Assessment of beech scale resistance in full- and half-sibling American beech families

Jennifer L. Koch, David W. Carey, Mary E. Mason, and C. Dana Nelson

Abstract: A beech bark disease infested American beech tree (*Fagus grandifolia* Ehrh.) and two uninfested trees were selected in a mature natural stand in Michigan, USA, and mated to form two full-sib families for evaluating the inheritance of resistance to beech scale (*Cryptococcus fagisuga* Lind.), the insect element of beech bark disease. Four half-sib families from both infested and uninfested trees were also evaluated for resistance. Using an artificial infestation technique, adult and egg count data were collected over 2 years and analyzed with generalized linear mixed methods to account for non-normal distributions of the response variables. A significant effect for family was found for each variable. Family least squares means were computed as a measure of resistance and repeatabilities were calculated to provide an upper limit estimate of broad-sense heritability. The two families that ranked highest for resistance were the full-sib family from two uninfested parents and the half-sib family from a stand where all diseased trees had been removed. Together, the results suggest that selection and breeding may be an effective means to improve populations for artificial regeneration, and silvicultural treatments may provide an effective management option for mitigating beech bark disease through managing the genetic composition of natural regeneration.

Résumé : Un hêtre à grandes feuilles (*Fagus grandifolia* Ehrh.) infecté par la maladie corticale du hêtre et deux hêtres sains ont été sélectionnés dans un peuplement naturel mature de l'État du Michigan, aux États-Unis. Ces arbres ont été croisés pour obtenir deux descendances biparentales dans le but d'évaluer le caractère héréditaire de la résistance à la cochenille du hêtre (*Cryptococcus fagisuga* Lind.), l'insecte associé à la maladie corticale du hêtre. La résistance de quatre descendances uniparentales provenant du hêtre infecté et des hêtres sains a également été évaluée. À l'aide d'une technique d'infestation artificielle, des données de dénombrement d'adultes et d'œufs ont été collectées pendant 2 ans et analysées au moyen de modèles linéaires généralisés mixtes pour tenir compte du fait que la distribution des variables de réponse n'était pas normale. L'effet des descendances était significatif pour chaque variable. Le moindre carré moyen des descendances a été calculé en tant que mesure de résistance et la répétabilité a été calculée pour fournir une estimation de la limite supérieure de l'héritabilité au sens large. Les deux descendances qui avaient la plus forte résistance étaient la descendance biparentale provenant des deux parents non infectés et la descendance uniparentale provenant d'un peuplement où tous les arbres malades avaient été éliminés. Globalement, les résultats indiquent que la sélection et l'amélioration génétique peuvent être des moyens efficaces pour améliorer les populations pour la régénération artificielle et que les traitements sylvicoles peuvent fournir une option efficace d'aménagement pour atténuer l'impact de la maladie corticale du hêtre via la gestion de la composition génétique de la régénération naturelle.

[Traduit par la Rédaction]

Introduction

Beech bark disease has been killing American beech (*Fagus grandifolia* Ehrh.) trees since the accidental introduction of the beech scale insect (*Cryptococcus fagisuga* Lind.) in Nova Scotia, Canada, around 1890 (Ehrlich 1934; Houston 1994). As the beech scale insect feeds, groups of host parenchyma cells collapse and die, resulting in the production of small fissures in the bark (Ehrlich 1934). These fissures provide an entryway for fungal inoculation with either *Neonectria ditissima* (Tul. & C. Tul) Samuels & Rossman or *Neonectria faginata* Castl. & Rossman (Castlebury et al. 2006). As the fungal mycelia grow, large areas of tissue become weakened and die. Eventually, complete girdling of the tree may result. Disease-damaged trees become prone to snapping during high-wind events, leaving high stumps and snags. Mortality levels in the first wave of the disease can be as high as 50% (Miller-Weeks 1983). Often cankers form, resulting in stem defects and a reduction in wood product value. Many severely deformed American beech trees persist in long-affected stands and their propensity for root sprouting results in the formation of "thickets" that prevent other species from establishing, offering little economic or ecological value.

Received 14 May 2009. Accepted 5 November 2009. Published on the NRC Research Press Web site at cjfr.nrc.ca on 30 January 2010.

J.L. Koch[1] **and D.W. Carey.** US Forest Service, Northern Research Station, 359 Main Road, Delaware, OH 43015, USA.
M.E. Mason. The Ohio State University, Ohio Agriculture Research and Development Center, Department of Entomology, 1680 Madison Avenue, Wooster, OH 44691, USA.
C.D. Nelson. US Forest Service, Southern Research Station, Southern Institute of Forest Genetics, 23332 Old Mississippi 67, Saucier, MS 39574, USA.

[1]Corresponding author (e-mail: jkoch@fs.fed.us).

Fortunately, an estimated 1% of American beech trees remain disease free in forests long-affected by beech bark disease (Houston 1983). Insect challenge experiments have demonstrated that such trees are resistant to the scale insect and extensive *Neonectria* infections typically are not observed without prior scale infestation (Houston 1982). These resistant trees are commonly found in close proximity. This indicates that they may be 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 and Houston 1990). Studies using isozymes (Houston and Houston 1994, 2000) have confirmed close relationships between some resistant trees within a stand. However, little is known about the inheritance of the scale resistance phenotype.

We hypothesize that the close relationships between resistant trees indicate a genetic basis for resistance to beech bark disease and that the proportion of resistant individuals within a family can be increased through the breeding of select trees. To test this hypothesis, an artificial infestation technique (Houston 1982) was used to compare beech scale resistance between progeny from two full-sib families and four half-sib (open-pollinated) families (Koch and Carey 2004).

Materials and methods

Study area and plant material

Breeding experiments were carried out at Ludington State Park, Ludington, Michigan, USA, where beech bark disease was first reported in 2000 (O'Brien et al. 2001) and heavy levels of beech scale infestation are currently observed. Two infested trees (1506 and 1510) and two uninfested trees (1504 and 1505) were selected as parents for scale resistance studies. Using an artificial infestation procedure in the field, scale eggs were applied directly to the bark of parent trees to confirm their scale-resistant/susceptible phenotype. Controlled cross-pollinations and seed germination were carried out as described previously (Koch and Carey 2004). All individuals from full-sib families were screened with six SSRs to confirm their parentage (data not shown). Uninfested parent 1504 was used as a pollen parent for both full-sib families, pollinating both an uninfested maternal parent (1505) and an infested maternal parent (1506). Half-sib families were produced from open-pollinated seed collections from Michigan parents 1504, 1506, and 1510.

A half-sib family (MExOP) grown from seed collected from a single uninfested maternal tree in Sebois County, Maine, USA, also was included in the scale resistance screening studies. The stand in Maine has been managed for beech bark disease through the removal of all diseased American beech trees in 1991 (Houston 2001; Farrar and Ostrofsky 2006), so the only possible paternal parents (i.e., pollen donors) are the remaining uninfested and presumably resistant trees. All seed was collected in the fall of 2001 and germinated in the winter of 2002.

Seedlings were maintained in 2-gallon pots and transferred to 5-gallon pots at 2 years of age. The potting media was Metromix 510 (Scotts, Marysville, Ohio) amended with Micromax micronutrients (Scotts) at a rate of 1.06 and 3.53 $g \cdot L^{-1}$ Osmoscote (14–14–14) (Scotts). Seedlings were tagged with a code number that contained no information identifying their parentage and then arbitrarily grouped. Throughout the study period, the seedlings were kept in a shade house, hand-watered as needed throughout the growing season, and transferred to a controlled-temperature cold storage facility (4 °C) from November until April. In April, the plants were top-dressed with 62 g per 5-gallon container of Nutricote CRF Type 180 (18–6–8) (Sun Gro Horticulture, Bellevue, Washington). The potted seedlings were moved several additional times each year due to space considerations, and as a result, no single tree had the same position or the same surrounding trees for the duration of the experiment, providing the randomization requirement of the completely randomized design (described below).

Screening for beech scale resistance

The artificial infestation technique developed by Houston (1982) was used to test both full- and half-sib families for resistance to the beech scale insect. The half-sib families tested included 1504xOP (n = 39 individuals), 1506xOP (n = 96), 1510xOP (n = 22), and MExOP (n = 73). Full-sib families tested included 1506x1504 (n = 53) and 1505x1504 (n = 49). All seedlings were 2 years old at the start of the infestation experiments.

Infestation experiments were initiated in 2004 and repeated in 2005. Insect eggs were collected as described in Koch and Carey (2005). The eggs were kept on ice and stored at 4 °C until used, but not longer than 2 days. Prior to use, the eggs were sieved through 200 μm nylon mesh to separate the eggs from debris, adult insects, and other contaminating insects. A subset of the eggs was kept at room temperature in a Petri dish to confirm viability (>75% hatching). Using a dissecting microscope, 100 eggs were counted out and placed on pieces of moistened polyurethane foam measuring 3 cm × 7 cm. The foam was affixed to the stem of the seedlings using plastic-coated wire, with the eggs directly facing the bark. In a few cases, excessive moisture accumulated in the foam pads, resulting in blackening of the foam and bark, mortality of the test insects, and mortality of some seedlings. These foam pads were removed and the data were not included in the analyses. To avoid this situation in the second test year (2005–2006), squares of Tyvek (DuPont, Wilmington, Delaware) were wrapped around the foam and affixed to the tree just above the foam pad with waterproof silicone and left open at the bottom. The Tyvek allowed moisture to escape the pad while diverting water from rain and irrigation away from the pad. In both years, the pads were applied during the second week of July. In the second year, the new pads were placed above the original pad, which was removed 5 weeks later.

After 57 weeks, the scale pads were removed and scored. The number of live adult scale insects on the foam pad and the tree was counted. To account for reproductive success of the scale population, the number of egg clusters and Form I nymphs (the single mobile phase of the life cycle that follows egg hatch) was counted on both the foam pad and the tree. Due to the small size of the insects, hand lenses were used to count both insects and eggs on trees, and the foam pads were counted under a dissecting microscope. The height of the tree was recorded in 2005, and the height and diameter were recorded in 2006.

Statistical analysis

Adult and egg cluster counts were analyzed as generalized linear mixed models with the SAS procedure GLIMMIX (http://support.sas.com/rnd/app/da/glimmix.html). Adult counts were analyzed as a binomial variable where $n = 100$, the number of eggs placed on each tree, and events = number of adults emerging and surviving at 57 weeks. Egg cluster counts at 57 weeks were analyzed as a Poisson variable, both with and without the tree's adult count serving as a covariate to account for differences in number of adults emerging and surviving from the initial 100 eggs. Statistical models for nymphs failed to converge under several different modeling strategies. Nymphs were extremely difficult to count due to their small size (≤0.1 mm) and mobility and were judged too variable (i.e., high measurement error) to include in the analysis of scale resistance. The following models were used to study the family and age effects:

- Adult (binomial distribution; $n = 100$, events = number of adults) = family + age + family × age + residual
- Egg (Poisson distribution; egg = number of egg clusters) = adults + family + age + family × age + residual
- Egg (Poisson distribution; egg = number of egg clusters) = family + age + family × age + residual

For all models, family was considered a fixed effect, while age and family × age were considered random effects. Age was treated as an independent test (i.e., replicate), separated by 1 year, of the same genotype. For the second model, adult count was used as a covariate variable. We used several of the variance component estimators provided in PROC GLIMMIX and found them to provide similar results. We report the results for the Cholesky root (type = chol), as it converged on all analyses and invokes the least assumptions on the covariance structure.

Least-squares means and differences were computed in the GLIMMIX model using the LSMEANS and PDIFF commands. They are estimates of marginal means over a balanced population and are computed on the model scale (where the model effects are additive), not the data scale. Least-squares means were taken as measures of scale resistance for the tested families. Pairwise differences in least-squares means were used to separate families into resistant, intermediate, or susceptible family groups. Bonferroni adjustment was used to correct for multiple comparisons when assessing statistical significance.

The limited availability of test families was not sufficient in the present study to provide estimates of heritability or parental breeding values; therefore, repeatability was used to estimate an upper bound for broad-sense heritability (Falconer and Mackay 1996). Repeatabilities (individual tree between years) for adult and egg (with and without covariate) were estimated using a model where all genetic effects (i.e., family, family × age, and tree) are contained in the among-tree variation (Roberds and Strom 2006). The following models were used:

- Adult (binomial distribution; $n = 100$, events = number of adults) = age + tree + residual
- Egg (Poisson distribution; egg = number of egg clusters) = adults + age + tree + residual
- Egg (Poisson distribution; egg = number of egg clusters) = age + tree + residual

In these models, age was considered a fixed effect and tree and residual were considered random effects.

The repeatabilities were calculated two ways: ratio-of-variance components and covariance (i.e., correlation) between years (Roberds and Strom 2006). We used several of the variance component estimators provided in PROC GLIMMIX and found them to provide similar results, although type = cs (compound symmetry) converged on all analyses attempted, so we report these results. As expected, the repeatability estimates (R) were virtually identical, so we report the ratio-of-variance component version only:

$$R = (G + E_g)/(G + E_g + E_s)$$

where G is the total genetic variance component due to evaluating the same tree (i.e., same genotype) between years, E_g is the general environmental variance component due to evaluating the same tree (i.e., same growing conditions, pot, and space occupied), E_s is special environmental variance component due to experimental and measurement errors (e.g., randomness in screening system and errors in counting and recording data, respectively) (Falconer and Mackay 1996). In this experiment, G and E_g cannot be separated, as they are both part of the among-tree variance; thus, our estimate is $R = T/(T + W)$, where T and W are the among and within tree variance components, respectively.

In addition, we used the relationship between repeatability, number of replicates, and relative error variance (Roberds and Strom 2006) to estimate the effects of replication on error and predict sufficient sample size for future experiments.

Results

Height, adult scale, and egg cluster data

The distribution by family of adult scale count, egg cluster, and juvenile nymph (first plus second instars) count is shown in Fig. 1. Family effects for height, height increment, and diameter (data not shown) were found to be significant ($p < 0.001$), indicating that the family size is sufficient for the detection of these genetic effects. Early analysis showed that height and diameter had no effect on scale population, so they were not included in the final models. The box plots of both scale count and egg cluster count show that the 1505x1504 family and the MExOP family have lower means and a smaller interquartile range than all other families. Families resulting from open pollination of an infested tree (1510xOP and 1506xOP) have the highest means for adult scale, egg cluster, and nymph variables, indicating that these families support a larger scale population and this is reflected in all three stages of the insect life cycle.

Statistical modeling of adult scale and egg cluster count data

Generalized linear mixed models were fit separately for adult scale proportion (binomial variable, where each egg placed is a trial and each egg producing an adult scale is a successful event) (Table 1*a*), egg cluster count (treated as a Poisson count) with adult scale number as a covariate

Fig. 1. Distribution of scale counts by family as box plots of scale count data. The box plots show the middle 50% of data observations (25th to 75th percentile) as a shaded box and the median as a line in the box (the median may be superimposed on the 25th percentile for some families). The vertical lines represent the degree of spread of the rest of the data. Outliers are included in the computations but not graphed. Data for 2005 (top panels) and 2006 (bottom panels) are shown for adults (left panels), egg clusters (center panels), and nymphs (right panels).

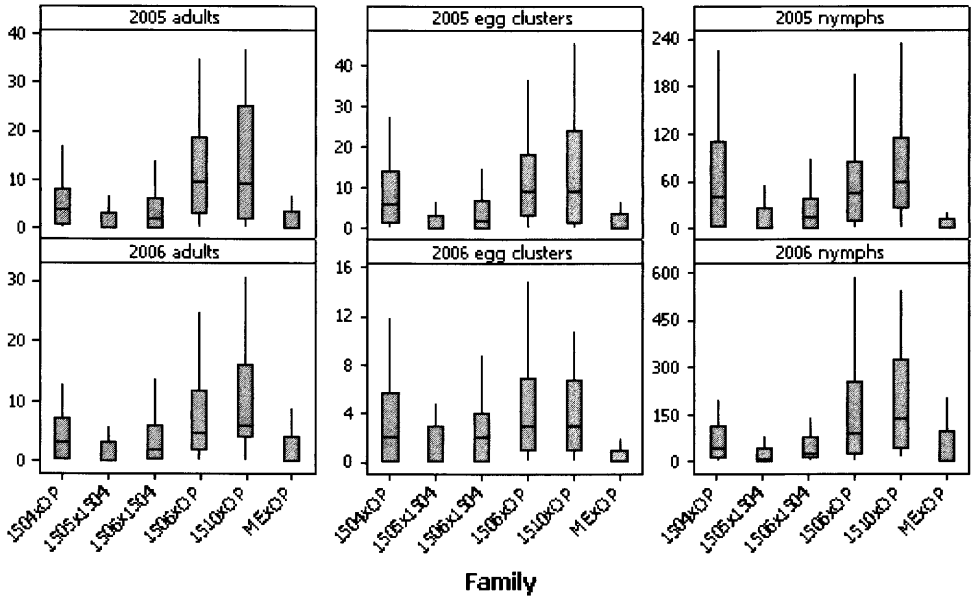

Table 1. Model test of effects and family least-squares means for adult scale count.

(*a*) Type III tests of effects for adult scale proportion

Effect	Numerator df	Denominator df	*F*	*p*
Family	5	323.5	13.21	<0.001
Age	1	315.0	0.89	0.346
Family × age	5	314.6	0.79	0.559

(*b*) Family least-squares means and SE for adult scale

Family	Mean	SE
1510xOP	0.1306a	0.01912
1506xOP	0.09281ab	0.008603
1504xOP	0.05662bc	0.01078
1506x1504	0.04038c	0.007509
1505x1504	0.03187c	0.007109
MExOP	0.03244c	0.006136

(*c*) Age least-squares means and SE for adult scale

Age (years)	Mean	SE
2	0.05857a	0.004878
3	0.05325a	0.004895

Note: Means that share the same letter are not significantly different.

Table 2. Model test of effects and family least-squares means for egg cluster count with adult count as a covariate.

(*a*) Type III test of effects for scale egg cluster count

Source	Numerator df	Denominator df	*F*	*p*
Adult	1	496.4	609.33	<0.001
Family	5	353.6	9.66	<0.001
Age	1	327.9	49.03	<0.001
Family × age	5	323.7	2.11	0.065

(*b*) Family least-squares means and SE for egg cluster count with adult covariate

Family	Mean	SE
1510xOP	2.2575c	0.3574
1506xOP	4.4686b	0.3446
1504xOP	6.0484a	0.6529
1506x1504	4.4913ab	0.5105
1505x1504	2.8213bc	0.4138
MExOP	2.8678bc	0.4035

(*c*) Age least-squares means and SE for egg cluster count with adult covariate

Age (years)	Mean	SE
2	14.9219a	0.3099
3	2.6493b	0.2145

Note: Means that share the same letter are not significantly different.

(Table 2*a*), and egg cluster count without adult scale number as a covariate (Table 3*a*). The family effect was highly significant ($p < 0.0001$) for all models. For the adult scale model, neither age nor interaction effects (family × age) were significant. For the egg cluster count model, the age effect was highly significant ($p < 0.001$) and the interaction effect marginally significant ($p = 0.0415$) but was disregarded due to its small contribution relative to family and age effects. For the egg cluster count with adult covariate model, the age effect and covariate were highly significant, but the interaction (family × age) was not significant. The significance of the adult covariate in the egg cluster model was expected, as egg laying is dependent on adult scale presence.

The age effect (significant only in the scale egg models) may be more properly thought of as a year effect. Differences in the age of the trees, differences in the egg batch used

Table 3. Model test of effects and family least-squares means for egg cluster number without adult count as a covariate.

(*a*) Type III tests of effects for scale egg cluster count

Effect	Numerator df	Denominator df	*F*	*p*
Family	5	324.1	6.67	<0.001
Age	1	328.1	24.96	<0.001
Family × age	5	321.2	2.34	0.042

(*b*) Family least-squares means and SE for egg cluster count

Family	Mean	SE
1510xOP	9.4282a	1.8737
1506xOP	7.4248a	0.8678
1504xOP	7.2479a	1.3232
1506x1504	4.1716ab	0.8215
1505x1504	2.8248b	0.7162
MExOP	2.6334b	0.6373

(*c*) Age least-squares means and SE for egg cluster count

Age (years)	Mean	SE
2	6.9638a	0.6487
3	3.5992b	0.4233

Note: Means that share the same letter are not significantly different.

from year to year, and any phenological differences in development of the scale population between years would be captured in the age effect. Age-dependent differences in the tree response are unknown. Attempts were made to minimize differences in the scale population from year to year by collecting the eggs from nearby trees in the same stand and testing them to confirm an acceptable level of viability (>75%). Beech scale is parthenogenetic, so the genetic composition of scale eggs collected from the same tree in different years is not expected to differ. However, there is evidence of phenological differences in the scale population between the 2 years, despite the pads being applied and scored for the same length of time and same time of year. The mean number of eggs differed between years (Tables 2*c* and 3*c*), and the mean number of juvenile stage nymphs differed between years (Fig. 1), suggesting that a different stage of reproduction may have been counted in the 2 years. The lower number of eggs counted in the second year is likely a result of eggs hatching prior to the count, as reflected in the higher nymph counts the same year. We were unable to confirm statistical differences in nymph counts because the statistical models failed to converge under several different modeling strategies (data not shown).

Tests of family differences

Differences between the families were further investigated for all of the models by computing least-squares means and using them to rank and determine significant differences between families in pairwise comparisons (Tables 1*b*, 2*b*, and 3*b*). The rankings of the families based on the adult variable (Table 1*b*) and the egg cluster count without adult covariate are consistent with each other and with the field assessment of the relative scale infestation of the parent trees. In contrast, the results for the egg cluster with adult covariate model produce a different ranking (Table 2*b*). In this model, the families MExOP, 1506x1504, and 1504xOP are in the same relative order, but the family MExOP is intermediate instead of low, 1506xOP is intermediate instead of high, and, most dramatically, 1510xOP is lowest instead of highest in rank. The model can be interpreted as evaluating the number of egg clusters per adult, which is clearly different from either adult count or total number of egg clusters. The total number of egg clusters is dependent on the total number of adults whether or not they survived to be counted at the time of data collection.

Least-squares means were also used to evaluate the age effect in the three models (Tables 1*c*, 2*c*, and 3*c*). In the adult scale model, the least-squares means are not significantly different, consistent with the age effect not being significant. For both egg cluster count models (with and without adult as covariate), the age least-squares means are significantly different, reflecting the age effect and the phenological differences discussed above.

Since the egg cluster count with adult covariate seems to assess a slightly different trait than the other two models, and the adult model and egg cluster count model (without adult covariate) are consistent, we examined pairwise differences between families more closely in the egg cluster count model only. The differences and *p* values of the test for equivalence for all pairwise comparisons between families are shown in Table 4. The families can be considered to form three groups. There is a resistant group made up of 1505x1504 and MExOP and a mutually exclusive susceptible family group including 1504xOP, 1506xOP, and 1510xOP. The family 1506x1504 forms an intermediate group not statistically different from either the resistant or the susceptible groups. It is interesting to note that MExOP, which is a half-sib family produced in the silvicultural treated stand where all beech bark diseased trees were removed, is not statistically different from the resistant × resistant full-sib family 1505x1504. Also of note is the fact that all three open-pollinated families from Michigan (1504xOP, 1506xOP, and 1510xOP) are not statistically different, regardless of whether the maternal tree was rated as infested or uninfested.

Repeatability

Repeatabilities for the three traits (adult, egg cluster with adult covariate, and egg cluster without covariate) are shown in Table 5. The repeatability for the adult and egg (no covariate) models are high enough to indicate a sufficiently large degree of genetic determination for these traits to suggest that tree improvement through selection and clonal propagation or breeding should lead to genetic gain in scale resistance. The repeatability for egg cluster count modeled with adult count as a covariate is smaller, suggesting that reproductive efficiency may be under less genetic control than the number of adult scale or total scale reproduction.

Repeatability was also used to investigate the expected result of different levels of replication on the assay for the adult count and egg count variables. We computed expected relative error variance (*E*) given our calculated repeatability and increasing numbers of replicates. Increasing the number of pads to three or four should provide good control of relative error variance for both adult ($E = 0.523$ with one pad, $E = 0.1743$ with three pads, and $E = 0.137$ with four pads) and

Table 4. Differences of family least-squares means for egg cluster count model without adult count as a covariate.

	1505x1504	1506xOP	1506x1504	1510xOP	MExOP
1504xOP	0.9423	–0.02412	0.5524	–0.2630	1.0124
	0.042*	1.000	0.608	1.000	0.014*
1505x1504		–0.9664	–0.3899	–1.2053	0.07018
		0.010*	1.000	0.004*	1.000
1506xOP			0.5765	–0.2389	1.0366
			0.185	1.000	0.003**
1506x1504				–0.8154	0.4600
				0.058	1.000
1510xOP					1.2754
					0.001**

Note: Values listed are the pairwise difference (on top, column family minus row family) and p value (below) (H_0: difference = 0). *Significant difference and **highly significant difference (using Bonferroni adjustment for multiple comparisons).

Table 5. Repeatability estimates and standard error for each model.

Trait	Model	Error distribution	R	SE
Adult	No covariate	Binomial	0.477	0.0438
Egg	Covariate = adult	Poisson	0.148	0.0575
Egg	No covariate	Poisson	0.308	0.0511

eggs (E = 0.692 with one pad, E = 0.231 with three pads, and E = 0.173 with four pads).

Discussion

Utility of the artificial infestation technique for screening seedlings

The artificial infestation technique used to assess scale resistance in this study is an adaptation of the technique developed by Houston (1982). We reported data from 2004 for the same families studied here that were also screened using Houston's technique as 1-year-old seedlings (Koch and Carey 2005). In the current study, the pads were scored after a longer interval, 57 weeks compared with 42 weeks in Koch and Carey (2005) and 52 weeks in Houston (1982) and Ramirez et al. (2007), to score both adult scale and reproduction.

Despite efforts to control as many variables as possible, the insect populations did not always develop synchronously, and populations in the 2 years were apparently at different points in their phenology when scored. This observation was not unexpected, as previous evidence that climate differences impact insect phenology through an inverse relationship between temperature and the period of egg incubation prior to hatching was reported in Ehrlich (1934). In addition, tree genotype and differences in health and vigor may also contribute to tree to tree variation in insect phenology. To avoid the significant year to year variation, more recently initiated experiments are using larger trees on which multiple pads can be placed and data collected in a single year. Repeatability-based calculations indicate that two to four pads per tree should give a reasonable balance between reducing relative error variance and increasing time, labor, and costs of screening. Future modifications that further reduce error variance will likely be identified, but the scale artificial infestation procedure as optimized for this study is sufficient to identify scale-resistant families and individuals. The scale and egg count distributions, outliers excluded, range from zero to approximately 40 adults and from zero to 60 for egg clusters, as illustrated in Fig. 1. Outlying data points not shown in Fig. 1 were as high as 62 for adults and 185 for egg clusters. Individuals that had zero adults and zero egg clusters in both years are the best candidates for resistant trees. However, the influence that variation between susceptible counts may have on long-term levels of beech bark disease resistance/susceptibility is currently unknown. For example, it is not known whether a seedling that supports only a handful of scale insects will go on to develop beech bark disease or if there is some threshold level of insect infestation required for *Neonectria* infection to occur. It is also possible that low level infestations in young saplings may equate to scale resistance or scale susceptibility as the tree matures. Further experiments are planned to assess the scale resistance phenotypes (and correlation with beech bark disease) of these families and individuals in a long-term field planting.

Utility of different response variables for scoring of scale infestation

Three different traits or combinations of traits were modeled in the analysis of the beech scale challenge data. Scale eggs placed on the trees at the beginning of the study hatch and the resulting Form I nymphs disperse locally and attach to the tree. The majority of nymphs stay close to or even under their parents, but observations of movement of more than 2 m have been reported (Ehrlich 1934). Following a molt, the nymphs overwinter as Form II second-instar nymphs and then molt again to become egg-laying adult insects by June (Ehrlich 1934). Adult scale may lay several batches of eggs in clutches of 8–10 before they die (Ehrlich

1934; author observations). Depending on what point in the life cycle the scale population is captured at when the challenge pad is removed, late-stage nymphs and young adults to nearly exhausted adults and their egg clusters and second-generation nymphs may be observed. Clearly, heavy infestation on a tree in nature requires both successful development to adulthood of the initial crawler infestation and the successful reproduction of the adults. Pads were removed and scored during the reproductive phase, so adults, eggs, and nymphs were all present on the trees. We attempted to analyze each of these stages as a response variable to determine the best scale stage to score trees as resistant and to look for potential differential impacts on reproduction (resistance as a lack of reproduction rather than a simple lack of adult scale).

The first model looks at adult only, or more specifically the proportion of eggs that hatch (out of 100) and mature into adults. Although use of surviving adult scale count as a response variable for resistance seems straightforward, it is possible that some adults are destroyed as pads are removed from the tree and subsequently cannot be counted. Shriveled dead adult scales were observed on some foam pads, which is consistent with observations reported by Ramirez et al. (2007). Some of these adults may have already completed their life cycle and died after 57 weeks.

The second model looks at egg cluster number using the adult count as a covariate. This model examines potential family effects on reproduction by essentially examining the number of egg clusters per adult. Houston (1983) and Ramirez et al. (2007) both reported occasional observations on some trees of eggs that would hatch into nymphs and mature into adults, but the adults would be unable to produce viable progeny. After a year, they would shrivel and die without ever reproducing. These results suggest that if the inability to produce viable young is stronger (or weaker) in a family, it would have on average less (or more) egg clusters per adult. The least-squares means based rankings for family based on egg cluster with adult covariate show a markedly different and inconsistent ordering of families when compared with the adult or egg cluster only models. Most notably, 1510xOP went from being the most susceptible family based on adult scale count to the most resistant family based on egg cluster count with adult covariate (egg per adult count). Several other families shift their relative ranking in unexpected ways as well.

There are several alternative explanations for this inconsistent ranking. Any adults that may have finished laying eggs and died are not counted. Occasional dead adults are observed on the foam pads, so there may be a bias attributing a higher number of egg clusters per adult in families with faster phenology (i.e., more dead adults at the time of scoring). Alternatively, it is possible that genetic factors of the tree affect not just the ability to support scale development but also influence the number of eggs that an adult scale can produce and the rate at which the scale population develops. If this is the case, limiting or delaying the reproductive capacity of scale populations may be a secondary mechanism of disease resistance. Smaller or later developing populations of scale may be more susceptible to extirpation in a bad environmental year for scale development (Thomsen et al. 1949; Houston and Valentine 1988). To better understand the role of genetics in reproduction and phenology in resistance, it may be necessary to model both nymphs and eggs. Although we were unable to model nymph data, our models failed during the estimation of maximum likelihood equations, so it is possible that other nymph data sets will be amenable to modeling.

The third model examines resistance by looking at the total number of egg clusters. We feel that this variable is the most complete look at insect colonization because it includes the egg clusters of existing, countable adults and also any absent, dead adults that already reproduced. Therefore, reproduction is measured directly and the total adult number is indirectly included due to the dependence of reproduction on the presence of adults. Trees on which scale is not able to mature or is not able to reproduce will score low for this variable, while trees with robust, fecund scale populations will score markedly higher. Family rankings based on this model are consistent with the adult variable model and in the expected order based on the field scoring of the parental trees. Because the egg cluster count model without the adult covariate is consistent with the adult scale model, yet indirectly includes a measure of both adult survival and reproduction, we consider this model as the best estimate of resistance for a 57-week test.

Patterns of inheritance and implications for American beech breeding and management

The low level of resistance observed in families with only one uninfested parent tree (1504xOP and 1506x1504) is consistent with the low levels of resistance reported in natural stands (Houston 1983) and suggests that susceptibility to scale infestation is dominant to resistance or that there is a threshold of quantitative factors necessary for resistance. Therefore, selection criteria for parents in a breeding program (or trees left in thinning operations) should be stringent. Selection based on field observation may not be sufficient due to the natural fluctuation of scale populations, which is influenced by climate (Houston and Valentine 1988). Screening open-pollinated families (wind pollinated in unthinned stands) for scale resistance will not be sufficient due to expected low levels of resistance in half-sib progeny as was reported in 1504xOP, 1506xOP, and 1510xOP. A more effective strategy for breeding American beech is to screen full-sib progeny resulting from controlled cross-pollinations between uninfested parents, which is supported by the family pairwise comparisons (Table 4). Careful selection and breeding of American beech should be an effective means to produce improved populations for artificial regeneration.

Attempts to genetically improve American beech for beech bark disease resistance need not be limited to traditional tree improvement through seedling planting. It may be more cost effective and operationally feasible to follow silvicultural guidelines for the management of beech bark disease, which include removal of diseased trees (Farrar and Ostrofsky 2006; Leak 2006). The performance of the MExOP family, which did not differ significantly from that of the full-sib family from the two uninfested parents (1505x1504) (Table 4), provides support for the idea that improvement of American beech is possible through such silvicultural management of natural regeneration. This is in

agreement with Leak (2006) who reported that 50 years of single tree selection (removal of diseased trees) resulted in an increase of basal area per acre in clean (uninfested, no disease symptoms) beech trees to 15% in managed stands compared with only 3.5% in similarly aged, unmanaged stands. In addition, current best practices to reduce stump and sucker sprouting, especially from cut susceptible trees, should continue to be incorporated into silvicultural genetic improvement efforts (Houston 2001). The repeatabilities and family effects reported in this paper indicate that the degree of genetic determination of beech scale resistance is sufficient to realize genetic gain, whether through traditional tree improvement, silvicultural methods designed to manipulate stand genetics, or a combination of both.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Jany Chan, Bill Sickinger, and Donna Wilburn in carrying out this work. We also would like to thank Warren Mullen, Robert Heyd, and others from the Michigan Department of Natural Resources for all of their assistance in locating disease-free trees, assisting us in the field portion of this work, and allowing us access to study sites in Ludington State Park. We are indebted to Henry Trial of the Maine Department of Conservation for supplying us with the beech seed for the Maine open-pollinated family and Consumers Energy Company (Muskegon Heights, Michigan) for assistance in the use of their bucket truck. We thank the Holden Arboretum (Kirtland, Ohio) for the donation of space and volunteers to carry out the artificial infestation experiments and for their assistance in plant rearing and maintenance. We gratefully acknowledge Therese Poland (Northern Research Station, USDA Forest Service) and Keith Woeste (Hardwood Tree Improvement Center, USDA Forest Service) for their critical review of a previous version of this manuscript. This work was partially funded through a grant from the Special Technology Development Program of Forest Health Monitoring, USDA Forest Service.

References

Castlebury, L.A., Rossman, A.Y., and Hyten, A.S. 2006. Phylogenetic relationships of *Neonectria/Cylindrocarpon* on *Fagus* in North America. Can. J. Bot. **84**(9): 1417–1433. doi:10.1139/B06-105.

Ehrlich, J. 1934. The beech bark disease: a nectria disease of *Fagus*, following *Cryotococcus fagi* (Bair.). Can. J. Res. **10**: 593–692.

Falconer, D.S., and Mackay, T.F.C. 1996. Introduction to quantitative qenetics. Edition 4. Longmans Green, Harlow, Essex, U.K.

Farrar, A., and Ostrofsky, W.D. 2006. Dynamics of American beech regeneration 10 years following harvesting in a beech bark disease-affected stand in Maine. North. J. Appl. For. **23**: 192–196.

Houston, D.B., and Houston, D.R. 1994. Variation in american beech (*Fagus grandifolia* Ehrh.): isozyme analysis of genetic structure in selected stands. Silvae Genet. **43**: 277–284. Available from http://www.bfafh.de/inst2/sg-pdf/43_5-6_277.pdf.

Houston, D.B., and Houston, D.R. 2000. Allozyme genetic diversity among *Fagus grandifolia* trees resistant or susceptible to beech bark disease in natural populations. Can. J. For. Res. **30**(5): 778–789. doi:10.1139/cjfr-30-5-778.

Houston, D.R. 1982. A technique to artificially infest beech bark with the scale, *Cryptococcus fagisuga* (Lindinger). U.S. For. Serv. Res. Pap. NE-507.. Available from http://www.fs.fed.us/ne/newtown_square/publications/research_papers/pdfs/scanned/OCR/ne_rp507.pdf.

Houston, D.R. 1983. American beech resistance to *Cryptococcus fagisuga*. *In* Proceedings, IUFRO Beech Bark Disease Working Party Conference. U.S. For. Serv. Gen. Tech. Rep. WO-37. pp. 38–41.

Houston, D.R. 1994. Major new tree disease epidemics: beech bark disease. Annu. Rev. Phytopathol. **32**(1): 75–87. doi:10.1146/annurev.py.32.090194.000451.

Houston, D.R. 2001. Effects of harvesting regime on beech root sprouts and seedlings in a north-central Maine forest long affected by beech bark disease. U.S. For. Serv. Res. Paper NE-717. Available from http://www.fs.fed.us/ne/newtown_square/publications/research_papers/pdfs/2001/rpne717.pdf.

Houston, D.R., and Valentine, H.T. 1988. Beech bark disease: the temporal pattern of cankering in aftermath forests of Maine. Can. J. For. Res. **18**(1): 38–42. doi:10.1139/x88-007.

Koch, J.L., and Carey, D.W. 2004. Control cross-pollinations with American beech trees that are resistant to beech bark disease. *In* Proceedings of the 14th Central Hardwoods Forest Conference. U.S. For. Serv. Gen. Tech. Rep. NE-316. Available from http://www.fs.fed.us/ne/newtown_square/publications/technical_reports/pdfs/2004/316papers/KochGTR316.pdf.

Koch, J.L., and Carey, D.W. 2005. The genetics of resistance of American beech to beech bark disease: knowledge through 2004. *In* Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium. *Edited by* E.C. Evans, J.A. Lucas, and M.J. Twery. U.S. For. Serv. Gen. Tech. Rep. NE-331. Available from http://www.fs.fed.us/ne/newtown_square/publications/technical_reports/pdfs/2005/331papers/koch331.pdf.

Leak, W.B. 2006. Fifty-year impacts of the beech bark disease in the Bartlett Experimental Forest, New Hampshire. North. J. Appl. For. **23**: 141–143.

Miller-Weeks, M. 1983. Current status of beech bark disease in New England and New York. *In* Proceedings, IUFRO Beech Bark Disease Working Party Conference. U.S. For. Serv. Gen. Tech. Rep. WO-37. pp. 21–23.

O'Brien, J.G., Ostry, M.E., Mielke, M.E., Mech, R., Heyd, R.L., and McCullough, D.G. 2001. First report of beech bark disease in Michigan. Plant Dis. **85**(8): 921. doi:10.1094/PDIS.2001.85.8.921A.

Ramirez, M., Loo, J., and Krasowski, M.J. 2007. Evaluation of resistance to the beech scale insect (*Cryptococcus fagisuga*) and propagation of American beech (*Fagus grandifolia*) by grafting. Silvae Genet. **56**: 163–169. Available from http://www.bfafh.de/inst2/sg-pdf/56_3-4_163.pdf.

Roberds, J.H., and Strom, B.L. 2006. Repeatability estimates for oleoresin yield measurements in three species of the southern pines. For. Ecol. Manag. **228**(1–3): 215–224. doi:10.1016/j.foreco.2006.03.005.

Thomsen, M., Buchwald, N.F., and Hauberg, P.A. 1949. Angreb af *Cryptococcus fagi*, *Nectria galligena*, og andre parasiter paa bog I Danmark 1939–43. Forstl. Forsogsvaes. Dan. **18**: 97–326.

Tubbs, C.H., and Houston, D.R. 1990. *Fagus grandifolia* Ehrh.: American beech. *In* Silvics of North America. Vol. 2. Hardwoods. *Edited by* R.M. Burns and B.H. Honkala. U.S. Dep. Agric. Agric. Handb. No. 654. pp. 325–331.