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Repeatability estimates for oleoresin yield measurements in three species of the southern pines

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Abstract

Repeatability was estimated for constitutive oleoresin yield measurements in 10 stands of three species of pines native to southeastern United States. Trees of these species that discharge large quantities of oleoresin upon wounding are considered to be most resistant to attack by southern pine beetle (*Dendroctonus frontalis* Zimmermann). Oleoresin yield is usually measured from one or more small surface wounds cut into the bole at breast height. Because multiple measurements per tree are possible, phenotypic variation in oleoresin yield includes among- and within-tree components. Thus it is of interest to determine the repeatability or relative contribution of variation among trees to phenotypic variation in oleoresin yield observed in populations. In the sample of stands studied, repeatability estimates were moderate to moderately high ($r = 0.43$–0.77), with no stands displaying low repeatability. For most stands estimates were greater than 0.5, implying that variation among trees is greater than variability within trees. These results suggest that for most purposes, two measurements per tree are adequate for estimating tree resin yields at breast height. Because initial attacks of *D. frontalis* are believed to most frequently occur at bole heights of 3–5 m, measurements taken on trees in two loblolly pine stands were used to develop equations for predicting oleoresin yields at a height of 4.5 m from assessments made at 1.5 m. To obtain reasonably good predictions it was necessary to develop a unique equation for each site. In addition, a new expression for approximating the standard error of repeatability estimates is introduced.

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Keywords: Resin flow; Sample size; Within-tree variability; *Pinus*; *Dendroctonus frontalis*; Allometry; Host resistance

1. Introduction

When more than a single measure of a trait is available for individuals, the total or phenotypic variability within a population can be separated into within- and among-individual components. Such a partition makes it possible to study the relative contribution of each component to phenotypic variability in the trait. Repeatability is a population parameter that provides useful information for investigations of this type. It is defined as the intraclass correlation (r) between two independent measurements made on individuals and can be expressed as the ratio of variation among individuals to existing phenotypic variation (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Values of this parameter range from zero to one, with zero indicating that phenotypic variance consists entirely of variation within individuals, and one denoting perfect repeatability, i.e., no variation within individuals. A value of 0.5 signifies that phenotypic variance is composed of variance among and within individuals in equal parts.

Estimates of repeatability may be used to determine the number of measurements required per individual to obtain adequate estimates of individual trait means and breeding values, and to provide estimates of an upper bound for broad-sense heritabilities (Falconer and Mackay, 1996; Mrode, 1996). A population's among-individuals component of variance is composed of its entire complement of genetic variance as well as environmental variance existing among individuals. The within-individuals component, however, consists only of the fraction of environmental variance that occurs within individuals. As a result, the population's phenotypic variance consists of within-individuals environmental variance plus among-individuals environmental variance as well as genetic variance. Because the repeatability parameter includes among-individuals environmental variance as a component of its numerator term, whereas heritability parameters do not, repeatability is an upper bound for broad-sense heritability.

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Repeated measures on individuals may also be used to develop equations to predict trait performance for one part of an organism based on measurements taken elsewhere on the same individual. For large plants such as trees, it is sometimes desirable to determine trait performance at bole positions some distance above ground level, i.e., performance that is inconvenient to measure but that is ecologically important. If suitable prediction equations can be developed, measurements taken at a standard, accessible bole locus can be used to estimate trait values at the desired points. This procedure invokes prediction across spatial domains and is analogous to the temporal prediction procedure described in Falconer and Mackay (1996).

In forest genetics applications, repeatability concepts have principally been employed to determine clonal repeatability, which likewise is generally interpreted as an upper bound for broad-sense heritability. This type of repeatability is estimated from clonal studies (in which measurements are taken on multiple ramets from each clone) and differs from individual or organismal repeatability in the way environmental variance is partitioned. Clonal repeatabilities have been studied for a number of traits in tree species including oleoresin yield in slash (*Pinus elliottii* Engelm. var. *elliotii*) and maritime (*Pinus pinaster* Ait.) pines (Peters, 1971; Tadesse et al., 2001) and tracheid cell wall microfibril angle in radiata pine (*Pinus radiata* D. Don) (Donaldson and Burdon, 1995). In this paper the focus is on organismal repeatability as opposed to clonal repeatability. Results are reported for this type of repeatability for constitutive oleoresin yield (resin flow) in three species of pines native to the southeastern United States. Hereafter the terms resin and oleoresin are used interchangeably to indicate constitutive oleoresin.

Constitutive oleoresin flow in trees of the genus *Pinus* is considered to be a trait of primary importance in tree resistance to attack by bark beetles. In pines of the southeastern United States, trees that rapidly dispense large quantities of resin are considered to be most resistant to infestation by the southern pine beetle, *Dendroctonus frontalis* Zimmermann, a primary insect pest of pines in the region (Hodges et al., 1979; Cook and Hain, 1987; Strom et al., 2002; Tisdale et al., 2003). A tree's resin producing capability, and thus to some degree its ability to ward off attack by *D. frontalis*, is most often assessed by measuring the resin yield from one or more small surface wounds made at or near breast height. Logistical convenience requires that measurements be made near ground level, despite evidence that initial *D. frontalis* attacks usually occur higher on the tree bole (3–5 m; Coster et al., 1977). As yields are expected to vary among wounds opened on the same tree, it is necessary to determine whether this variability contributes substantially to error in determining mean yield values for each tree. Because little is known about within-tree sampling variability for oleoresin yields obtained from small wound samples, and because this methodology is being used increasingly, research was initiated to study repeatability for this trait in three species of the southern pines. Estimates of this parameter are presented for breast-height sampling in stands of loblolly (*Pinus taeda* L.), longleaf (*Pinus palustris* Mill.) and slash pines. In addition, an estimator for the approximate standard error of repeatability estimates is derived, the effect of estimated repeatabilities on sampling error associated with within-tree sample sizes is evaluated, and equations to predict oleoresin yields at a bole height of 4.5 m from yields measured at 1.5 m are described.

2. Materials and methods

2.1. Stands sampled

Repeatability was estimated for breast-height oleoresin yield measurements in 10 stands of southern pines growing on sites in the central Gulf Coastal Plain of the southeastern United States. This collection consists of a sample of stands drawn from three species and includes five stands of loblolly pine, four stands of longleaf pine and one of slash pine. Characteristics of each stand together with details of sampling are presented in Table 1.

Table 1
Features and sampling statistics for stands sampled for repeatability determinations

Stand	Location	Age (years)	Trees sampled	Samples per tree	Mean dbh (cm)	Date sampled
Loblolly Pine						
Rapides 1	Rapides Parish, LA	Early 20's	31	8	–	May 1999
Rapides 2	Rapides Parish, LA	Early 20's	35	8	27.0	July 2002
Rapides 3	Rapides Parish, LA	Mid 20's	40	10	24.1	June 2004
Santa Rosa	Santa Rosa County, FL	10–11	1131	2	18.3	September 1999, May 2000
Wilcox	Wilcox County, AL	12–14	1619	2	19.8	August 2003, May 2004
Longleaf Pine						
Harrison 1A	Harrison County, MS	27	40	10	20.4	April 2004
Harrison 1B	Harrison County, MS	28	40	10	21.0	May 2005
Harrison 3	Harrison County, MS	21	40	10	19.5	May 2005
Natchitoches	Natchitoches Parish, LA	42	40	10	53.5	June 2005
Rapides 4	Rapides Parish, LA	Mid 30's	32	8	21.0	July 2002
Slash Pine						
Harrison 2	Harrison County, MS	Mid 20's	40	10	19.6	April 2004

Note: Ages approximated from ring counts made on breast height increment cores are represented as a range of ages, e.g., Early 20's.

Two of the loblolly pine stands, Santa Rosa and Wilcox, were established as field experiments consisting of replicated full-sib family plots. At the time of measurement, tree ages in these experiments ranged from 10 to 14 years with the older trees occurring in the Wilcox experiment (Table 1). Each planting was treated as a replication-in-blocks incomplete block experiment configured so that trees of different ages occupy different blocks. A detailed description of this experimental design as applied to the Santa Rosa progeny trial is given in Roberds et al. (2003). This particular experiment contained progeny from 72 families produced by mating 48 parents whereas the Wilcox progeny trial consisted of 95 families generated from crosses made among a different collection of 48 parents. In both experiments resin yields were assessed during early spring (Spr RY) and late summer (Sum RY). Two samples per tree were taken at each assessment, except that only a single measurement was taken in the spring assessment of nine trees in the Santa Rosa experiment. Resin samples were collected from wounds made on opposing sides of the tree bole in the neighborhood of breast height. Although the number of measurements evaluated per tree was restricted to the minimum number necessary to assess within-individual variation, trees in these experiments were young and of small size (averaging less than 20 cm dbh), and thus had relatively small bole surface areas. Moreover, tree sample sizes were large, extending well beyond 1000 individuals in each experiment, making precise estimation of repeatability possible (Table 1).

Eight stands in central Louisiana and southern Mississippi containing trees of unknown pedigree were also sampled for repeatability estimation. Stands of all three of the pine species studied are represented in this group. Within each stand, 31–40 trees were chosen for oleoresin yield evaluation. Eight or 10 yield measurements, depending on the stand, were made on each selected tree, with the occasional exception of an added or missing measurement. Oleoresin yields were assessed from wounds administered more or less evenly around the tree bole in the neighborhood of breast height. Trees in these stands ranged in age from the early 20's to 42 years, and therefore were older than those in the experimental plantings. Ages of individual stands at time of measurement are given in Table 1.

Resin yield data from two additional loblolly pine stands were used to evaluate the prospect for predicting yields at a bole height of 4.5 m from measurements taken at 1.5 m. The upper height of 4.5 m falls in the 3–5 m bole height zone considered significant with respect to attack by *D. frontalis*. As no one height within this zone is believed to be of greater import than any other with regard to preference for *D. frontalis* attack, a point 3 m above the base height of 1.5 m was chosen as a convenient reference height for upper bole resin yields. The stands selected for sampling, Rapides 5 (sample size 37 trees) and Rapides 6 (sample size 33 trees), are located in central Louisiana (Rapides Parish), and like the other Louisiana and Mississippi stands studied, contain trees of unknown pedigree. Their ages at time of resin sampling, the middle twenties, are comparable to those of the Louisiana loblolly stands sampled for repeatability estimation. The two stands differ from each other in that only three weeks separated the last prescribed burn and resin sampling for Rapides 5 while 13 months elapsed between the last fire and resin sampling in Rapides 6. In other respects, the fire histories of the two stands are similar. Regression analysis, based on mean resin yields at the two bole heights for each tree was used to develop empirical prediction equations for each stand. Almost all of these means were computed on the basis of four measurements, but six means were estimated from only three measurements.

2.2. *Resin yield measurements*

Oleoresin yields were measured as the resin mass (in grams) that flowed from wounds to bole surfaces over a fixed period of time. Wounding followed methodology described in Strom et al. (2002) and involved extracting all tissue from a ~1.27 cm diameter wound extending inward through the tree bark, phloem, and cambium to the surface of the xylem. During wounding, care was taken to minimize scoring of the xylem face and, although some scoring is inevitable, any wound that extracted a portion of the sapwood was not used. Resin from each wound was funneled into a container attached to the tree bole. Samples were collected 24 h (±1.5 h) following wounding in all stands except one. In Rapides 1, resin sampling was discontinued 8 h after wounding to prevent rainwater contamination. When multiple samples were obtained from a small bole surface area, care was taken to position wounds at least 5 cm apart in an effort to reduce the impact of adjacent wounding on yields.

2.3. *Statistical analysis*

Repeatability was estimated using two procedures: ratio-of-variances estimation and correlation coefficient estimation. Each was applied in the sampling situation for which it was the simpler method of estimation. The ratio-of-variances method was used for stands having trees with more than two yield measurements, whereas the correlation method was adopted for stands having only two measurements per individual.

The ratio-of-variances method requires that variance components be estimated for observations both within- and among-individuals in stands. Repeatability then can be estimated as:

$$\hat{r} = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_w^2}, \tag{1}$$

where σ_a^2 indicates variance among individuals, σ_w^2 indicates variance within individuals, and carets represent estimates (Falconer and Mackay, 1996). Note that the denominator in this ratio is an estimate of the phenotypic variance (σ_P^2). To compute repeatability from (1), variance components were estimated from a mixed model using the REML procedure. Computations were carried out utilizing PROC MIXED in SAS (release 8.1 for windows, SAS Institute Inc., Cary, NC).

For the Santa Rosa and Wilcox experimental plantings, repeatability was estimated as the correlation between the two measurements made on each tree. Taking advantage of the experimental design in place for these experiments, repeatabilities were computed as correlations over trees within replications. A mixed model analysis was employed in which block and replication-in-block effects were treated as random effects and correlations were estimated using repeated measures analysis executed from PROC MIXED of SAS.

2.4. Approximate standard error

The reliability of repeatability estimates can be assessed by evaluating their standard errors. Becker (1984) describes a method for determining approximate standard errors based on the analysis of variance approach for estimation of variance components and the formula for the approximate variance of a ratio. Here we present a new expression for the approximate standard error of repeatability estimated as a ratio of variances. Like Becker's approximation, it is based on the approximate variance for a ratio; however, our expression can be used for any method of variance component estimation provided that estimates are available for the variances of the variance component estimates and of the covariance between them. Taking the square root of the right side of (A.3) in Appendix A yields the following estimator for the approximate standard error for repeatability estimates:

$$\mathrm{S.E.}(\hat{r}) \cong \frac{\sqrt{(1-\hat{r})^2\,\widehat{\mathrm{Var}}(\hat{\sigma}_{\mathrm{a}}^2) - 2\hat{r}(1-\hat{r})\,\widehat{\mathrm{Cov}}(\hat{\sigma}_{\mathrm{a}}^2,\hat{\sigma}_{\mathrm{w}}^2) + \hat{r}^2\,\widehat{\mathrm{Var}}(\hat{\sigma}_{\mathrm{w}}^2)}}{\hat{\sigma}_{\mathrm{a}}^2 + \hat{\sigma}_{\mathrm{w}}^2}. \tag{2}$$

Approximate standard errors for repeatabilities determined using the ratio of variances method were computed using (2). Values for the necessary variance components plus estimates of the variances and covariance for these components were computed using the REML procedure in PROC MIXED of SAS.

For repeatabilities estimated as correlations, approximate standard errors were computed from the formula for the approximate variance for correlation estimates given in Stuart and Ord (1994). Estimates of correlation coefficients, variance and covariance components, and of the variances and covariances of the component estimates were obtained from repeated measures analysis conducted using the REML method in PROC MIXED of SAS.

2.5. Relative error variance

Variation among means of individuals determined from n measurements can be partitioned into true variance among individuals (σ_{a}^2) and $1/n$ times the variance among observations within individuals (σ_{w}^2). This latter term may be viewed as the error variance contribution (σ_E^2) to variance among means of individuals, and thus can be written as:

$$\sigma_E^2 = \frac{\sigma_{\mathrm{w}}^2}{n}. \tag{3}$$

When estimating means for individuals, it is desirable to control σ_E^2, keeping its value small relative to the phenotypic variance (σ_{P}^2). Letting E represent this error variance expressed as a proportion of phenotypic variance, and referring to it as relative error variance, the following explicit representation for this normalized error variance is obtained,

$$E = \frac{\sigma_E^2}{\sigma_{\mathrm{P}}^2}. \tag{4}$$

Because repeatability as a parameter is defined as the following ratio of variances,

$$r = \frac{\sigma_{\mathrm{a}}^2}{\sigma_{\mathrm{P}}^2}$$

(Becker, 1984; Falconer and Mackay, 1996), and since $\sigma_{\mathrm{P}}^2 = \sigma_{\mathrm{a}}^2 + \sigma_{\mathrm{w}}^2$, the antithesis of repeatability, $1 - r$, is given by:

$$1 - r = \frac{\sigma_{\mathrm{w}}^2}{\sigma_{\mathrm{P}}^2}. \tag{5}$$

Solving for σ_{w}^2 using (5) and inserting the result into (3) leads to an expression for σ_E^2 in terms of repeatability. Substitution into (4) then produces the following equation:

$$E = \frac{1 - r}{n}. \tag{6}$$

This result demonstrates that relative error variance is a function of repeatability and the number of measurements per individual. For fixed $r < 1$, it is clear from (6) that E represents the general term for a decreasing sequence in n with limit, $\lim_{n\to\infty} E = 0$. Moreover, for constant n, E is also a decreasing function of repeatability. These properties of E are illustrated in Fig. 1.

When r is known, Eq. (6) suggests a method for determining the minimum number of measurements per individual required to keep relative error variance at or below an

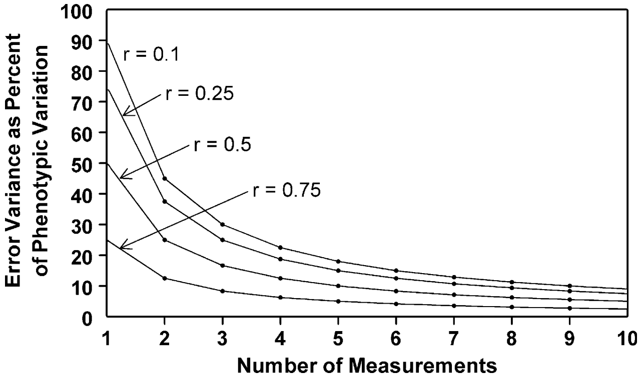

Fig. 1. Error variance as percentage of phenotypic variance plotted over number of measurements per individual. Plotted points (dots) designate values computed from Eq. (6) in the text multiplied by 100. Each graph represents results for a different repeatability (r) as indicated.

Table 2
Resin yield repeatability estimates obtained as the ratio of among-tree variance to phenotypic variance and estimates of other selected stand parameters

Stand	Trait	Mean (g)	Phenotypic variance	Repeatability		CV_A	CV_W
				r	S.E.		
Loblolly Pine							
Rapides 1	Spr RY	2.79	2.3105	0.67	0.07	44.6	31.5
Rapides 2	Sum RY	3.36	4.1755	0.64	0.06	48.5	36.7
Rapides 3	Spr RY	2.07	1.9128	0.71	0.05	56.5	37.5
Longleaf Pine							
Harrison 1A	Spr RY	1.28	1.0635	0.46	0.07	54.4	59.1
Harrison 1B	Spr RY	1.51	2.1123	0.47	0.07	66.0	70.3
Harrison 3	Spr RY	0.66	0.4343	0.43	0.07	65.2	75.4
Natchitoches	Spr RY	7.31	38.8703	0.77	0.04	75.0	40.7
Rapides 4	Sum RY	2.80	3.7403	0.65	0.07	55.9	40.7
Slash Pine							
Harrison 2	Spr RY	0.43	0.2956	0.55	0.06	94.8	85.4

Note: Spr RY, spring resin yield; Sum RY, summer resin yield; CV_A, among-individuals coefficient of variation; CV_W, within-individuals coefficient of variation.

acceptable value. Let $E_m > 0$ represent the maximum acceptable relative error variance and n_m be the minimum number of requisite measurements. Then, from (6) it is clear that $E_m \geq (1 - r)/n$ is a required condition. Rearranging terms in this inequality shows that n_m is the smallest positive integer such that:

$$n_m \geq \frac{1 - r}{E_m}. \tag{7}$$

3. Results

3.1. Repeatability determinations

Repeatability estimates for the ten stands investigated are presented in Tables 2 and 3. These results indicate that, for the three pine species studied, variation among trees contributes substantially to phenotypic variation in oleoresin yield at breast height. Coefficient of variation (CV) estimates for among and within individuals corroborate the repeatability findings. Standard errors determined for the repeatability estimates indicate that these statistics are estimated with good precision. Values are less than 20% of the repeatability estimates, which is evidence that sample sizes were large enough to provide reliable estimates for the range of repeatabilities observed. The standard errors estimated for the Santa Rosa and Wilcox sites are about one-third of those estimated for the other sites, and are likely a reflection of the much larger number of trees evaluated at these two experimental sites.

In the loblolly pine stands, variation among trees makes up roughly two-thirds of the total variability among measurements, thus denoting moderately high repeatability. Repeatability estimates extend from $r = 0.64$ to 0.71 and CV values for the among-trees component of variation range from about 45% to a little over 65% (Tables 2 and 3). In line with the repeatability estimates, values for the within-trees CV's are somewhat lower, in the range of 30–45%. Estimates across stands are remarkably consistent. This is especially significant given the varying characteristics of the stands and the differing times (years and seasons) in which resin yields were evaluated (Table 1).

Repeatability estimates varied widely for the longleaf pine stands studied, with values ranging from a low of $r = 0.43$ for Harrison 3 to a high of $r = 0.77$ for the stand in Natchitoches Parish (Table 2). Values for the two central Louisiana stands, like those for the loblolly pine stands, are substantially greater than one-half, implying that variation among trees is considerably larger than variation within trees. In contrast, the estimates for the south Mississippi stands are homogeneous but occur in the interval $r = 0.4$–0.5 indicating that within-tree

Table 3
Loblolly pine resin yield repeatabilities estimated as intraclass correlation coefficients plus estimates of other selected stand parameters

Stand	Trait	Mean (g)	Phenotypic variance	Repeatability		CV_A	CV_W
				r	S.E.		
Santa Rosa	Sum RY	1.51	1.1747	0.70	0.02	60.0	39.2
	Spr RY	1.90	1.6199	0.64	0.02	53.5	40.4
Wilcox	Sum RY	1.58	1.2530	0.68	0.02	58.5	40.2
	Spr RY	1.01	0.6448	0.68	0.01	65.7	45.3

Note: Phenotypic variance estimated as $\hat{\sigma}_1\hat{\sigma}_2$ where $\hat{\sigma}_i$ indicates estimate of the standard deviation for the ith measurement on an individual; CV_A, among-individuals coefficient of variation estimated as $100\sqrt{\mathrm{C\hat{o}v}(1,2)}/\bar{x}$ where $\mathrm{C\hat{o}v}(1,2)$ represents estimate of covariance between two measurements made on a common individual and $\bar{x}$ represents estimate of the trait mean; CV_W, within-individuals coefficient of variation estimated as $100\sqrt{\hat{\sigma}_1\hat{\sigma}_2 - \mathrm{C\hat{o}v}(1,2)}/\bar{x}$.

variability in resin yield is greater than variation among trees. For the Harrison 1 stand, values were estimated from different trees in 2 consecutive years, represented here as Harrison 1A and 1B. Although the phenotypic variance for Harrison 1B is twice that of Harrison 1A, repeatability estimates for the two samples agree closely. Comparison of the within-tree and among-tree CV values for the Louisiana and Mississippi stands reveals that the major reason for lower repeatabilities in the Mississippi stands is greater within-tree variability as opposed to lower variation among trees. At present, there is no satisfactory explanation to account for the difference in within-tree variability observed between stands in Louisiana and Mississippi.

The lowest phenotypic variability, as well as the lowest mean oleoresin yield of all the stands included in this study, was found in the single slash pine stand investigated (Harrison 2). Mean yield for Harrison 2 was less than 0.5 g, whereas mean values for the loblolly and longleaf stands investigated were higher, reaching magnitudes greater than 2.0 g for several stands (Table 2). This pattern agrees with results reported by Hodges et al. (1977), who found that slash pine had a lower mean resin yield than the other major southern pines when yields were evaluated 8 h following wounding. Despite low mean yield and low phenotypic variance, the repeatability estimate for this stand is only slightly lower than the values obtained for the loblolly stands, and intermediate to those determined for the longleaf stands. Moreover, since the estimated repeatability is larger than $r = 0.5$, variation among trees is greater than variation within trees. Finally, values obtained for the CV statistics are considerably larger than values estimated for the loblolly and longleaf stands. This suggests that for oleoresin yield, slash pine has higher variability relative to the mean than the other two species on both within-tree and among-trees bases.

3.2. Within-tree sampling

For most applications, interest in resin yield centers primarily around evaluating individual tree (mean) performance. When repeated measures on individual trees are possible for a trait, tree means are an appropriate measure of tree value. When means are to be computed, the number of measurements per tree required to adequately estimate this parameter must be determined. The inequality in (7) demonstrates that the number needed depends on the maximum acceptable error variance relative to phenotypic variance (E_m) and the repeatability for the trait. The extent in which error variance, expressed as a percentage of phenotypic variance, decreases with increasing numbers of measurements is exhibited for a selection of repeatabilities in Fig. 1. Using the ratio of variance among individual means to phenotypic variance, Falconer and Mackay (1996) show that with high repeatability little advantage is gained by taking more than a few measurements per individual; however, if repeatability is low, additional measurements are necessary to obtain suitable accuracy. This pattern is also evident in the graphs of percent error variance presented in Fig. 1. Unless repeatability is low, it is clear that little additional accuracy is obtained when means are based on more than two or three measurements—the same conclusion given in Falconer and Mackay (1996). All but two of the stands studied have resin yield repeatabilities greater than 0.5 (Tables 2 and 3). For stands with repeatabilities of this magnitude, two measurements per individual provide reasonably accurate estimates of tree means (percent error variance values are below 25%; Fig. 1). These results suggest that two measurements per tree are sufficient to evaluate breast-height resin yield for most purposes. For applications that require estimation of tree means with a high level of precision, or when repeatability is very low, four measurements per tree may be necessary. Very little additional benefit, however, is achieved by taking more than four measurements.

3.3. Prediction of resin yield at bole height of 4.5 m

Operational considerations dictate that resin yield determinations be based on measurements taken from the ground. Usually these assessments are based on measurements made in the vicinity of breast height. Data presented in Coster et al. (1977), however, suggest that initial southern pine beetle attacks occur most frequently at bole heights ranging from 3 to 5 m above ground level. Such a property of *D. frontalis*' attack behavior suggests that tree defense in this region of the bole is especially important. For this reason, and because sampling for resin is most conveniently done at an easily accessible bole height, it is desirable to accurately estimate resin yields in the region of 3–5 m above ground from measurements taken in the neighborhood of breast height. Here estimates of parameters are reported for equations developed to predict resin yields at a bole height of 4.5 m from measurements made at 1.5 m. Because this is a first attempt to develop equations to predict resin yield at an upper bole location from resin measurements taken on the lower bole, these results must be considered preliminary. They, however, do reveal previously unknown features of the relationship between resin yields in these bole regions. Estimates of sample statistics and parameters for the prediction equations for two central Louisiana stands, Rapides 5 and 6, are presented in Tables 4 and 5. Mean resin yields for the two bole heights do not differ significantly in either stand ($p = 0.146$ and 0.095, LR test) although means and standard deviations estimated at 4.5 m are slightly smaller than those computed for 1.5 m. In this regard our results are in agreement with those observed by Schmitt et al. (1988), who found no significant differences in mean resin yields from 10 bole heights

Table 4
Resin yield summary statistics for loblolly pine stands sampled to develop prediction equations for resin yield at 4.5 m

Stand	Bole height (m)	*n*	Mean (g)	S.D.	Min. (g)	Max. (g)
Rapides 5	1.5	37	2.94	2.15	0.13	9.39
	4.5	37	2.56	1.53	0.43	6.10
Rapides 6	1.5	33	3.32	1.62	0.60	7.82
	4.5	33	3.12	1.44	0.76	6.78

Note: n represents number of trees sampled.

Table 5

Statistics from regression analysis of resin yield at bole height 4.5 m on resin yield at bole height 1.5 m for two stands of loblolly pine

Statistic	Stand	
	Rapides 5	Rapides 6
Correlation coefficient[a]	0.72****	0.84****
Intercept (ln *a*)	0.299***	0.111ns
Regression coefficient (*b*)[b]	0.592****	0.845****
Residual SS	5.2720	2.0297
PRESS	6.3735	2.2195
R^2	0.68	0.76
$R^2_{\text{prediction}}$	0.62	0.73

Unless noted, values were estimated from data transformed using natural logarithms. ns, not significantly different at 5%.

[a] Estimated from nonlogarithmic data.

[b] For test of the hypothesis $b < 1$, t significant at $p < 0.0001$ in Rapides 5; at $p = 0.0403$ in Rapides 6.

*** t significant at 0.1%.

**** t significant at 0.01%.

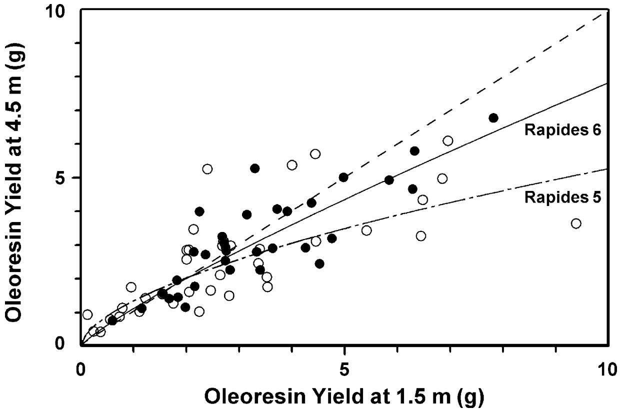

Fig. 2. Oleoresin yields at bole heights of 1.5 and 4.5 m on trees in loblolly pine stands, Rapides 5 and 6. Open circles indicate results for Rapides 5 ($n = 37$ trees) and filled circles indicate results for Rapides 6 ($n = 33$ trees). The curves represent fitted allometric equations for the stands, and the dashed straight line represents equivalent yields for the two bole heights.

in loblolly pines of varying age classes, but are incongruous with those obtained by Tisdale and Nebeker (1992) who reported evidence for variation among resin yields assessed at differing bole heights.

If trees have equivalent resin yields at heights of 1.5 and 4.5 m, a linear prediction equation with zero intercept and regression coefficient equal to one would be expected to provide the best fit to yield data collected from these different bole positions. Exploratory analysis, however, revealed that the linear model:

$$\ln Y_i = \ln a + b \ln X_i + e_i \tag{8}$$

fits the sample data more closely. Here *Y* and *X* represent resin yields at heights of 4.5 and 1.5 m, respectively, *e* represents random error, *i* designates trees, and ln indicates the natural logarithm. This equation is the linearization of the bivariate allometric model $Y_i = aX_i^b$, which has been used widely to investigate relationships between two size variables for individuals (Gould, 1966; Sprent, 1972). In the analyses described here, the model is additionally assumed to have multiplicative error. As with simple linear prediction, if resin yields at the two heights are equivalent in each tree, estimated values for the regression coefficient (*b*) and intercept parameters (ln *a*) in (8) are expected to be one and zero, respectively.

Mean resin yields at the two heights for trees in Rapides 5 and 6 fit the model in (8) reasonably well ($R^2 = 0.68$ and 0.76). Although untransformed mean yields at the two positions are highly correlated in both populations (Table 5), values estimated for the *b* parameter are significantly less than one but significantly greater than zero, indicating the presence of negative allometry. Furthermore they imply that trees with high resin yields at 1.5 m tend to have higher yields at that height than at 4.5 m. Estimated intercept values (ln *a*) are positive for both stands, but only the intercept for Rapides 5 is significantly larger than zero. Because $\ln a > 0$ implies $a > 1$, these results indicate that trees with low resin yields in the Rapides 5 population tend to have higher yields at 4.5 m than at 1.5 m. However, the estimated intercept is small, so these yield differences are not large. Curves representing the fitted nonlinear equations are shown in Fig. 2.

Tests for homogeneity of parameters indicate that the b parameter differs from stand to stand ($0.04 < p < 0.05$), but that the hypothesis that there is a common intercept cannot be rejected ($p = 0.217$). Given the heterogeneity in the b parameters, relationships between the two yield variables in Rapides 5 and 6 could not properly be described by a common equation. Therefore separate prediction equations were necessary for each stand.

Because data are not available from independent samples to validate the two prediction models, a validation method based on the Prediction Error Sum of Squares (PRESS) statistic was used to evaluate model quality. PRESS is a sample reuse statistic that indicates how well a regression model will perform in making predictions (Weisberg, 1985; Montgomery et al., 2001). A good prediction model has a low value for PRESS. To provide a benchmark for evaluation, the residual sums of squares obtained by fitting the model to the original sample data may be used. From PRESS, $R^2_{\text{prediction}}$ (Montgomery et al., 2001) was computed and used as an indicator of model predictive capacity. These statistics are presented in Table 5 for each of the prediction equations developed. Estimates for the PRESS statistic are not unduly greater than the Residual Sums of Squares; values are 21 and 9% higher than this benchmark for the Rapides 5 and 6 models, respectively. The $R^2_{\text{prediction}}$ values (Table 5) indicate that the prediction models can be expected to account for roughly 61.5 and 73.4% of the variability in newly observed resin yields for trees in the two stands. These values are far below a desired predictive capacity of above 90%, but compare favorably with the 68.2 and 75.7% of the variability explained by the fit of the models to the sample data sets used to estimate the model parameters. Although a large part of the among-tree variation in resin yield at 4.5 m is accounted for by the fitted models, a substantial portion is not because variability exists among-trees in the relationship occurring between resin yields at the two bole heights. The small differences between ordinary and prediction R^2 values, and between PRESS statistics and Residual Sums of Squares, suggest that it is this unaccounted for variation, rather than prediction error, that chiefly limits the predictive capacity of the fitted models. Thus, individual tree variability in the relationship between oleoresin yields at the two bole heights substantially limits the accuracy of predictions made using the developed equations. In summary, the equations can be expected to account for only about 62 and 73% of the among-tree variation in oleoresin yield at the 4.5 m height in the two stands.

4. Discussion

Repeatability studies can provide a great deal of information about variation among and within individuals in populations when repeated measures are possible for a trait. Such is the case for oleoresin yield in pines. Because estimates of repeatability for populations can be obtained from measurements made on random samples of individuals, controlled mating experiments with progenies partitioned according to a known family structure are not required. Therefore, repeatability can often be determined for traits and populations for which it is difficult to acquire reliable estimates of heritability. Some information related to heritability, however, can be gleaned from the repeatability estimates in these cases because the values may be interpreted as upper bounds for broad-sense heritability. This is the situation for oleoresin yield in pine species that do not have appropriate experiments available from which precise estimates of genetic variances can be obtained. Fortunately, repeatabilities can be estimated for oleoresin yield with relatively modest effort, making it feasible to determine values for multiple populations within a species, and thus providing the opportunity for estimation across a broad span of environments. Results from this study suggest that sample sizes of 30–40 trees per population, with yields evaluated on 8–10 resin samples per tree, are sufficient to provide reasonably precise estimates for this parameter.

A significant finding of this research is that two measurements per tree are usually adequate for assessing individual tree resin yields in the neighborhood of breast height. Results presented here provide the first quantitative support for adoption of this sample size. Previously, little information was available regarding amounts of variability occurring within trees relative to variation existing among trees in stands. For the three species studied, the results obtained suggest that the prevalent condition is either for variation among trees in stands to be greater than variability within trees (most common), or for variation within trees to be only slightly larger than that among trees. With such partitions of phenotypic variability, a single measurement is not sufficient to characterize tree resin yields, yet large numbers of measurements are not required. Although the observed patterns of variability may predominate for breast-height resin yields, there also may be environmental influences that cause variability within trees to exceed variability among trees, thereby fostering low repeatability. In situations where such factors exert a strong influence, more than two measurements per tree will be necessary for adequate estimation of individual tree yields.

The negative allometry observed in loblolly pine between tree resin yields at a bole height of 4.5 m and those at a height of 1.5 m indicates a nonlinear relationship between these two variables. Over the range of mid- and upper-level yield values, this nonlinearity takes the form of declining growth in yield at 4.5 m with increasing yield at 1.5 m. For low yields, however, it reflects higher values at 4.5 m than at 1.5 m (Fig. 2). Because of this relationship, trees with high resin yields at a height of 1.5 m tend to have considerably lower yields at a height of 4.5 m. Yield standard deviations for the two heights differ in a pattern that is expected for this type of allometry (Table 4).

To account for the observed allometry, we suggest that effects associated with differing environments at the two bole heights are important determinants, although differences in tree physiology (e.g., maturity of resin-producing tissues) cannot be ruled out as contributing factors. We conjecture that environments at the two bole heights act differentially on resin production depending on the magnitude of tree resin yield. Ground fire and lower bole lacerations are obvious factors that have the potential to cause effects consistent with this hypothesis.

Low-intensity prescribed fire and simulated basal logging injuries have been shown to increase resin yields in the bole area near breast height (Santoro et al., 2001; Nebeker and Hodges, 1983). We speculate that basal injuries or heat trauma from such fires may have larger effects on resin producing tissues near ground level than on those located higher on the tree bole. With low ground fire and basal lacerations, resin producing capacity at a height of 4.5 m is less likely to be affected, leaving resin yields at this height at or near levels realized in the absence of these traumas. We hypothesize that these events cause resin production at a bole height of 1.5 m to be stimulated progressively as resin producing capability increases among the higher yielding individuals, but act to retard resin production in trees of low producing capacity. According to this hypothesis, factors that cause these effects at 1.5 m are less influential at 4.5 m, resulting in oleoresin yields at this upper bole height that tend to "regress" toward the mean when compared with yields at 1.5 m. The allometric relationships observed in this study are consistent with such behavior.

The recent fire history in loblolly pine stands Rapides 5 and 6, considered in light of the time resin yield assessments took place, provides some support for this hypothesis. Rapides 5, the stand that showed pronounced negative allometry between resin yields, was subjected to prescribed fire three weeks prior to resin sampling. In Rapides 6, resin sampling occurred only two weeks after sampling in Rapides 5, but over 13 months had elapsed between the last prescription burn and the time of resin sampling. Fire effects in Rapides 6 would probably have decreased during this 13-month period. The weaker negative allometry observed between resin yields at the two heights in this stand is consistent with such a decline. The coincidence of strong negative allometry with more recent prescribed fire and the presence of weak negative allometry with a longer interval between prescribed fire and measurement of oleoresin yield are clearly evident in our results. Such a pattern suggests that low-intensity ground fire might well significantly affect resin yields in the lower bole region of loblolly pines, while having little or no effect on yields at a height of 4.5 m.

These findings raise the question of whether substantial population-to-population variability exists in the relationship between breast height oleoresin yields and those at points higher on the tree bole; for example, in the region where initial southern pine beetle attacks most frequently occur. Additional research is needed before a clear indication can be obtained about the magnitude of this variability and how it is affected by environmental conditions. Of especial interest is the association between these yield variables in stands that have not recently been subjected to fire or lower-bole injuries. In the absence of such disturbances, the relationship between resin yields in the lower mid-bole and breast height regions would be expected to differ from the relationships observed in the two stands studied.

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Appendix A

An expression for the approximate variance of repeatability estimates computed using the ratio of variances method can be obtained from the formula for approximate variance of a ratio. Stuart and Ord (1994) show that the variance of a ratio can be approximated as:

$$\mathrm{Var}\left[\frac{x}{y}\right] \cong \left[\frac{E(x)}{E(y)}\right]^2 \left[\frac{\mathrm{Var}(x)}{E^2(x)} + \frac{\mathrm{Var}(y)}{E^2(y)} - \frac{2\mathrm{Cov}(x,y)}{E(x)E(y)}\right], \quad \text{(A.1)}$$

where E indicates expectation, Var represents variance, and Cov represents covariance.

Let $\hat{r} = \hat{\sigma}^2_{\mathrm{a}}/(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}})$ be the estimate for repeatability where $\hat{\sigma}^2_{\mathrm{a}}$ is the estimate for variance among individuals and $\hat{\sigma}^2_{\mathrm{w}}$ is the estimate for variance within individuals, then substitution for x and y in (A.1) yields,

$$\mathrm{Var}(\hat{r}) \cong \left[\frac{E(\hat{\sigma}^2_{\mathrm{a}})}{E(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}})}\right]^2 \left[\frac{\mathrm{Var}(\hat{\sigma}^2_{\mathrm{a}})}{E^2(\hat{\sigma}^2_{\mathrm{a}})} + \frac{\mathrm{Var}(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}})}{E^2(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}})} - \frac{2\mathrm{Cov}(\hat{\sigma}^2_{\mathrm{a}}, \hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}})}{E(\hat{\sigma}^2_{\mathrm{a}})E(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}})}\right] \quad \text{(A.2)}$$

The following identities can be used to simplify the right side of (A.2):

$$\mathrm{Var}(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}}) = \mathrm{Var}(\hat{\sigma}^2_{\mathrm{a}}) + \mathrm{Var}(\hat{\sigma}^2_{\mathrm{w}}) + 2\mathrm{Cov}(\hat{\sigma}^2_{\mathrm{a}}, \hat{\sigma}^2_{\mathrm{w}}),$$

$$\mathrm{Cov}(\hat{\sigma}^2_{\mathrm{a}}, \hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}}) = \mathrm{Var}(\hat{\sigma}^2_{\mathrm{a}}) + \mathrm{Cov}(\hat{\sigma}^2_{\mathrm{a}}, \hat{\sigma}^2_{\mathrm{w}}),$$

$$E(\hat{\sigma}^2_{\mathrm{a}}) = \sigma^2_{\mathrm{a}} = r(\sigma^2_{\mathrm{a}} + \sigma^2_{\mathrm{w}}),$$

$$E(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}}) = \sigma^2_{\mathrm{a}} + \sigma^2_{\mathrm{w}}.$$

Making substitutions in (A.2) and combining terms gives:

$$\mathrm{Var}(\hat{r}) \cong \left[\frac{\sigma^2_{\mathrm{a}}}{\sigma^2_{\mathrm{a}} + \sigma^2_{\mathrm{w}}}\right]^2 \left[\frac{(1-r)^2\mathrm{Var}(\hat{\sigma}^2_{\mathrm{a}}) - 2r(1-r)\mathrm{Cov}(\hat{\sigma}^2_{\mathrm{a}}, \hat{\sigma}^2_{\mathrm{w}}) + r^2\mathrm{Var}(\hat{\sigma}^2_{\mathrm{w}})}{(\sigma^2_{\mathrm{a}})^2}\right].$$

Cancelling terms and replacing parameters by their estimators produces:

$$\mathrm{Var}(\hat{r}) \cong \frac{(1-\hat{r})^2\widehat{\mathrm{Var}}(\hat{\sigma}^2_{\mathrm{a}}) - 2\hat{r}(1-\hat{r})\widehat{\mathrm{Cov}}(\hat{\sigma}^2_{\mathrm{a}}, \hat{\sigma}^2_{\mathrm{w}}) + \hat{r}^2\widehat{\mathrm{Var}}(\hat{\sigma}^2_{\mathrm{w}})}{(\hat{\sigma}^2_{\mathrm{a}} + \hat{\sigma}^2_{\mathrm{w}})^2}. \quad \text{(A.3)}$$

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