Fundy Model Forest

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"The Fundy Model Forest (FMF) is a partnership of 38 organizations that are promoting sustainable forest management practices in the Acadian Forest region."

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Fisheries and Oceans Canada
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American Beech Vegetative Propagation and Genetic Resistance Testing

Final Report to Fundy Model Forest, 2004-2005

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Introduction:

Until the last century, American beech was an important component of late-successional hardwood and mixed-wood forests of eastern North America. Seed and buds are important sources of food for a number of wildlife species. During the past 100 years however, almost all beech trees in the northeastern portion of the species range have been severely damaged or killed by beech bark disease. Until recently, the northward spread of the disease has apparently been limited by cold climate. With global warming, the diseased beech is likely to persist and spread further throughout the northern part of its range exacerbating already severe problems.

One of the challenges facing forest managers in the Fundy Model Forest is hardwood management where there is a high frequency of beech. Almost all the beech is heavily damaged by beech bark disease. Diseased beech does not produce much seed but it sprouts readily from damaged roots or stumps, so after a harvesting operation in tolerant hardwood, beech is often more frequent in the regenerating stand than previously, but more than 95% of it is likely to become diseased within a few years.

Beech bark disease is caused by a combination of a scale insect (*Cryptococcus fagisuga*) and a fungus, one of two species of *Nectria*. The insect and one of the fungal species are exotic species that have caused immense damage to eastern Canadian forest ecosystems. Sustainable management of the Acadian forest requires an ecologically acceptable means of dealing with American beech. Herbicides are not likely to provide a long-term solution; breeding for resistance to the scale (conferring resistance to the disease) is an ecologically acceptable, long-term solution, which will allow restoration of healthy beech to FMF forests. This project is the first step toward restoration of healthy tolerant hardwood forest.

We have been working on a multi-year research program aimed at developing the capacity for managing forest types having a component of beech through understanding the dynamics of the disease, and by developing protocols for producing disease-resistant beech trees. Present forestry practices are moving toward eliminating the species from their natural ecosystems. Our long-term strategy to develop and introduce disease-free trees has the potential of maintaining the species presence in its natural range, while also enabling commercial utilization.

Though vegetative propagation of American beech has proven very difficult, we are working to develop dependable methods for vegetative propagation of disease-resistant beech. We have begun to study the mechanisms of resistance and its mode of inheritance. These steps are necessary in establishing a dependable long-term source of disease-resistant beech for restoration of tolerant hardwood forest.

Objectives:

Goal: To develop a means by which disease resistant American beech can be made available to forest managers.

Specific objectives:

1. to identify an effective method for vegetative propagation of American beech
2. to understand the mode of resistance to the beech scale and its inheritance
3. to establish reliable sources of scale resistant beech for vegetative propagation and breeding
4. to understand the status of beech bark disease in southern New Brunswick

Methods and Materials:

Disease and Regeneration Surveys

The frequency of disease-free beech trees in heavily diseased areas is low, but has not previously been quantified. Surveys were conducted along 1km transects in selected sites to evaluate the severity of the disease and frequency of disease-free trees (Fig. 1).

Material

Seed was made available from the National Tree Seed Centre for growing rootstock from diseased and disease-free trees. Locations of more than 30 disease-free beech trees in southern and central New Brunswick, including several in Fundy National Park, have been documented. In summer 2003 and 2004, seedlings were grown in the Canadian Forest Service greenhouse for use as rootstock in 2004 and for next year. Scions were collected in spring, 2003, and 2004 from 22 identified disease-free trees as well as 5 diseased trees, for grafting. At the same time, at least 200 buds were collected from each tree for micropropagation. All material for propagation was collected from the lower part of the crown to maximize juvenility. In late spring, branches (approximately 2 cm in diameter) were collected from a subsample of 10 disease-free trees for induction of epicormic shoots; and roots (1 – 3 cm in diameter) were collected for production of suckers.

Grafting

Scions collected from disease-free and diseased trees were grafted onto wild rootstock (650 plants) in the spring of 2003 using standard top-cleft grafting techniques, wrapping the juncture with elastic bands and sealing with grafting wax. Grafts were maintained in a greenhouse under a watering and misting regime designed for grafted hardwood species. About one-third of the grafts were successful. Additional scions were collected and grafted in 2004 (with lower success rate, probably related to greenhouse temperature). The material produced in the trials is used for screening for resistance. Additional grafts will be produced in 2005 to screen a wider genetic base for resistant genotypes.

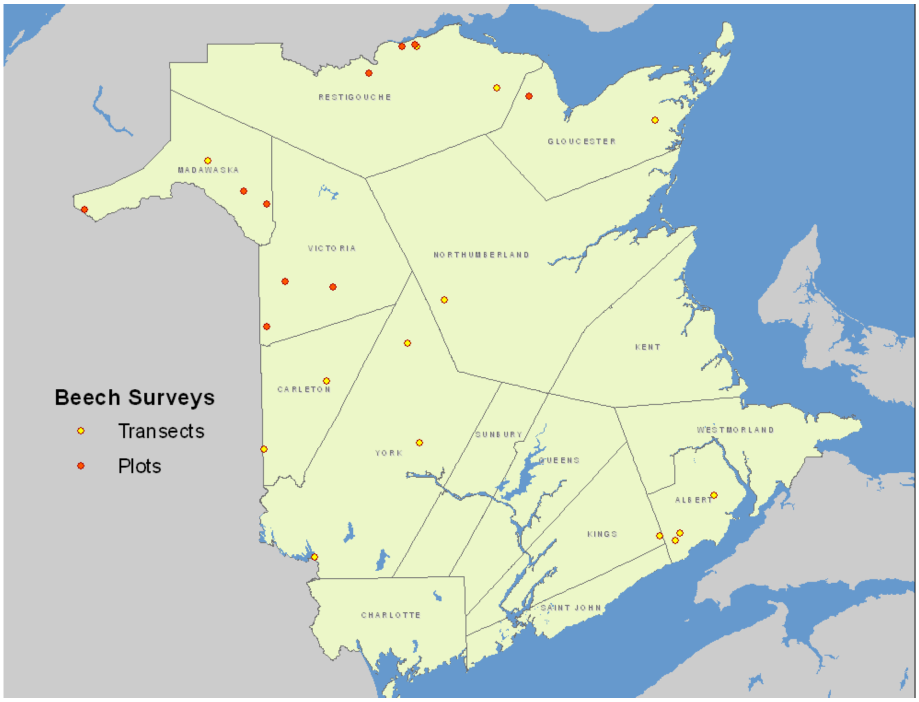

Figure 1. Location of 1 km transects (yellow) and 30-tree plots to assess proportion of disease-free trees in New Brunswick

Resistance screening

In July, 2003 and in 2004, beech scale eggs were collected from diseased trees. The eggs were placed on foam, which was wrapped around grafts from diseased and undiseased trees, and secured with strips of wire. The trees were individually watered to avoid wetting the foam. And they were maintained at 5°C over winter. After 12 months the foam was removed and stems were examined for evidence of scale-insect colonies. Successful establishment of scale colonies indicates susceptibility while the reverse indicates resistance.

Micropropagation

A number of tissue types and techniques have been used to produce micropropagules of disease-free beech. Experiments conducted in 2003/04 were more successful than previous attempts to produce plants through vegetative means. The most troublesome steps in micropropagation were identified and were focused on in 2004/05. Contamination is still problematic because of the difficulty of completely cleaning all the crevices in the buds. A high proportion of clones produced some roots in culture but plantlets did not continue to develop normally. In 2005/06, buds will be collected from grafts and from suckers in the field at later stages of development. In addition to vegetative propagation of buds, the potential for embryogenesis from non-embryonic tissue will be explored

The transfer from sterile culture to the greenhouse

To date, only two studies reported a modest-scale success with micropropagation of American beech (Barker at al. 1997; Simpson, 2001) but in neither case it was possible to establish the plantlets in the soil. We have not yet been successful in transferring plantlets to soil. The roots may begin deteriorating before the transfer or buds may not fully develop prior to the plantlets shutting down. Roots were examined in winter, 2004 and fixed for further microscopy work. We continue working to refine the procedures for transferring material from culture to non-sterile media testing several hypotheses generated during the course of the past two years.

Rooted cuttings

Greenwood cuttings were taken from suckers in the field and from suckers forced in the greenhouse and rooting was attempted in a hydroponics system. None of the cuttings produced roots. Plans for 2005/06 include collecting cuttings earlier, using woody material formed in 2004. A hydroponics system will be attempted again and this will be compared with cuttings initiated in a soil median with and without bottom heating.

Controlled crossing

Controlled crossing within and between disease-prone and disease resistant trees followed by disease-resistance testing of the progeny is necessary to elucidate the mode of inheritance of the resistance. In spring 2004, controlled crosses were conducted among 4 putatively resistant and 2 susceptible trees at each of 2 locations with the following crossing design:

Disease-free x disease free – 24 crosses
Disease-free x diseased – 6 crosses
Diseased x disease-free – 6 crosses
Diseased x diseased – 4 crosses

Seed was collected from all crosses and will be grown in 2005/06 for resistance screening. Mode of inheritance will be determined by examining ratios of resistant to susceptible seedlings. If there are flowers this year, pollen will be collected, and optimum processing and storage methods will be developed to build up a pollen bank for future use.

Results:

The frequency of disease-free trees was found to be 4.5% with a high degree of consistency across the province (Table 1). A few transects in Nova Scotia, where the disease has been present the longest, indicate a lower frequency. Likewise, frequency appears to be lower in PEI. It is unknown whether the difference represents a higher frequency of escapes in NB or if it is caused by different silvicultural practices, but it may be due to the ownership of land surveyed and the different treatment afforded these different types of ownership. In NB, most of the areas surveyed were fairly inaccessible, and located in provincial protected areas. The transects on PEI and NS were located on private land, where there is a higher probability of disease-free trees being harvested.

Table 1. Frequency of disease-free beech in New Brunswick, based on 1 km transects

Location	# trees assessed	% Disease-free
Jacquet River	354	4.5
Kennedy Lakes	280	3.6
Spednic Lake	184	3.8
Caledonia Mountain	253	4.7
McCoy Brook	154	4.5
McManus Hill	299	4.0
Sugar Loaf	323	4.0
FNP Laverty Falls	358	3.6
FNP Caribou Plains	155	5.2
Dunbar Road	247	2.0
Falls Brook	380	7.9
Haysville	299	6.3
Hovey Hill	233	4.7
Hatheyville	284	3.2
Mean		4.5

Plantlets have been produced by micropropagation, but they are still not viable in soil. Several hypotheses have been generated to explain the problems with establishment in soil. Activities this year (2005-06) include (1) collection of material from grafts to capitalize on induced juvenility, later in the spring , (2) transfer of plantlets to rooting media with a solid matrix to avoid damage to newly developing roots, (3) examining roots from plantlets to determine whether structure is altered in culture and (4) attempting embryogenesis with bud tissue.

The hydroponics system may have failed in 2004 because of timing. Twigs may have been collected too late for root production. The system will be tested again in 2005 with two collection dates, one just as buds are beginning to flush and a second date when buds have fully flushed but before leaves have expanded. Cuttings will be tested in soil with bottom heating to assess the potential of warming the soil to produce roots. Root segments will be tested for their potential to produce viable roots and shoots, as well.

Initial results indicate that the challenge test works well. The challenge experiment was partially replicated in 2004 and results will be known in summer 2005. Of the 22 putatively resistant trees challenged, 20 of them appear to be resistant based on one year of results (Table 2). When the foam was removed from the grafts, all four of the susceptible clones had well developed colonies of scale insect under the foam, and some of the ramets of two of the putatively resistant clones also had well developed colonies. These two are probably escapes, rather than resistant genotypes. In some cases, the only surviving scale insects were on the graft union, indicating that the rootstock was susceptible and the scion was resistant. For example, of the nine surviving grafts of clone C1, only two had any established scale insects, and all of them were on the graft union. Likewise, for clone G1, one ramet had 52 established scale insects under the foam, but all were on the graft union.

Table 2. Challenge test results for 22 putatively resistant genotypes and four known susceptible genotypes. Results are for one year only (2004) and the number of scale insects found on the graft union or on the rootstock is not included in the average.

Putatively Resistant Genotype	No. grafts	Mean number of scale	Susceptible Genotype	No. grafts	Mean number of Scale
A1	11	4.1	A3	8	49.4
A2	10	1.6			
B1	2	3.5			
B2	2	0			
B3	2	0	B4	4	25.5
C1	9	0			
C2	9	1.0			
D1	4	0.5			
D2	16	2.1			
D3	6	0.8			
E1	8	2.0			
E2	5	0.4			
F1	5	1.2	F2	7	72.4
F3	5	1.8			
G1	6	0.2			
G2	1	1.0			
G3	5	19.0*			
G4	4	4.5			
G5	3	2.7			
H2	1	0	H1	9	48.1
I1	1	0			
12	7	30.6*			

Figures 2 and 3 illustrate the appearance of the resistant and susceptible grafts, respectively. Note the location of the white "cotton" on the resistant tree, only present at the graft union. There is much more cotton on the susceptible tree and individual scale insects are visible above the graft union.

Fig. 2. Graft from putatively resistant beech tree, with evidence of the scale insect only present at the graft union (notice where scalpel is pointing) one year after inoculation with 100 beech scale eggs.

Fig. 3. Graft from a susceptible tree one year after inoculation with beech scale eggs. Note the white "cotton" produced by mature scale insects.

The challenge testing was repeated in summer 2004, with newly grafted material and two-year old grafts from 16 of the trees. The results will verify the practical value of the challenge testing procedure for determining resistance of beech genotypes to the beech scale. The numbers of new and second-time grafts used in the challenge tests in 2004 are listed in Table 3.

Controlled crosses among putatively resistant trees, between putatively resistant and susceptible trees and among susceptible trees were successful (Table 4). Crosses were made in crowns of roadside mature trees at two locations: Noonan Research Forest and Dean Toole's woodlot in Sussex. Pollination bags were replaced with mesh bags in early summer, to prevent insect damage, and seed was collected in October. The seed was stratified in bags of moist peat moss at a temperature just above freezing, and germination began in March. Each germinated seed was removed from stratification and planted into individual containers and placed in a heated greenhouse, when the radicle was visible.

Table 3. Number of grafts challenged for resistance to beech scale in summer, 2004, by genotype.

Genotype	# Inoculated	# Reinoculated
A1	4	6
A3	4	6
B4	3	4
C1	5	5
C2	5	5
D2	2	8
D3	2	5
E1	3	7
E2	5	5
F1	3	2
F3	3	3
G1	2	6
G3	6	4
H1	2	8
I	5	5
total	54	79

Germination of the controlled cross seed was 80%, which is higher than is typical for beech seed, and seedlings are being grown in a greenhouse for challenge testing. If they attain sufficient size, they will be inoculated with beech scale eggs in August, 2005. Resistance : susceptible ratios will be calculated for each group of progeny: resistant x resistant, resistant x susceptible and susceptible x susceptible, to determine the mode of inheritance. A set of progeny will be monitored over time to determine the point at which the scale dies on the resistant progeny. This will provide a basis for determining the mechanism of resistance.

Parenchyma samples will be taken from known resistant and susceptible trees for analysis of protein profiles and metabolites. If differences can be found between resistant and susceptible trees, it will lead to an understanding of the mechanism of resistance to the beech scale, which effectively provides resistance to beech bark disease.

Table 4. Results of controlled crosses conducted in Sussex area and Noonan in spring, 2004.

Cross Type	Number of Crosses	Number of Seedlings
R x R	22	976
R x S	11	385
S x S	4	120

R – putatively resistant to scale
S – susceptible