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VEGETATIVE PROPAGATION OF AMERICAN BEECH
(*Fagus grandifolia*)

by

James Ian Simpson

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ABSTRACT

Vegetative propagation of American beech (*Fagus grandifolia*) was explored for potential use in restoring American beech to forests affected by beech bark disease. Three techniques were tested: micropropagation of dormant buds, softwood shoot cuttings from root suckers (over 1 year old), and softwood cuttings of forced root sprouts (1 to 2 months old) from root sections.

Each technique produced rooted cuttings. Sucker shoot cuttings and root sprout cuttings were acclimatized to the non-mist greenhouse environment; micropropagated plantlets, however, were not. Root length varied significantly by tree for both shoot cutting sources, and by indole-3-butyric acid (IBA) concentration for sucker shoot cuttings. Root sprout cuttings produced the largest root systems, whereas sucker shoot cuttings produced the longest buds. No cuttings survived overwintering.

Both root sprout and sucker shoot cuttings show promise for future beech propagation. Further testing must be carried out to determine an effective overwintering technique for rooted cuttings.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Chapter 1. INTRODUCTION

This study was carried out to develop a protocol for the vegetative propagation of American beech trees (*Fagus grandifolia*) that show resistance to the beech bark disease. This protocol is intended for use in restoring healthy beech in forests affected by beech bark disease. Successful vegetative propagation of American beech has not been reported (Dirr and Heuser 1987; Barker *et al.* 1997).

Beech bark disease is an introduced disease complex afflicting beech throughout much of its range, including southern New Brunswick (Houston 1980). The disease progressively infects the tree, killing the phloem and cambium, leading to girdling and death (Shigo 1972). The disease has economic and ecological ramifications: it renders the tree unusable for lumber production, and reduces growth rate and nut (mast) production (Houston 1999). Disease-free trees found in the wake of the disease have been shown to be resistant to the disease (Houston 1983), and this resistance is believed to be of genetic origin (Houston and Houston 1987).

A number of propagation techniques were explored, and three approaches were settled on for further testing in the current study: (1) micropropagation of dormant bud tissue from mature trees, (2) semi-developed (*i.e.* softwood) shoot cuttings from beech suckers, and (3) softwood cuttings of root sprouts from roots brought into the greenhouse. In this study, 'suckers' refer to vegetatively produced stems more than one year old arising from roots *in situ* (Fig. 2.2); 'sprouts' refer to forced shoots 1 to 2 months old from sections of roots brought into a greenhouse (Fig. 2.5). Factors tested on the micropropagation material were source tree effect and rooting medium; date-of-cut and

exogenous auxin (IBA) concentration were tested for the sucker shoot cuttings; and source tree effect and exogenous auxin concentration were tested for the root sprout cuttings.

Objectives of the study were as follows:

1. to determine whether buds from mature beech could be micropropagated and the plantlets successfully acclimatized to the greenhouse environment; and

2. to evaluate factors affecting rooting of softwood root sprout cuttings (less than three months old) and softwood sucker shoot cuttings (more than one year old), in order to develop a protocol for American beech propagation through cuttings.

1.1 The Beech Tree

> "Beech ... is one of the most grand and lovely of all the forest trees, whether we consider its stately trunk, its smooth silvery rind, its glossy foliage, or graceful spreading pendulous branches. Virgil was right in choosing the beech for its shade, for no tree forms so complete a roof..." Perley (1847).

American beech was a major and valuable component of the Acadian forest before the arrival of beech bark disease around 1890 (Houston 1980). As a very shade-tolerant and long-lived tree, beech was often a climax species on fertile soils throughout the Acadian Forest region, along with sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*) and hemlock (*Tsuga canadensis*) (Farrar 1995). Its favoured habitats are moist, well-drained slopes and rich bottom-lands (Farrar 1995). Names of the beech tree in the Acadian Forest region include the French *hêtre americain* and *hêtre à grandes feuilles*, the Maliseet *mihihqimus* and *soomosi*, and the Mi'kmaq *mimgwaganimusi* (Hinds 2000). Beech is a member of the Fagaceae (Beech) family.

Beech can reach 21 to 25 m in height and 60 to 100 cm in diameter on good sites (well-drained loam soils) (Farrar 1995). In addition to its naturally smooth grey bark, beech is known for its copper-coloured fall foliage, which persists through the winter on saplings and lower branches of older trees – a striking hue against a background of snow. Beech is monoecious, that is, male and female flowers occur on the same tree.

Beech trees produce large seed crops every few years, starting when they reach about 40 years (Farrar 1995). The beech genus, *Fagus*, comes from the Greek word *fago*, meaning to eat, and the nutritious beechnuts are eaten by many, including members of the squirrel family (red squirrel (*Tamiasciurus hudsonicus*) and chipmunk (*Tamias striatus*), for example), white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), ruffed grouse (*Bonasa umbellus*) and bluejays (*Cyanocitta cristata*). Black bears (*Ursus americanus*) also feast heavily on the nut; mast failures have been shown to adversely affect bear fecundity and subsequent survival of young in northern forests where beech mast is the primary fall food (Houston 1999). In northern Maine, the 2-year reproductive cycle of black bear has been shown to be timed to the heavy mast years of beech (Houston 1999).

Humans have made use of beech in a number of ways. In addition to eating beechnuts raw, people have ground nuts for flour, pressed them for oil and even used them to make a coffee-like drink (Ritchie 1996). Leaves were used as well: mattresses were stuffed with them as they did not mildew or crumble like hay (Ritchie 1996). The wood has a variety of uses. Its high density makes it nearly without equal as fuel, and its toughness and ability to wear smooth make it a first choice for toys, furniture, tool

handles, and flooring (Barker *et al.* 1997).

In addition to reproduction by seed (which are spread widely by bluejays), beech reproduce prolifically through root and stump sprouting (Farrar 1995). This is evidenced by thickets of beech sprouts found in many beech-dominated stands. Root sprouts tend to arise from lateral roots near or at the soil surface. Root injury stimulates root sprouting, but is not required (Jones and Raynal 1988). Usually only a few trees in a stand produce sprouts, with the number of sprouts positively related to the size of the parent (Jones and Raynal 1988). Sprouts may have better survival than seedlings due to a faster growth rate and greater ability to withstand browsing (Ward 1961). Root sprouting appears to be the main mode of beech regeneration on specific sites and in certain areas of its range (Houston and Houston 1994). The range of root sprouting, however, is much more limited than the range of seed dispersal; Jones and Raynal (1986) found that 99% of root sprouts still attached to the parent tree occurred within a 10 m radius of the parent tree. Thus potential for clones to spread is limited over the short term. Jones and Raynal (1986) also noted that sprouts generally do not become functionally independent until they are over 10 years of age.

Initial work on the genetic diversity of beech has been carried out. Houston and Houston (1994) found that many of the beech trees in their two study stands were ramets of existing clones, that is, had identical genotypes, or had very similar genotypes and were related by descent. They found that the mean number of alleles per locus averaged 2.9 and the proportion of polymorphic loci was 89%, comparable to diversity values for *Populus tremuloides* and *Gleditsia triacanthos*, and exceeding values for *Quercus*

macrocarpa and *Q. gambelii.*

Houston and Houston (1994) stated that the genetic variation observed in their sample of American beech is higher than that reported for many conifer species and most angiosperms. Beech has life-history traits that are associated with high allozyme diversity, including large range, high fecundity, outcrossing modes of reproduction, wind pollination, and long generation times (Hamrick and Godt 1989).

1.2 Beech Bark Disease – A History

Nature of the Disease

Beech bark disease results from infection by *Nectria* fungi, especially *N. coccinea* var. *faginata* (Lohman, Watson and Ayers), of trees infested by scale insects, especially *Cryptococcus fagi* (Baer) (Houston 1980). Colonies of the beech scale (0.5-1 mm, soft-bodied, wingless insects) become prominent on beech trunks before any disease symptoms develop, and their presence is often noticed by woolly wax the insect secretes over its body. This woolly wax forms irregular white lines along the bole of the tree and eventually covers the bark as the infestation increases (Shigo 1972).

The insect lays its eggs between mid and late summer, and nymphs are hatched in late summer to early winter (Hawboldt 1944). Some nymphs begin to feed immediately, while others may crawl some distance before feeding (Hawboldt 1944). Before becoming sedentary, eggs and nymphs may be dispersed to other trees by air currents, and this is believed to be the main distribution mode as the disease has advanced steadily and evenly, without instances of distant, isolated outbreaks (Hawboldt 1944).

The most common, and usually only predator of *C. fagi,* is *Chilocorus stigma*

(Say), the twice-stabbed ladybird beetle (Houston 1980). Parasitic and predaceous mites have also been found associated with immature *C. fagi* (Mayer and Allen 1983), but no predators have proven effective in controlling *C. fagi* (Houston 1980).

The principal pathogen involved in the disease, *Nectria coccinea* var. *faginata*, can be identified by its white sporodochia in the summer (although the sporodochia can be confused with the scale insect) and its red perithecia in the fall (Shigo 1972). *N. coccinea* var. *faginata* spores are easily transported by wind. Other *Nectria* species involved in the disease are *N. galligena* (most common in Europe, but also has been reported in North America), and *N. ditissima*, which also appears to be associated with the disease (Shigo 1964). *Nectria coccinea* var. *faginata* is attacked by the mycoparasite *Gonatorrhodiella highlei* (A.L. Smith), but as with the scale insect, is not significantly affected by predators or parasites (Houston 1980).

The scale insect feeds on the cambium, thereby providing numerous minute entries to infection by the fungus. The fungus progressively infects the tree, killing the phloem and cambium, eventually leading to girdling and death of the tree. The infection severely cankers the bark, and is easily noticed (Fig. 2.3; Methods Section). Although the scale insect and the fungus can damage the tree separately, the damage is minor compared to the damage inflicted when the two are present, and the symptoms of the disease occur only after both organisms have attacked the tree (Shigo 1964). Contrary to Shigo (1964) and Houston (1980), Londsdale (1980) suggested that sources of stress other than the beech scale insect (drought or nutritional deficiency) may predispose beech to heavy infection by *Nectria* fungi.

Initial infection by the *Nectria* fungus is followed by a host of other fungi and insects targeting the bark patches killed by the *Nectria*, leading to further decline and degradation of the tree (Shigo 1964). For a more complete description of the role of insects and fungi associated with the beech bark disease, and their interaction with the beech bark disease, see Shigo (1964).

The disease has acted as a partial disturbance, in which a number of trees are killed quickly, while many manage to survive in a weakened, defective state, and a dense understory of susceptible beech becomes established throughout the diseased stand (Twery and Patterson III 1984; Ostrofsky and McCormack 1986). In this situation, the number of beech stems may actually increase due to the disease.

The loss of mature beech from a stand also can affect succession. Hemlock tends to benefit from the loss of mature beech, likely due to the antagonistic relationship between beech and hemlock, while mature yellow birch is adversely affected, likely due to its inability to withstand the higher solar intensity (Twery and Patterson III 1984). Mortality of beech because of the disease is greater in hemlock-dominated stands. This may be coincidental (hemlock tend to occur on sites that are inherently stressful for beech), but it may also be due to the fact that hemlock is a very good root competitor (Twery and Patterson III 1984). The result is that in some cases the loss of beech from the overstory may lead to softwood-dominated stands (Twery and Patterson III 1984).

Left to natural stand development, the proportion of resistant trees may increase after several generations, if diseased beech are less fit than non-diseased beech. Human activities could, of course, change this situation. Indiscriminate cutting of all beech may

lead to extensive suckering, producing stands of predominately susceptible beech (Twery and Patterson III 1984).

Introduction to Canada

The beech scale and, most likely, the associated fungus were introduced from Europe to Halifax at about 1890 on a shipment of ornamental beech trees (Houston 1975). L.O. Howard, one of the first to verify the identity of the insect, wrote in 1913 to R.W. Braucher "so far as I am aware, this is the first report of the occurrence of this coccid [*Cryptococcus fagi*] in North America.... Doubtless it has been imported on European stock, and measures should be taken to prevent its becoming widely distributed" (Braucher 1914). In reply to Braucher, C.G. Hewitt, Dominion Entomologist in Ottawa in 1914, wrote with respect to the discovery of the beech scale that is it possible the insect is native, and that "it is unlikely that this pest will be introduced on beech trees imported into Canada from Great Britain and other parts of Europe, as all such trees are carefully inspected" (Hewitt 1914). The disease was firmly established in stands of American beech close to Halifax some 20 years after its arrival (Ehrlich 1934).

The disease complex has now spread throughout the Maritimes, New England and New York, into Quebec as far north as Rimouski, west to Montreal and Ohio, and south into Pennsylvania and West Virginia (Houston 1999). Southern New Brunswick was heavily infested by the late 1930s (Hawboldt 1944).

A 1980 survey of New Brunswick shows that the disease is still prevalent: 81% of the beech surveyed had some degree of dieback, and 15.7% had more than half of their crowns dead (Magasi and Newell 1983). These figures are the provincial average;

dieback and mortality are generally more severe in southern New Brunswick. The survey also found that the mortality rate generally decreased with increasing size. The reason is unknown, but it is plausible that only trees with some degree of resistence to the disease can survive long enough to attain large size (>25cm). One barrier to the rampant spread of the disease appears to be cold winter temperatures. The scale insect cannot survive temperatures lower than -34 °C (Houston and Valentine 1988). The incidence of cankering in New Brunswick is lowest in the northwest (Magasi and Newell 1983), which is also the coldest region of the province.

Resistance to Beech Bark Disease

Mature disease-free beech can often be found singly or in clumps from both seed and root sprout origin in stands wherein all other mature beech trees are infected (Shigo 1972; Houston 1983) (Fig. 3.2; Methods Section). A number of disease-free mature trees have been documented in Nova Scotia (Houston 1983) and Southern New Brunswick (Moore 1998). In Nova Scotia, Houston found 12-15 disease-free trees per hectare (most of which were clumped) in two heavily diseased stands, which represented less than one percent of the total beech stems in the two stands.

In stands severely hit by the disease, it is likely that disease-free trees are genetically resistant to the disease, rather than escapees (Shigo 1972). Houston (1983) has shown clear (symptom-free) beech to be resistant to *C. fagi* (the scale insect most closely associated with the disease). In his study, *C. fagi* succeeded in completing its life cycle on susceptible trees. One year after eggs were introduced, abundant, healthy, mature females had become established and had produced copious numbers of eggs. In

contrast, *C. fagi* failed to establish on most resistant trees. The few insects that became established failed to resume development the following spring. No eggs were laid, and all introduced insects were dead or moribund after one year. This condition held for a second year.

Houston and Houston (1987) demonstrated that clumps of disease-free trees are either families of seedling origin or groups of clones. They concluded that resistance is likely genetic, given that these closely related, disease-free individuals are surrounded by diseased trees. Additionally, trees Houston had found to be disease-free were still disease-free after 20 years.

The actual nature of the resistance is not known. Houston (1983) postulated that the resistance could be due to the presence of a toxin, absence of some required substance, or a complete or partial anatomical barrier. Wargo (1988) found that the bark of resistant trees contains significantly lower total and amino nitrogen than that of susceptible trees.

Londsdale (1983) suggested that stone cells in the outer bark act as a barrier to stylar penetration. He showed that in trees appearing resistant, either the outermost layer of lignified cells was strongly developed or the depth of unlignified parenchyma was small compared to that in susceptible trees.

There appears to be a gradient in the level of infection – from complete girdling and quick death for some trees to a rather mild infection, with just a few erumpments, in others. For the purpose of this study, trees that show any sign of the disease (cankers, evidence of the scale insect) were considered susceptible.

1.3 Vegetative Propagation of Beech

1.31 Micropropagation

Micropropagation is a wide and diverse field, with a myriad of strategies and techniques employed to proliferate multiple plantlets from a single donor plant meristem under aseptic conditions (Pierik 1987; Bonga and von Aderkas 1992). Micropropagation can be separated into three primary stages, each with its own challenges and specific techniques (Pierik 1987). The first stage is the establishment of an aseptic explant on a growing medium. Effective disinfection of the explant is essential to success in this stage. The second stage is the multiplication of shoots or other structures on the explant that, once cut and induced to root, will produce complete plants. Multiplication is aided by addition of plant growth regulators such as cytokinins and auxins in varying concentrations. The most effective type and concentration of plant growth regulators can be specific to species and tissue as well as the genotype of an individual clone (Dirr and Heuser 1987). Moving the explants from a state of depending on nutrient medium to a self-sustaining state is the third stage, and depends on initiation of functional roots and hardening of the plantlets to the relatively harsh greenhouse environment.

Micropropagated plantlets can be very difficult to transfer successfully from an *in vitro* to an *in vivo* environment (Dirr and Heuser 1987; Bonga and von Aderkas 1992). The plantlets are tender due to under-developed epicuticular wax, stomata (Bonga and von Aderkas 1992), photosynthetic ability, vascular connections, and root systems (Pierik 1987). These conditions may result in dehydration and leaf burning, as well as reduced nutrient uptake and gas exchange. The plantlets must be acclimatized slowly through

gradual humidity reduction and increase of light intensity, giving enough time for new, functional leaves to develop or for the current leaves to adapt. Griffis *et al.* (1983) suggested giving 100% humidity initially and gradually reducing it over 1 to 4 weeks along with reducing shade from 90% initially to 30% after several weeks.

Explants may enter a natural dormant period during which shoot production ceases and buds are formed that do not immediately flush. Such explants need a cold rest period (that is, a certain length of time at a certain temperature) before they will break bud and grow again. Two degrees Celsius (Aitken-Christie and Singh 1987) for 1000 hours (Lewis[1], personal communication, 2000) has been suggested as an appropriate chilling regime for northern deciduous trees, but no specific light regime has been recommended: some do well in complete darkness while others respond to partial light (Aitken-Christie and Singh 1987).

Another factor affecting explant survival is the age of the culture medium; it should be no older than 2-3 weeks at the time explants are placed in storage (Aitken-Christie and Singh 1987). Length of time explants are in storage can also affect survival. Aitken-Christie and Singh (1987) reported that apple explants had 95-100% survival after 1 to 6 months, but only 55% after 12 months. They also mentioned, however, that other species had no survival decline and that survival during cold storage may depend largely on the explant's vigor before storage: large, healthy explants tend to do best.

Micropropagation of American Beech

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[1]J. Lewis, Greenhouse Manager (retired) Canadian Forest Service, Fredericton, NB

The only known reported American beech micropropagation work was carried out by Barker *et al.* (1997). This group successfully initiated root growth on bud and shoot tip explants, but they were not able to establish the plantlets in soil. Barker *et al.* experimented with micropropagation of actively growing shoots from seedlings to determine the better of two culture media, Wolter and Skoog (WS) and Aspen Culture (AC), and the best combination of the two hormones 6-benzyladenine (BA) and naphthalenenactic acid (NAA).

They applied these findings to the micropropagation of buds and actively growing shoot tips of root sprouts, both from mature, disease-free trees. Seventy-three percent of the mature beech tree genotypes produced root sprouts and 39% of these were successfully micropropagated (that is, explants with roots, at the *in vitro* stage). The use of forced buds was less successful, with only 15% of the genotypes surviving to the rooting stage. Also, the ones that did survive grew more slowly than the cultures produced from the root sprouts. Rooting success with explants from both the root sprouts and the forced buds ranged from 48 to 97% for the group of source trees. However, all plantlets eventually died.

Micropropagation of European Beech

Mircopropagation of European beech (*Fagus sylvatica*) has received more attention than that of American beech, but has yet to prove completely successful (Ahuja 1984; Meier and Reuther 1991; Vieitez and San-Jose 1996). Two comprehensive micropropagation studies have been reported. The first (Vieitez *et al.* 1993) used stem and axillary shoots of 8- to 9-week-old *in vitro* established plantlets from embryonic axes

of embryos extracted from beech seeds. Success was achieved with shoot multiplication, plantlet regeneration and transfer to the greenhouse. Seventy-two percent of the plantlets placed in a greenhouse in a humidity tunnel survived after 8 weeks. No information on survival after this point was reported.

The second major study (Meier and Reuther 1994) used buds from mature trees. The researchers were able to successfully culture 18% of the mature genotypes used. Meier and Reuther (1994) reported that culturing actively growing tissue was not possible due to contamination problems as disinfectants strong enough to be effective harmed the tissue. Disinfection of buds with a 3-second dip in 70% ethanol, followed by 5 minutes in 5% NaOCL proved 70% successful. Surface disinfection by flaming (ethanol dip followed by passing through an open flame) was the most effective method, and caused no negative effect on *in vitro* development.

The time of bud collection was found to have an effect: the closer to bud flush the more successful the micropropagation (Meier and Reuther 1994). The position on the shoot and size of the bud also was found to have a significant effect on propagation success. Apical buds performed better than axillary buds, and buds longer than 2 cm produced more shoot-forming explants and a higher multiplication rate than shorter buds (Meier and Reuther 1994). Age of stock plant was also important, as increasing age adversely affected the propagation response (Meier and Reuther 1991; Vieitez and San-Jose 1996). Meier and Reuther (1994) found that grafting mature material (35 years old) onto juvenile root stock resulted in significant increase in microshoots on excised buds and multiplication rate of bud explants.

The most effective group of root promoting chemicals is the growth hormones known as auxins, occurring naturally in developing leaves and buds of plants (Dirr and Heuser 1987). Work to improve upon the natural auxins has resulted in the development of the synthetic auxins IBA (indole-3-butyric acid) and NAA (α-naphthaleneacetic acid), which have proven the most effective in inducing root growth, with IBA being the most universally effective (Hartmann and Kester 1983; Dirr and Heuser 1987). Species respond to auxin treatments in different ways, and each appears to have an ideal hormone concentration range (Dirr and Heuser 1987). An important consideration is that the absolute auxin level given to a plant, or produced by the plant itself, does not necessarily correlate with rooting success (Stoltz 1967). Auxin must be accompanied by other rooting factors, including carbohydrates. The influence of the various factors on root formation is not well understood at the mechanistic level (Dick and Dewar 1992).

Woody Pant Medium (WPM) proved the best culture medium (Vieitez *et al.* 1993; Meier and Reuther 1994), and a variety of types and levels of medium supplements has been tested. Vieitez *et al.* (1993) settled on 6-benzyladenine (BA) (0.5 mg/l); naphthalenenactic acid (NAA) (0.2 mg/l); and zeatin (2 mg/l), while Meier and Reuther (1994) used 4.5 mM BA; 1.5% fructose; 0.1% polyvinylpyrrolidone (PVP); 1 g/l casein hydrolysate and 100mg/l myo-inositol. Meier and Reuther (1994) also found that multiplication rate improved when explants were transferred to fresh medium every 2 weeks, and that the subcultured basal nodal segments of microshoots responded better than microshoot tips.

The concentrated IBA dip method (30 seconds) induced rooting most

successfully. Vieitez *et al.* (1993) and Meier and Reuther (1994) both used a concentration of 1 g/l IBA. Vieitez *et al.* (1993) also found that a 7-day dark period immediately after the IBA dip significantly improved rooting success.

Micropropagating leaf explants from young seedlings (2 months to 3-years-old) has also been explored. There has been some success in inducing adventitious buds with this method (Vieitez and San-Jose 1996).

1.32 Propagation through Cuttings

Cuttings can be taken from various parts of a plant, including leaves, roots and stem. There are three types of stem cuttings: softwood, semi-hardwood and hardwood, each defined by the maturity of the shoot being cut. Of these approaches, softwood stem cuttings were used in this study, and are generally the most successful in propagating difficult-to-root species (Dirr and Heuser 1987), such as beech. Softwood shoots are the newly emerging shoots on shrubs, trees and perennial herbs. The shoots are tender and wilt easily when cut. The softwood condition lasts for 2 to 8 weeks for most woody plants (Dirr and Heuser 1987). The window for successful rooting within this span can be very small, and will vary from year to year depending on weather conditions (Dirr and Heuser 1987). Cuttings should be 5 to 12 cm long, and should not wilt immediately when cut (which would indicate the shoot is too young and propagation is much more difficult). Ideally cutting is done in early morning, or failing that, in late evening. Cuttings must be kept cool and moist during transport (Dirr and Heuser 1987).

A number of endogenous and exogenous factors affect the success of rooting cuttings, and the relative importance of each factor varies by species. Endogenous factors

include the following: species and genotype; juvenility; type, size and position on source tree of cutting; physiological condition of cutting and stock plant; and preconditioning of stock plant. Exogenous factors include rooting medium, water supply, nutrient supply, rooting hormones, and collection timing.

Rooting success varies from species to species, and among genotypes within a species. Some species are easier to root than others; willow and poplar root with ease while beech and some maples are much more recalcitrant to rooting. Beech is considered very difficult-to-root (Gaspar and Coumans 1987; Menzies 1992). Older plants and older parts of plants tend to be much more difficult to root than young plants or parts of plants, and this is particularly true with difficult-to-root species. The reasons for this are not clear: "Juvenility is attributed to many causes but the real reasons are largely deep, dark secrets" (Dirr and Heuser 1987). Coppicing, pruning and hedging can be applied to develop or maintain juvenility of stock plants and are often used with difficult-to-root species (Dirr and Heuser 1987).

Cuttings from branches generally root better than cuttings from the main stem, and rooting ability decreases the higher up the tree the collection is made; this is likely related to the above-mentioned maturation effect (Dirr and Heuser 1987). Cuttings from a few plants show a topophysis effect, that is, cuttings from different parts of the plant show different growth characteristics (e.g. lateral growth as opposed to vertical growth). This is not a problem for most plants from which cuttings are taken (Dirr and Heuser 1987).

An important consideration is whether leaves are to be left on the cutting, and if

so, how much leaf area should be maintained. Although leaves contribute to the desiccation of the cutting through transpiration, auxins and other root promoting substances are produced in the leaves (van Overbeek and Gregory 1945; Stoltz and Hess 1966). Auxin alone will not induce root growth, but must be accompanied by other substances (especially carbohydrates) that are produced in leaves and buds (Dick and Dewar 1992). Thus maintaining leaves on cuttings generally increases rooting success.

The physiological health of cuttings is directly related to the health of the donor plant. Of particular importance is the nutrition of stock plants: vigorous and healthy plants give better cuttings. High carbohydrate levels generally have a direct, positive influence on rooting success, and, along with either endogenous or exogenous auxin, largely determine whether rooting will be successful (Stoltz 1967). Plants can be preconditioned to enhance rooting by removing the terminal buds to promote the accumulation of carbohydrates and other nutrients in the rooting zone at the base of the shoots (Dirr and Heuser 1987). Another technique is to remove a ring of bark from a shoot while still attached to the parent plant, and adding a moist peat pack with rooting hormone to the girdled area; the shoot is severed below the treated area 6 to 8 weeks later (Dirr and Heuser 1987).

Developmental stage of shoots at time of cutting can be crucial when working with difficult-to-root species. The window of opportunity can be as little as 7-10 days (Dirr and Heuser 1987). Generally, softwood cuttings of deciduous species are taken in the spring after the leaves are fully expanded and the shoots are partially mature.

The best rooting medium varies somewhat by species, but a commonly used

medium is two-parts perlite to one-part peat, by volume (Dirr and Heuser 1987). Another researcher (Chadwick 1949) reported that a mixture of one-half silica sand and one-half vermiculite gave the best rooting results for softwood cuttings of certain deciduous shrubs. Sand may lead to a coarse root structure for cuttings of some species (Dirr and Heuser 1987), while peat tends to produce slender, flexible, well branched roots (Long 1932). Long (1932) also noted that some plants do not root well in pure peat. The varying results may be due to the difference in aeration and moisture provided by peat and sand. Peat contains more than twice as much air and three times as much moisture as sand on a volume basis, but actual aeration of the roots is much higher with sand, given the close adherence of the peat moss particles to the roots.

The technique of mist propagation (Gardner 1941), is used to ensure that cuttings never experience water stress, an important consideration for difficult-to-root species. Cuttings are generally kept within an enclosed bench space and are misted just enough to maintain moist leaves. This treatment does not seem to contribute to fungal growth, and has proven quite useful in increasing rooting success (Gardner 1941). Wells (1985) stressed that adding nutrients to the striking medium before roots have developed is of no advantage and may even slow the rooting process. It also encourages the development of algae on the surface of the rooting bench. However, some propagation studies have noted nutrient deficiencies when propagating under mist, due to leaching by the mist and/or growth of the cuttings during propagation.

Wott and Tukey (1966) compared the effect of a straight water mist with a fertilizer water solution (23/19/17, NPK; 42.6 g/100 litres) mist on ornamental shrub

cuttings. Misting was done for 12 seconds every 2½ minutes during the day. The researchers found that softwood cuttings showed an increase in dry matter weight, increased N, P, and K content and increased rooting success and root quality under the nutrient mist. This held for most of the species used, although a few responded better under the straight water mist. Sanitation is of greater concern when a nutrient mist is used due to increased threat of algal and fungal growth.

The method of applying the auxin can affect rooting success. Auxin applied in a talc form has proven less effective than hormone applied in a liquid form, either in water (if the salt form is used) or alcohol (if the acid form is used) (Dirr and Heuser 1987). This is likely because the auxin must be dissolved to be taken up by the cutting. Further, better results are achieved with a high concentration of alcohol (95%) than with water or lower concentrations of alcohol (50%) (Dirr and Heuser 1987). A higher concentration of alcohol is less damaging due to its faster evaporation (Bonga[1], personal communication, 1999). Cooper (1944) reported no adverse effects due to the use of alcohol (25-95%) in the hormone solution. Exposure time to auxin is also important, especially when applying the auxin in an alcohol solution. Dirr and Heuser (1987) reported that a 5-second dip was as effective as a 160-second dip, and that a 320-second dip decreased rooting.

Cuttings that are rooted under shade and mist are quite tender and must be hardened off to the relatively harsh conditions of the regular greenhouse environment.

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[1]Dr. J. Bonga, research scientist, Canadian Forest Service, Fredericton, NB

This is achieved by gradually increasing light and ventilation, and decreasing misting and humidity (Dirr and Heuser 1987).

Certain species require a flush of growth following rooting in order to survive overwintering (Dirr and Heuser 1987). This flush (breaking bud and growing before entering the overwintering stage) is necessary to provide the plant with sufficient carbohydrate and nitrogen reserves to serve as an energy source during the winter and to break bud in the spring. The flush may be achieved by supplying supplemental lighting (60 - 100 watt; placed 1 m apart, 1 m above plants) either continuously or from 10 p.m. to 2 a.m. (Dirr and Heuser 1987), for 6-8 weeks, starting either during or immediately after the rooting period (Smalley and Dirr 1986). Temperature should be maintained above 13 °C during the extended photoperiod (Smalley and Dirr 1986).

Softwood Cuttings of American Beech

There is only one report on rooting American beech cuttings (Reid 1984). Reid (1984) tested the effect of date-of-cut on rooting success, whether stump shoots or root suckers were the better source for cuttings and the importance of an enclosed propagation bench. Cuttings were taken monthly for 6 months (June 15 to Nov. 21) from an area clearcut 3 years prior. The propagation bench enclosure consisted of two layers of shade cloth and a layer of plastic erected in tunnel fashion. All cuttings were wounded (bark on base of cuttings sliced) and given Seradix #3 rooting hormone (active ingredient 0.8% IBA) in powder form, and fungicides and fertilizer were applied weekly. The rooting medium was sifted vermiculite.

Reid concluded that stump sprouts were the best for producing rooted cuttings

(26% versus 12% rooting success). Cuttings collected after July did not root and that almost all of the rooting took place within the first 10 weeks of striking (the first point at which rooting was assessed). Reid demonstrated the importance of the enclosed propagation bench by carrying out an additional collection (date not reported) and placing half within the enclosure and half outside the enclosure. None of the cuttings outside the enclosure rooted, while 10% of those within did. Overwintering was not reported.

Softwood Cuttings of European Beech

Studies with European beech cuttings achieved some success. Spethmann and Hamzah (1988) were able to achieve 55% rooting success with the use of 0.5% IBA and 10% Euparen (a fungicide) in a peat and sand medium. Cuttings were taken from 2-year-old plants and placed in a plastic tunnel within a greenhouse and watered with a fog machine. The timing of the cuttings and the method of auxin application were not reported. Obdržalek and Pinc (1995) achieved up to 93% rooting success using cuttings from 3-year-old European beech, treated with 2% IBA and placed under a fog mister. Schachler *et al.* (1991) achieved 98.6% rooting success using auxin, fungicide and a rock wool and nursery soil medium.

In a related study, Psota *et al.* (1995) experimented with the application of cytokinins to the leaves of European beech donor plants. They found that the application of cytokinins, particularly as a concentration of marine algae (Bio-algeen S-90), dramatically increased leaf retention on cuttings. Rooting success ranged from 12% in their control to 52% in the sample with the Bio-algeen treatment, and no auxin treatment was given. These results show the importance of leaving leaves on cuttings when struck.

Chapter 2: METHODS

2.1 Study Area and Sample Trees

Field, greenhouse and lab work took place over the spring and summer of 2000 and the spring of 2001; a pilot study was carried out over the spring and summer of 1999 to explore possible propagation techniques. Sixteen trees were used for the study (although not all 16 trees were used for each method tested). Eleven trees were located within the Fundy Model Forest area, three near Fredericton, and one each at Central Hampstead and at Hillsborough (Fig. 2.1). Trees were located in different forest stands except for two near Fredericton (approximately 100 m apart) and two in Fundy National Park (approximately 75 m apart). Descriptions and locations of sample trees are found in Appendix I, as well as bud and root collection dates.

Sample trees where chosen based on the following criteria: (1) no visible signs of beech bark disease (presence of cankers, the scale insect, or the *Nectria* fungus) (Fig. 2.2; note, Figs. 2.2 to 2.10 are found on pages 35-39); (2) diameter at breast height equal to or greater than 10 cm (some trees smaller than this do not show susceptibility to the disease); and (3) located in southern New Brunswick (progression of the disease is slowed in northern New Brunswick due to the adverse effect of cold temperature (-34 °C) on the scale insect). Figure 2.3 illustrates the remains of a beech afflicted by the beech bark disease. Again, in this study, 'suckers' refer to vegetatively produced stems more than one year old arising from roots *in situ* (Fig. 2.2); 'sprouts' refer to forced shoots 1 to 2 months old from sections of roots brought into a greenhouse (Fig. 2.5).

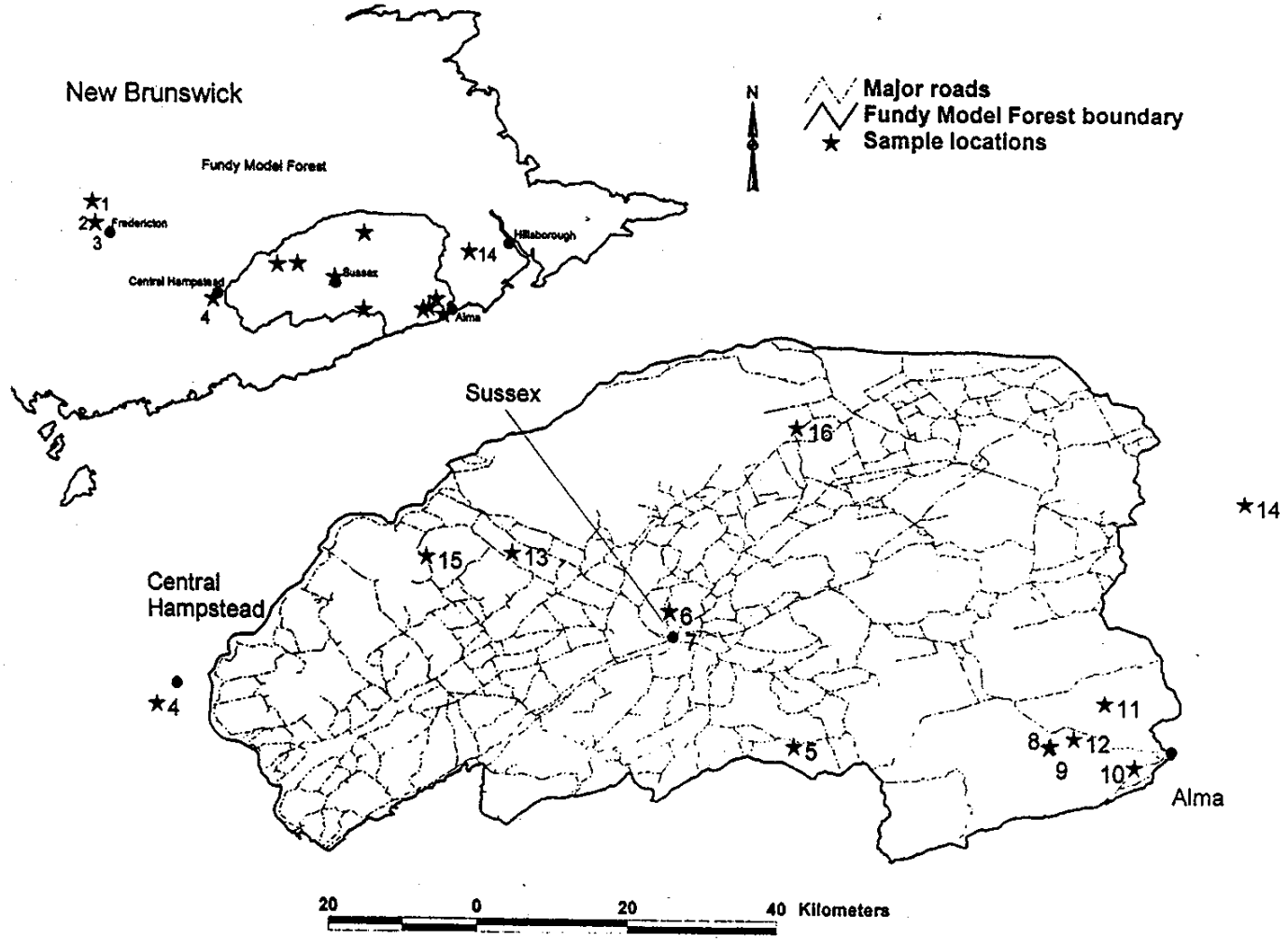

Fig. 2.1. Sample site locations in New Brunswick and the Fundy Model Forest

2.2 Pilot Study

A pilot study was undertaken in order to determine the best explant source and micropropagation procedure. Micropropagation work was carried out to determine the best method for excising propagation material from dormant buds, and to determine whether three other explant materials could be utilized for micropropagation: callous formed over root wounds and root ends; young, actively growing shoot tips of root sprouts; and actively growing shoot tips from forced buds of mature trees. None of these explants could be established on culture due to contamination. Barker *et al.* (1997) reported success in establishing root sprout shoot tips, but other studies (Meier and Reuther 1994), including this one, found that the strongest disinfectant that could be used without damaging tissue did not prevent contamination. Another culture medium, Woody Plant Medium (WPM), was also tested, but did not prove better than ACM (average shoot production per explant for 16 trees was 0.47 for WPM and 0.53 for ACM).

Several acclimatization approaches for micropropagated plantlets were tried during the pilot study, including placing individual plantlets in sealed plastic bags, which were opened for increasingly longer periods of time; placing clear plastic containers over the plantlets; as well as placing them in the greenhouse with no coverings. Shade cloth was used in all cases. None of the techniques proved successful.

Several cutting methods were investigated in the pilot study: root sections were potted with and without sprouts and with and without IBA treatment, and semi-hardwood shoot cuttings from young trees and softwood root sprout cuttings were struck with and

without IBA treatment. The only cuttings to root were the root sprout cuttings with IBA treatment (6 of 16 cuttings rooted), prompting further experimentation with softwood cuttings.

As a result of the pilot study, it was decided to use dormant buds as the explant source for micropropagation, and ACM as the culture medium. The entire bud axis minus only the bud scales was found to be the best excising method. With respect to rooted cuttings, it was decided to use IBA-treated softwood sucker shoot cuttings and root sprout cuttings. The lack of acclimatization success with all cuttings prompted the need to try an enclosed misting bench.

2.3 Micropropagation

Branches with a combined total of at least 120 buds were collected from each of 15 mature trees showing no sign of the beech bark disease. The lowest branches on the trees were sampled as these tend to be the easiest to propagate (Dirr and Heuser 1987). See Appendix I for sampling dates by tree. Approximately 30 cm of the base of the branches were cut off before the branches were placed in water in a cold room set at 3 °C until they could be used (maximum 2 weeks).

Aspen Culture Medium (ACM), as described by Ahuja (1983) (Appendix II), was used, except the iron component was Na_2.EDTA and $FeSO_4$ rather than NaFe EDTA, as the latter chemical was not available, and the two have been used interchangeably (K. Forbes[1], personal communication, 1999). This change in medium is not likely to have

[1]K. Forbes, Forest Genetics Lab Technician; Canadian Forest Service, Fredericton, NB

affected explant growth, as the iron component of $FeSO_4$ will oxidize to Fe^{3+} (*i.e.* ferric state), and SO_4 is present in other medium ingredients. The medium was gelled with Agar and supplemented with BA and NAA as described by Barker *et al.* (1997) (Appendix II).

Test tubes were sterilized before use (autoclaved) and the medium was cooked in an Agaramat (pressure cooker). In the Agaramat, the medium was raised to 81 °C, then lowered to 76 °C and set to the correct pH (5.5-5.6) using hydrochloric acid. The medium was then raised to 121 °C for 15 minutes and dispensed (12 ml per test-tube) at 56 °C. Filled test tubes were set at an angle (approximately 50 degrees) while the medium gelled.

Buds were removed from the twigs and disinfected for 5 minutes on a stir plate using diluted bleach (0.5% hydrochloric acid initially, and then increased to 1.6% when contamination was found to be a problem). Bud scales were removed in a laminar flow hood using sterile utensils. The entire bud, minus bud scales and any of the subtending shoot, was placed on ACM in a test tube, on its side, with the proximal end set lightly into the medium. The test tubes were then placed in a growth room under flourescent light (55 $\mu mol\ m^{-2}s^{-1}$), at 26 °C and 16-hour days.

For three of the trees (sites 4, 9, and 14), many or all of the 120 buds were flower buds, resulting in lower numbers of vegetative buds for culturing than expected. For trees at sites 9 and 14, 20 of the flower buds were cultured as an alternative. Only 108 vegetative buds were sampled from tree 4. In three other cases, a number of buds were too small to culture, and thus fewer than 120 were cultured.

In spite of precautions taken, contamination was a major problem with explants. Salvaging contaminated explants was attempted by removing the infection with a scalpel in a 4 to 1 water/bleach mix. This procedure was not particularly successful as only about 10% were saved. Severely contaminated explants (ones with fungus or bacteria covering most or all of the medium and explant) were discarded (Table 3.1).

Surviving explants were transferred to clear plastic containers (Magentas) containing 40 ml of medium after approximately one month (as they outgrew the test-tubes). Two to four explants were placed in each Magenta, and were transferred to fresh medium at least monthly.

Shoots at least 2 cm in length were cut from the explants, dipped (for 10 seconds) in 2,500 ppm IBA (indole-3-butyric acid; crystalline, FW 203.2) and placed in a Horticube (a foam-like rooting medium; Smithers-Oasis, Kent, OH) saturated with 100 ml of ½ ACM / Magenta, as described by Barker *et al.* (1997). Four Horticubes were placed in each Magenta, which were then placed under the same conditions as described for the test-tubes.

Plantlets with roots growing through the Horticubes (Fig. 2.6) were transferred to the greenhouse, potted in 200 ml Rigi-pots (IPL Inc., Quebec) and placed within a misting enclosure. The enclosure consisted of white plastic fastened to metal half hoops placed over the greenhouse bench (Fig. 2.7). The overhead shade cloth was used and a shade cloth was placed over the misting enclosure to reduce temperature and amount of light. Several slits were made in the top of the enclosure to release heat. The greenhouse in which the misting enclosure was located was set for 22 °C. The temperature within

the misting enclosure was monitored, and fluctuated between 22 and 29 °C. Misters were placed through the plastic at intervals insuring misting of the entire bench surface. The daytime misting frequency was 10 seconds every 15 minutes from 7 a.m. to 8 p.m. and thereafter for 10 seconds every hour. Moisture levels were checked to ensure this misting regime maintained a constant film of water on the leaves of the plantlets.

Two potting mixtures were tested: straight Pro-mix BX (a peat-based potting medium; Premier Horticulture Ltd., Quebec) and a mix of Pro-mix, vermiculite and perlite in equal parts. Pro-mix containes 75-85% peat with perlite, vermiculite and limestone making up the rest. The peat proportion of the second mixture is decreased from approximately 80% to 25% and thus has greater air space and drainage. The second mixture also has more potassium and magnesium due to the greater proportion of vermiculite.

The potted plantlets were left in the enclosure for one week, after which misting and humidity were decreased and light increased, based on recommendations by Griffis *et al.* (1983). This was achieved by gradually raising the plastic covering on one side of the misting bench while gradually reducing the misting frequency over the course of a week. Thus, by the end of the second week, the plastic was fully raised from one side of the misting bench, and misting was reduced to 10 seconds every 2 hours.

The rooted plantlets were placed in a greenhouse when the acclimatization period was over. The overhead shade cloth was drawn and the plantlets were watered to ensure the medium was kept moist. Fertilizer (8/20/30 NPK; 35 ppm) was applied with each watering. The greenhouse was allowed to cool naturally to about 5 °C, and this

temperature was maintained for the winter.

Explants on ACM that formed buds in the growth room were put into cold storage for 42 days (1000 hours) in an effort to satisfy the chilling requirement for breaking bud dormancy. Half of the explants from each tree were placed in dark cold storage, while the other half were given 8-hour days with 50% light in an effort to determine if light affected survival during the dormancy period. Temperature for all explants was 3-5 °C. Once the cooling period was over, all explants were given 16-hour days (55 $\mu mol\ m^{-2}s^{-1}$) at about 20 °C.

2.4 Sucker Shoot Cuttings

Only three clear beech trees were found with suckers of sufficient size for sampling, and two were sampled (Trees 10 and 16). The third tree (Tree 9) had abundant shoots for cutting, but very few of the shoots were long enough to meet the minimum cutting length requirement (approximately 4 cm). Thus, this third tree was not included as a sample tree.

Two factors were tested: time of collection (measured by cumulative growing degree days; data provided by the Atlantic Climate Centre) and rooting hormone (IBA) concentration. (Cumulative growing degree days is the cumulative number of hours per day above 5 °C multiplied by the number of degrees above 5 °C.) Collection times were June 5th and 6th, June 19th and 20th, July 4th and 5th, and July 17th (Tree 16 only). See Table 3.2 for the cumulative growing degree days for each collection date. IBA concentrations were 2,500, 5,000 and 10,000 ppm (0.25%, 0.5% and 1.0% IBA; or 12.3 mM, 24.6 mM, and 49.2 mM). Cuttings were struck in a wet sand: perlite: vermiculite

mix (1:1:1 by volume). (Note, the June 5th and 6th collection experienced a failure of the misting system. All cuttings died, which may have resulted from lack of misting.)

Tree 10 had one root sucker (approximately 3 m tall), which served as the sole source of cuttings. Tree 16 had numerous root suckers and several were sampled in order to take sufficient cuttings. The suckers from Tree 16 averaged approximately 1.5 m and were thus likely younger than the sucker from Tree 10. Thirty shoots were collected from each of the two sites, at each collection time, except for the June 19th and 20th collection, for which only 24 cuttings were collected from each of the sites due to short supply.

Branches with a basal diameter up to approximately 1½ cm were taken equally from the top, middle and bottom portions of the suckers as far as possible. In general, however, there were far fewer shoots of sufficient length on the lower branches of the suckers, thus shoot collection was biased toward upper branches. As cuttings were to be struck with only the uppermost two leaves attached, all other leaves were removed to reduce water loss. The branches were misted with water before being placed in a cooler containing ice and water for transport to the greenhouse.

At the greenhouse, shoots of at least 4 cm in length were cut for striking (Fig. 2.8). First-order terminal shoots (terminal shoots of branches extending directly from the bole of the tree) were not used as they are less likely to root than lateral shoots, as reported for some species (Dirr and Heuser 1987). Shoots were cut with a scalpel to include a portion of the stem (1-2 mm) just below the bud scale scar (Peck[1], personal

[1]D. Peck, Greenhouse Manager (retired); University of New Brunswick, Fredericton, NB

communication, 2000). Before striking, both the length from the bud scale scar to the end of the shoot and the basal diameter of the cutting (just to the distal side of the bud scale scar) were measured. The cuttings were given a 10-second dip in one of the three IBA concentrations (IBA dissolved in 95% ethanol), air dried for a few seconds, and then struck. Cuttings were struck into individual Ray Leach Stubby Cone-tainers™ (50 ml; Stuewe & Sons Inc., OR) containing a sand: perlite: vermiculite mix, 1:1:1 by volume, and were placed in seedling container holders (Fig. 2.9). Cuttings were randomized among and within container holders with use of a random numbers table. Use of rooting enclosure, misting, and overhead shade cloth was the same as described for micropropagation work.

Cuttings were treated with one of three fungicides (Captan, Rovral or Daconil). All cuttings received at least one fungicide application except for the July 17th cuttings (fungicide treatment was mistakenly suspended for this week). No fungal contamination was noticed on any cuttings.

Rooting success was assessed after 6 weeks and every 2 weeks thereafter up to 16 weeks. Shoots were recorded as rooted, callused, swollen, damaged, dead or a combination thereof. A cutting was considered rooted if it had at least one root longer than 2 mm. Note, cuttings that were obviously rooted were not removed from the medium until they were potted. Rooted cuttings were potted 2 weeks after rooting was noted (200 ml Rigi-pots; Pro-mix BX potting mixture). The number of roots per shoot and lengths of the six longest roots were measured during potting.

Rooted cuttings were acclimatized to the non-mist environment in the same

manner as described for the micropropagated plantlets, that is, by gradually raising the plastic on one side of the enclosure and decreasing the misting frequency over one week. Rooted cuttings were then transferred to another greenhouse with 70% humidity and overhead shade cloth in place. Water was given as needed (ranging from three times per week to once per week as the fall progressed) and a finishing fertilizer (8/20/30 NPK; 35 ppm) was applied with each watering. Temperature was allowed to fall naturally to about 5 °C, where it was maintained for the winter.

Cuttings were placed in a warm greenhouse on March 6th (daytime temperature 22 °C, nighttime temperature 20 °C, 16-hour days, relative humidity 60% minimum). Fertilizer was applied with each watering, starting March 29th (8/20/30; 100 ppm).

2.5 Root Sprout Cuttings

Roots with a diameter ranging from 0.5 cm to 3.5 cm were collected from 15 locations: 14 were the same as those sampled for buds and one new tree was added (tree 16). Total length of roots collected per tree is found in Table 3.5. Roots were cut in 15-30 cm lengths and placed into trays (roots from one tree in each tray) containing a peat-vermiculite mix (1:1 by volume) (Fig. 2.4). Small wounds covering approximately 10% of the surface area were made through the cambium layer of each root by scraping with a knife. Trays were placed on a greenhouse bench and watered as needed to keep the medium moist.

Sprouts were cut from the roots with a scalpel once they reached at least 7 cm in height, dipped (10 seconds) in one of three IBA concentrations (2,500, 5,000, or 10,000 ppm, in 95% ethyl alcohol), and struck in a sand: perlite: vermiculite medium, 1:1:1 by

volume (50 ml Ray Leach Stubby Cone-tainers™). Length and basal diameter of cuttings were recorded.

Rooting success was assessed after 6 weeks and every 2 weeks thereafter. Once rooted, the lengths of the six longest roots were measured, and the cuttings were potted (200 ml Rigi-pots, Pro-mix BX) (Fig. 2.10). Root growth was assessed by the same method again 6 weeks after cuttings were potted. Care was taken not to damage the root systems, many of which were fibrous. Rooting enclosure was the same as described for the micropropagation material; acclimatizing method, watering and fertilizer regimes, overwintering period and spring growing conditions were the same as described for the sucker shoot cuttings.

A second root collection was carried out in late fall of 2000 (November 30th and December 1st) to test if fall collected roots could be overwintered and produce cuttings in the spring. Three sites were sampled: Sites 5, 7, and 10. Two trees showing no disease symptoms were sampled from each, except for Site 10, for which only one clear beech could be located.

Roots were placed in trays in a similar fashion as the previous root collection, and overwintered in the same greenhouse as described for the rooted cuttings. The greenhouse was warmed as described for the rooted cuttings, at which point the roots were wounded as described for the previous root collection. Roots were watered as required to ensure the medium remained moist.

Fig. 2.2. Beech tree with no disease symptoms and abundant suckers.

Fig. 2.3. Remains of a beech tree afflicted by beech bark disease.

Fig. 2.4. Beech root collection in greenhouse.

Fig. 2.5. Beech root sprouts in greenhouse.

Fig. 2.6. Rooted beech plantlet in Horticube.

Fig. 2.7. Misting enclosure for cuttings.

Fig. 2.8. Beech sucker shoot cutting ready for striking.

Fig. 2.9. Beech sucker shoot cuttings in seedling containers and trays.

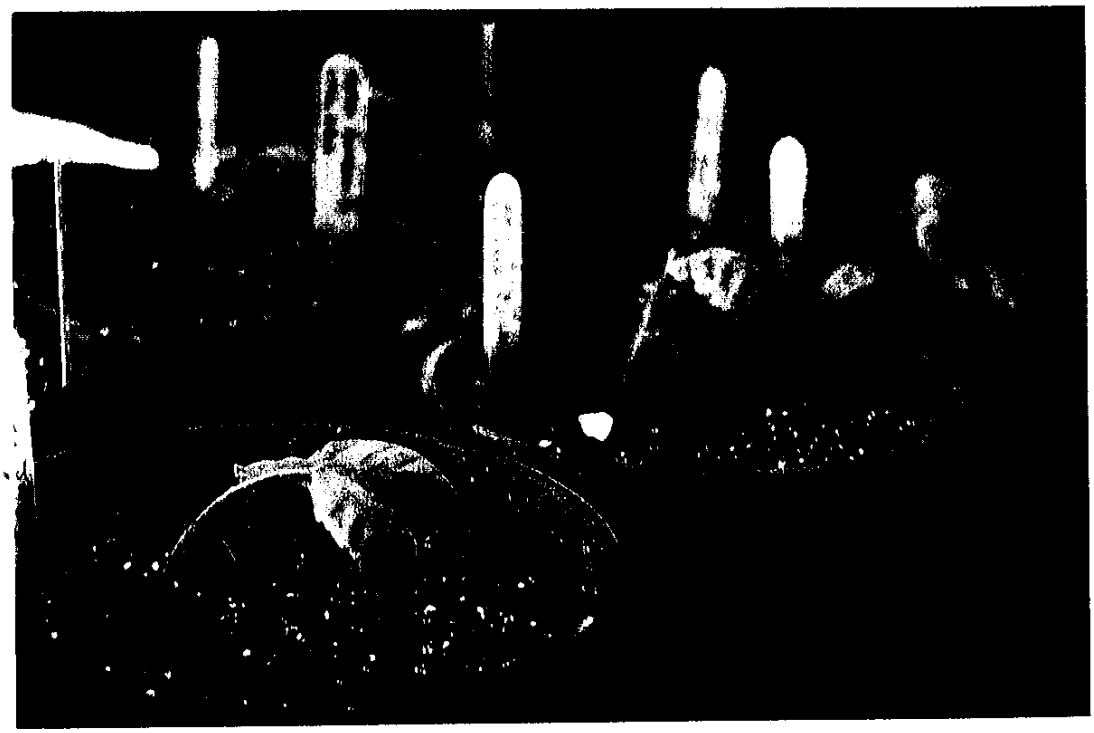

Fig. 2.10. Rooted and potted sprout cuttings (from pilot study; pot size decreased in current study, see page 33).

2.7 Statistical Analysis

The experimental unit for both root sprout cuttings and sucker shoot cuttings was the individual cutting. Micropropagated plantlets were not statistically analyzed as none were successfully acclimatized to the non-mist environment, and thus no data collection was made. Cuttings from both sources were randomly placed among the seedling container holders (30 cuttings per holder), and all container holders were placed on one bench under a common misting enclosure. A block design was not used as the misting enclosure environment was believed to be relatively homogenous (misting was observed to ensure the entire area within the enclosure received misting).

Variables measured for cuttings were rooting percentage, total number of roots and cumulative length of the six longest roots for each rooted cutting. Rooting percentage data were analyzed with a binary logistic regression model (MiniTab © 13.1 statistics software), as the response variable was binary (a cutting rooted or it did not).

Summary of Experimental Design:

Sucker shoot cuttings:

- number of trees sampled: 2; number of collection dates: 3 for tree 10 and 4 for tree 16; number of IBA concentrations: 3; number of cuttings per date and IBA concentration: 8-10;

Root sprout cuttings:

- number of trees sampled: 15; number of IBA concentrations: 3; number of root sprout cuttings per tree used in the statistical analysis: 7 - 20.

Hypotheses Tested:

Sucker shoot cuttings:

1. Ho: there is no date of cut effect on rooting percentage or root length;

Ha: there is a date of cut effect.

2. Ho: there is no IBA concentration effect on rooting percentage or root length;

Ha: there is an IBA concentration effect.

Root Sprout cuttings:

1. Ho: there is no tree effect on rooting percentage or root length;

Ha: there is a tree effect.

2. Ho: there is no IBA concentration effect on rooting percentage or root length;

Ha: there is an IBA concentration effect.

Note: micropropagation experiment was not statistically tested; see Chapter 4 Discussion.

The regression model was as follows:

$Y = \phi tree + \phi I\hat{B}A + \varepsilon$;

where Y = dependant variable;

$\phi tree$ = tree factor effect (random factor);

ϕIBA = IBA factor effect (fixed factor);

and ε = random error.

A general linear model (MiniTab © 13.1 statistics software) was used for the analyses of variance of the root length data to allow for imbalanced data. A residuals versus fitted values graph was used to estimate whether the data were normally distributed. If fanning of the points occurred, data were transformed by taking their log. This transformation sufficed in each case that data transformation was deemed necessary.

The models used in the analyses of variance were as follows:

1. Sucker shoot cuttings: $Y_{ik} = \mu + C_1 + C_2 + G_i + \varepsilon_{ik}$;

2. The date-of-cut factor was added to GLMs for individual trees:

$Y_{ijk} = \mu + C_1 + C_2 + G_i + D_j + \varepsilon$;

3. Root sprout cuttings: $Y_{ijk} = \mu + C_1 + C_2 + G_i + T_j + GT + \varepsilon$;

where Y = dependant variable (root length);

C_1 = shoot length covariate;

C_2 = shoot diameter covariate;

G = growth hormone (IBA) concentration ($_i = 1 - 3$);

T = tree effect ($_j = 1 - 15$); GT = IBA concentration x tree interaction;

D = date-of-cut ($_j = 1 - 3$);

and ε = within plot error.

IBA was a fixed factor, and tree and date-of-cut were random factors. Interaction effect between IBA concentration and date-of-cut in number 2 above was not included due to rank deficiency.

Chapter 3: RESULTS

3.1 Micropropagation

Contamination ranged from 20% (Tree 14) to 97% (Tree 3) of the cultured buds by the first transfer (Table 3.1). Within each tree, the ratio of shoots produced to the number of explants ranged from 0 to 1 (not including Tree 9, for which the one explant transferred produced 2 microshoots). Seven of the 15 trees produced rooted explants; 3 of the 15 produced 3 or more rooted explants.

No correlation was found between the diameter of sample trees and microshoot production or rooting success rate ($r = 0.02$ in both cases) (diameter of trees can be found in Table 3.5).

Table 3.1. Explant transfer success, microshoot production, and rooting success

Tree	#Buds	#Trans.	#Shoots	Shoots/explant	#Rooted	#Rooted/#Shoots	#Rooted/#Buds
1	70	14	6	0.43	1	0.17	0.01
2	120	16	2	0.13	0	0	0
3	120	4	0	0	0	0	0
4	108	15	5	0.33	2	0.4	0.02
5	120	27	7	0.26	2	0.29	0.02
6	120	68	20	0.29	3	0.1	0.03
7	99	46	46	1.00	11	0.24	0.11
8	120	16	1	0.06	0	0	0
9	20[a]	1	2	2	0	0	0
10	89	11	0	0	0	0	0
11	120	64	4	0.06	0	0	0
12	120	32	4	0.13	0	0	0
13	120	20	10	0.50	1	0.1	0.01
14	20[a]	16	0	0	0	0	0
15	120	65	53	0.82	17	0.32	0.14

[a] flower buds

Testing of cold storage technique for explants that had produced buds showed that 1,000 hours at 3 °C, with no light, induced explants to flush and produce shoots. No

explants kept under 8-hour days during the cold period flushed. Note, the two batches of explants were kept in separate storage areas for the 1000 hour cold treatment.

None of the rooted plantlets placed in the greenhouse and overwintered flushed in the spring. All of the buds were small (no more than half the length of buds on healthy seedlings) and did not show signs of swelling. Note, there were accidental interruptions of the acclimatization periods (interruptions of misting schedule), possibly affecting results.

3.2 Sucker Shoot Cuttings

Rooting percentages varied between trees, among dates and among IBA concentrations (Table 3.2). The highest rooting percentage by date was 45.8%, for tree 10, date 1. The highest rooting percentage by date and IBA concentration was 75%, for tree 10, date 1, and 5,000 ppm IBA. Rooting percentage generally decreased with increasing growing degree days (Fig. 3.1). However, no factor showed a statistically significant effect on rooting percentage (as tested by a binary logistic regression model).

Table 3.2. Rooting success (%, after 14 weeks)

TREE #10	n	Rooting (%)	Rooting by Date (%)
June 19th (298 g.d.d.[a])			
IBA Conc. (ppm)			
2,500	8	12.5	
5,000	8	75	45.8
10,000	8	50	
July 4th (466 g.d.d.)			
2,500	10	30	
5,000	10	40	33.3
10,000	10	30	
TREE #16			
June 19th (343 g.d.d.)			
2,500	10	0	
5,000	10	30	13.3
10,000	10	10	
July 4th (558 g.d.d.)			
2,500	10	0	
5,000	10	0	20
10,000	10	60	
July 17th (726 g.d.d.)			
2,500	10	20	
5,000	10	0	16.7
10,000	10	30	

[a]growing degree days (Atlantic Climate Centre, 2000)

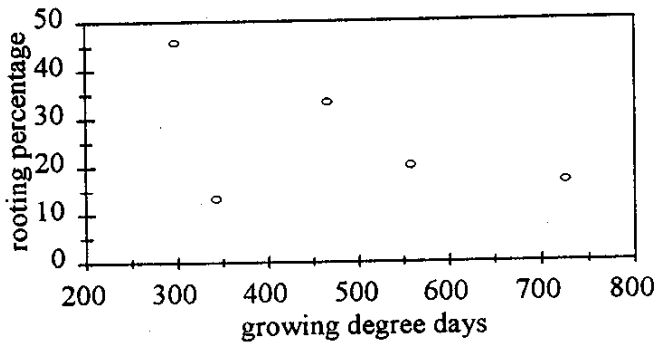

Fig. 3.1. Rooting success with increasing growing degree days

Of the cuttings that rooted, 83% rooted within 10 weeks of striking, 97% did so within 12, and the remaining 3% rooted within 14 weeks.

Analysis of variance showed that IBA concentration had a significant effect on root length (Table 3.3); mean root length was highest with 2,500 ppm IBA (Table 3.4). Note, interactions could not be included in the test due to rank deficiency. Covariates 'cutting length' and 'cuttings diameter' were statistically insignificant and thus removed from the model. The factor 'date' was included in analyses of the individual trees, but was not statistically significant in either case. Although buds of several cuttings elongated, none of the cuttings survived to flush in the spring.

Table 3.3. Factor effect on root length of sucker cuttings

Source	DF	MS	F	P
tree	1	2.5469	3.06	0.091
IBA conc.	2	3.2462	3.90	0.031*
error	30	0.8331		
total	33			

*significant at $P < 0.05$ level

Table 3.4. Mean root length and 95% confidence intervals by IBA conc.

IBA	n	mean (cm)	95% C.I.
1	5	4.5	2.0 - 7.0
2	13	2.2	0.9 - 3.5
3	16	2.1	0.8 - 3.5

3.3 Root Sprout Cuttings

The number of cuttings produced per metre of root varied among trees (Table 3.5). Only tree 16 produced sprouts that were too small for cutting. Ten of the 15 trees sampled produced root sprouts of the quality necessary for cutting, 8 of which produced rooted cuttings. Average length of cuttings was 6.2 cm (95% C.I. = 5.8 - 6.7 cm). The

high variation in total root lengths collected per tree is due to differing accessibility of roots among trees. Also, some trees had been sampled during the pilot study and thus had fewer roots available the following year.

Table 3.5. Production of cuttings per metre of root and tree diameter

Tree	Total Root Length (cm)	Cuttings Produced	Cuttings Rooted	Cuttings/ m of Root	Tree DBH[a] (cm)
1	331	10	6	3.02	26
2	413	6	2	1.45	24
3	450	3	2	0.67	27
4	441	0	0	0	20.5
5	762	21	18	2.76	13.5
6	437	0	0	0	14.5
7	na	na	na	na	14
8	96	7	6	7.29	14.5
9	72	0	0	0	44.5
10	494	20	19	3.85	10
11	333	7	5	2.10	13
12	266	0	0	0	14
13	178	1	0	0.56	26
14	229	2	1	0.87	22.5
15	350	3	1	0.86	18.5
16	282	0	0	0	68

[a] diameter at breast height

Rooting percentage was negatively correlated with the diameter of the sample trees ($r = -0.808$), indicating that rooting success of sprout cuttings generally decreases with increasing tree size.

For trees that produced 7 or more cuttings, 83.1% of cuttings rooted (Table 3.6). Highest rooting by tree was 95% (tree 10), and by IBA concentration was 87% (5,000 ppm). Differences in rooting percentages among IBA concentrations were not significant when tested with a binary logistic regression model; however, rooting percentage for Tree 10 was significantly higher than the other trees ($P=0.034$).

Table 3.6. Proportion of cuttings struck that rooted by tree and IBA conc. (ppm)

Tree[a]	IBA Concentration						Total rooted	%
	n	2,500	n	5,000	n	10,000		
1	3	0.67	4	0.75	3	0.33	6	60
5	8	0.75	7	0.86	6	1.0	18	85.7
8	3	1.0	3	1.0	1	0.0	6	85.7
10	7	1.0	7	1.0	6	0.83	19	95
11	3	0.67	2	0.5	2	1.0	5	71
total	20/24		20/23		14/18		54	83.1%
(% =	83.3		87.0		77.8)			

[a] trees that produced seven or more cuttings

The majority of rooting was complete within 6 weeks of striking. Of the 58 cuttings that rooted, 78% rooted within 6 weeks, and 93% rooted within 8 weeks. The remaining 7% rooted within 10 weeks. However, none of the rooted spout cuttings flushed in the spring after the cold treatment was complete.

For the trees used in Table 3.6, average number of roots per rooted cutting ranged from 2 (95% C.I. = 0.8-3.2) (tree 1) to 8.8 (3.8-13.9) (tree 8). With respect to IBA concentration, average roots per rooted cutting were 3.9 (95% C.I. = 2.7-5.1; 2,500 ppm), 5.1 (2.6-7.5; 5,000 ppm) and 4.9 (3.1-6.6; 10,000 ppm).

Root lengths were measured twice, once as rooted cuttings were potted and again 6 weeks later. The percent with fibrous roots increased dramatically over this time (Table 3.7). Cuttings treated with 2,500 ppm achieved the highest percentage with fibrous roots.

Table 3.7. Percent of cuttings with fibrous roots by IBA conc. (ppm) and time of measurement

IBA Conc.	% Fibrous roots	
	Day 1	Day 42
2,500	52	71
5,000	26	56
10,000	6	62

With respect to root length, 'Tree' was statistically significant in the first

measurement data, once the insignificant covariates were removed (Table 3.8). IBA concentration was not statistically significant for any of the trees. (Note, tree 8 was not included as the 10,000 ppm IBA cell was empty.)

Table 3.8. Factor effect on root length, first measurement

Source	DF	MS	F	P
IBA conc	2	0.1337	0.27	0.767
tree[a]	3	2.3762	5.97	0.025
IBA conc.*tree	6	0.3857	0.49	0.810
error	36	0.7843		
total	47			

[a] trees that produced seven or more cuttings except tree 8

Both 'tree' and the covariate 'shoot diameter' were statistically significant in the analysis of variance for the second root length measurement data (42 days after potting) (Table 3.9). The covariate 'shoot length' was insignificant and thus not included in the model. First and second root length measurements (total length of a cutting's 6 longest roots) for each batch of cuttings is given in Table 3.10.

Table 3.9. Factor effect on root length, second measurement

Source	DF	MS	F	P
shoot diameter	1	987.6	6.59	0.015
tree	3	409.2	4.11	0.025
IBA conc.	2	71.6	0.72	0.504
IBA conc.*tree	6	81.3	0.54	0.772
error	31	149.9		
total	43			

Table 3.10. Sample size and mean root length (six longest roots, cm) for each measurement

Tree	n	Day 1	n	Day 42
1	6	2.3	5	8.5
5	18	8.5	15	22.9
8	6	10.2	6	34.5
10	19	10.3	19	24.7
11	5	10.3	5	29.1

Root material collected in the fall and overwintered produced sprouts when placed in a warm greenhouse with supplemental lighting in early March. Sprout production began by mid-March and cuttings were available by mid-April. Number of sprouts per metre of root averaged 4.6, compared to an average of 2.3 sprouts per metre of root for the spring-collected roots.

3.4 Comparison of Cutting Sources

Root sprout cuttings had significantly longer root lengths than the sucker cuttings ($P = 0.001$); mean root length was 5.6 cm (4.4-6.9, 95% C.I.) for the root sprout cuttings and 2.5 cm (1.7-3.3, 95% C.I.) for the sucker cuttings. However, no root sprout cuttings could be obtained from tree 16, while the tree did produce rooted sucker cuttings. There was no statistically significant interaction between the method used and IBA concentration (ANOVA, General Linear Model).

The buds of the sucker cuttings were significantly longer than those of the sprout cuttings ($P < 0.001$). Mean bud lengths were 1.2 cm (1.0 - 1.5 cm, 95% C.I.) for the sucker cuttings and 0.4 cm (0.4 - 0.5, 95% C.I.) for the sprout cuttings.

Chapter 4. DISCUSSION

4.1 Micropropagation

Of the three propagation approaches tested, micropropagation of American beech has been given the most attention by other researchers. Barker *et al.* (1997) developed a suitable culture medium, including the optimal amounts of BA and NAA supplements, and a suitable IBA concentration for micropropagating meristem material from mature buds and the shoot tips of root sprouts. Unfortunately, no plantlets were established in soil during their study.

Results of the micropropagation work in this study differed somewhat from Barker *et al.* (1997). In the present study, 80% of the trees produced shoots, compared to 15% by Barker *et al.* (1997). Forty-seven percent of the trees in this study produced rooted plantlets, but this statistic was not reported in the Barker *et al.* study. Potential causes of differences in rooting success among source trees include tree age, physiological condition of the tree, and genetic effects. Tree age can roughly be approximated by diameter, and no statistically significant correlation was found between tree diameter and shoots produced per explant or explant rooting percentage. All source trees were generally healthy with robust crowns and no obvious differences in physiological condition (except for the trees producing flower buds). Thus genetic effects are probably most important in explaining rooting success differences among trees.

Survival following acclimatization has proved most problematic. In addition to shading, a misting tent and two different potting media were used in the present study to

address the problems of dehydration and inadequate drainage during the pilot study. In spite of these conditions, no plantlets resumed growth after acclimatization or survived the overwintering period.

Further work with micropropagation

Micropropagation would be a valuable beech propagation method if perfected. The tissue material required is more easily collected than the material required for the other methods, and micropropagation has proven a rapid multiplication technique for some species (Bonga and von Aderkas 1992). Micropropagation success could be improved by reducing contamination. Much material in this study was lost to contamination, despite use of several recommended techniques that have been used successfully in culturing buds of other species in the same lab as the present study (K. Forbes, personal communication, 1999). The flaming method (Meier and Reuther 1994) might solve this problem (buds are dipped in ethanol and then passed through an open flame).

The most pressing challenge is to ensure that rooted plantlets survive overwintering. This may be achieved by increasing the vigour of plantlets before they enter dormancy by ensuring high quality bud material and possibly by allowing plantlets to grow to a larger size *in vitro* before potting in a greenhouse. Ensuring high quality of the bud material could also improve micropropagation success. Meier and Reuther (1994) suggested (for European beech micropropagation) (a) using the youngest source trees possible, (b) taking buds just before bud flush, (c) using apical buds in preference to

axillary buds and (d) using buds at least 2 cm in length. Sampling from the lowest possible branches on the tree can be added (Dirr and Heuser 1987). It was not possible to use young source trees in this study because susceptibility to the disease is sometimes not apparent until the trees are over 10 cm in diameter. Both apical and axillary buds were used in this study as Pijut[1] (personal communication, 1999) found no difference between the two in a previous American beech micropropagation study (Barker *et al.* 1997).

4.2 Softwood Cuttings

Little previous work has been reported on rooting American beech cuttings. Reid (1984) worked with sucker and stump sprout cuttings, but did not test tree effect or IBA concentration. Use of forced root sprout cuttings has not been reported in the available literature. The effect of tree was tested in the root sprout experiment because of its importance in determining the number of source trees required to obtain the desired number of clones, and was found to be statistically significant in terms of root length growth. This result was expected given the widely documented range of rooting success among clones of many species (Dirr and Heuser 1987). In the present study, 8 of the 15 trees sampled (53%) produced rooted cuttings.

Tree effect could not be tested with the sucker cuttings because only 2 trees were sampled. Considering that there is likely a tree effect, generalizations cannot be made from this study of sucker cuttings. However, both sample trees produced rooted cuttings, suggesting that this technique is potentially effective.

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[1]Dr. P. Pijut, research scientist, USDA Forest Service, St. Paul, MN

IBA is the most universally effective auxin (Dirr and Heuser 1987), but plants respond differently to IBA concentration, thus experimentation was required to determine the best concentration for American beech. The statistically significant effect of 2,500 ppm IBA concentration on root lengths of sucker cuttings (Tables 3.3 and 3.4), indicates that no more than 2,500 ppm is needed to induce rooting; further testing could determine whether another concentration (between 0 and 5000) is optimum.

The effect of IBA concentration on root length of root sprout cuttings was not statistically significant, nor were there any obvious trends in rooting success, either among all trees or within individual trees: it appears that the three IBA concentrations are equally effective. This result is unexpected given the number of reports on the parabolic effect of IBA on rooting (Dirr and Heuser 1987). However, this result shows that a concentration greater than 2,500 ppm is not necessary to induce rooting.

Finally, developmental stage of shoots has been shown to be critical in rooting difficult-to-root species, and the window of opportunity for taking cuttings differs from species to species (Dirr and Heuser 1987). Growing season was not measured by the calender date, but rather by cumulative growing degree days (the cumulative number of hours per day above 5 °C multiplied by the number of degrees above 5 °C). Growing degrees (Atlantic Climate Centre, 2000) were used in this study to give an actual account of the spring growing season in each of the two locations that sucker shoots were collected.

The lack of statistical significance of date-of-cut on rooting percentage and root length of sucker cuttings indicates that beech cuttings may be taken over a period of at

least 1 month (the period over which cutting collections were carried out). It is important to note that the first collection of shoots from the Fundy National Park site (198 growing degree days; June 5th, 2000), which were not included in the study due to a greenhouse watering failure, wilted slightly shortly after striking (prior to the watering failure), whereas the cuttings collected from the same site after 298 growing degree days (June 19th, 2000) showed no early wilting signs. This early wilting likely would have resulted in poor rooting (Dirr and Hueser 1987) should the experiment have continued, indicating a possible lower limit on the timing of shoot collection.

The statistical significance of the root sprout cuttings' diameter on the second measurement of root sprout root lengths is likely because the greater shoot diameter allows for a larger vascular system, thus greater nutrient flow and more root growth. The larger shoot diameter may also provide more carbohydrate and nitrogen reserves to fuel root growth. The change in statistical significance between the root length measurement dates (day 1 versus day 42) was possibly due to an increase in shoot diameter importance as root systems develop.

Several other factors have been shown to affect rooting success. Rooting medium (Dirr and Heuser 1987) and various nutrient (Wott and Tukey 1966) and cytokinin (Psota *et al.* 1995) leaf sprays have been shown to affect rooting success and root quality. These were not tested simply due to the size of the experiment; only so many factors could be tested, and the ones tested were believed to be the most critical (Peck, personal communication, 2000). A sand, perlite, vermiculite medium (1:1:1 by volume) was used as it was recommended as a good, general medium for rooting softwood cuttings (Peck,

personal communication, 2000), and because a peat-based medium appeared to lack sufficient drainage when used under the misting regime.

The rooting enclosure was used to optimize the environmental conditions for rooting. The enclosure provided shade and a humid environment for cuttings, both of which are important in rooting difficult-to-root species.

Shoot cuttings source comparison

Root samples could be collected from many more trees than could sucker cuttings, due to difficulty in locating suckers, especially ones large enough to provide a sufficient number of cuttings. The two sources also differed in their rooting response: root sprout cuttings had a higher rooting success, developed longer root systems, and developed more fibrous root systems than sucker cuttings. This may be due to the juvenility factor: the root sprouts were less than 2 months old, while the sucker cuttings were taken from suckers at least several years old. Dirr and Heuser (1987) suggest that the age of the vegetative sprout reflects the juvenility state of cuttings, rather than the age of the source tree; the act of vegetative reproduction involves a partial return to juvenility.

The sucker cuttings, however, formed larger buds than the root sprout cuttings, as might be expected given the larger diameter of the sucker cuttings, and thus greater reserves to put into bud production, and because they were struck earlier, giving them more time to develop before short days resulted in shut-down.

4.3 Recommended Further Work with Cuttings

The factors tested in the present study were chosen because they were considered most important based on the literature. However, a number of other factors warrant

testing, especially those that may improve overwintering success of rooted cuttings.

The challenge with root sprout cuttings is to achieve overwinter survival. One approach is to increase bud and root size by starting the rooting procedure earlier in the year. A side experiment of the present study showed that roots collected from disease-free beech in late November and early December 2000 and stored at 5 °C will produce root sprouts when placed in a warm greenhouse with supplemental lighting in late winter (March 6th). Average sprout production per metre of root was not lower than that for spring-collected roots. Striking cuttings by mid-April would add 3 months to the growing time available to cuttings in the present study. This increased growing time before bud set is likely to result in larger buds and root systems with greater reserves, which may increase overwinter survival.

Smalley and Dirr (1986) stressed the importance of giving cuttings an extended photoperiod (75-100 watts; continuously or 10 p.m. to 2 a.m.) for 6-8 weeks starting during or immediately after rooting, in order to increase cuttings' carbohydrate and nitrogen reserves and thus achieve overwinter survival. Temperature during the extended photoperiod should be above 13 °C. Obdržalek and Pinc (1995) found that inducing flushing by giving an extended photoperiod before overwintering improved survival of European beech cuttings. Obdržalek and Pinc (1995) also found that use of an insulated hotbed improved overwintering success. They noted that an insulated hot bed allowed for an air temperature of -7 °C with no harm to cuttings. Winter air temperature in the present study was kept at approximately 5 °C, which may not have been low enough, considering Obdržalek and Pinc's (1995) study. Additionally, Smalley and Dirr (1986)

recommended an overwinter storage temperature of 1 °C for several deciduous tree species.

Some species (certain deciduous shrubs, for example) respond poorly to transplanting directly after rooting (Dirr and Heuser 1987). Overwintering success may be improved if rooted cuttings are not transplanted in the fall, but rather are left in their rooting medium and transplanted in the spring once new growth has developed. This procedure was not tested in the current study as transplanting was necessary to measure root lengths of the cuttings.

Testing whether increased humidity provided by the rooting enclosure is necessary is also warranted; misters and overhead shade cloth alone may be adequate. Not using the plastic enclosure would help make the procedure more suited to large-scale operation.

Use of nutrient mist on leaves of cuttings may also improve rooting. Wott and Tukey (1966) showed that adding fertilizer (23/19/17 NPK; 42.6 g/100 L) to mist water improved rooting, root quality and shoot growth for softwood cuttings of certain ornamental species (beech was not included in the test).

Testing also could be carried out to determine the optimum rooting medium. Only one medium was used in this study (equal parts sand, perlite, and vermiculite by volume) as other factors were given priority for testing. Rooting media such as pure vermiculite and peat-based media could be tested for their effect on rooting and root quality, as media have differing effects on species (Dirr and Heuser 1987).

Improving rooting success of sucker cuttings would be easier after an orchard of

clear beech has been developed. Once an orchard is available for use, effort should be focused on ensuring optimum physiological condition of source plants, including proper nutrition, watering and light conditions. Juvenility should be addressed by hedging or coppicing young orchard trees. However, it is not known how long these practices will maintain juvenility.

Increased rooting success may also be achieved by using etiolation before cuttings are taken (Maynard and Bassuk 1987). Although no etiolation work with American beech is known (Bassuk[1], personal communication, 1999), this technique has been used with some success with European beech, where Bassuk *et al.* (1984) were able to improve rooting success from 5% for control cuttings to 68.5% for etiolated cuttings.

Cytokinin pre-treatments of orchard trees may enhance rooting. Psota *et al.* (1995) showed that spraying an alginate solution (Bio-algeen S-90; 18 ml/l) on 3-year-old European beech seedlings significantly improved rooting and root length. The sprays were given three times, approximately once a month up to 2 weeks before taking cuttings. Such pre-treatment may also increase rooting success of root sprout cuttings.

4.4 Strategy Toward Beech Restoration

Once overwintering success has been achieved, the following steps can be taken to fulfil the goal of planting resistant beech into forests affected by the beech bark disease:

1. use rooted cuttings from disease-free trees to develop an orchard of beech;

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[1]Dr. N. Bassuk, research scientist; Cornell University, Ithaca, NY

2. use orchard as source of cuttings for further propagation testing, as well as root collections from healthy beech;

3. test the propagated trees for resistance to the beech bark disease; and

4. field test planting resistant beech into woodlots affected by the disease.

Grafting scions of mature disease-free beech onto beech rootstock may help to establish an orchard of resistant trees. The portion of stem below the graft would require protection (mechanical or chemical) from the disease. The grafted portion may retain its maturity and thus produce seed once crossed with other grafted trees. Seed produced in this manner would be grown out and the young trees tested for resistance to the disease.

Restoration should also be attempted through silviculture, by reducing the number of diseased beech while promoting the growth and reproduction of resistant trees. Although no controlled breeding experiments have been conducted to determine how the resistance is inherited, it is likely that crosses between resistant trees will result in seedlings with increased levels of resistance (Houston 1999). Also, an unpublished study by Houston shows that more root sprouts tend to develop around resistant trees than around susceptible trees.

Dealing with unwanted suckering of susceptible beech is a challenge. Intuitively, cutting beech in late summer when most of the trees' energy is above ground seems appropriate, and will avoid disturbance to the roots during spring, which is the time of greatest sprout production and survival from wounded roots (Jones and Raynal 1988). Applying herbicide to stumps of susceptible beech after cutting may be a silviculturally valid option for controlling beech regeneration. Glyphosate has been tested on beech and

has proved an effective herbicide (Ostrofsky and McCormack 1986). Clear beech and their suckers would have to be marked and avoided. Girdling susceptible trees before cutting may also reduce suckering by reducing root system vitality (Salonious[1], personal communication, 2001).

[1]Dr. P. Salonious, research scientist; Canadian Forest Service, Fredericton, NB

Chapter 6: CONCLUSION

This study has demonstrated that American beech softwood root sprout cuttings and softwood sucker shoot cuttings can be successfully rooted (rooting percentages from 60 to 95 and 13 to 46, respectively) and acclimatized (with continued root growth) to a non-mist greenhouse environment. This is the first attempt known to propagate American beech through root sprout cuttings, and the first known study to document acclimatization of rooted cuttings (from any source) to a greenhouse environment outside a rooting enclosure. Micropropagation of buds from mature American beech was not successful beyond that achieved by other researchers (Barker *et al.* 1997). Plantlets were produced, but these were not acclimatised to a non-mist greenhouse environment.

The maximum IBA concentration necessary for rooting softwood cuttings from these sources was 2,500 ppm. The lower growing degree day limit on taking sucker cuttings is likely between 200 and 300 growing degree days (June 2nd to June 16th for the Havelock site (tree 16), and June 5th to June 19th for the Alma site (tree 10)). Rooting success and root length varied among trees: eight of fifteen trees produced root sprout cuttings that rooted, and root lengths differed significantly by tree. None of these factors had been tested for American beech prior to this study.

Further experimentation is required to successfully overwinter rooted beech cuttings. This might be achieved by (a) collecting root material in the fall so that cuttings are taken earlier in the spring, (b) not transplanting cuttings in the fall but rather waiting until they have put on growth in the spring, and (c) giving cuttings an extended photoperiod for 6-8 weeks.

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APPENDICES

APPENDIX I

COLLECTION SITES

Site 1: Dunbar Road (46 07 16 N, 66 44 15 W)

Stand: yellow birch, beech, maple

DBH: 26 cm

Height: approximately 15 m

Collection dates: April 10 for buds; May 29 for roots

Notes on location: Tree is located about 100 m into the forest on the right side of the Dunbar road at a point approximately 3 km from the highway.

Site 2: Odell Park (46 00 41 N, 66 43 52 W)

Stand: beech, maple, hemlock

DBH: 24cm

Height: approximately 15 m

Collection dates: April 10 for buds, May 29 for roots

Notes on location: Enter the park through the Robie St. entrance (by the church and water tower). Walk along the trail running perpendicular to Robie St. This trail will cross another trail; continue on until a large clear beech with a broken top can be seen on the right. The beech sampled is on the left side of the trail just before this large beech.

Site 3: Odell Park (46 00 41 N, 66 43 52 W)

Stand: beech, hemlock, maple

DBH: 27cm

Height: approximately 15 m

Collection dates: April 10 for buds, May 29 for roots

Notes on location: Enter the park as for site two, but stop at the first bench encountered, which will be on the right. Turn right into the woods at the bench and walk about 25 m.

Site 4: Larry Slipp (488-8904), Central Hampstead, (45 38 52 N, 66 08 31 W)

Stand: beech, maple, balsam fir

DBH: 20.5 cm

Height: 14 m

Collection dates: April 12 for buds, May 30 for roots

Notes on location: Contact owner to reach site.

Site 5: Crown Land (45 36 16 N, 65 23 31 W)

Stand: beech, maple

DBH: 13.5 cm

Height: approximately 10 m

Collection dates: April 12 for buds, May 30 for roots

Notes on location: Drive along Shepody Rd. (from its western entrance) and watch for a tower on your right. Take the next logging road on your left after the tower. Follow this road up over a hardwood ridge. The tree is down about 50 m from the top of the ridge and on the right of the road about 10 m into the woods.

Site 6: Dean Toole (433-2105) (45 45 31 N, 65 32 26 W)

Stand: balsam fir, maple

DBH: 14.5 cm

Height: approximately 10 m

Collection dates: April 12 for buds, May 30 for roots

Notes on location: Drive up the logging road next to Dean Toole's house. The tree is on the left of the road a few hundred metres up this road.

Site 7: Dean Toole (433-2105) (45 45 37 N, 65 32 16 W)

Stand: beech, maple, balsam fir

DBH: 14

Height: approximately 12 m

Collection dates: April 12 for buds, roots not collected

Notes on location: Drive up the logging road as previously mentioned. A number of clear beech are located where the road splits. The original tree sampled for buds could not be re-identified.

Site 8: Bennet Brook Trail, FNP (45 36 47 N, 65 04 47 W)

Stand: beech, yellow birch, red spruce

DBH: 14.5 cm

Height: approximately 12 m

Collection dates: April 13 for buds, June 5 for roots

Notes on location: Walk down the Bennet Brook Trail from Bennet Lake until you come to a large clear beech on your left (about 15 minutes). Tree 8 is found about 40 m up the

hill behind this large clear beech.

Site 9: Bennet Brook Trail (45 36 49 N, 65 04 41 W)

Stand: beech, yellow birch, red spruce

DBH: 44.5 cm

Height: approximately 20 m

Collection dates: April 13 for buds, June 5 for roots

Notes on location: This tree is the large clear beech mentioned in the description for Site 9 above.

Site 10: Shady Maple Trail (45 35 20 N, 64 59 16 W)

Stand: maple, beech, red spruce

DBH: 10 cm

Height: approximately 8 m

Collection dates: April 13 for buds, June 1 for roots

Notes on location: Drive up Maple Drive and stop at the Shady Maple Trail head. The trail does a loop and ends up a short distance further up the road. Entre the trail at the upper end and look for the tree on your right just a few metres in.

Site 11: Laverty Tower (45 39 19 N, 65 01 08 W)

Stand: maple, beech, yellow birch, red spruce

DBH: 13 cm

Height: approximately 12 m

Collection dates: April 13th for buds, June 1 for roots

Notes on location: Upon entering the Laverty Tower parking lot, park immediately to

your right. The tree is about 30 m into the wood from this corner of the parking lot. Note, there are a number of other clear beech around this parking lot.

Site 12: Caribou Plain (45 37 17 N, 65 03 29 W)

Stand: yellow birch, maple, beech, red spruce

DBH: 14 cm

Height: approximately 12 m

Collection dates: April 13 for buds, June 1 for roots

Notes on location: Start along the trail clockwise, i.e. opposite to the trail head sign. Walk until you see the interpretive sign reading "between snow melt...." The tree is a few metres off the trail on your left at this point.

Site 13: Jack Crealock (433-2491) (45 49 42 N, 65 43 25 W)

Stand: maple, beech

DBH: 26 cm

Height: approximately 18 m

Collection dates: April 28 for buds, May 30 for roots

Notes on location: A logging road is located on the right about ½ km before Mr Crealock's house. Drive down the logging road until you notice a mature hardwood stand on your left. Park here and walk down the logging road leading into this stand. Take your first left and look for the tree on the edge of the right side of the road.

Site 14: Herb Sobeck (734-2045) (45 53 37 N, 64 51 01 W)

Stand: beech, maple

DBH: 22.5cm

Height: approximately 18 m

Collection dates: April 28 for buds, June 1 for roots

Notes on location: The tree is on the left of the road a few metres across the property line into Mr Sobeck's property. Contact owner to access tree.

Site 15: Hazen Hughes (45 48 54 W, 65 48 58 N)

Stand: maple, white birch, beech

DBH: 18.5

Height: approximately 12 m

Collection dates: May 1 for buds, May 29 for roots

Notes on location: contact owner to access tree.

Site 16: Brian Hicks (534-2429), Havelock (45 58 34 N, 65 22 40 W)

Stand: balsam fir, white ash, popular, white birch

DBH: 68cm

Height: approximately 18m

Collection dates: June 6 for roots

Notes on location: Contact owner to access tree.

APPENDIX II

ACM RECIPE

ACM recipe

1. Macronutrients	g/l
NH_4NO_3	0.4g
$CaNO_3.4H_2O$	0.556g
K_2SO_4	0.99g
$CaCl_2.H_2O$	0.096g
$MgSO_4.7H_2O$	0.37g
KH_2PO_4	0.17g

2. Sugars and Agar	
Agar	8.0g
Myo-inositol	0.1g
Sucrose	20.0g
(Lysine	0.1g)

3. Iron	100x stock (g/100ml; use 10ml/L)
$Na_2.EDTA$	0.373g
$FeSO_4$	0.278g

4. Micronutrients	1000x stock (g/100ml; use 1ml/L)
$MnSO_4.H_2O$	2.23g
$ZnSO_4.7H_2O$	0.86g
H_3BO_3	0.62g
KI	0.083g
$NaMOO_4.2H_2O$	0.025g
$CuSO_4.5H_2O$	0.0025g
$CoCl_2.6H_2O$	0.0025g

5. Vitamins	1000x stock (g/100ml; use 1ml/L)
Thiamine.HCl 0.01g	

Nicotinic.HCl	0.05g
Pryidoxine.HCl	0.05g
Lysine	(not used in stock, see #2 above)
6. Hormones	1000x stock (g/100ml; use 1ml/L)
BA	0.02005g
NAA	0.00502g

VITA

Candidate's Full Name:

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