozyme loci were scored (Kitamura and Kiwano 2001). They reported a very high G_{ST} value (0.168), indicating a strong degree of differentiation between populations. Mean expected heterozygosity was relatively low at 0.186. Thirteen polymorphic loci were scored in another study by Kitamura *et al.* (2000), which compared populations having varying amounts of regeneration by root suckers in Quebec and Pennsylvania. They reported similar levels of genetic diversity among populations. The final study

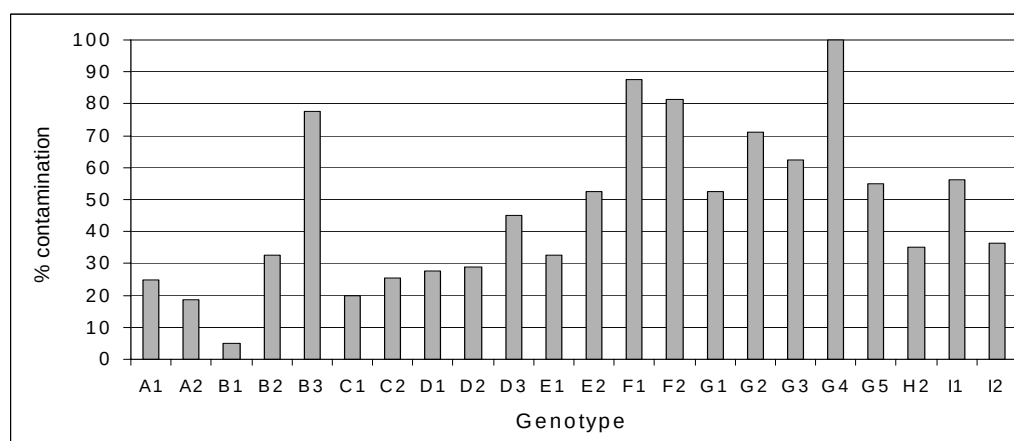


Figure 2a.—Percent contamination of bud cultures by genotype for buds from 22 trees representing 9 locations (A to I) in southern New Brunswick.

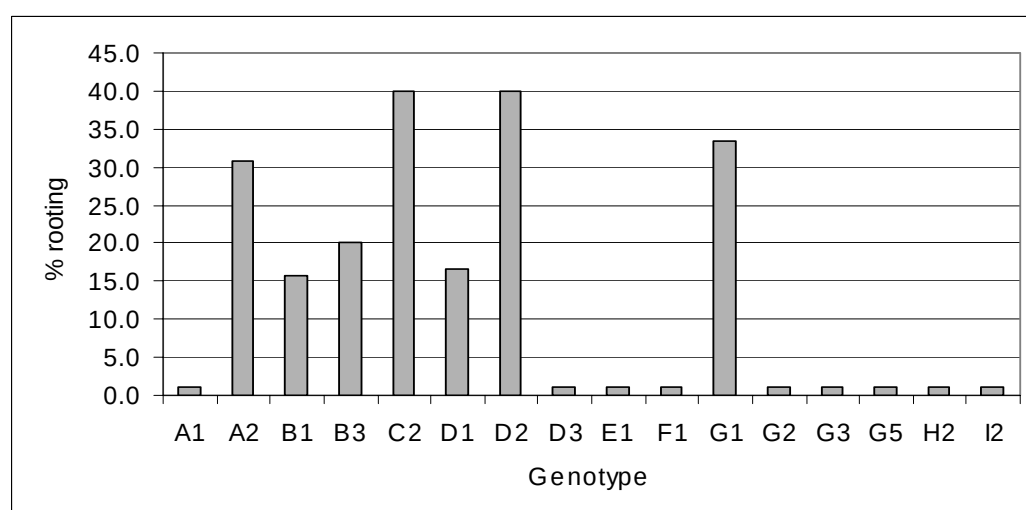


Figure 2b.—Percent of cultured shoots that produced roots by genotype for buds from 16 trees that proliferated shoots in culture.

(Kitamura *et al.* 2001) compared populations with and without vegetative regeneration by root suckers, and found a homozygote excess in the three populations with clonal reproduction and a heterozygote excess in the population that originated only from seed.

Houston and Houston (2000) sampled 9 stands from Prince Edward Island in eastern Canada to West Virginia and they mapped the susceptible and putatively resistant trees in four stands. They scored 23 allozyme loci and found that though the levels of diversity were within the range expected for forest trees, they were lower than reported for most other hardwood species. Resistant and susceptible subsets of populations were very similar in most diversity measures; however, resistant trees exhibited a deficiency of heterozygotes.

Resistance Screening and Breeding

Recently, small-scale programs have been initiated in Ohio (Koch and Carey 2004b) and in New Brunswick at the Canadian Forest Service Atlantic Forestry Centre, to screen disease-free mature trees for resistance to the scale insect, and to produce crosses for challenge tests and genetic analysis. Grafted stock has been screened at both locations using the technique developed by Houston (1982) (Fig. 5). Results of challenge tests on grafted trees in New Brunswick are preliminary (only one year of data is available yet) but indicate strongly that most disease-free trees in natural stands are genetically resistant.

The results of challenge tests on seedlings produced by controlled crossing among resistant and susceptible trees carried out in Ohio are reported in these proceedings (J.

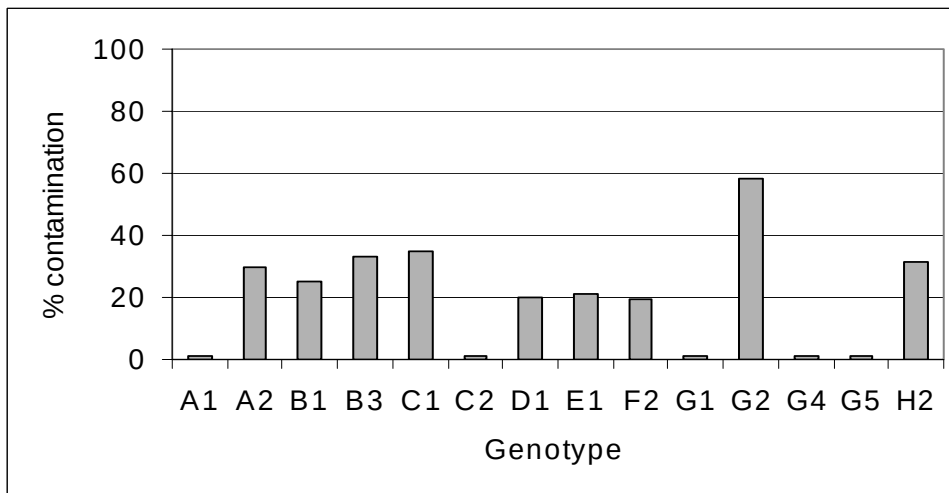


Figure 3a.—Percent contamination of stem section cultures by genotype for suckers produced in a greenhouse from 14 trees representing 8 locations (A to H) in southern New Brunswick.

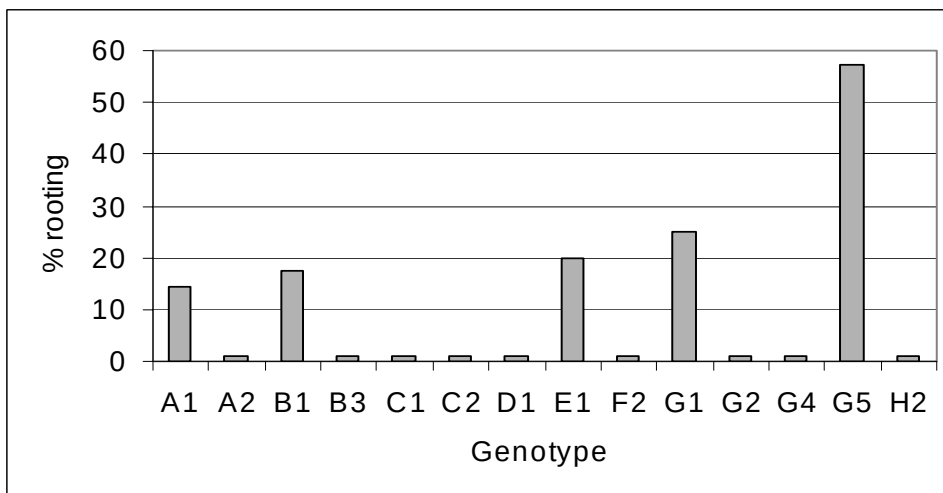


Figure 3b.—Percent of cultured shoots that produced roots by genotype for stem sections from 14 trees that proliferated shoots in culture

Koch). Controlled crosses were conducted in New Brunswick as well, using four resistant and two susceptible trees at each of two locations in spring 2004. Seed was set for all crosses but results are not yet available. Resistance screening will be carried out starting in summer 2005, with results expected in summer 2006.

Conclusions

None of the conventional vegetative propagation techniques work well for beech. Cuttings have been successfully rooted but the challenge of over-wintering remains. Micropropagation works best with shoot segments from root sprouts but rejuvenation remains problematic, and none have successfully over-wintered.

Grafting is a useful technique for resistance screening and developing seed orchards. It is not a solution for mass production of scale-resistant genotypes.

Several general conclusions may be drawn from the body of existing literature and recent results of studies. Any two resistant trees in a beech stand may be identical or closely related, emphasizing the need to sample broadly in a breeding program for resistance. It appears that sufficient genetic diversity exists in resistant trees, scattered across populations, to conduct a breeding and restoration program. Initial indications imply that populations differ sufficiently to require sampling over broad geographic areas for conservation of the resistant

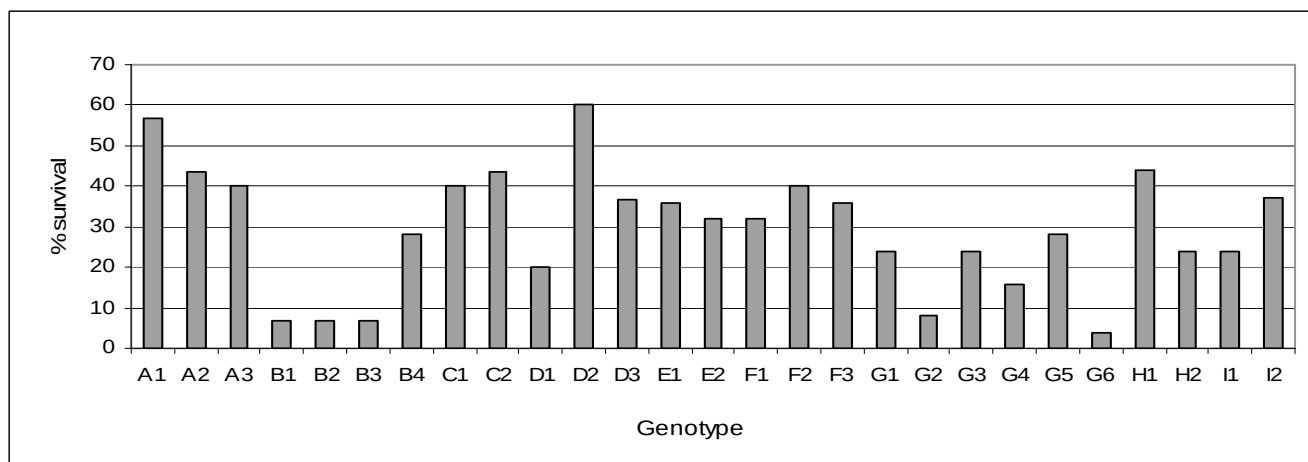


Figure 4a.—Grafting success for 22 genotypes representing nine locations (A—I) in southern New Brunswick.

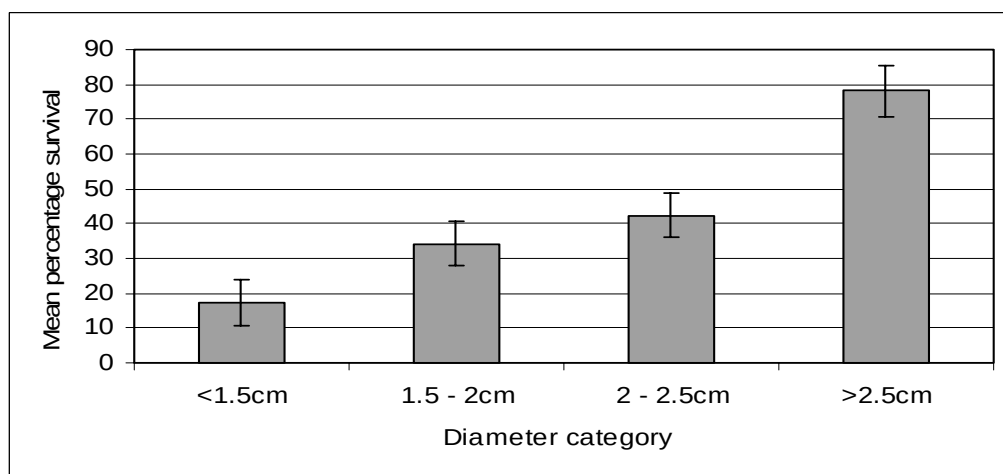


Figure 4b.—Survival of grafts with scions of different diameters; grafting success appears to be related to scion diameter.

gene pool. We know little, however, about genetic structure over intermediate geographic distances. Gaps and discrepancies between studies results imply a need for further work.

Breeding for resistance to the beech scale appears to be technically feasible, but more complete results of challenge tests on recently completed crosses are required before embarking on a breeding program. The parallel and complementary work undertaken in the two research programs (Ohio and New Brunswick) will enable comparison of genetic control of resistance near the point of introduction and near the new killing front of the disease.



Figure 5.—Beech bark disease screening on grafted stock from disease free mature trees.

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Contains invited papers, short contributions, abstracts, and working group summaries from the Beech Bark Disease Symposium in Saranac Lake, NY, June 16-18, 2004.

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