

Predicted genetic gains and testing efficiency from two loblolly pine clonal trials

Fikret Isik, Barry Goldfarb, Anthony LeBude, Bailian Li, and Steve McKeand

Abstract: Clonal field trials were established at two sites using rooted cuttings from 450 clones of eight full-sib families of loblolly pine (*Pinus taeda* L.). Height, survival, fusiform rust infection (caused by *Cronartium quercuum* (Berk) Miyabe ex Shirai f.sp. *fusiforme*), bole straightness, and diameter were measured after four growing seasons. There were significant differences among full-sib families and among clones within families for all traits studied. Moderately high within-family repeatabilities of clone means (0.50 to 0.75) for growth traits and a very high within-family repeatability of clone means (0.94) for fusiform rust infection were estimated. When the best eight clones were selected regardless of family structure, the volume yield was 52% greater than that of the unimproved seedlings at two sites. Selection of the best two clones from each of four families produced only slightly lower estimated genetic gains than the above scenario. The probability of fusiform rust infection ranged from 0.08 to 0.93 among clones at the South Carolina site. Predicted genetic gain for rust resistance was relatively insensitive to selection intensity, as there were numerous clones with high apparent resistance. The number of ramets per clone necessary to reliably characterize performance on one site was estimated to be between four and six. These results contribute to estimates of the gains available from clonal forestry and will help guide clonal testing and selection programs. Implementation of clonal forestry and cost issues are discussed.

Résumé : Des tests clonaux ont été établis sur deux sites à partir de boutures racinées représentatives de 450 clones provenant de huit descendance biparentales de pin à encens (*Pinus taeda* L.). Après quatre saisons de croissance, les caractères suivants ont été mesurés : la hauteur, la survie, l'infection par la rouille-tumeur des chesnaies (causée par *Cronartium quercuum* (Berk) Miyabe ex Shirai f.sp. *fusiforme*), la rectitude du fût et son diamètre. Des différences significatives ont été remarquées pour tous les caractères étudiés parmi les descendance biparentales et parmi les clones d'une même famille. Des valeurs modérément élevées de stabilité intra-familiale des moyennes clonales (0,50 à 0,75) ont été estimées pour les caractères de croissance. Il y avait aussi une très grande stabilité intra-familiale (0,94) des moyennes clonales pour l'infection par la rouille-tumeur des chesnaies. La sélection des huit meilleurs clones, sans tenir compte de la structure familiale, s'est traduite par un rendement en volume 52 % plus élevé que celui de semis non améliorés dans les deux sites. La sélection des deux meilleurs clones pour chacune des quatre meilleures familles a produit un gain génétique estimé qui était juste légèrement inférieur à celui obtenu avec le scénario précédent. La probabilité d'infection par la rouille-tumeur des chesnaies variait de 0,08 à 0,93 parmi les clones du site situé en Caroline du Sud. Le gain génétique prédit pour la résistance à la rouille était relativement peu affecté par l'intensité de sélection puisqu'il y avait de nombreux clones dont la résistance apparente était élevée. Le nombre de ramets par clone nécessaire pour caractériser de façon fiable la performance sur un site a été estimé entre quatre et six. Ces résultats s'ajoutent aux estimations de gains découlant de la foresterie clonale et permettront d'orienter les programmes d'essais et de sélection des clones. Le déploiement de la foresterie clonale et les questions de coût sont abordés.

[Traduit par la Rédaction]

Introduction

The use of vegetative propagation to produce planting stock from elite genotypes for reforestation can improve forest plantation productivity and quality (Zobel 1992). Two strategies for using vegetative propagation are common (Frampton et al. 2000): (1) multiplying (bulking-up) limited quantities of full-sib seed derived from crossing parents of

known genetic value, and (2) choosing clones from within families for multiplication and deployment. The latter approach is often referred to as clonal forestry, a term that assumes the performance of the clones has previously been assessed and found to be desirable (Libby and Ahuja 1993). There is a great deal of interest in using either rooted stem cuttings or somatic embryogenesis for clonal forestry in the southeast United States (Stelzer and Goldfarb 1997).

Currently, the most reliable method for identifying superior clones is to assess performance in replicated field tests. Theoretical approaches have been used to determine the optimal distribution of testing effort between the number of clones and the number of ramets per clone (Shaw and Hood 1985; Russell and Libby 1986) and the assessment of clonal performance in different environments (Burdon and Shelbourne 1974). Frampton and Foster (1990) reviewed approaches for

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using vegetative propagules for different testing objectives, including comparisons of propagule types, evaluation of clonal performance, assessment of clone by environment interactions, and estimation of genetic parameters. In addition, results have been reported for empirical trials containing clones in several tree species, including *Pinus radiata* D. Don (e.g., Burdon 1976; Burdon et al. 1992), *Eucalyptus grandis* W. Hill ex Maiden (Osorio et al. 2003), *Larix laricina* (Du Roi) K. Koch (Park and Fowler 1987), *Picea mariana* (Mill.) BSP (Mullin et al. 1992), *Tsuga heterophylla* Sarg. (Foster et al. 1985), *Populus nigra* L. (Isik and Toplu 2004), and *Pinus taeda* L. (Frampton and Huber 1995; Paul et al. 1997; Isik et al. 2003a, 2003b). These trials have contributed genetic parameter information on which clonal selection programs can be based. Results from both the theoretical approaches and the empirical experiments have contributed much to our understanding of efficient clonal test design. However, many of these studies (and particularly those in loblolly pine) have included a large number of crosses, with relatively few clones per cross. In practice, one would probably use prior breeding information to choose excellent full-sib crosses and then test a relatively large number of clones from each cross. Recent improvements in loblolly pine propagation via stem cuttings (e.g., Murthy and Goldfarb 2001; Rowe et al. 2002; LeBude et al. 2004) have made this approach feasible.

In loblolly pine, breeding and testing have resulted in substantial population improvements and genetic gains from seedlings derived from seed orchards (Li et al. 1999). During testing, a large number of full-sib families have been produced and evaluated. These provide a valuable resource on which to base within-family selection for clonal deployment. In this study, we report on empirical trials designed to guide future clonal selection activities. Our objectives were to establish a clonally replicated test of loblolly pine with a relatively large number of clones from a few elite full-sib crosses and to use the early results to determine (1) the amount of genetic gain in height, volume, and fusiform rust (caused by *Cronartium quercuum* (Berk) Miyabe ex Shirai f.sp. *fusiforme*) resistance that can be obtained with clonal forestry, (2) the sensitivity of genetic gain to selection intensity, and (3) the number of ramets necessary to characterize clonal performance on a site.

Materials and methods

Plant material

Eight full-sib families were selected from the North Carolina State University – Industry Cooperative Tree Improvement Program on the basis of progeny tests of second-cycle parents. Because the objective of the study was to simulate a program to select clones for deployment, families were unrelated and chosen on the basis of rapid growth, good fusiform rust resistance, acceptable bole straightness, and seed availability. In October 1996 seeds from each family were sown in Ray Leach SuperCells™ (169 cm³). Approximately 100 seedlings (i.e., genotypes or clones) per family were grown continuously through the winter in a heated, polyethylene-covered greenhouse at the North Carolina State University Horticultural Field Laboratory (35°47'N, 78°39'W). Seedlings were transplanted in May 1997 into 12-L containers containing a medium of composted, shredded pine bark – sand (4:1,

v/v), top dressed with 51 g of Osmocote (18N–6P–12K; 8–9 months) controlled-release fertilizer (Grace-Sierra, Milpitas, California), and grown outdoors on a gravel container pad equipped with an overhead sprinkler system. These seedling stock plants were sheared to a height of 15 cm twice during the growing season in 1997. Hand weeding was used as necessary, and several pesticides were applied throughout the growing season to control tip moth (*Rhyacionia frustrana* (Comst.)) and fusiform rust. The container pad was covered with white polyethylene and left unheated to overwinter the hedged stock plants from December to April.

In February 1998, terminal stem cuttings were collected from the hedged stock plants, stored in insulated coolers, and placed in a cold room maintained at 4 °C. In mid to late March 1998, 15 stem cuttings per clone were set for rooting in a randomized complete-block design replicated 15 times with one cutting per clone per block. Stem cuttings were retrimmed to a length of 9 cm, inserted in 10 mmol/L 1-naphthaleneacetic acid (1.86 g·L⁻¹ in 30% ethanol, v/v) for 3 s, and set in Ray Leach SuperCells™ containing a medium of peat–perlite (2:3, v/v). Rooting of stem cuttings was conducted in a clear polyethylene-covered greenhouse under natural photoperiod and irradiance. Heating and cooling systems were set to maintain the daily air temperature between 23° and 26 °C and the night temperature between 20° and 23 °C. Mist was applied to stem cuttings intermittently at a variable frequency related inversely to the relative humidity within the greenhouse and modified by time of day. Mist frequency was calculated by the Gem3™ environmental software package (Q-Com Corp., Irvine, California) and delivered by a traveling gantry (boom) system (Solaris, McConkey Co., Mt. Puyallup, Washington). After 12 weeks (late June), rooted stem cuttings were removed from the greenhouse and grown at a spacing of 544 plants·m⁻² (49 plants·ft⁻²) on the gravel container pad until field planting. Plants were fertilized weekly until the time of planting with Peter's soluble fertilizer (20N–20P–20K) (The Scotts Co., Marysville, Ohio) at a rate of 100 ppm N.

Experimental design and data collection

Following hedge production and subsequent vegetative propagation of stem cuttings, 450 clones produced at least nine rooted stem cuttings for use in field tests. The first test plantation consisted of 282 clones from four crosses (F, H, I, K) planted in South Carolina (SC) in November 1998. The second test site was established in Florida (FL) in December 1998 with 168 clones from another four crosses (A, C, D, E). The field experimental design at both sites was a randomized complete block with nine blocks and one ramet per clone per block (i.e., single-tree plots). Seedlings from two seed lots were also planted as check lots at both sites to compare the growth of clones with that of the improved and unimproved seedlings. Seed lot UC is an unimproved seed source that has been included in loblolly pine progeny tests to estimate genetic improvement. Seed lot IC is a fast-growing, open-pollinated family that has been widely planted in the southern United States.

At ages 1 through 4, survival (%) was recorded and tree height (HT1–HT4) was measured at both the SC and FL sites. Fusiform rust infection was recorded as a binary variable at ages 3 and 4 at both sites. Rust-infected trees were

scored as “1”, and noninfected trees were scored as “0”. Trees that had died because of rust infection were also scored as “1”. Diameter at breast height (DBH4) of trees was measured at age 4. Individual-tree volume (VOL4) was estimated (cubic feet) according to Goebel and Warner (1966) and then converted to cubic decimetres. Each tree at the FL site was assessed for bole straightness (STR4) using a four-point scale where 2 is the most straight and 5 is the most crooked.

Statistical analysis of growth traits and bole straightness

Analyses of variance were conducted to test whether there were significant differences among families and among clones within families for the traits studied. Coefficients of variation based on the phenotypic variances of clone means were estimated as

$$CV_{\bar{c}(f)} = \frac{\sqrt{\sigma_{c(f)}^2 + \sigma_e^2 / b}}{\bar{X}} \times 100$$

where $\sigma_{c(f)}^2$ is the clone-within-family variance component, σ_e^2 is the error variance component, b is the number of ramets per clone, and \bar{X} is the site mean. Variance components were estimated for height, diameter at breast height, volume, and straightness for each site separately, fitting a general linear mixed model given in matrix form:

$$[1a] \quad \mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where \mathbf{y} is the column vector of individual phenotypic values for a response variable, \mathbf{X} is the incidence matrix of fixed effects and $\boldsymbol{\beta}$ is the column vector of fixed effects that includes the intercept and block effect, \mathbf{Z} is the incidence matrix of random effects, \mathbf{u} is the column vector of random effects that includes family and clone effects with $E(\mathbf{u}) = 0$, and \mathbf{e} is the column vector of residuals assumed to be randomly and independently distributed with $E(\mathbf{e}) = 0$. The covariance matrix \mathbf{V} for the vector of observations \mathbf{y} is $\mathbf{V} = \mathbf{Z}\mathbf{G}\mathbf{Z}^T + \mathbf{R}$ (Littell et al. 1996), where \mathbf{G} is the genetic covariance matrix of random genetic effects, \mathbf{R} is the diagonal matrix of residual errors, and \mathbf{Z}^T is the transpose of the design matrix (Lynch and Walsh 1998). Best linear unbiased estimates of fixed effects ($\hat{\boldsymbol{\beta}}$) and best linear unbiased predictors (BLUP) of random effects ($\hat{\mathbf{u}}$) were obtained for traits by solving the mixed-model equations (Lynch and Walsh 1998).

$$[1b] \quad \hat{\boldsymbol{\beta}} = (\mathbf{X}^T \mathbf{V}^{-1} \mathbf{X})^{-1} \mathbf{X}^T \mathbf{V}^{-1} \mathbf{y}$$

$$[1c] \quad \hat{\mathbf{u}} = \mathbf{G}\mathbf{Z}^T \mathbf{V}^{-1} (\mathbf{y} - \mathbf{X}\hat{\boldsymbol{\beta}})$$

Variance components and solutions of random and fixed effects were estimated without including the data from the two check lots (IC and UC). Adjusted clone genetic values were estimated by adding the grand mean (mean of all the clones) to the clone BLUP values. The MIXED procedure of SAS[®] and ASReml were used for analyzing all traits (SAS Institute Inc. 1996; Gilmour et al. 2002).

Statistical analysis of fusiform rust infection

Fusiform rust infection (RUST4) was observed as a binary trait (0, 1) that follows a Bernoulli distribution. RUST4 has a mean of $\pi = X/n$, and an estimated variance of $S^2 = \pi(1 - \pi)/n$, where X is the number of infected trees, and n is the to-

tal number of trees. The standard deviation (SD) and coefficient of variation (CV) for RUST4 were calculated as

$$SD = \sqrt{[\pi(1 - \pi)] / n}$$

and

$$CV = \frac{\sqrt{[\pi(1 - \pi)] / n}}{\pi} \times 100$$

The general linear mixed model fitted for the growth variables assumes that the errors are normally distributed, variances are homogeneous, and the response variable is a linear combination of random and fixed effects (Littell et al. 1996). Fitting a general linear model to a binary trait violates the assumptions of the standard linear mixed model and may produce estimates outside the range of permissible values (i.e., 0, 1). Thus, the probability of infection ($\hat{\pi}$) of a single tree was modeled with the generalized linear mixed model using a logit (canonical) link function:

$$[2] \quad \eta_{ijk} = \log[\pi / (1 - \pi)] = \mu + r_i + f_j + c(f)_{k(j)} + e_{ijk} \\ = \mu + \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where η_{ijk} is the link function $[g(\mu)]$, μ is the conditional mean, π is the proportion of infected trees, r_i is the fixed effect of the i th block, f_j is the random effect of the j th family with $N(0, \mathbf{I}\sigma_f^2)$, $c(f)_{k(j)}$ is the random effect of the k th clone within the j th family with $N(0, \mathbf{I}\sigma_{c(f)}^2)$, where \mathbf{I} is an $n \times n$ identity matrix with 1 in the diagonal and 0 in the offdiagonal, and e_{ijk} is the random residual with $N(0, \mathbf{I}\sigma_e^2)$. The vector of random effects, \mathbf{u} , was assumed to be multivariate normal with a variance-covariance matrix of $\mathbf{G} = \text{var}(\mathbf{u})$ (SAS Institute Inc. 1996). The validity of the model fitted to the rust data and the predicted values of clones are closely related to the average rust infection. We assumed that the average rust infection (38%) at the SC site was within acceptable boundaries but that the average infection (5.4%) at the FL site was not. Thus, fusiform rust infection was not analyzed for the FL site but was analyzed for the SC site.

Because linear predictors for rust infection were computed on a logit scale, the solutions from the generalized linear mixed model are difficult to interpret. Therefore, predicted probabilities ($\hat{\pi}$) of the clones were calculated by applying the inverse of the link function and using the BLUP of the random solution vector (Littell et al. 1996):

$$[3] \quad \hat{\pi} = \frac{[\exp(\mathbf{X}\hat{\boldsymbol{\beta}} + \hat{\mathbf{u}})]}{[1 + \exp(\mathbf{X}\hat{\boldsymbol{\beta}} + \hat{\mathbf{u}})]}$$

where \mathbf{X} is the design matrix for fixed effects, $\hat{\boldsymbol{\beta}}$ is the solution for fixed effects (i.e., the intercept and block effects), and $\hat{\mathbf{u}}$ is the solution for random effects (i.e., BLUP clone rust infection probability values). Clone rust infection probability values (p) range between 0.0 and 1.0. A high probability value for a clone indicates a high probability of disease infection. These values are BLUPs for clones. The generalized linear model was fitted to the data twice, first excluding data from the check lots (IC and UC) and then including them in the analysis.

Within-family repeatabilities of clone means and correlations

Within-family repeatabilities of clone means ($H_{\bar{c}(f)}^2$) for all traits were estimated according to eq. 4:

$$[4] \quad H_{\bar{c}(f)}^2 = \frac{\sigma_{c(f)}^2}{\sigma_{c(f)}^2 + \sigma_e^2 / b}$$

where $\sigma_{c(f)}^2$ is the clone-within-family genetic variance, σ_e^2 is the error variance, and b is number of ramets per clone. Standard errors of the within-family repeatabilities of clone means were estimated using the Delta method (Lynch and Walsh 1998) based on the covariance matrix of the univariate model using a SAS/IML code (SAS Institute Inc. 1996). Genetic correlations (r_G) between pairs of traits were estimated using the following equation and by applying bivariate models using the ASReml software (Gilmour et al. 2002):

$$[5] \quad r_G = \frac{\sigma_{c(f)ij}}{\sqrt{\sigma_{c(f)i}^2 + \sigma_{c(f)j}^2}}$$

where $\sigma_{c(f)ij}$ is the clone-pooled within-family genetic covariance between traits i and j , $\sigma_{c(f)i}^2$ and $\sigma_{c(f)j}^2$ are the pooled clone-within-family genetic variances for traits i and j , respectively. Phenotypic correlations between pair of traits were estimated based on clone means across families using the MEANS and the CORR procedures of SAS (SAS Institute Inc. 1996). The standard errors of the correlations were estimated using the Delta method (Lynch and Walsh 1998).

Clone selection scenarios and number of ramets per clone

Four clonal selection scenarios were implemented to estimate genetic gains for VOL4 and RUST4 using adjusted clonal genetic values: (1) clones were ranked at each site, and genetic gains were estimated by selecting the best 1, 2, 3, 4, 8, 16, and 24 clones, regardless of family; (2) family was taken into consideration, and genetic gains were estimated by selecting the top 1, 2, 3, 4, and 6 clones from each of the four families at each test site; (3) the number of clones selected was weighted by the family ranking, and 10 clones were selected: four from the best family, three from the second best, two from the third, and one from the lowest ranking family; and (4) clone genetic values were compared with those of the unimproved (UC) and the improved (IC) check lots to estimate genetic gains. In calculating genetic gains relative to the check lots, least-squares means for check lots were used.

Using computer simulations, we investigated how the number of ramets sampled affects the estimation of clone means. A Markov-Chain Monte-Carlo sampling strategy was used to estimate clone means and their 95% confidence intervals based on different numbers of ramets (Gilks et al. 1996). For each sample size (n = number of ramets), 500 random samples were generated. The mean for each clone and the endpoints of its confidence interval were calculated for each sample. Samplings were carried out with a SAS macro code using unrestricted random sampling with replacement and the equal probability option of the SURVEYSELECT procedure (SAS Institute Inc. 1996). The clone means and the

endpoints of their confidence intervals were plotted against the sample size (number of ramets). Spearman rank correlations were calculated between the genetic values of clones based on randomly selected subsets of ramets (two to eight ramets) and the genetic values based on nine ramets (the full number of ramets considered). Rank correlation coefficients were plotted against the numbers of ramets for each site.

Results

Survival, growth, and rust infection

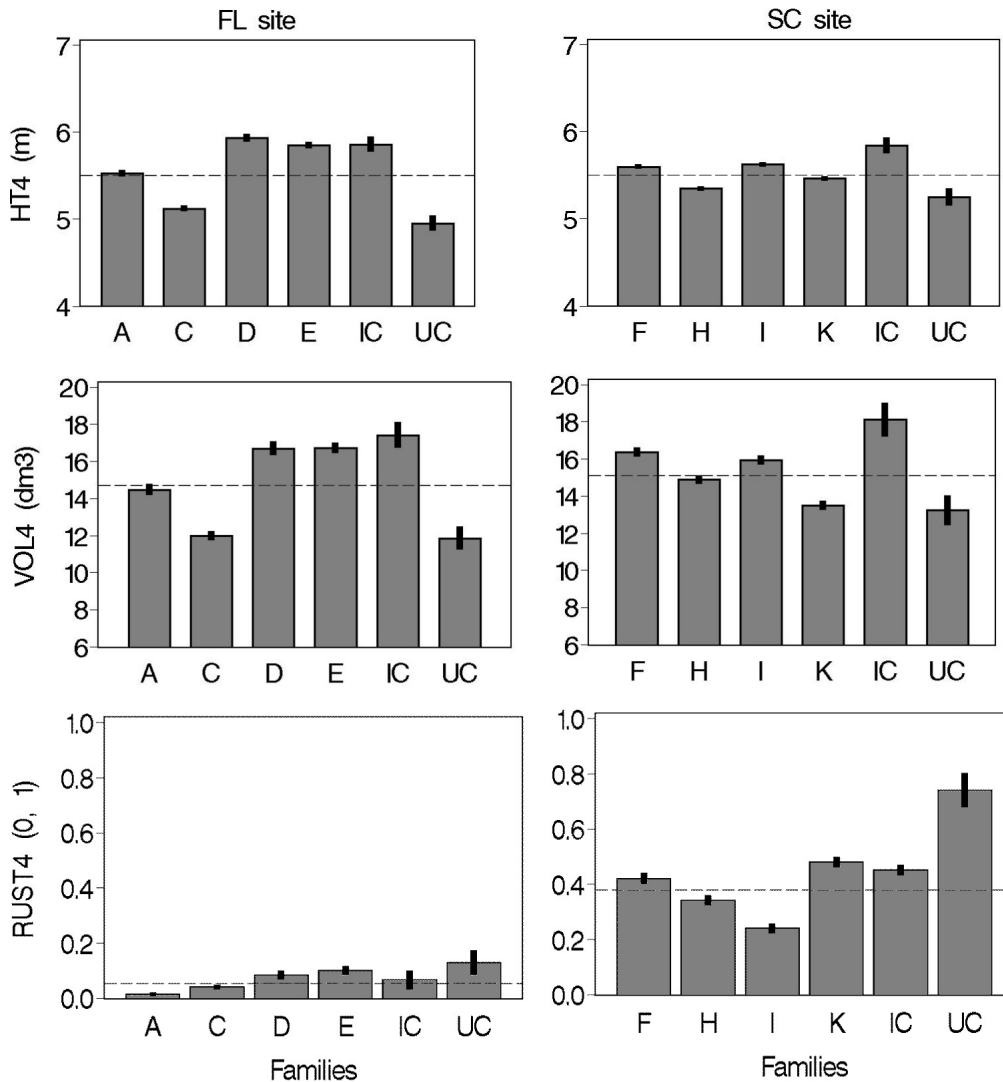
Survival at age 2 was high at both sites, 93% at FL and 95% at SC. Survival at age 3 dropped to 88% at FL, but did not change considerably at SC (94%). HT4 was 5.51 m at SC and 5.55 m at FL (Fig. 1). The two sites had similar values for VOL4 (~15 dm³/tree) at the end of the fourth growing season. Analyses of variance results revealed significant differences ($P < 0.001$) among full-sib families and among clones within full-sib families for HT4, DBH4, and VOL4 at both sites (data not presented). There were also significant differences ($P < 0.001$) among families and among clones for STR4 at the FL site. The unimproved check lot (UC) seedlings were the slowest growing at both sites, whereas the improved check lot (IC) seedlings were among the fastest growing entries (Fig. 1). Within-family clone-mean phenotypic variances for the growth traits were slightly greater at the SC site than that at the FL site, as shown by the coefficients of variations (Table 1).

At age 4, there were significant differences ($P < 0.001$) among families and among clones within families for fusiform rust infection at the SC site (Fig. 1). Average fusiform rust infection at SC was 24% at age 3 and increased to 38% at age 4. Family I had the lowest infection (24%) among the four crosses and two check lots tested at SC. The unimproved check lot UC had the highest rust infection rate (74%) among the seed sources (Fig. 1), with almost twice the rust infection rate of the SC site mean. IC seedlings had approximately 45% rust infection at SC by age 4. The mean rust infection rate at FL was near zero at early ages and increased to only 5.4% at age 4. Thus, rust infection in FL was omitted from further analyses.

Genetic variation and within-family repeatabilities of clone means

Variance components, their standard errors, within-family repeatabilities of clone means ($H_{\bar{c}(f)}^2$), and coefficients of variation based on the phenotypic variances of the clone means ($CV_{\bar{c}(f)}$) are presented in Table 1. As shown by the $CV_{\bar{c}(f)}$ values, clones had greater variability at the SC site than at the FL site. At SC, clonal differences for growth traits explained greater percentages of total phenotypic variance than did family differences. In contrast, the clone-within-family variance components at FL were smaller than the family variance components. For bole straightness at FL, the clone variance component was considerably greater than the family variance component. The $H_{\bar{c}(f)}^2$ values for growth traits were moderately high at SC, but were lower at FL. STR4 had moderate $H_{\bar{c}(f)}^2$ values at FL. For all traits, the family variance components were associated with higher standard errors than were the clone variance components. The standard errors of the variance components and their

Fig. 1. Least-squares means and one standard error of the means for full-sib families, the improved check lot (IC), and the unimproved check lot (UC) for height (HT4), volume (VOL4), and rust infection (RUST4) at the Florida (FL) and South Carolina (SC) sites at age 4. The horizontal broken line across the bars is the site mean for each trait.



functions are crude approximations from restricted maximum likelihood analysis and need to be considered cautiously, since the underlying distributions of the variance components are not known.

Differences among clones in fusiform rust infection explained a much greater percentage of the phenotypic variance than did differences among families at the SC site. High genetic variation among clones was reflected in a high $H_{\bar{e}(f)}^2$ for RUST4 at SC. The distribution of clone genetic values within families was more bimodal than unimodal, particularly for families F and K (Fig. 2).

Predicted genetic gains from clonal selection

Estimated genetic gains for VOL4 from selecting the best clones were considerable at both sites. When the best eight clones were selected regardless of family structure (scenario 1), volumes were 27% and 31% greater than the mean volume of all clones at SC and FL, respectively. Selection of the best two clones from each of four families (scenario 2) produced only slightly lower estimated genetic gains than

scenario 1 (Fig. 3). Genetic gains for scenario 1 and scenario 2 decreased gradually at both sites as the number of clones selected increased. Selection of 10 clones weighted according to family ranking (scenario 3) yielded an estimated 30% and 26% gain in VOL4 at SC and FL, respectively. These gains were similar to those estimated with scenarios 1 and 2 when 10 clones were selected.

Selection of the best eight clones for VOL4 in scenario 4 resulted in volumes that were approximately 53% (SC) and 52% (FL) greater than those of the unimproved check lot UC (Fig. 4). Predicted genetic gains over UC decreased gradually when 24 clones were selected. As expected, genetic gains over the improved IC seedlings were smaller than the gains over UC seedlings for the same selection intensities.

Considerable genetic gains for rust infection were predicted when scenario 1 was used at SC. Selecting the best eight clones for rust resistance produced a probability of infection that was 0.30 below that of the site mean (Fig. 5a). In contrast to growth traits, the gain in rust resistance was

Table 1. Variance components (estimate), their standard errors (SE), within-family repeatabilities of clone means ($H_{\bar{c}(f)}^2 \pm \text{SE}$), phenotypic coefficients of variation for clone means ($\text{CV}_{\bar{c}(f)}$), and site means (\pm standard deviation (SD)) at two sites.

(A) South Carolina site.								
Source	HT4 (m)		DBH4 (cm)		VOL4 (dm ³)		RUST4 (0,1) ^a	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
F	0.02	0.014	0.23	0.194	1.69	1.483	0.31	0.287
C(F)	0.11	0.013	0.58	0.075	6.18	0.824	2.02	0.241
Residual	0.30	0.009	2.05	0.065	26.30	0.830	1.00	0.000
$H_{\bar{c}(f)}^2 \pm \text{SE}$	0.75 \pm 0.024		0.70 \pm 0.028		0.66 \pm 0.031		0.94 \pm 0.006	
$\text{CV}_{\bar{c}(f)}^b$	6.8		9.9		20.3		2.48	
Mean \pm SD	5.51 \pm 0.656		9.25 \pm 1.682		15.1 \pm 5.93		0.38 \pm 0.0096	

(B) Florida site.								
Source	HT4 (m)		DBH4 (cm)		VOL4 (dm ³)		STR4	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
F	0.14	0.112	0.27	0.229	4.95	4.169	0.02	0.021
C(F)	0.07	0.013	0.26	0.060	4.26	0.805	0.07	0.015
Residual	0.33	0.014	2.15	0.090	23.04	0.969	0.52	0.022
$H_{\bar{c}(f)}^2 \pm \text{SE}$	0.65 \pm 0.043		0.50 \pm 0.061		0.60 \pm 0.048		0.51 \pm 0.059	
$\text{CV}_{\bar{c}(f)}^b$	6.2		8.1		18.3		10.6	
Mean \pm SD	5.55 \pm 0.764		9.05 \pm 1.662		14.7 \pm 5.82		3.45 \pm 0.780	

Note: F, family; C, clone; HT, height at age 4; DBH4, diameter at breast height at age 4; VOL4, individual-tree volume at age 4; RUST4, fusiform rust infection at age 4; STR4, bole straightness at age 4.

^aRUST4 was not analyzed at the Florida site because of low average infection at age 4 (0.054).

^b $\text{CV}_{\bar{c}(f)}$ values were calculated as $\text{CV}_{\bar{c}(f)} = \left[\sqrt{\sigma_{\bar{c}(f)}^2 + \sigma_{\bar{c}}^2 / b / \bar{X}} \right] \times 100$, except for RUST4. Standard deviation and $\text{CV}_{\bar{c}(f)}$ for

RUST4 were calculated as $\text{SD} = \sqrt{[\pi(1-\pi)] / n}$ and $\text{CV} = [\sqrt{(\pi(1-\pi) / n)} / \pi] \times 100$.

relatively insensitive to selection intensity. Moreover, the gain in rust resistance was similar for scenario 2, when clones were evenly selected from all four families. The improvements in rust resistance compared with that of the UC and IC seedlings were considerable in scenario 4. The best eight clones had probabilities of infection that were 0.61 and 0.33 below those of the UC and IC seedlings, respectively (Fig. 5b). Again, the estimated gain in rust resistance was not strongly affected by selection intensity.

Effects of the number of ramets on clone-mean estimation

The number of ramets had a considerable effect on the rank correlations of clone genetic values for HT4. As the number of ramets increased, the rank correlation of clone genetic values also increased (Fig. 6). The increase in rank correlations was more gradual after five ramets per clone. For example, the rank correlations obtained between two and nine ramets were approximately 0.70 and 0.60 at SC and FL, respectively. Rank correlations increased to 0.92 and 0.90 when six ramets were used.

The relationships between the endpoints of the 95% confidence intervals for clone means as a function of sample size (number of ramets) were graphed (Fig. 7). The number of ramets had a significant effect on the interval coverage of the clone means. When two ramets were used for estimation, the intervals had a large spread of upper and lower endpoints. As the number of ramets per clone increased, the interval

endpoints narrowed sharply and flattened at approximately four ramets. At FL, some clones had large confidence intervals between two and six ramets; however, interval endpoints did not change for the vast majority of clones after four ramets.

Trait-trait correlations

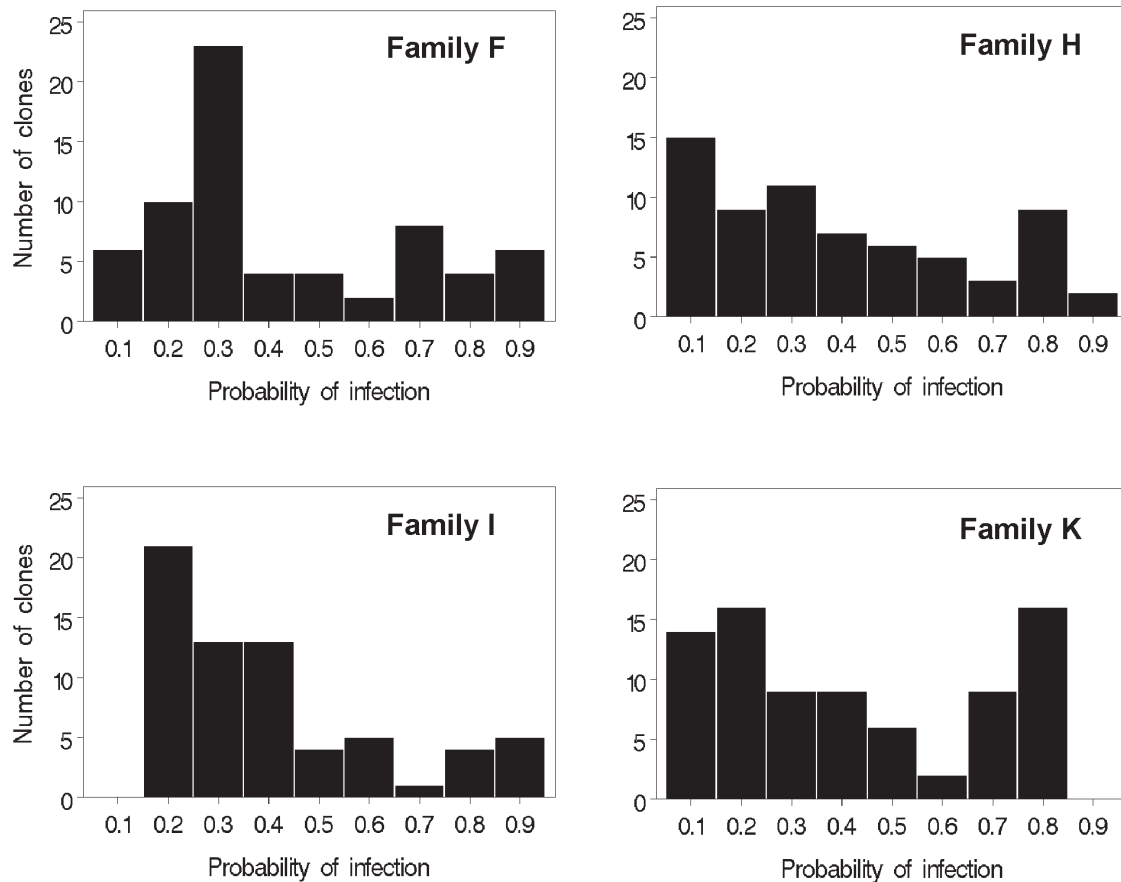
Genetic correlations among HT4, DBH4, and VOL4 were strong at both sites (Table 2). Rust infection had moderate negative (favorable) genetic correlations with growth traits at SC. Clone-mean phenotypic correlations were similar to genetic correlations between growth traits and RUST4 at SC. Clones with slower growth tended to have slightly straighter stems, as indicated by moderately low positive (unfavorable) genetic correlations between STR4 and growth traits at FL. However, there were no significant phenotypic relationships between STR4 and the growth traits at FL. All genetic and clone-mean phenotypic correlations were associated with relatively small standard errors, except for genetic correlations with RUST4 in SC and with STR4 in FL.

Discussion

Genetic variation and gain in growth traits

We found considerable differences for growth traits among clones within families of loblolly pine. Clonal differences explained greater percentages of observed variances than did

Fig. 2. Frequency distributions of rust infection genetic values for clones within each full-sib family at the South Carolina site at age 4.



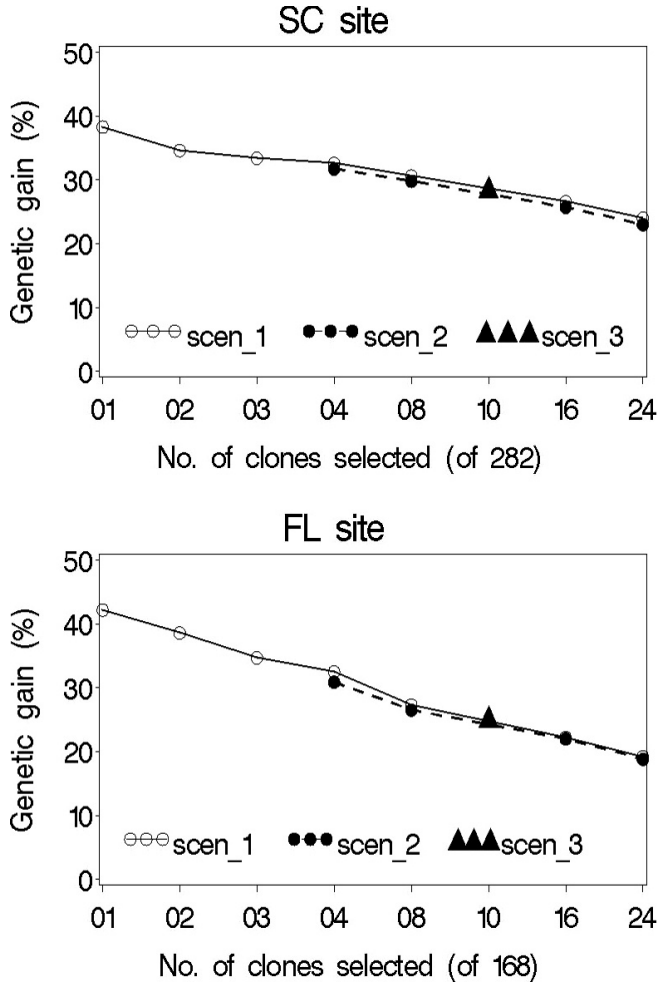
family differences at the SC site, but this was reversed at the FL site. Moderately high within-family repeatabilities of clone means ($H^2_{c(f)}$) for growth traits (0.50 to 0.75) suggest reasonable efficiency of selection for superior clones within families of loblolly pine in similarly designed clonal tests. These repeatability estimates may be biased upward because clones were tested on only one site. Moreover, clonal ("C") effects, e.g., common environmental factors associated with rooting and location of the cutting on the tree (Libby and Jund 1962), may have inflated the repeatabilities. However, these estimates are in close agreement with those reported by Frampton and Huber (1995) for height (0.52) and Isik et al. (2003b) for volume (0.70) from two independent multiple-site clonal trials of loblolly pine. Because the family structure differed considerably among these three studies, we can reasonably conclude that growth traits in loblolly pine are under moderate to strong genetic control at the clonal level.

Clonal selection yielded considerable genetic gains for volume over the means of all the clones at both the SC and FL sites. At age 4, a volume gain of approximately 30% was obtained by selecting the best eight clones on both sites (scenario 1). When family was taken into consideration (scenario 2), predicted genetic gains from selecting eight clones were equal to those of scenario 1. While estimates of volume gains from early field results may be inflated (Li et al. 1999; Carson et al. 1999), it is clear that gains from selecting the best clones could be substantial. This is demon-

strated by the even larger gains (53%) estimated for the best eight clones over the unimproved seed lot (UC). Improvements in value, by incorporating better form and disease resistance, are expected to be even greater (Li et al. 1996).

Volume gains over the improved check lot (IC) estimated from choosing the best eight clones were smaller (4%, 13%) than the gains over the unimproved check lot (UC) (~53%; Fig. 4) at both sites. The family chosen for the IC seed lot in this study does not represent an average open-pollinated family, but is one of the best in the breeding program after two cycles of improvement. The full-sib families in this study were chosen from preliminary tests in the diallel testing program and were constrained by the availability of seed; thus, faster-growing full-sib families are available today. This is shown by the result that the IC seedlings had a greater mean volume than any of the full-sib families. One might expect gains from selecting clones within the IC family to be similar in magnitude to those obtained by selecting clones within the full-sib families in this study. One explanation for the lower gains compared with the IC family would be that rooted cuttings may not perform as well as seedlings from the same families. However, several studies in loblolly pine have shown that if rooted cuttings are obtained from relatively juvenile hedges (as was the case in this study), the growth of seedlings versus rooted cuttings from the same families is indistinguishable (Stelzer et al. 1998; Frampton et al. 2000; Frampton et al. 2002). A more likely explanation

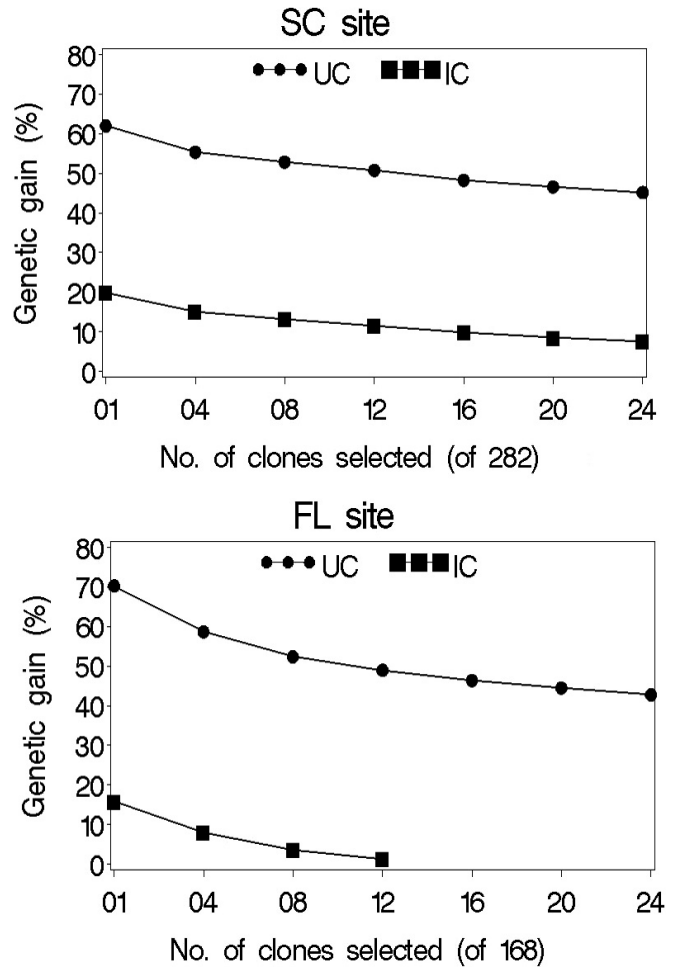
Fig. 3. Genetic gain (%) for volume at age 4 from various clonal selection strategies at the South Carolina (SC) and Florida (FL) sites. Open circles (solid line) are for selection of the top clones regardless of family (scenario 1), the solid circles (broken line) are for selection of clones from each family (scenario 2), and the solid triangles are for proportional selection of clones from all families (scenario 3).



for growth differences is initial stock size. In this study, the IC seedlings were substantially larger than the rooted cuttings at the time of planting. The effects of unequal stock size on early growth was discussed by Ritchie et al. (1993) and Stelzer et al. (1998). Thus, the gains compared with the IC seed lot may have been underestimated in this study. As the number of years in the field increases, these comparisons will be more meaningful.

One important consideration in clonal selection programs is the cost of maintaining genetic diversity among the selected clones. To test this, we initially selected clones from full-sib families that were unrelated. When using identical selection intensities, selecting clones that were equally distributed among the families (i.e., two clones per family) yielded similar gains (~30%) as selection scenarios that placed no restriction on relatedness (~31%). This will likely differ according to the relative breeding values of the families chosen for clonal selection. In this study, one or more clones

Fig. 4. Genetic gain (%) for volume at age 4 from various numbers of clone selections over an unimproved check lot (UC) and improved check lot (IC) at the South Carolina (SC) and Florida (FL) sites (scenario 4).



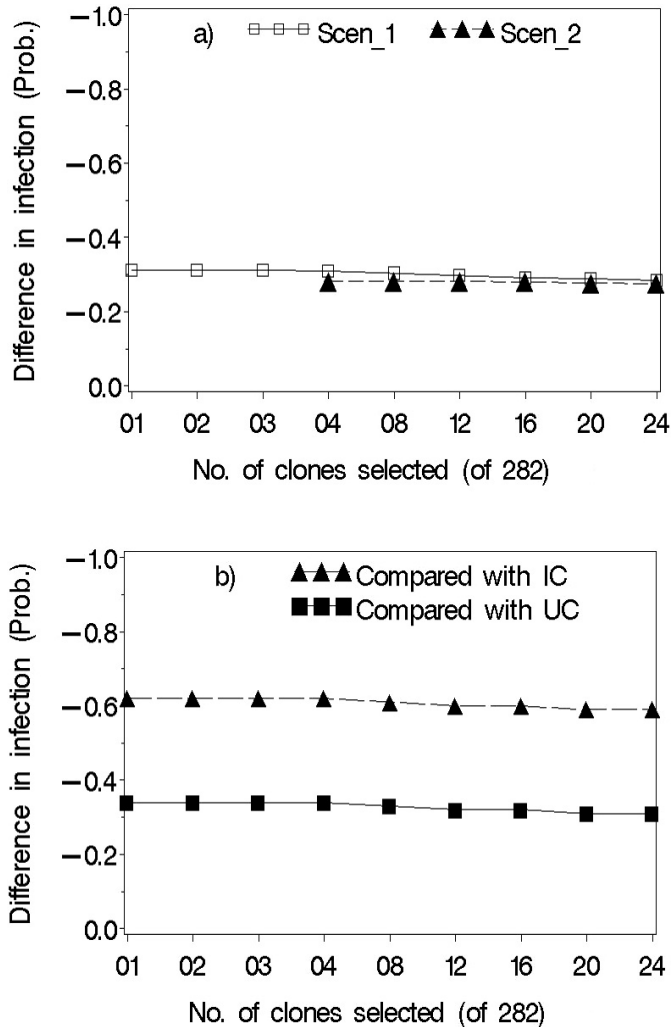
from each full-sib family were represented in the group of fastest growing clones. From this population, breeders could deploy a relatively diverse group of clones (i.e., eight clones) and realize genetic gains that are very similar to those obtained when selecting only the top-ranked clones.

Genetic variation and gain in rust resistance

Considerable variation in rust infection was observed among clones within families. While the four families planted at SC had predicted probabilities of infection ranging from 0.23 to 0.49 (IC = 0.46, UC = 0.74), there were numerous clones in each family that had a probability of infection below 0.18 or above 0.78. The clone genetic values (probability of infection) were not normally distributed in any of the four families (Fig. 2). The log-normal or bimodal distributions may be indicative of major gene effects or qualitative inheritance, particularly in family K, which had a bimodal distribution. At least six markers linked to major genes have been detected for fusiform rust resistance in loblolly pine, and others likely exist (Wilcox et al. 1996; Amerson et al. 1997).

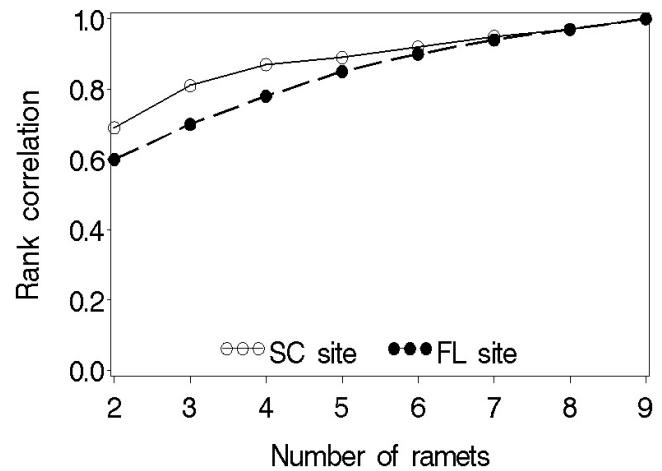
We estimated a high within-family clone-mean repeatability ($H^2_{\bar{c}(f)}$) for rust infection in this study. This estimate

Fig. 5. The reduction in the probability of fusiform rust infection versus the number of clones selected under various scenarios at the South Carolina site. (a) The difference between the selected clones and the site mean (over four families). The solid line (with open squares) shows the predicted change in infection probability resulting from selecting clones regardless of family (scenario 1), the broken line (with solid triangles) shows the predicted change in infection probability when clones are selected from each family (scenario 2). (b) The difference in probability of rust infection resulting from selection of clones compared with the improved (IC) check lot and the unimproved (UC) check lot (scenario 4).



could be biased upward because of clone by environment interactions. Clones planted on only one site may have been exposed to a limited pathogen population and might not show the same level of resistance to other populations. However, Isik et al. (2003b) also reported high within-family clone-mean heritabilities for rust infection in a genetic test of clones replicated across two widely separated sites. Several studies have reported that loblolly pine trees derived from vegetative propagules have lower levels of rust infection than do seedlings from the same families (McKeand 1985; Frampton et al. 2000). This has been attributed to physiological or epigenetic resistance mechanisms, and it is

Fig. 6. The change in rank correlations between best linear unbiased predictors (BLUP) clone genetic values based on testing different number of ramets from a simulation study for height at age 4 at the South Carolina (SC) and Florida (FL) sites. The original BLUP genetic values were based on nine ramets. The rank correlations between the original genetic values and the simulated genetic values increase as more ramets are used to predict the genetic values.



unclear how heritable these mechanisms may be. Also, genetic variances due to clones may be influenced by C effects, such as common environmental factors, particularly, at early ages. The high clone-mean heritabilities reported here suggest that field testing clones as rooted cuttings should provide useful information for rust resistance assessment, provided the clones are exposed to representative inocula and conducive environmental conditions for rust infection.

Genetic gains in rust resistance from selecting clones were large. Unlike growth traits, the genetic gain was not very sensitive to selection intensity. The reductions in probability of infection over the mean of all the clones were in the range of 0.30 when one through 24 clones were selected. Similarly, the reduction in probability of infection compared with the IC and UC check lots remained about 0.40 to 0.60 when selecting the same numbers of clones. This is because of the large number of clones expressing high levels of resistance (Fig. 2). This, combined with the negative (favorable) phenotypic and genetic correlations between rust infection and growth traits, indicates that rust resistant clones can be selected, without sacrificing growth for this collection of clones. Correlations in other populations may be substantially different.

Number of ramets per clone

The reliability of clone-mean estimates depends on the number of ramets used in field tests. Using a small number of ramets may decrease both the accuracy and precision of clone mean estimates. On the other hand, increasing the number of ramets increases the cost of testing and may limit the number of clones that can be tested, thus decreasing selection intensity. Using a theoretical approach, Russell and Libby (1986) suggested that the optimal number of ramets per site ranged from two to six, depending on the heritability of the trait. In an empirical trial with loblolly pine, Isik et al. (2003b) determined that as few as two ramets per clone

Fig. 7. Clone means (middle line) and the endpoints of their 95% confidence intervals (open circles) for volume at age 4 (VOL4) based on various numbers of ramets at the Florida (FL) and South Carolina (SC) test sites. Spline curves were fitted for the means and confidence intervals endpoints.

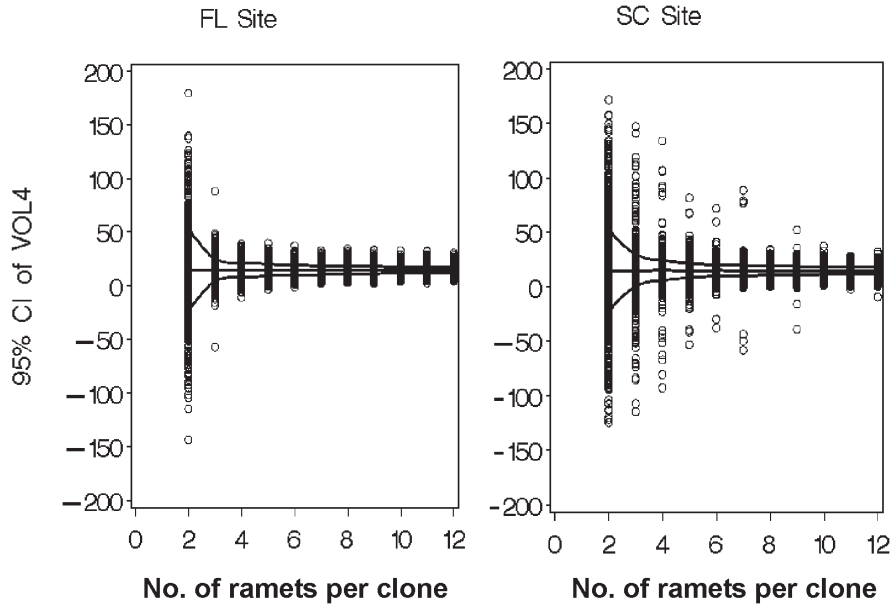


Table 2. Genetic correlations (\pm standard error (SE)) (below diagonals) and clone-mean phenotypic correlations (\pm SE) (above diagonals) between height (HT4), diameter (DBH4), volume (VOL4), straightness (STR4), and rust infection (RUST4) at age 4 at two sites.

(A) South Carolina site.				
	HT4	DBH4	VOL4	RUST4 ^a
HT4	—	0.83 \pm 0.008	0.87 \pm 0.059	-0.21 \pm 0.019
DBH4	0.87 \pm 0.022	—	0.97 \pm 0.002	-0.19 \pm 0.020
VOL4	0.90 \pm 0.018	0.97 \pm 0.006	—	-0.14 \pm 0.021
RUST4	-0.17 \pm 0.087	-0.20 \pm 0.084	-0.22 \pm 0.080	—
(B) Florida site.				
	HT4	DBH4	VOL4	STR4 ^a
HT4	—	0.78 \pm 0.012	0.86 \pm 0.009	0.09 \pm 0.032
DBH4	0.87 \pm 0.030	—	0.95 \pm 0.003	0.00 \pm 0.030
VOL4	0.93 \pm 0.020	0.99 \pm 0.006	—	0.02 \pm 0.031
STR4	0.28 \pm 0.110	0.12 \pm 0.131	0.15 \pm 0.120	—

^aNo data were available to estimate correlations for rust infection at the Florida site and for straightness at the South Carolina site.

yielded the greatest amount of within-family genetic gain if the total number of trees in progeny tests for a breeding program was held constant.

Using these empirical data, we also examined the question of the optimal number of ramets by treating experimental clones as a selection and deployment population. Spearman rank correlations were calculated between the genetic values of clones based on randomly selected subsets of ramets (two to eight ramets) and the genetic values based on nine ramets (the full number of ramets considered). The upper and lower endpoints of 95% confidence intervals for each clone mean were calculated using different numbers of ramets by resampling. Increasing the number of ramets from two to six increased the clone genetic value rank correlations substantially (Fig. 6). However, after six ramets, the increase was more gradual. End-

points of intervals stabilized at four ramets on both sites, although some outlying values were observed with greater numbers of ramets on the SC site (Fig. 7). Thus, our results suggest that an optimal ramet number for testing clones on one site is in the range of four to six. It should be noted that this holds for tests where survival and uniformity are acceptable.

Based on clone means, negative genetic and phenotypic correlations between fusiform rust infection and growth traits were weak but favorable. The estimates in this study were similar to those reported by Isik et al. (2003a) based on a limited number of seedlings and rooted cuttings per family. Although genetic correlations between growth traits and bole straightness were unfavorable, they were weak and were associated with high standard errors. C effects may inflate the correspondence between genetically related clones, particu-

larly in early stages of clonal trials (Borrhalho and Kanowski 1995). Nevertheless, C effects, particularly propagation effects, may not be considerable after age 4 (McRae et al. 1993).

Implications for clonal forestry

The results from this study show that significant gains can be achieved by the implementation of clonal forestry for loblolly pine in the southern United States. There is substantial genetic variation and moderately high heritabilities among clones within families for growth and fusiform rust infection. Progress in producing rooted cuttings of loblolly pine (Goldfarb et al. 1998; Frampton et al. 2000; LeBude et al. 2004) has made cloning more economically feasible. The eight full-sib families cloned in this study were selected somewhat for availability of seed and not strictly for elite performance. If only the best crosses from the second-generation loblolly pine breeding population could be used for clonal testing and selection, expected genetic gains should be substantially higher than those reported based on the population means (Li et al. 1999). These predicted gains are comparable with gains from long-term gain trials established in New Zealand using *Pinus radiata* D. Don. (Carson et al. 1999). An average of 34% gain in volume was reported for selection of a control-pollinated family over moderately selected seed lots in mid-rotation ages (Carson et al. 1999).

This study and previous studies (i.e., Frampton and Huber 1995; Isik et al. 2003b) suggest that rust infection of loblolly pine can be reduced considerably by clonal selection for deployment. Improvement in rust resistance for the second cycle of the coastal breeding population of loblolly pine was about 30% (Li et al. 1999). In this study, selection of the top 24 clones for rust resistance (scenario 1) gave 31% and 59% improvement over the genetically improved (IC) and unimproved (UC) check lots, respectively. A selection scenario combining the best families from the second-generation seed orchards with the top eight clones could potentially reduce rust infection by at least 90% compared with UC in rust hazardous sites. Moreover, the lack of unfavorable correlations between growth traits and rust infection indicates that selecting for either variable should not adversely affect gain in the other.

The optimal level of non-relatedness among deployed clones is an important question, but difficult to quantify. A balance must be struck between increasing gains from choosing only the best clones and maintaining diversity to guard against largely unknown risks. We used several clonal selection scenarios in this study to provide data for making informed choices. We found little loss of genetic gain when clones were selected from all unrelated families. Moreover, selecting clones from all families was as successful as selection based purely on ranking of clones, regardless of family information. We suggest that starting a clonal selection program with the four best unrelated families from an elite population and selecting the best three clones from each family would capture considerable gains, yet preserve an adequate level of genetic diversity in an overall plantation program. When compared with existing *Populus* and *Eucalyptus* clonal deployment programs (Ahuja and Libby 1993), which are often planted as exotics, the suggested approach could even be considered conservative.

Our study demonstrates that substantial gains in volume and rust resistance can be achieved by selecting and deploying superior clones of loblolly pine. The prospects for operational clonal forestry in the southeast United States with this species, however, also depend on other factors. First, the costs of clonal field tests must be considered. Second, current methods for producing clonal planting material, whether by rooted cuttings or somatic embryogenesis, are more costly than producing seedlings from seed orchards. Although field tests are one-time investments, and the actual cost would be smaller as rooted cutting technology progresses, both of these additional costs must be weighed against the gains in volume and rust resistance. Similarly, the economic value of clonal stands in the future is not certain. First, market prices for wood fluctuate widely, making it unclear what the value will be in any given year in the future. Second, it may be that volume is not the trait that confers the greatest value. For example, gains in better form and wood quality could potentially be more important than growth rate. However, in the current economic system, it is not clear whether these gains would be appropriately valued by wood processors. Third, while difficult to quantify with current data, it is reasonable to expect that monoclonal stands would be considerably more uniform than stands derived from seedlings. However, although uniformity appears to be desirable, there is no current market mechanism to value it. Thus, while the prospects for obtaining better and more wood from clonal forestry appear to be bright, important questions remain about the economic valuation of clonal stands and the overall cost-effectiveness of clonal selection, production, and deployment in the wood products industry of today and in the future.

Acknowledgements

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