Validation of Predicted Breeding Values for Slash Pine (*Pinus elliottii* var. *elliottii*) Using Field Trials Planted in Large Block Plots

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Abstract: Predicted breeding values were validated using realized gains estimated from large-rectangular-plot field trials from the first generation breeding population of slash pine (*Pinus elliottii* var. *elliottii* Engelm.) in the Southeast. These 43 realized gain trials consisted of three types: 1) rust resistant and rust susceptible material growing in high rust hazard sites in the Best Management Practices study (5 trials), 2) material selected for growth by the Cooperative Forest Genetics Research Program at the University of Florida (19 trials), and 3) Improved and unimproved material established by the Plantation Management Research Cooperative at the University of Georgia (19 trials). All trials contained slash pine seedlots collected from unrogued or lightly rogued first generation seed orchards. Multiple regression analyses were conducted to validate predicted breeding values calculated for each seedlot considering pollen background. Observed realized gains for each seedlot were used as the dependent variable, while site variables (site index and rust hazard) along with the predicted breeding values were used as independent variables. BLP values predicted for rust resistance were reasonably accurate, and most of the known variation in rust incidence was accounted for by the predicted breeding values. Conversely, validation of BLP-predicted volume breeding values was difficult due to excessive noise in the data for individual tree volume and stand yield. The use of highly replicated medium-size rectangular plots is suggested to overcome this problem of imprecise field data from realized gain trials.

Keywords: Tree improvement, realized gains, breeding values, validation, block plots, slash pine, *Pinus elliottii*.

INTRODUCTION

The underlying breeding value of an individual is the sum of the average effects of the alleles that it carries (Falconer and Mackay 1996). Breeding values are predicted from genetic trails containing an observed sample of offspring from a given parent using the mean value of that progeny (Falconer and Mackay 1996). Thus, progeny tests provide estimations of how future offspring will perform under operational conditions (White and Hodge 1987). The estimation of breeding values is an important and useful tool in tree improvement programs, where having accurate and precise predictions is fundamental in

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making reliable decisions to maximize genetic gains (White and Hodge 1987, White and Hodge 1988, Hodge and White 1992).

The Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida planted many open-pollinated (OP) and control-pollinated (CP) progeny tests in the southeastern USA in order to predict breeding values of their first-generation selections of slash pine (*Pinus elliottii* var. *elliottii* Engelm.). In 1995, volume and rust resistance breeding values for 2,491 first-generation selections were predicted using best linear prediction (BLP) and using data from about 500 OP and CP progeny tests planted from the 1960's to 1990, generally in randomized complete block designs with row plots (White *et al*. 1996).

Because volume breeding values are meant to accurately predict genetic gains for a given progeny above unimproved controls under operational conditions, it is desirable to validate these predictions using a large set of realized gain trials established with large rectangular plots. Unlike row-plot or single-tree-plot trials, large rectangular-plot trials resemble operational planting conditions where all entries compete evenly across time (Foster 1989, Lambeth *et al*. 1994). However, rectangular plots have low statistical precision (Dhakal *et al*. 1996), and many trials are needed for reliable validation of *a priori* expectations.

Slash pine breeding value predictions have not been extensively validated using large rectangular plots. However, several studies have compared average realized and expected gains, especially in rust resistance. Comparisons of realized gain against conventional predicted values (Sohn and Goddard 1979), against 1988 BLP breeding values (Hodge *et al*. 1993) and against 1995 BLP breeding values (Lopez-Upton *et al*. 2000, Vergara *et al*. in review, Vergara *et al*. in preparation) have been conducted. One in-depth validation study in slash pine using data from 175 CP row-plot and single-tree-plot trials (Dhakal 1995) found that 1988 BLP breeding values in rust resistance and volume over-estimated the realized gains by 31% and 47%, respectively. These findings were used to adjust the 1995 BLP predictions (White *et al*. 1996).

The aim of this study is to validate the 1995 BLP breeding values for rust resistance and volumes using observed rust incidence and stand yield realized gains estimated for 187 seedlot-trial combinations from a total of 43 field trials established with large block plots. The specific objectives were to: 1) Validate the accuracy of rust resistance breeding value predictions (R50s values); 2) Validate the accuracy of individual tree volume breeding value predictions (BVVs); and 3) Assess the impact of rust hazard and site index on the accuracy of breeding values.

MATERIALS AND METHODS

Realized Genetic Gain Trials

The database for validating slash pine BLP predicted breeding values included realized gain trials planted in the Southeast with large rectangular plots of genetically improved

material and unimproved controls. The 43 trials were planted between 1977 and 1987 by the Best Management Practices study (5 BMP trials), CFGRP (19 trials), and the Plantation Management Research Cooperative at the University of Georgia (19 PMRC trials). All trials contained first-generation several types of OP slash pine progenies from unrogued or lightly rogued seed orchards (Table 1). Experimental design, age, location, site index, and rust hazard information of each trial are described by Vergara *et al.* (in review) for the BMP trials and by Vergara *et al.* (in preparation) for the CFGRP and PMRC trials.

Type of Progenies	# improved seedlots/trial	Unimproved controls	# Trials	# Reps	BreedingValue[1]	
					R50	BVV
Single OP-families (CFGRP trials)	19-22	UF checklot	3	3	43.9	12.3
	10-12	UF checklot	3	3-4	42.3	11.6
	9-10	None	4	2-4	42.4[2]	6.1[2]
Bulk seed orchard mixtures (CFGRP trials)	1-3	UF checklot and/or others	9	4-10	48.3	9.1
Mixture of 6 rust-resistant families (BMP trials)	1	Rust susceptible mixture	5	3	31.4	2.5
Mixture of 6 families + 1 OP-family (PMRC trials)	2	Unimproved bulk seedlot	19	1	39.3	15.3

Table 1. Slash pine progenies in 43 realized gain trials used to validate the 1995 BLP breeding values.

[1]Adjusted BLP-breeding values (White *et al.* 1996) in percentage, averaged across all seedlots from a specific type taking into account pollen background (R50=rust incidence breeding value and BVV=volume breeding value). [2]Value adjusted to the difference between each family and the breeding value of the family taken as the unimproved control.

Calculation of Realized Genetic Gains

Plot-level data were used to obtain least square means (LSM) for each improved seedlot (*I*) in each trial. Each record also had the LSM for unimproved value (*U*) averaged across all the controls in the trial. The final database included 187 records containing LSM for percentage rust incidence (RUST), average volume of living trees (TREEVOL), and mean annual increment (MAI, extrapolated to a per-area basis) for both improved and unimproved material. To compute RUST, data from the youngest measurement age were used, since the effect of mortality could bias the RUST estimations (Anderson *et al.* 1986, Schmidt and Allen 1997). For TREEVOL and MAI, data from the measurement age closest to rotation age were used in each trial. Additionally, the trial's rust hazard (RHAZ, estimated as RUST from the unimproved control(s)) and site index (SI, at 25 years as estimated from the data for the unimproved control(s)) were included in the final database (Vergara 2003).

Percentage of realized gain in rust resistance was calculated as the incidence on each improved seedlot, I, adjusted to the rust hazard level in each site, as follows. RUST on I was transformed to adjusted incidence (I50) using the hypothesis of proportional resistance (Hodge *et al*. 1993) through the equation I50=(I*50)/rust hazard, where I was average RUST on the improved seedlot and rust hazard was the RUST value on U, the unimproved control. Thus, I50=(I/U)*50, where I50 was the realized rust incidence adjusted to an environment in which unimproved material would have 50% rust incidence. Therefore, an I50 smaller than 50% means positive realized gain in rust resistance. I50s were estimated only for sites with a rust hazard greater than 15% (Lopez-Upton *et al*. 1999), because low rust incidence levels have small variances (White and Hodge 1987) and because the proportional resistance hypothesis is not applicable on low-rust-hazard sites (Hodge *et al*. 1993).

Realized gains for TREEVOL and MAI were estimated as percentage gains (G_TREEVOL or G_MAI=(($I-U$)/U)*100, respectively), and as the non-percentage difference between improved and unimproved material (DIF_TREEVOL or DIF_MAI= $I-U$ in m^3 and $m^3 ha^{-1} year^{-1}$, respectively).

<u>Predicted Breeding Values</u>

The first-generation R50s and BVVs currently used in slash pine were predicted in 1995 by the CFGRP using about 500 OP and CP progeny tests planted from the 1960's to 1990 (White *et al*. 1996). R50s were expressed as the predicted percentage of infection when unimproved material would have a 50% rust incidence (50% rust hazard environment); consequently, low values of R50 indicate high rust resistance. BVVs were expressed in percentage superiority above unimproved material.

To assign the correct predicted breeding value for every seedlot used in the validation, it was necessary to consider that improved seedlots (I) such as single families, family mixtures, or bulk-seed-orchard seedlots were obtained from wind-pollinated first-generation seed orchards. Thus, the seedlot's predicted breeding values were affected by pollen from other clones in the orchard and/or by pollen from external unselected populations. When reliable information was available, the seed orchard pollen contribution was calculated by averaging breeding values across the clones that were present at the time that the seed was collected, weighted according to the number of ramets of each clone. When information was not available, R50=50% and BVV=10% were used for the male contribution to the seedlot.

Pollen coming from external sources was considered to be undomesticated with R50=50% and BVV=0%, the same as unimproved controls (U). This contaminating pollen also affects the adjusted breeding value of an orchard wind-pollinated seedlot. Pollen contamination in fully productive seed orchards may be between 5% and 50% with values commonly from 20-40% in different pine species (Wang *et al*. 1960, Friedman and Adams 1985, El-Kassaby *et al*. 1989, Lai and Chen 1997). Thus, 30% pollen contamination was assumed in this study, and the computation of all adjusted breeding values reflected this value. For example, for seed from a single mother with

R50=35% and BVV=19%, in the seed orchard, the adjusted R50 would be 35% x 0.5 (mother contribution) + 50% x 0.5 x 0.7 (pollen from the seed orchard) + 50% x 0.5 x 0.3 (pollen from external sources) = 42.5%. Likewise, the adjusted BVV would be 19% x 0.5 (mother contribution) + 10% x 0.5 x 0.7 (pollen from the seed orchard) + 0% x 0.5 x 0.3 (pollen from external sources) = 13%, assuming an orchard in which the clones averaged R50=50% and BVV=10%. Henceforth, the adjusted R50s and BVVs for single-family, mixed-family, and bulkseed orchard collections are denoted simply as R50s and BVVs, respectively.

Validation of Breeding Values Using Regression Analysis

If parental BLP-breeding values are precise and accurate, offspring performance should be directly predictable by the adjusted breeding values of each seedlot evaluated in realized gain trials (White and Hodge 1989, Mrode 1996). To validate current breeding values predicted by the CFGRP (White *et al.* 1996) and to examine the influence of site index and rust hazard on the realized gains, multiple regression analyses (Rawlings *et al.* 1998) were conducted with PROC GLM in SAS (SAS 1990). Realized rust infection in percentage (I50), TREEVOL realized gain in percentage (G_TREEVOL), and MAI realized gain in percentage (G_MAI) were regressed against BVV, R50, SI, RHAZ, and the two, three, and four way interactions among those variables. Also, variables DIF_TREEVOL (TREEVOL *I-U* difference in m^3) and DIF_MAI (MAI *I-U* difference in m^3 ha^{-1} year^{-1}) were regressed against all variables except BVV, which was replaced by predicted deviation in volume between *I* and *U* in m^3, (DEV using age 15 data with mean individual volume = 0.108 m^3, White *et al.* 1996). Each regression using growth-related variables as dependent variables had 187 observations, one for each seedlot-trial combination from 43 trials with 146 single-family lots, 24 mixed families, and 17 bulk seed orchard collections. Regressions using I50 as dependent variable had only 140 observations, with 122 single-family lots, 10 mixed families, and 8 bulk seed orchard collections, because seedlots growing in trials with rust hazard <15% were not used in the analysis.

"Backwards elimination" stepwise regression (Rawlings *et al.* 1998) was used to choose the most parsimonious model as follows: 1) Start with the full model (including all main effects and their interactions) and drop non-significant effects of the highest order interactions; 2) Run the reduced model dropping all non-significant terms; and 3) Continue this process until all terms were significant and the model had a reasonable biological interpretation. Type III sums of squares were utilized to define non-significant effects with *p*>0.1. Adjusted R^2 (adj-R^2) was used to compare models with different number of parameters (Rawlings *et al.* 1998).

RESULTS AND DISCUSSION

Validation of R50s

The regression analysis relating the observed I50 values for each seedlot to their predicted R50s and other site factors provided a final model that included R50, BVV,

RHAZ, and the interaction BVV x RHAZ with an adj-R^2=0.386. However, BVV and BVV x RHAZ effects were not biologically interpretable, meaning that graphical analyses did not reveal any logical or important trends. So, a new backwards elimination round was conducted starting with a full model excluding BVV. The new final model I50=1.169*R50-0.1839*RHAZ was the most parsimonious explanation for I50 with an adj-R^2=0.361, similar to that obtained for the model including the BVV effects. In this new model, the general intercept was not significantly different from zero (*p*=0.3135). Therefore, it was dropped and the intercept changed across different levels of rust hazard, although having the same slope among those levels (Figure 1a).

In the final model, R50s alone did not adequately predict observed I50s. Rather, RHAZ was required to predict R50 in different rust hazard environments (Figure 1a). Also, the slope of 1.169 was significantly different from the expected value of 1.000 if R50s unbiasedly predict field rust resistance expressed as I50. So, the accuracy of predicting field performance by BLP R50 values is compromised by rust hazard of the site and a slight over prediction of I50 by R50. Interestingly, the most accurate predictions occurred when R50 values were near the same rust hazard level. For example, R50s between 15 and 30% predicted accurately the I50 values at rust hazard = 20% but were biased at 60%. Similarly, R50s between 60 and 70% were accurate in predicting I50s at rust hazard = 60% but biased at 20% (Figure 1b). I50 values are calculated by adjusting a given rust incidence to a 50% rust hazard environment using the hypothesis of proportional resistance (Hodge *et al*. 1993), and the accuracy of R50 predictions rests on the reliability of this hypothesis. Therefore, these results are evidence that rust resistance is not completely proportional across different rust hazard environments. The difference between predicted and realized rust incidence values (R50=31.4% and I50=21.9%) found by Vergara *et al*. (in review) in the BMP trials could be explained in part by this bias, where the R50=31.4% might be biased in predicting the I50, under predicting rust resistance by approximately 3%, since the average rust hazard was 43% in the BMP trials (Figure 1).
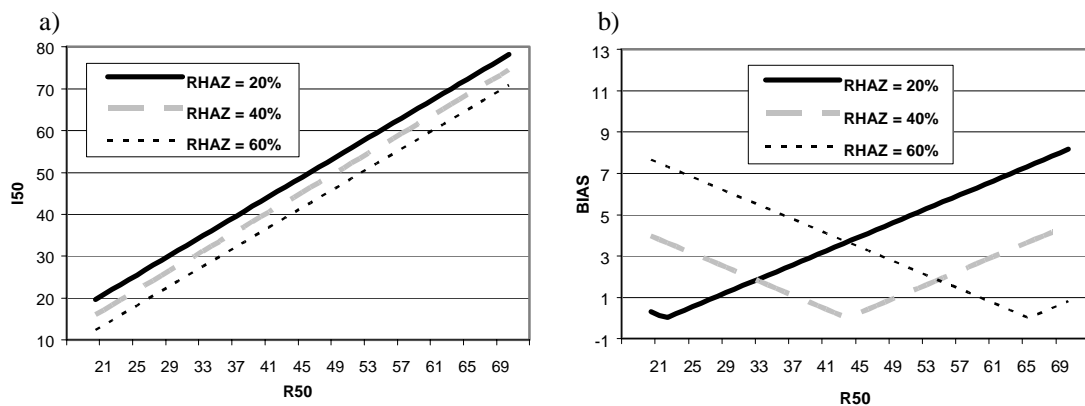

Figure 1. Relationship between realized (I50) and predicted (R50) percentage rust incidence at three levels of rust hazard (RHAZ) representing approximately one standard deviation below the mean (20%), the mean (40%), and one standard deviation above the mean (60%) rust hazard for the 43 trials: a) Regression of I50s on R50s according to I50=1.169*R50-0.1839*RHAZ (adj-R^2=0.361), b) Bias of the predictions, measured as the absolute difference between R50 and I50.

Additionally, to validate the general predicting quality of R50s, the relationship between R50 and I50 was assessed through the simple linear regression model $I50 = \beta_0 + \beta_1 * R50$. The analysis showed a moderately positive correlation between R50 and I50 (r=0.563). This model's low adj-R^2=0.317 indicated that the precision for predicting I50 values was low and influenced by factors other than R50s; however, the highest adj-R^2 obtained from the multiple regression analysis was 0.386, indicating that other unknown factors may be involved in rust resistance and/or that a higher experimental precision must be achieved by using more and better replicated trials. Although the I50 equation (I50=-13.18+1.29*R50) had intercept and slope significantly different from the expected values for accuracy (β_0=0 and β_1=1, respectively), the predictions were reasonably unbiased (Figure 2). I50 was slightly over predicted for R50s smaller than 45%, meaning R50 predicts that a seedlot will get slightly more rust than actually observed, and slightly under predicted for seedlots with R50s greater than 45%. In other words, at R50 <45%, R50s predict seedlots to be slightly more susceptible than observed and vice versa above 45%. However, the bias was never more than a few percent.

In general, most studies comparing predicted and realized rust incidence in slash pine have been done across different levels of rust hazard, and most of them have demonstrated that average I50 values are very well predicted by R50s, with differences always smaller than 4% (Sohn and Goddard 1979, Hodge *et al*. 1993, Lopez-Upton *et al*. 2000, Vergara *et al*. in review, Vergara *et al*. in preparation). These previous studies agree with the general results found here. Even though the 1995 R50s (White *et al*. 1996) seem reasonably accurate, adding weight to the hypothesis of inflated variances in the 1988 R50s (Dhakal 1995), further research must inspect the behavior of rust resistance at different levels of rust hazard to confirm or to correct the hypothesis of proportional resistance.
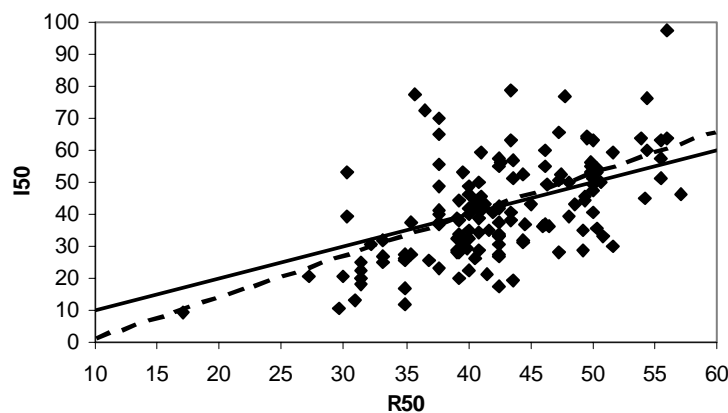

Figure 2. Comparison of observed (I50) versus predicted (R50) percentage rust incidence. The solid line represents the ideal relation between realized and predicted values with 100% accuracy. The dashed line is the actual regression of I50s on R50s according to I50=-13.18+1.29*R50 (adj-R^2=0.317).

<u>Validation of BVVs</u>

When G_TREEVOLs were regressed against BVV and other regressors including RHAZ, SI, R50, and all two, three, and four-way interactions, the final model including only significant terms had 12 effects including R50 and some of its interactions with a low adj-R^2=0.221. Because this model did not have a reasonable biological interpretation, a new full model was analyzed deleting R50 and all its interactions. In this new analysis, the most parsimonious model was:

G_TREEVOL=5.744*BVV + 0.0645*SI - 0.23202*BVV*SI
       with an adj-R^2=0.214.

This adj-R^2 was similar to the final model obtained using all the variables, showing either that the terms that were dropped, while statistically significant, contributed very little toward explaining the variability of G_TREEVOL or that BVV and R50 were colinear in explaining the variation of G_TREEVOL (i.e., high covariance between BVV and R50).

Using the latter model, BVV predicted G_TREEVOL very well at some SIs, but not well in others (Figure 3a). To determine the slope of each regression line, the model was solved for SI=18, 21 and 24 m (approximately –1, 0 and +1 standard deviations from the mean SI of 43 trials), resulting in the following partial models;

    When SI is 18 m, G_TREEVOL=1.16+1.57*BVV,
    When SI is 21 m, G_TREEVOL=1.35+0.87*BVV, and
    When SI is 24 m, G_TREEVOL=1.55+0.17*BVV.

The best predictions were at SI=21m, with a slope close to 1 (0.87). At low SIs (i.e., SI=19m), BVV underestimated G_TREEVOL, and at high SIs (i.e., SI=24m) BVV strongly overestimated G_TREEVOL (Figure 3b). Consistently low G_TREEVOLs for good sites, regardless of seedlot BVV, might be related to having selected trees in low SI stands in the 1950s through 1960s, as the selections may have been adapted to restricted nutritional conditions and high competition. When growing in near optimal conditions, with fertilizer, mechanical soil preparation and/or herbicide, the genetic advantages of the selected trees may not be displayed.

Another possible explanation is the scale effect of measuring gains in percentage. If there is a constant gain in TREEVOL across site indices, the percentage gain would be smaller when site indices are larger and larger in poor site indices, as is observed in Figure 3a. To test this idea, DIF_TREEVOL (TREEVOL *I-U* difference in m^3) was regressed against same former variables, after replacing BVV with DEV (predicted deviation in volume between *I* and *U* in m^3). In the final model, DIF_TREEVOL=0.5076*DEV (adj-R^2=0.048), SI was not significant in explaining absolute gains and therefore confirmed some scale effect in measuring gains as a percentage.
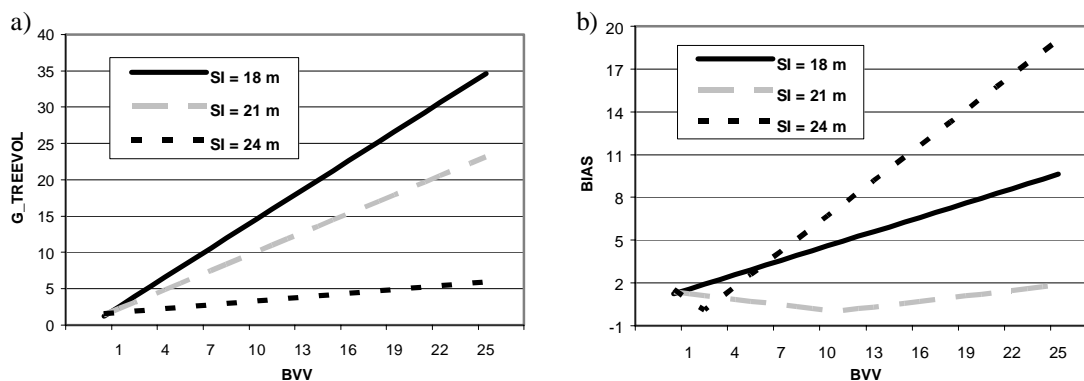
Figure 3. Relationship between realized gain in individual tree volume (G_TREEVOL) and predicted volume breeding values (BVV) at three levels of site index (SI) representing approximately one standard deviation below the mean (18m), the mean (21m), and one standard deviation above the mean (24m): a) Regression of G_TREEVOL on BVV according to G_TREEVOL=5.744*BVV + 0.0645*SI - 0.23202*BVV*SI (adj-R^2=0.214), b) Bias of the predictions, measured as the absolute difference between G_TREEVOL and BVV.

Since all regressions of realized gains in individual tree volume had low adj-R^2 values, two factors that could confound both the assessment of individual tree volume gains and the prediction of BVV through BLP should be considered: 1) since rust mortality favors individual tree volume by providing more space to the living trees, BVVs from row-plot data could be impacted by a seedlot's rust resistance and observed realized gains in block plots could also be impacted, and 2) the measurements of G_TREEVOL from the 10 to 18 years used in the analysis could confound realized gains if age affects performance.

The full model regression of realized gains in stand yield (G_MAI) on BVV and RHAZ, SI, R50, and all two, three, and four-way interactions produced an extremely low adj-R^2=0.07. As for G_TREEVOL, the DIF_MAI (MAI *I-U* difference in $m^3\,ha^{-1}\,year^{-1}$) was regressed against the same regressors (except replacing BVV by DEV), and the adj-R^2 was again very low. Thus, these results provide little insights about the relationships among the studied variables.

Most of the variation found in G_MAI in this study is likely due to experimental noise. Realized gains in MAI were not significantly influenced by any of their most probable predictors such as BVV, R50, RHAZ, or their interactions. Mortality not due to rust infection, such as from poor microsites, plantation effects, lightning, and other causes probably greatly influenced the realized gains in stand yield. With the database analyzed here, it was not possible to validate or determine if the BVVs are accurately predicting stand yield.

Variation in microsites can greatly affect within-replication uniformity in field trials. To compare the volume performance of seedlots, progeny tests should have a replication size smaller than 0.1 ha (Matheson 1989). These realized gain trials usually exceeded this limit, and replication numbers were low. Both problems clearly contributed to the

excessive noise found in this study. We suggest trials with medium-size plots (i.e., 36 to 49 total trees, 16 to 25 measurement trees), no more than six entries per replication, and at least 10 replications to overcome these problems. However, a simulation study should be done before to estimate a sufficient design to maximize the amount of information obtained and statistical precision.

CONCLUSIONS

Validation of predicted rust resistance breeding values by comparing expected to realized rust incidence (R50 to I50) in 43 trials suggests that the 1995 R50s are reasonably accurate, although they are not very precise. Also, there is evidence that higher rust incidence is predicted by R50 in low rust hazard sites than in high rust hazard sites, which suggests that the hypothesis of proportional resistance should be revised. Most of the known variation in rust incidence can be assigned to the R50s; however, the influence of rust hazard levels should not be ignored when making new predictions.

Validation of BVVs was difficult using either individual tree volume or stand yield realized gains in large rectangular plots. BVVs were better correlated with realized gains in individual tree volume than with realized gains in stand yield. Realized gains in individual tree volume were predicted imprecisely by BVVs, with very different accuracies depending on the level of site index, being most accurate for seedlots tested in trials with average site indices (approximately 21 m) but less accurately for trails with low or high site indices.

The large amount of noise associated with stand yield made it impossible to validate volume predictions using realized gains in stand yield. This experimental error was likely due to mortality from rust infection and other sources, along with the inherent imprecision of large rectangular plots. This last issue could be improved by optimizing trial design.

ACKNOWLEDGMENTS

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