Screening for Resistance to Beech Bark Disease: Improvements and Results From Seedlings and Grafted Field Selections

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Abstract

Beech bark disease (BBD) is an insect-disease complex that has been killing American beech (*Fagus grandifolia* Ehrh.) trees since the accidental introduction of the beech scale insect (*Cryptococcus fagisuga*) to Canada around 1890. Insect infestation is followed by infection with *Neonectria ditissima* or *N. faginata*. Mortality levels in the first wave of the disease can be as high as 50 percent, with consequent loss to stand health, merchantable timber, and many wildlife and ecosystem services. It is currently estimated that between 1 and 5 percent of the native American beech are resistant to beech bark disease, and resistance has been shown to be to the insect part of the complex.

Recent work has shown that artificial infestation techniques can be used to screen seedlings for scale resistance. Here we present results from additional beech insect resistance screening experiments, including additional seedling families, grafted parental ramets of seedlings, and nonnative beech species. Results further confirm the utility of the screen to allow selection of better performing individuals, even within families that perform poorly overall, and to rank families for overall performance. When full-sibling families using parents of known scale phenotype were screened, an enriched proportion of resistant progeny were observed only in families with two resistant parents.

Trees selected in the field can be grafted and the assay is useful to confirm the field-assessed scale resistant phenotype. We are currently identifying, grafting, and testing scale-resistant beech trees as part of a multi-state, multi-agency cooperative effort. Confirmed resistant genotypes will be used to establish seed orchards to supply regionally adapted disease-resistant beechnuts for use in restoration plantings and BBD management. The use of resistant parents is necessary to produce significant improvement over unselected seed lots.

Key words: beech bark disease, beech scale, *Cryptococcus fagisuga*, *Neonectria*, scale resistance

Introduction

Beech bark disease (BBD) was introduced into North America in Nova Scotia in the late 1890s and has been steadily spreading south and west over the last 120 years (Ehrlich 1934). Beech bark disease is caused by an insect-fungal complex. The beech scale insect (*Cryptococcus fagisuga*) is the insect component, and a canker-causing *Neonectria* (either *Neonectria ditissima* or *N. faginata* in North America) is the fungal component (Castlebury et al. 2006). Insect infestation appears first and is believed to predispose trees to fungal infection of insect-damaged bark (Ehrlich 1934). Many forests where American beech is the dominant component of stands are already heavily impacted, with mortality in the 'killing front' as high as 50 percent followed by significant additional damage due to beech snap (Miller-Weeks 1983, Papaik et al. 2005). Residual and regenerating stands may be dominated by susceptible beech, with susceptible beech root sprouts capable of forming thickets that prevent regeneration of resistant beech or other species, and offer no economic and severely reduced ecological value as beech bark disease continues to kill susceptible beech over time (Morin et al. 2007).

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In native beech populations heavily infested with BBD, there are an estimated 1 to 5 percent of trees that remain healthy (Houston 1983). These apparently resistant beech trees are frequently found in clusters suggesting they may be clonal or related seedlings. Houston (Houston 1982) developed a scale infestation technique and used it to demonstrate that these healthy trees are actually resistant to the scale insect component of BBD. To investigate the genetic control of resistance to beech scale in American beech, its inheritance, and its potential for improvement through breeding, the U.S. Department of Agriculture, Forest Service (USDA FS) has been conducting beech scale resistance studies. Koch and Carey (2005) selected and tested scale-resistant American beech trees in the field (to determine if they were resistant to scale (R) or susceptible to scale (S)) and performed controlled cross pollinations that produced RxR and RxS seedling families (Koch et al. 2006). Houston's (Houston 1982) artificial infestation technique was adapted to screen the seedlings and significant family effects were found for scale resistance (Koch et al. 2010). In this paper, we present results from several experiments conducted over several years, including:

- Screening cultivars and seedlings.
- Screening grafted trees and improving the screen.
- Screening families of seedlings.
- Development of regional seed orchards for breeding resistant beech.

Methods and Materials

Plant Materials and Growth Conditions

Beech plants were grown in containers, either in a greenhouse or shaded outside growing areas, as previously described (Koch et al. 2010), and repotted to larger containers as needed. In general, seedlings were screened at 1 to 2 years of age and grafts were screened 1 to 2 years post-graft. The European beech seedlings and cultivars were purchased from Lawyer's nursery (Plains, Montana). Scion was grafted using either top (cleft) grafts or side-veneer grafts and utilizing a 'hot-callous' system to warm the graft union relative to the rootstock and scion (Koch et al. 2006). Rootstocks were grown from unselected seed collected in Ohio. Grafts were maintained in a greenhouse for the first year and received intensive aftercare (rewrapping grafting rubber, pruning rootstock, etc.). After the first year, plants were grown outside under shade (approximately 50 percent).

Grafted trees may produce flowers, either from mature scion in the year of grafting or after resuming normal cyclical flowering; these flowers were utilized in control pollinations. Flowering ramets used as female parents were isolated and emasculated to prevent rare self-pollination, then pollinated using camel hair brushes when receptive (Koch and Carey 2004). Flowering ramets used as male parents were isolated, and male flowers were collected when dehiscent. Pollen was collected and passed through fine mesh screen to remove any anthers or other debris, then stored at -80 ^{o}C over desiccant until use. Ramets carrying nuts were maintained in the greenhouse and protected from insect infestation.

Beech nuts were collected before being shed from the burrs. The burrs were allowed to dry and open in the laboratory, and the nuts were then separated and stratified 90 days in Banrot® wetted peat moss at 4 to 10 ^{o}C. When radicals began to emerge, nuts were transferred to soil-less media in the greenhouse to complete germination and begin growth.

Scale Screening

When grafted ramets and seedlings were of sufficient size to support the required number of scale screening pads, they were transferred to the screening facility at Holden Arboretum (Kirtland, Ohio). Scale pads were applied as previously described (Koch et al. 2010) including the use of Tyvek® covers to prevent excess moisture from reaching the foam pads. Field scale pads were established in a stand of susceptible beech trees at Holden Arboretum to provide a consistent supply of eggs for use in screening. One hundred eggs (through 2006) or 150 eggs (2007 and thereafter) were placed on each pad prior to the pad being affixed to the tree (seedling or grafted ramet). Viability assays were conducted on each batch of eggs prior to use for quality control. Scale pads were scored 52 weeks after application to maximize the number of scale adults and egg clusters and to minimize the number of mobile juvenile nymphs at the time of scoring due to the difficulty in counting them (Koch et al. 2010). Trees were overwintered with scale test pads affixed either in a 4 °C cold storage facility or a heated poly-house maintained just above freezing.

Scale pad data was examined for quality prior to further analysis and inclusion in statistical datasets. Multiple pads were reviewed for consistency and any obviously discordant pads were either discarded (for three or more pads total, leaving at least two), or the ramet or seedling was rescreened. For seedlings or ramets with only two pads, an additional quality control metric was calculated to confirm the two pads were consistent. The quality control metric is adjusted difference:

Adjusted difference = (pad 2 value – pad 1 value)/(pad value mean + 0.0001).

An adjusted difference of zero corresponds to two identical pads, while an adjusted difference of one corresponds to the difference between the two pads being equal to the mean. Ramets or seedlings with an adjusted difference greater than 1.5 were considered to be inconsistent and were rescreened. The cutoff value of 1.5 was originally chosen by graphing the adjusted difference values for the 2009 dataset, and observing that there was a break in the distribution of adjusted difference around 1.5, with approximately 90 percent of values below 1.5. Adjusted difference has been similarly distributed in later years.

Scale pads were scored resistant if they had zero (or occasional one small or unhealthy) scale adults and no egg clusters. Susceptible pads had both healthy scale adults and egg clusters, often in equal or greater number than the scale adults count indicating a robust and reproductive scale population. Pads were scored intermediate if they had only a few scale adults, or mainly unhealthy scale adults and no or only a few egg clusters (many less egg clusters than scale adults, e.g., a pad with 6 small adults and one egg cluster would be considered intermediate). Intermediate classification was less stringently defined, and when stringent classification was desired, these pads were classified as susceptible. Grafts or seedlings were scored resistant, intermediate, or susceptible based on the score of all the pads, with a resistant score being most conservative. A resistant genotype had only resistant pads. A genotype with a mix of resistant and intermediate pads was scored intermediate (or, more stringently, susceptible). A susceptible genotype had susceptible pads or a mix of susceptible and intermediate pads. Additional exploratory data analysis including calculation of summary statistics and graphing were carried out using Minitab®.

Statistical Analysis

Repeatability (for fig. 3, section titled Screening Grafted Trees and Improving the Screen) was estimated by r, the ratio of the variance attributed to genotype over the total variance. Minitab® was used to fit an analysis of variance (ANOVA) with model:

Scale adults = intercept + Genotype (Sample) + Error

Model fit was assessed by examination of residuals. The use of ANOVA was appropriate here because we were modeling the scale adults over the full experiment (essentially averaging), and the model fit was confirmed by examination of residuals. Variance components were reported for genotype, sample, error, and total, and used to compute r. Expected relative error variance was calculated using the relationship:

$E=(1-r)/n$.

For family analysis (for fig. 5, table 4, and section titled Screening of Families of Seedlings), SAS® was used to fit a generalized linear model using the GENMOD procedure. Model fit was assessed by examination of residuals and alternate models were compared using deviance/degrees of freedom, AIC, AICC, and BIC scores, and graphing of residuals supplied by GENMOD. Model variation was guided by exploratory data analysis and included models with different distribution and link function options as well as models with and without interactions and main effects. The best-fit model was selected and the differences of least square means computed and interpreted only for the best fit model. Actual p-values are reported rather than an a priori cutoff or adjustment. The current dataset contained many susceptible trees, so it was not as severely zero-inflated as data containing more highly resistant families. Therefore, the current data was sufficiently normalized by applying square root transformation prior to analysis. The best fit model was:

Square root (sum of adults) = intercept + family + scorer + year + error;

specifying tree (genotype within family) as the subject of repeat observations to correctly specify the covariance structure and account for repeated measure on individual seedlings. Replication in the model comes from evaluating multiple pads per genotype.

Results and Discussion

Screening Cultivars and Seedlings

Koch et al. (2010) described an artificial scale infestation technique and reported data from several families screened using one pad per seedling per year and combining analysis over 2 years to obtain statistically analyzable results. In this paper, we report data from several smaller groups of seedlings that were also screened using one pad per seedling per year. Table 1 shows a summary of the results of these screenings. Results are consistent with those reported in Koch et al. (2010) in that only families produced from crosses where both parents were scored resistant had an enriched number of resistant seedlings. Families with a beech scale susceptible parent and open-pollinated (all male parents presumed susceptible) families had only a few, if any, resistant progeny.

Table 1—Beech seedlings and families screened by USDA Forest Service

Entry	Type	Source[a]	No. R trees	No. S trees	Avg adults	Avg eggs	Year(s) screened
DSP1973xOP	seedling	open-pollinated family, S mother	0	6	25.5	25.6	2004, 2005
1505xOP	seedling	open-pollinated family, R mother	2	14	32.21	33.24	2008
1505xSebois23	seedling	control cross, RxR’	17	7	14.8	15.86	2008
1505xSebois85	seedling	control cross, RxR’	2	9	3.22	15.33	2008
1520xOP	seedling	open-pollinated family, S mother	0	21	45.71	52.95	2008
DNxOP	seedling	open-pollinated family, S mother	0	50	6.25	8.73	2005, 2006

[a] Source is either control-cross pollinated or open-pollinated family with parents field scored as R=resistant in field test, R’=field selected as healthy with no visible scale in post-aftermath forest, presumed scale resistant, S=susceptible in field test.

A number of exotic species and selections of beech are commercially available in the United States, and a sample of these were purchased and screened in 2005 and 2006 (table 2). Similar to observations with American beech (*Fagus grandifolia* Ehrh.), the additional species had both resistant and susceptible individuals. Several of the cultivars of European beech (*Fagus sylvatica* L.) appear to be resistant to beech scale. Also similar to the American beech data, susceptible trees of the different *Fagus* species showed a range in the size of the scale population they supported (fig. 1).

Table 2—Beech (*Fagus*) cultivars screened by USDA Forest Service

Entry	Type	Source	No. R trees	No. S trees	Avg adults[a]	Avg eggs[a]	Year(s) screened
F. orientalis[b]	Asian species	commercial horticulture	5	0	-	-	2006
F. sylatica v. *cristata*	European cultivar	commercial horticulture	6	0	-	-	2006
F. sylatica v. *rotundafolia*	European cultivar	commercial horticulture	9	0	-	-	2006
F. sylvatica v. *spaethiana*	European cultivar	commercial horticulture	6	4	0.7	3.0	2006
F.sylvatica v. *asplendafolia*	European cultivar	commercial horticulture	1	0	-	-	2006
F. purpurea	European sub-species	commercial horticulture	7	19	10.3	8.4	2008
F. sylvatica	European sub-species	commercial horticulture	15	19	24.0	27.8	2008

[a] Average of the susceptible trees only: reflects the relative degree of susceptibility of the S population. The value cannot be calculated (is undefined) if all trees are resistant.
[b] *F. orientalis*, *purpurea*, and *sylvatica* are species within *Fagus*. The other groups are horticultural selections or sub-species of *Fagus sylvatica.*

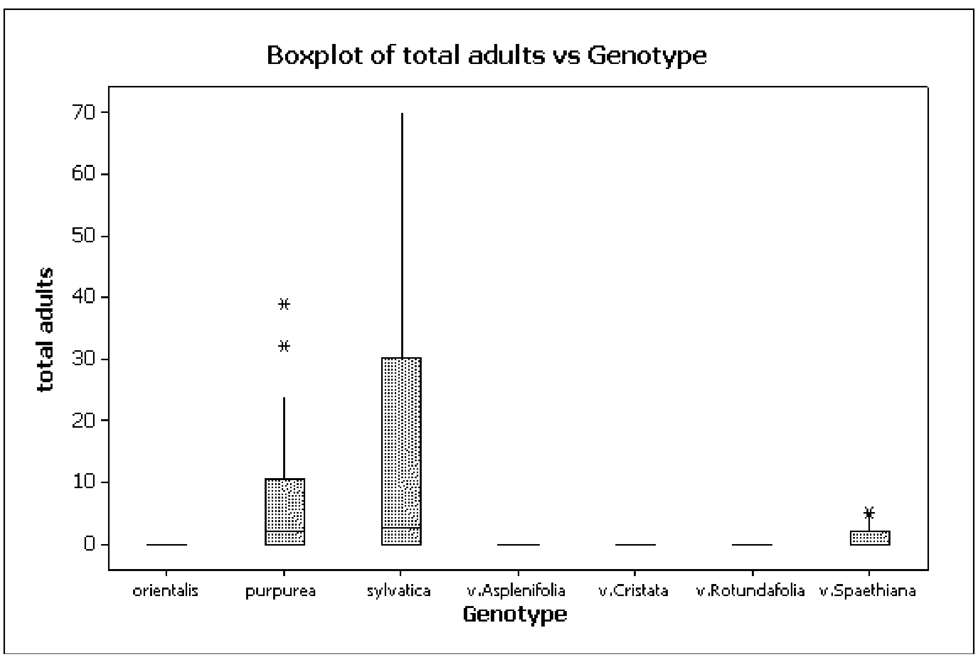

Figure 1—Boxplot of the distribution of beech scale adults count of several exotic species and cultivars of beech screened by the USDA Forest Service. Box: first to third quartile, line: median, 'whiskers:' upper (or lower) quartile plus (minus) 1.5 times the inter-quartile range, asterisks: outliers.

Screening Grafted Trees and Improving the Screen

Parent trees and other field selected trees were grafted, and ramets were screened to confirm the greenhouse screening with the field selection phenotypes. Early screens (2006) used one pad per ramet and relied on screening multiple ramets to determine screening result (R or S). This replication of the genotypes allows the reproducibility of the screen to be assessed. An examination of the screen data showed generally consistent results, but problems were identified as well (discordant ramets; table 3). Examination of the notes taken during scoring revealed several problems with early screens. Often small side branches needed to be pruned in order to place the screening pads and Tyvek® protectors on the tree. Scale insects appear to establish more readily on this freshly wounded tissue fig. 2A), sometimes even on normally resistant trees. To avoid the impact that pruning wounds have on scale tests, pads are now installed either away from the wound entirely or with the foam facing the unwounded side of the branch only. An additional problem in scoring was a concomitant infestation of oyster shell scale (fig. 2B), including adults and juveniles present on and under beech scale test pads. While the adult stage is not difficult to distinguish, the egg and juvenile phases of the two scale insects are much more similar in appearance and can be confused. It is unknown what impact the two scale infestations may have on each other, but oyster shell scale infestation appeared to cause seedling mortality at the infestation levels present in the experiment, so it was likely having a significant impact on overall tree health during the beech scale screen.

Table 3—Performance of grafted ramets of trees in different years with different numbers of pads

Genotype	Source	Year	Pads per ramet	No. R ramets	No. S ramets	No. discordant pads/total pads	Avg. adults	Avg. eggs
1503	selection	2006	1	6	1*	n/a	1	1
1504	selection	2006	1	2	3*	n/a	0.67	1
2179	unselected	2006	1	1	2	n/a	2.5	0
2189	unselected	2006	1	1	1	n/a	4	2
PA2691	selected	2006	1	3	2*	n/a	2	0.5
PA2692	selected	2006	1	6	1*	n/a	0	1
WV193	unselected	2006	1	0	1	n/a	7	2
WV963	unselected	2006	1	0	1	n/a	9	9
WV964	unselected	2006	1	1	2	n/a	7.5	5.5
WV966	unselected	2006	1	0	2	n/a	8	7.5
WV969	unselected	2006	1	0	2	n/a	8.5	2.5
WV970	unselected	2006	1	0	3	n/a	11.6	2
1504	selection	2009	1-2	3	0	1/5[a]	0	0
1505	selection	2009	2-3	5	0	1/13[b]	0	0
1506	selection	2009	1-3	0	4	1/8[c]	23.4	23.4
1520	selection	2009	2	0	1	0/2	15.5	23
DN00726	unselected	2009	2-3	0	5	1/13[c]	14.1	13.5
DN00740	unselected	2009	1-3	0	4	0/8	37.6	32.5
BEWL01	selected	2009	3	1	0	0/1	0	0

*discordant ramets likely mis-scored due to interfering conditions (insects, wounds, failed pad).
[a] One pad with five adults and six egg clusters.
[b] One pad with six adults and no egg clusters.
[c] One pad with no adults and no egg clusters.

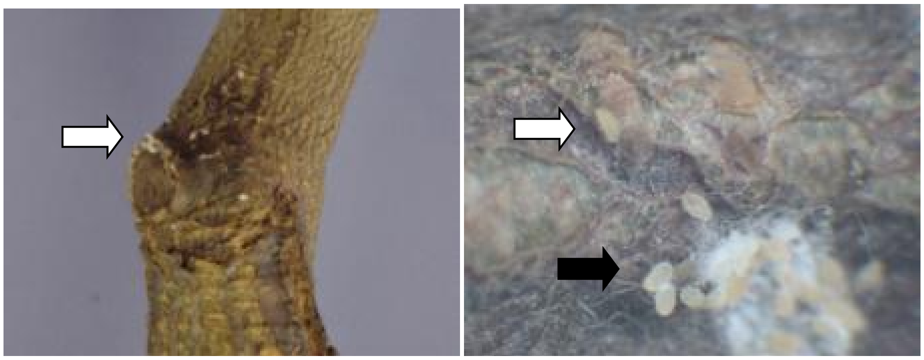

Figure 2—Beech scale infestation complications. Left (A): beech scale established around pruning wound (white arrow). Right (B): oyster shell scale adult and juvenile (white arrow) in area of beech scale juveniles and eggs (black arrow) on a tested tree. Note the very similar size and morphology of the juveniles.

The use of multiple pads per tree has made it possible to identify occasional discordant screening pads (pads inconsistent with the set of pads on the genotype). These discordant pads fall into two types: R pads on apparently S trees, and S or I pads on apparently R trees. The first conditions (R pad on S tree) can be considered a failed pad. A number of potential causes of pad failure have been identified including pads not receiving eggs and pads being placed backwards, or drying out before attachment to the tree during setup. Occasionally pads became loose over the duration of the experiment and/or the Tyvek® covers also became loose resulting in the pad being compromised. In the second case (S pad on R tree) the causes are potentially less clear, but a number of factors that impact tree health either locally or systemically can be supposed to compromise a score pad. Poor overall tree health or infection with other insects or fungi and damage or broken bark, especially fresh pruning wounds, are all noted on scoring sheets associated with discordant pads. Pads inadvertently placed below or straddling the graft union are an easily recognized case of S pads on R trees, and are discarded. With multiple pads per tree or ramet, discordant or failed pads can be easily detected and the data either carefully scrutinized for consistency or discarded.

Koch et al. (2010) used the relationship between expected relative error variance and number of pads (replicates) for a set of beech seedlings screened with only one pad from 2004 to 2006 to predict the number of pads that would improve control of error variance and improve the overall accuracy of the screen while maintaining a manageable workload. Increasing from one pad per tree to three to four pads per tree was estimated to give the best improvement of error variance. We calculated the same value for a set of grafted ramets screened in 2009 with multiple pads per ramet (fig. 3) and found a similar value of three to five pads per tree to give the optimal reduction in error variance (estimated by the inflection point of the curve, with pads above five giving only marginal improvement in error variance for each additional pad).

Based upon the 2006 results, and the predicted improvement based on increased replication, a new set of grafts was screened beginning in 2009 with a goal of screening three ramets with three to four scale pads each for a total of nine reps per genotype. The improvement in the ability to identify and discard discordant pads greatly improves the accuracy and confidence of the screen based phenotype (table 3). Figure 4 shows our standardized scale egg score pad attached to a tree (fig. 4A), a grafted ramet with the improved replication of three scale pads with Tyvek® covers (fig. 4B), and our improved scale screening facility at Holden Arboretum (fig. 4C) that provides consistent, quality, dedicated beech growing space. The new growing space is optimized for growing beech trees and isolation from other Holden Arboretum growing stock reduces insect problems. This screening intensity allows consistent phenotyping of selected genotypes in one year, even if there are problems with a particular pad or ramet. Seedlings are also now screened at higher replication of test pads, generally with two applied per seedling. After scoring the pads, we compute a pad consistency metric, adjusted difference, and rescreen any seedlings with values over a cutoff value of 1.5. Trees with only one pad scored due to a pad failure are also automatically rescreened. The improved screen replication, improved and consistent growing space, and adoption of quality control metrics combine to greatly improve the robustness and efficiency of the screening technique.

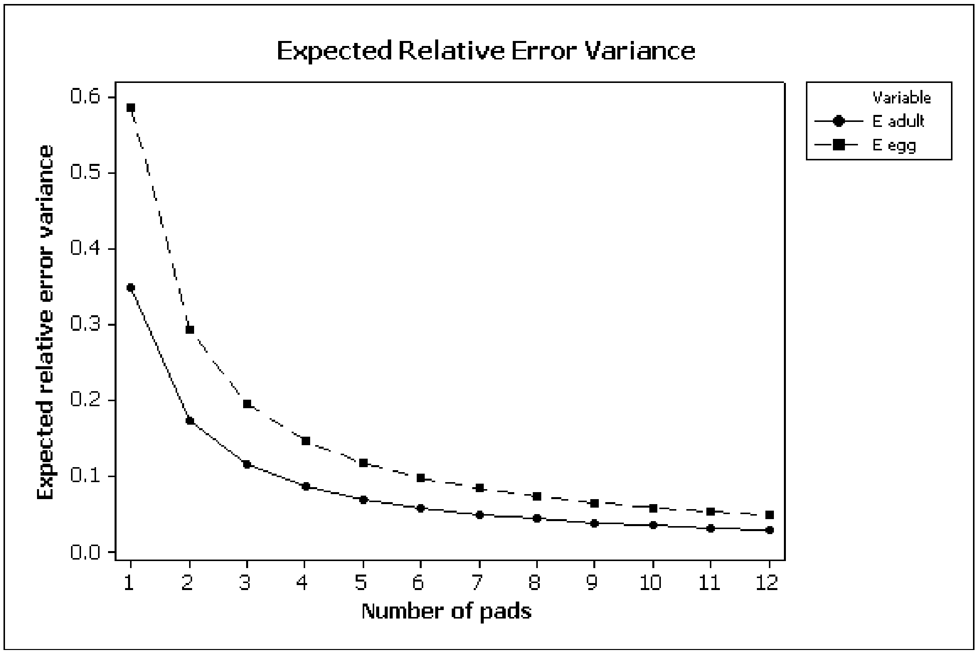

Figure 3—Expected relative error variance by number of pads screened. Expected relative error variance was calculated from the repeatability of grafts with two or more pads in 2009, based on the relationship E= (1-r)/n. Three to five pads is considered an optimal balance between the additional time and effort to add a pad and the reduction in expected relative error variance.

Screening Families of Seedlings

As field-selected trees are grafted for screening, grafted ramets occasionally flower. Any ramets that produce flowers are moved to the greenhouse and control pollinated to produce families of seedlings for screening. Ramets used as female parents are maintained in the greenhouse until nut collection, and this 'containerized seed orchard' approach is an efficient method to generate high quality controlled cross pollinated beechnuts (Koch et al. 2006). Ideally all trees grafted would flower and be used once or twice as a male parent and once or twice as a female parent to allow assessment of their suitability as R parents and to provide a source of improved seedlings for testing of planting requirements. A small group of five inter-related families were produced on flowering ramets in 2006 and the resulting seedlings were screened at the scale screening facility from 2008 to 2011.

The distribution of total scale adults per scoring pad is shown in figure 5 for all the pads on all the trees in the experiment and has the features typical of these datasets in all experiments. The zero 'spike' is typical for scale screen datasets and is more or less pronounced depending on how many trees in the dataset are resistant (in this case it is less pronounced). Due to the number of zeroes, the data are sometimes zero inflated relative to the distribution that accurately models the non-zero portion of the data, so zero-inflated models are considered in the analysis in addition to several transformations (either directly or by varying the distribution and link function in the generalized linear model specifications). Only the best fit model based on typical goodness of fit measures (e.g., deviance/df=1, or smallest AIC) is used to determine significance of effects and evaluate family rankings.

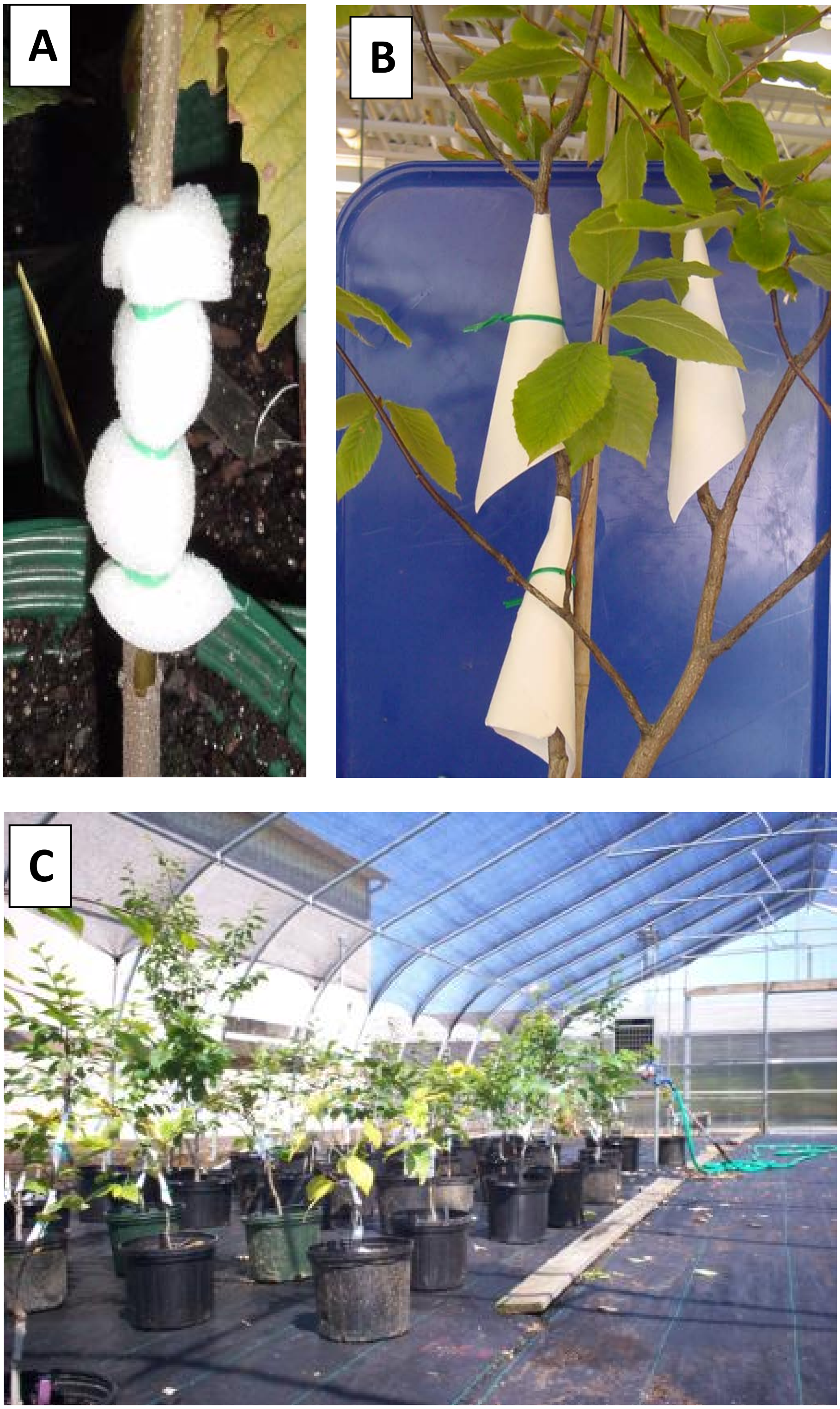

Figure 4—Scale screening pads and current improved screening facility. A: Foam pad with scale eggs is attached to the tree using wire ties. B: Several foam pads are placed on each tree and covered with Tyvek® to protect them from rain and irrigation water. C: Grafted ramets at the U.S. Department of Agriculture, Forest Service scale screening facility at Holden Arboretum.

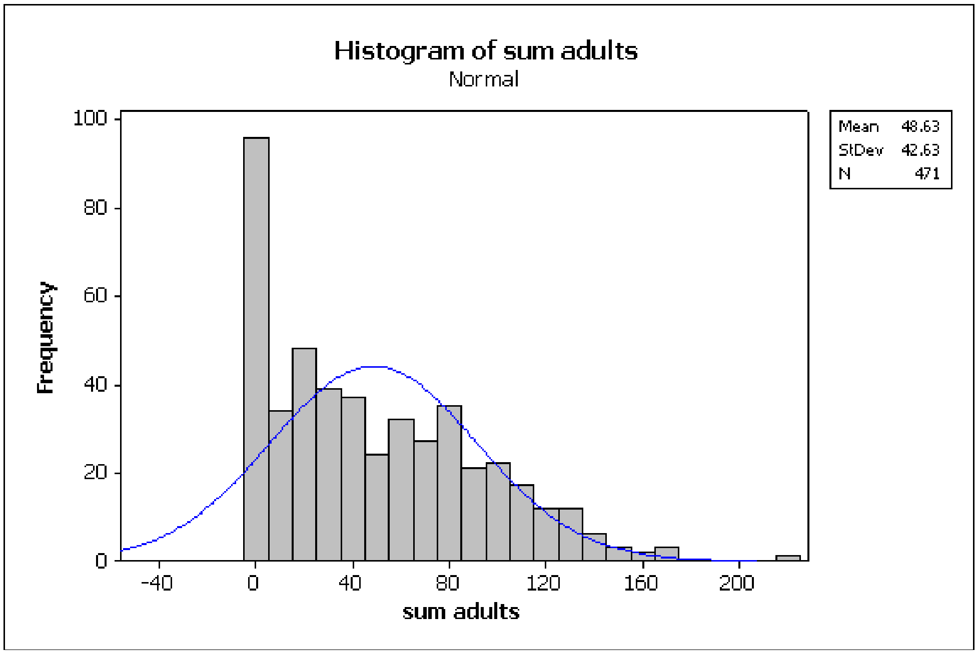

Figure 5—Histogram of scale adults per tree with normal curve. The normal curve based on the mean and standard deviation for the sample is superimposed over the histogram. Both the high proportion of zero counts and the lack of normality expected for count data can be seen in the chart. Depending on the proportion of zeroes in a given dataset, simple transformation may be insufficient to normalize the data and zero-inflated models should also be investigated.

For this experiment (dataset), the best model for the total scale adults per tree analyzed the square root of the total adults with main effects for family, year, and person scoring (scorer), and using a generalized linear model specifying the normal distribution, identity link function, and tree as the subject of repeated observations. Effects were tested using the Wald statistics. Family and year were significant (both $p<0.0001$), but scorer was not ($p=0.2049$). Least squares means were computed for family and year and are shown in table 4. Consistent with the previous results, only the RxR family had operationally reasonable numbers of resistant progeny (over half), while the SxR and SxS families were dominated by susceptible individuals. The scale population carried by the three groups of families (RxR, SxR, and SxS) differed in the expected way with fewer scale insects on families with both resistant parents. We also found that the least squares mean for SxR families is significantly ($p<0.0001$ for difference of LSM=0) less than those for SxS families. These families also had a small number of resistant progeny, consistent with estimates of resistance in natural stands. The YEAR effect was also significant. Relative rankings will probably be most robust if completed within a year and control trees or families should be screened each year for multi-year experiments (to allow differences in years to be estimated statistically). Five different individuals scored trees over the 3 years including the research scientist that developed the assay and summer interns. The lack of significance for scorer is reassuring that the screen and training is robust and should be transferable to other work groups or managers.

Development of Regional Seed Orchards for Resistant Beech

Early experiments (Koch et al. 2010) showed both the utility of the screen to identify beech scale resistant individuals and superior families and indicated that the degree of genetic determination of resistance to beech scale was sufficient to realize genetic gain through traditional tree breeding programs. However, these conclusions were based on the analysis of a small number of families. The findings presented here further support the conclusions of Koch et al. (2010) and Koch (2010), and

Table 4—Least squares means for family (A) and year (B)

A. Family	Field score of parents in cross	Proportion of resistant progeny	Least squares mean estimate for total adults	Significance
BEWL01x1504	R x R	0.607	1.4	A[a]
DNx1504	S x R	0.08	23.9	B
DNx1505	S x R	0.21	27.40	B
DNx1506	S x S	0	59.2	C
DNxDN	S x S	0	50.5	C

B. Year	Least squares mean estimate	Significance
2009	43.3	A
2010	15.0	B
2011	27.1	AB

[a] Means that share a letter are not significantly different (all significant differences had an unadjusted p<0.0001).

taken together provide the framework to develop a strategy for managing beech bark disease. A multi-state, multi-agency workgroup has been assembled to select, screen, and propagate beech scale-resistant American beech for use in the installation of regional seed orchards. The initial efforts have focused on both the Lower and Upper Peninsulas of Michigan, and central Pennsylvania. A number of beech trees have been selected, grafted, and screened (table 5). Resistant beech trees appear easier to detect during the killing front phase, and harder to detect in aftermath forest. Based on these early results, selected trees are being field tested before grafting.

Table 5—Regional seed orchard project (American beech trees were selected by local cooperators, then grafted and screened for beech scale resistance by U.S. Forest Service personnel at the screening facility at the Holden Arboretum)

Region	Beech bark disease status	Tree ID for screen result: resistant[a]	Tree ID for screen result: intermediate[b]	Tree ID for screen result: susceptible[c]
Michigan-Lower Peninsula	killing front	1503,1504,1505,1510		1506[d] 1520[d]
Michigan-Upper Peninsula	killing front	1201,1207,1208,1209,1210,1211,1213, 1214,1215,1217,1219,1220,1228,1229, 1230, 1231, 1301		1202 1216
Pennsylvania	aftermath	PA2661, PA2663, PA2665, PA2691, ANF4491304, ANF4491308, ANF4491321, ANF4693608, ANF4694223, ANF4693619, ANF4694209, ANF4714904, D-9-1, D-9-2	ANF4491304 ANF4694204 PA2692	PA2659 ANF4693615 ANF4693905 ANF4693622
West Virginia	killing front/ aftermath	MNF3839335 MNF3839557		
Ohio	uninfested			DN00726[d] DN00740[d]

[a] No more than 3 adults and/or one egg cluster.
[b] A few adult scale or mainly unhealthy adult scale, and only a few egg clusters.
[c] Robust scale infestation present with both adults and eggs.
[d] Originally selected as susceptible control.

Conclusions

We report results from screening several different sets of seedlings, including open-pollinated, controlled-pollinated, commercially available, and grafted ramets of American beech for resistance to the beech scale insect. Evaluation of these scale test results has guided improvement, quality control, and standardization (best practices) of the beech scale insect screening for both grafted trees and seedlings. These changes have also made the process of successfully scoring beech grafted trees and seedlings faster, more predictable, and more efficient. Analysis of family data continues to support the conclusions of Koch et al. (2010) that only families with two resistant parents have high numbers of resistant seedlings. Based on these finding, a pilot seed orchard project is being established where collaborators are identifying superior parents in their region for establishment in a seed orchard to supply resistant beech nuts for planting.

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