Advances in Breeding American Beech for Resistance to Beech Bark Disease

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Introduction

Beech bark disease (BBD) is a complex disease that is initiated by the feeding activities of the beech scale insect (*Cryptococcus fagisuga*). As the insects feed, groups of parenchyma cells collapse and die resulting in the development of small fissures in the bark (Ehrlich 1934). These provide entryway for fungal inoculation with either the native pathogen *Neonectria galligena*, or *Neonectria coccinea* var. *faginata*, a pathogen believed to have been introduced from Europe. As the fungal mycelia grow, large areas of tissue become weakened and die. Eventually complete girdling of the tree may occur. Often, cankers form resulting in wood defects. Large numbers of severely deformed American beech trees persist in forests long-affected by BBD, complicating stand management practices due to prolific formation of root suckers by these diseased trees. This can result in the formation of dense "thickets" of small diameter susceptible beech. Such thickets are of little value and may actually interfere with the regeneration of desired species (Houston & O'Brien 1983).

The spatial and temporal spread of BBD has been classified into three distinct stages (Houston & O'Brien 1983). The advancing front is the first stage of the disease and refers areas recently invaded by the beech scale insect. Large diameter, mature beech trees are normally the first to be infested. The second stage is termed the killing front and refers to the situation where extremely high levels of beech scale infestation are coupled with severe *Neonectria* attacks, resulting in heavy mortality. The last phase of the disease is termed the aftermath forest. This is an endemic stage persisting in regions where heavy mortality has occurred in the past and the remaining beech trees are mostly small, highly deformed and defective, and often of root-sprout origin. These younger trees provide a continual source of inoculum of both the scale insect and the *Neonectria* fungi.

Fortunately, even in aftermath forests long-affected by BBD, there are American beech trees that remain disease-free. Insect challenge trials have demonstrated that such trees are resistant to the scale insect and extensive *Neonectria* infections are typically not observed without prior scale infestation (Houston 1982). Resistant trees are commonly found clustered in close proximity to one another. This indicates they are likely related, originating either as clones of nondiseased individuals established through root- and stump-sprouting or as full- or half-sib seedlings clustered due to a limited radius of seed dispersal (Tubbs & Houston 1990). Currently, suggested management strategies for stands infested by or about to become infested by BBD include reducing the proportion of susceptible beech and retaining the resistant trees so that they may reproduce increasing the frequency of resistant individuals (Leak 2006). However, retaining only the estimated 2-5 percent of beech trees that are resistant may result in a significant loss of genetic variability due to the high degree of relatedness among resistant trees in a stand. The number of resistant genotypes in a stand could be far less than the number of actual resistant stems. In fact, studies using both isozymes (Houston & Houston 1994; Houston & Houston 2001) and RAPD markers (Carey et al. 2001) have confirmed the close relationships between resistant trees clustered in close proximity.

Therefore, conservation of genetically diverse resistant trees will be critical to the long-term survival of healthy, productive American beech. In addition to conserving the genetic mechanisms involved in resistance, it is of equal importance to maintain diversity related to adaptation to local environments. Development of a beech breeding program is an integral part of achieving these goals. Our early research has focused on developing tools required for investigating and eventually understanding the genetics behind the scale-resistance phenotype and to establish methods for preserving and propagating a genetically diverse array of resistant selections.

Controlled Cross-Pollinations in American Beech

The first step in developing a breeding program is to identify the desired traits and determine the underlying genetics responsible for the heredity of those traits. Due to the fact that *Neonectria* infections are not a significant problem in beech in the absence of the scale insect, our research has focused on the scale-resistant phenotype.

The parent trees used in our cross-pollination experiments were located in Ludington State Park, Ludginton, MI. This region is considered a *killing front* having an abundant scale population and the presence of *Neonectria coccinea* var. *faginata*. Although the disease at this location was first reported in 2001 (O'Brien et al. 2001), anecdotal evidence suggests the scale has been present for ten years or more. All parent trees have had their phenotype confirmed by using the artificial infestation technique (Houston 1982) that involves placing insect eggs directly on the bark of the tree. Productive controlled cross-pollinations were achieved between two resistant parents (R x R), and between a resistant and susceptible parent (S x R) (Koch & Carey 2004). Half-sib families were derived from seed collected from two of the parent trees as well as an additional susceptible tree. Also obtained was a half-sib family from a tree (ME) located in a stand in Maine that had been managed for BBD by removal of all susceptible beech stems (Farrar & Ostrofsky 2006). Because the specific trees that provided pollen were not identified nor was their resistance level confirmed, this cross is considered a R x pseudo R cross. A summary of the full- and half-sib families that were obtained is listed in Table 1.

Table 1. Summary of full- and half-sib families.

Female parent	Male parent or open-pollinated	Number of seedlings
1505 (R)	1504 (R)	47
1506 (S)	1504 (R)	50
1504 (R)	OP	33
1506 (S)	OP	89
1510 (S)	OP	22
ME (R)	OP (pseudo R)	71

R=resistant; S=susceptible; OP=open-pollinated

To determine the proportion of the scale-resistant phenotype among individuals in each of these families, the artificial infestation technique was carried out on one-year old seedlings in 2004 and the data have been reported (Koch & Carey 2005). Insect challenges were performed again in 2005 and 2006. The data from 2004 were collected during a different phase of the insect life cycle than those collected in 2005 and 2006. Despite this, the data from all three years did identify the same trends (Koch et al. in prep.). Data from the current year, 2006, are summarized in Figure 1. The largest proportion of resistant progeny is observed in both the R x R controlled-cross and the ME open-pollinated (R x pseudo R) population. Where there is only one resistant parent (S x R) the proportion of resistant offspring is smaller than that seen when both parents are resistant, but greater than what is observed in progeny from open-pollinated susceptible parents. Open-pollinated seed from a resistant parent (1504) yields a proportion of resistant to susceptible individuals much like the S x R cross. This is presumably because the majority of available pollen donors are susceptible, unlike the situation in Maine. The proportions of resistant individuals in the half-sib families from susceptible parents appear to be related to distance between the maternal tree and a potentially resistant pollen donor. For example, 1506 is located within 20 feet of a resistant tree while 1510 is at least 600 feet from the closest resistant tree. The effect of genotype or family on the number of scale insects populating an individual tree was determined to be significant ($p < .001$) based on an analysis of variance (Koch et al. in prep.). A separate breeding effort headed by Dr. Judy Loo of the Canadian Forest Service has produced seedlings that have been challenged and in the summer of 2007 will yield data available for comparison with the studies reported here.

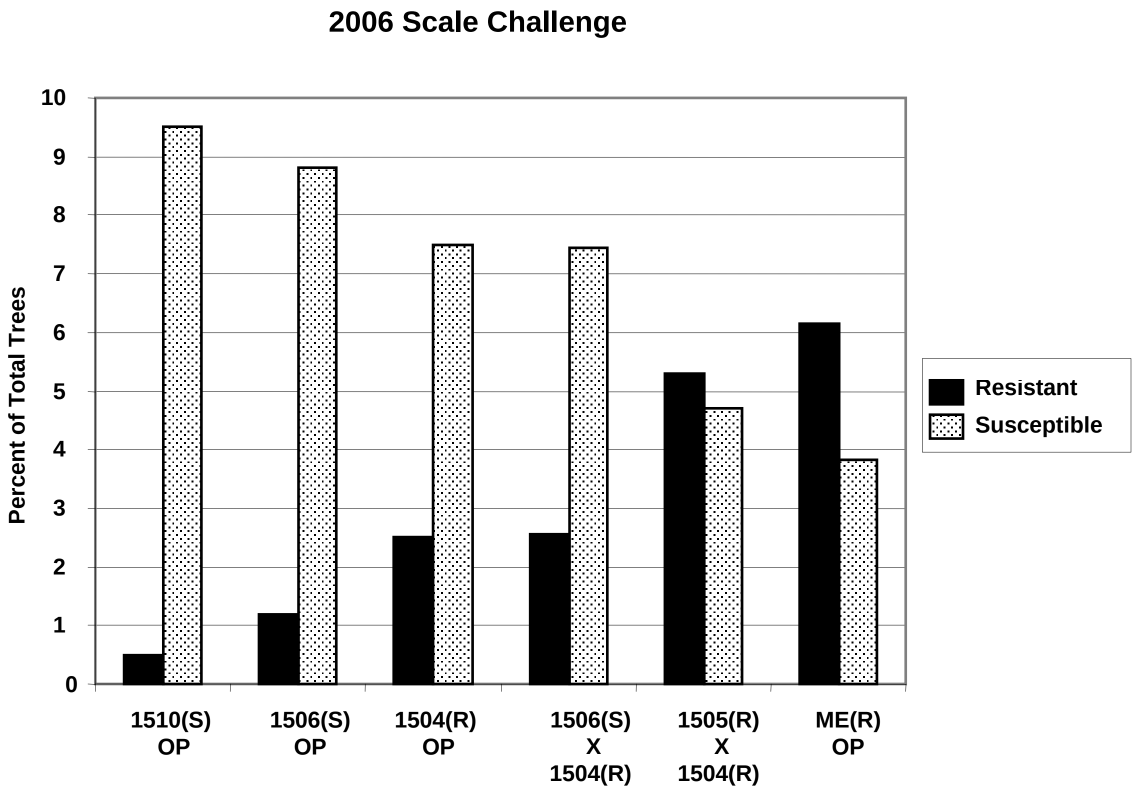

Figure 1. Results of 2006 scale challenge experiment of full- and half-sib families.

Containerized Greenhouse Seed Orchards

The scale-challenge data on the full- and half-sib families demonstrate that scale-resistance is a highly heritable trait with potential for considerable genetic gain in resistance. However, the establishment of seed orchards is a daunting task for several reasons. American beech does not reach sexual maturity until roughly 40 years of age at which point it can be upwards of 70-120 ft.

tall. Flowers are produced at sporadic intervals and good seed crops occur only once every 2 to 8 years (Tubbs & Houston 1990). To facilitate breeding efforts in American beech, effective methods for inducing flowering at younger ages, increasing successful reproduction and facilitating seed collection are needed. Furthermore, effective means to propagate selected parent trees are not available for this intractable species. Micropropagation techniques have been attempted and have proven to be inefficient (Barker et al. 1997; Loo et al. 2005). Traditional grafting methods have proven to be more successful, with a reported average success rate of 33 percent (Loo et al. 2005).

In an attempt to improve the efficiency of vegetative propagation of American beech, we adapted a hot-callus grafting system (Strametz 1984) for use with *Fagus grandifolia* (Carey & Koch in prep). Using this technique in the first year of grafting, 2005, successful graft rates were between 50-60 percent for both scion sources: seedlings (cross-progeny) and mature scion. In one case, several mature scions taken from the resistant tree 1505, produced flowers post-grafting. These flowers were successfully pollinated and went on to produce viable seed (Table 2.) In 2006 the seedling take rate was increased to 71 percent with the overall mature scion take rate of 60 percent. Graft success rates varied depending on the genotype and on the time of year the grafting was performed. Grafts from some genotypes are routinely 90-100 percent successful. In 2006, scions from 19 different mature resistant genotypes gave an overall grafting success rate of 85 percent. Of these 19 genotypes, 16 went on to flower post-grafting. All of the grafts that produced viable flowers were pollinated in the greenhouse and kept there until seed could be harvested. To date, a portion of this seed has begun to germinate and Table 2 demonstrates the significant increase in germination rates in containerized greenhouse pollinations compared to field pollinations. In 2004, resistant tree 1505 was pollinated in the field at Ludington State Park, MI, with pollen collected from Sebois County, Maine. The same pollen, Sebois 23, was also used in 2005 to pollinate containerized grafts of 1505 in the greenhouse. Only 14 percent germination was achieved in the seeds collected from the field pollinations, but 92 percent of the seed collected from the greenhouse pollinations germinated. In all cases, dramatic increases were observed when comparing germination rates of field-pollinated seed to greenhouse-pollinated seed. The highest germination rates obtained in the field were 30-32 percent, and the lowest germination rates reported to date for the greenhouse pollinations are 58-62 percent, representing a minimum twofold increase over the field pollinations.

Discussion

The developmental fate of the flower-producing buds was predetermined at the time the scion was harvested. At this time, it is unknown whether or not these grafts will continue to flower in future years or if grafting will have a rejuvenation effect delaying any subsequent formation of reproductive structures. If this is the case, future research will focus on developing methods to promote flowering. Regardless of the developmental fate of these mature scions once they are grafted, containerized greenhouse seed orchards will greatly expedite the American beech breeding program in a cost-effective manner. Travel costs and other expenses such as repeated bucket truck rental are reduced by greenhouse pollinations, and seedling yield is increased dramatically. This increase in high quality seed production can most likely be attributed to the controlled greenhouse environment where seed-producing grafts receive an adequate supply of water and nutrition which promote vigorous growth and prevent drought and nutrient stresses. Flowers are protected from damaging frosts and developing fruits from herbivore damage. The seedlings obtained from these greenhouse pollinations will be tested for scale-resistance. If they exhibit the same high proportion of resistant offspring as was that observed for field pollinations between two resistant parents, the greenhouse approach may be used in the future to supply landowners with a source of resistant seed or planting material. Resistant seedlings could be planted ahead of the killing front as a way to minimize the impact of beech bark disease and in aftermath forests for restoration purposes.

Table 2. Summary of results of field pollinations and greenhouse pollinations.

Female parent	Male parent	Year/field or greenhouse	# of grafts or branches	Total # of flowers	Total # of seed	# of seed germinated	Percent germination
1506 (S)	1504 (R)	2002/F	4	98	241	77	32
1504 (R)	1506 (S)	2002/F	6	249	675	11	2
1504 (R)	1501 (I)	2002/F	2	69	133	13	10
1505 (R)	1504 (R)	2002/F	2	115	231	51	22
1505 (R)	1503 (R)	2004/F	2	80	33	10	30
1505 (R)	Sebois 85 (R)	2004/F	5	260	206	23	11
1505 (R)	Sebois 52 (R)	2004/F	2	52	21	5	24
1505 (R)	Sebois 23 (R)	2004/F	4	228	109	18	14
1505 (R)	**Sebois 23 (R)**	**2005/GH**	**14**	**NA**	**48**	**44**	**92**
1201 (R)	**1208 (R)**	**2006/GH**	**17**	**71**	**134**	**87**[*]	**65**[*]
1209 (R)	**1219 (R)**	**2006/GH**	**16**	**39**	**128**	**80**[*]	**63**[*]
1211(R)	**1228 (R)**	**2006/GH**	**20**	**38**	**55**	**54**[*]	**98**[*]
1228 (R)	**1211 (R)**	**2006/GH**	**16**	**56**	**112**	**71**[*]	**64**[*]

[*] Seed are currently germinating, this number represents only those that have germinated to date. It is expected that this number will increase.

NA=data not available; S=susceptible; R=resistant; I=intermediate; F=field; GH=greenhouse

Previous work has shown that American beech does not self-fertilize readily and there are indications that closely related trees do not efficiently produce beechnuts (Nielson & De Muckadelli 1954; Koch & Carey 2004). The mature resistant trees that remain in aftermath forests are frequently found in clusters and have been shown to be closely related (Houston & Houston 1994, 2001; Carey et al. 2001). Presumably, these closely related clusters will not effectively interbreed. By planting resistant trees with a high level of genetic variation cross-breeding may be facilitated to promote natural regeneration. Further research is needed to determine the parameters that influence the ease with which resistant beech may be restored to aftermath forests.

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