SPACING AND FAMILY AFFECT FUSIFORM RUST INCIDENCE IN LOBLOLLY PINE AT AGE 17

Joshua P. Adams, Samuel B. Land, Jr., and Howard W. Duzan, Jr.[1]

Abstract—The effects of fuel reduction treatments, fire and mechanical understory removal (alone and in combination), were examined to determine changes in abundance and composition of woody regeneration in the Southern Appalachian Mountains. While mechanical treatment alone (M) had little effect on seedling density, burning (B) and mechanical treatment + burning (MB) produced a significant increase. Sapling density was greatly reduced in MB immediately following treatment, but 2 years later abundant sprouting was observed. Regeneration of shade-tolerant species was encouraged in M and B, whereas MB favored shade-intolerant species. Reponses of dominant tree species varied by treatment based on species' reproductive strategies. Red maple increased in B primarily through seed germination, whereas MB promoted sprouting. Yellow-poplar (*Liriodendron tulipifera* L.) showed rapid initial response to B and MB, but seedlings that germinated after fire in B did not persist. In contrast, seedlings germinating following treatment in MB thrived, quickly growing into the saplings. B and MB contained more than twice as many oak (*Quercus* spp.) seedlings recorded pretreatment, with the majority sprouting from remaining stems; however, a large acorn crop 1 year after burning contributed to seedling numbers observed the third year posttreatment. Shrub abundance was greatly reduced by M and MB, but sprouting in the understory layer was observed in all treatment units. Reducing fuel using techniques described here can affect woody species composition and potentially change development of mixed-oak forests in the Southern Appalachian Mountains.

INTRODUCTION

Fusiform rust disease is caused by the fungi *Cronartium quercum* (Berk) Miyabe ex Shirai f. sp. fusiforme. This disease affects loblolly pine (*Pinus taeda* L.) as well as slash pine (*P. elliottii* Englm.). Due to the detrimental effects this disease can have on these species, genetic improvement has been extensively studied in which family performance, geographic location effects, and interactions have been considered.

Genotype-by environmental interactions have been considered negligible in several studies (Hodge and others 1993, Kinloch and Stonecypher 1969, McKeand and Amerson 1999). On the other hand, others have shown that various families have differing responses to rust inoculums from various regions (Power and others 1977, Snow and others 1975), and thus interaction effects should be considered. Also, current molecular approaches have led to the identification of a few major genes which control resistance (Kubisiak and others 2004, Wilcox and others 1996). The possibility of the few resistance genes being overcome by a variant of the fungus from a different location has led to general recommendations of deploying several half-sib families in either pure or mixed family deployment (Bridgewater and others 2005, McKeand and others 2003).

A loblolly pine stand 17-years old is approaching, if it has not already reached, stand volume allowing a merchantable harvest. This study investigates virulence presence in living 17-year stems in half-sib loblolly pine families. Family and environmental effects are studied. However, environmental effects in this study are effects of scale (i.e., spacing). Better control of infection may be possible through use of spacings that allow the trees to maintain greater vigor. Furthermore, deployment effects will be analyzed to determine if decreased infection can be achieved through deployment of mixed families or single pure family plots.

METHODS

Plant Material and Experimental Design

Containerized seedlings of eight open-pollinated families in North Carolina (NC) and one open-pollinated "genetic check" (bulk seed lot) from east-central Mississippi (MS) and west-central Alabama (AL) were provided by Weyerhaeuser Company. Seedlings were planted during April 22 to May 7, 1985 at two sites on the John Starr Memorial Forest (Mississippi State University school forest) in Winston County located in east-central MS. The experimental design was a randomized complete block with four replications at each site. The two sites were an old field and a cutover site-prepared area. Treatments were arranged in split-split plots, where each rep was split into three spacings (5 by 5, 8 by 8, 10 by 10 feet). Each spacing was split into a mixed family subplot and a pure family subplot. The pure family subplot contained nine sub-subplots, each having one family or check. A single or double border row was planted around each sub-subplot. The interior trees of each pure family sub-subplot covered an equal area of 0.0367 acres so that there were 64, 25, 16 trees in each plot of the 5 by 5, 8 by 8, 10 by 10 feet spacings, respectively. The mixed family subplot did not contain the check and covered an area equal to the eight sub-subplots in the pure family subplot. In this mixed deployment, families were randomly planted while maintaining equal representation of each family. Survival, d.b.h., and total height were recorded at ages 5, 9, 13, and 17 and presence of stem fusiform rust galls were recorded at age 17.

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[1] Graduate Student and Professor, respectively, Mississippi State University, Starkville, MS; Research Manager, Weyerhaeuser Co., Columbus, MS.

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ANALYSIS

Presence or absence of fusiform rust galls per stem is a binary trait (0 or 1) and cannot be analyzed traditionally because it violates the assumption of residual normality and homogeneity in general linear modeling (GLM). Thus, this analysis used stems infected at age 17 divided by live stems at age 17 (percent infection) in each plot as the response variable. Use of this measurement in conjunction with the large sample size satisfies the assumptions and allows infection to be gauged on a stand level. Analysis of family and spacing differences was conducted only using pure family plots and the mixed model used was:

$$y_{j(i)l} = \mu + \tau_i + \beta_{j(i)} + \alpha_k + \phi_l + (\tau\alpha)_{ik} + (\tau\phi)_{il} + (\beta\alpha)_{j(i)k} + (\beta\phi)_{j(i)l} + (\alpha\phi)_{kl} + (\tau\alpha\phi)_{ikl} + (\beta\alpha\phi)_{j(i)kl} \begin{cases} i = 1,2 \\ j = 1,2,3,4 \\ l = 1,2,\ldots,9 \end{cases} \tag{1}$$

where y was the percent infection, μ was the overall population mean, τi was the effect of the ith site, βj(i) was the effect of the jth replication nested in the ith site, αk was the fixed effect of the kth spacing, and was the random effect of the lth family. Duncan's New Multiple Range Test was used to test for differences among individual families. Correlations between family means for growth traits (i.e., height, d.b.h., stem volume, and stand volume) and family infection percentages were calculated using Pearson's Correlation Coefficient. Analysis of deployment used the simpler fixed effects model:

$$y = \mu + \alpha_i + \beta_{j(i)} + \tau_k + \alpha\tau_{ik} + \beta\tau_{j(i)k} + \lambda_l + \alpha\lambda_{il} + \beta\lambda_{j(i)l} + \delta_m + \alpha\delta_{im} + \beta\delta_{jm} + \alpha\beta\delta_{ijm} + \tau\delta_{km} + \alpha\tau\delta_{ikm} + \beta\tau\delta_{j(i)km} + \lambda\delta_{lm} + e \begin{cases} i = 1,2 \\ j = 1,2,3,4 \\ k = 1,2,3 \\ l = 1,2 \\ m = 1,2,\ldots,8 \end{cases} \tag{2}$$

where y was percent infection, μ was the overall population mean, α was the effect of the ith site, β was the effect of the jth replication nested within the ith, τk was the kth spacing, λl was the effect of the lth deployment, and δm was the effect of the mth family. Three-way and four-way interactions between deployment-by-family and site, replication, and spacing were not significant and were subsequently pooled into the sub-subplot error term.

RESULTS AND DISCUSSION

Family and Spacing Effects

Average stand infection percentage per plot was 14.9 percent (s.d.= 0.05). A significant difference (p-value<0.0001) existed between the two sites where the old field had 18.3 percent infection and the cutover, site-prepared area had 11.5 percent infection. Both family and spacing independently affected the percent infection at the alpha=0.05 level (p-values= 0.0032 and 0.0087 respectively). Range among families was substantial, with a low of 8.4 percent for family NC8 and a high of 27.3 percent for family NC3 (table 1). Families such as NC2 and NC3 and the Check have been shown in previous studies (Adams and others 2006, Land and others 2004) to be relatively marginal in regards to stand volume and average height. In this study, these families show a greater infection percentage further decreasing their value. Interestingly, greater individual stem growth rates have generally been associated with greater infection incidences (Burton and others 1985, Eaton and others 2006, McNab and others 1990, Schmidt 1998). This has been attributed to rapid growth, in which new, rapidly dividing cells are more susceptible to fungal infection. On a tree level, this study supports this. Stems that produce large individual tree volumes (e.g., families NC2 and NC3 (Adams and others 2006)) had greater infection prevalence. However, on a stand level the fastest growth, greatest volume producing families had significantly lower infection percentages. Families NC4, NC7, NC1, and NC8 were all statistically the same in regards to infection percentage and comprised four of the top five stand volume producing families (Adams and others 2006).

Table 1—Duncan's test of ranked family and spacing infection percent means at age 17 for pure family deployment

Family	Mean[a] (%)	Grouping[b]
NC3	26.3	A
Check	21.7	AB
NC2	20.9	AB
NC6	17.6	B
NC5	16.7	B
NC4	10.6	C
NC7	10.5	C
NC1	10.3	C
NC8	8.4	C

Spacing (feet)	Mean[a] (%)	Grouping[b]
10x10	17.6	Y
8x8	15.7	YZ
5x5	13.7	Z

[a] Mean infection percent calculated from (# live trees infected at age 17)/(# live trees at age 17).

[b] Means followed by the same letter and case are not significantly different at the 0.05 probability level.

Spacing effect does seem to support the supposition that rapidly growing trees are more susceptible to infection. The 10 by 10-foot spacing, which provides the greatest potential for rapid tree growth, had the highest infection percentage (table 1). Percent infection decreased as the spacing size decreased from 8x8-foot to 5 by 5-foot spacing. Though this effect was significant, the difference between the 10 by 10-foot and the 5 by 5-foot spacing was only 3.9-percent. However, closer spacing may have less prevalence simply because infected trees have died prior to age 17.

Correlations between age 17 infection percentage and growth traits generally became significant with age. At age 13, height and stand volume were negatively correlated with infection (table 2). On the other hand, diameter growth was positively correlated with increases in infection percentage. Correlation with diameter became significant at the early age of five and remained strong through age 17. Tree volume was only significant at age five but was not strongly correlated after that. This is probably due to the inverse correlations that diameter and height, both used for calculation of stem volume, had with percent infection. The reverse correlations exhibited by diameter and height with infection percentage seem to counter one another. However, once survival is taken into account in calculating stand volume, the more vigorous trees at the stand level had fewer rust incidences. In this study, families with the largest diameters achieved this at the expense of surviving stems. Thus these families were, relatively sensitive to competition and had more prevalence of rust among surviving stems at age 17 while less competition-sensitive families produced greater stand volume and had fewer incidences.

Mix-family Versus Pure-family Deployment

Analysis with consideration for deployment types (i.e., mixed-family deployment and pure-family deployment) showed that there was significant interaction (p-value=0.05) between deployment type and family for rust infection. Most interaction (change in rank) was associated with the change in family NC1. Families NC2 and NC3, which had the highest infection percentages in the previous analyses of pure family deployment, had infection percentages significantly lower when deployed in a family mixture (fig. 1). Families NC5 and NC6, which also had high infection percentages in pure-family blocks, had decreased infection, though not significant, in the mixed deployment. Families with fewer rust incidences in pure-family subplots (e.g., NC1 and NC8) had slightly more, though not significant, infection in the mixed-deployment.

On the subplot level (i.e., mixed- versus pure-family deployment subplots), rust infection percentage at the a priori 0.05 alpha level was not significantly different by deployment. However, a p-value of 0.10 makes this factor at least highly influential with average infection percentage 14.27 percent and 15.16 percent for the mixed- and pure-family deployment subplots respectively. This lends support for the use of mixed-family deployment, not just as an added genetic safeguard, but as a direct way to minimize stand rust prevalence at age 17. Conversely, an argument could be made that less prevalence at age 17 is a function of more rust associated mortality (RAM) through the life of the mixed stand. However, overall survival across spacings and families in both deployments was 59 percent, and does not support the possibility of increased RAM.

Table 2—Pearson's Correlations Coefficients between infection percentage[a] at age 17 and growth traits for pure family deployment

Growth Trait	Age-5	Age-9	Age-13	Age-17
Height	0.06	-0.13	-0.24**	-0.15*
Diameter	0.23*	0.15*	0.12*	0.15*
Tree Volume	0.21*	0.11	0.08	0.11
Stand Volume	0.00	-0.13	-0.29**	-0.28**

* =Coefficient is significant at the 0.05 level.

** =Coefficient is significant at the 0.01 level.

[a] Mean infection percent calculated from (# live trees infected at age 17)/(# live trees at age 17).

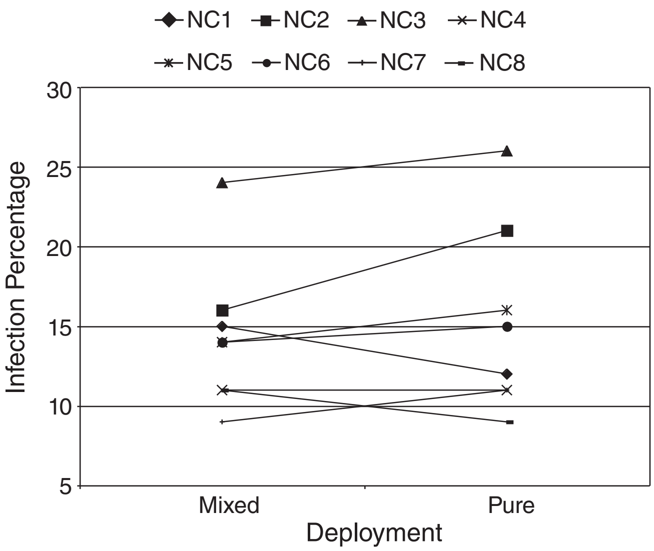

Figure 1—Infection percentage change across deployment in a 17-year old pine stand.

The actual cause of the rust prevalence difference by deployment is not clear and two possible factors could be the cause. First, spatial influences could be at work in which more resistant families act as buffers for less resistant trees. A second theory is that rust infection in this study is related to the domination or suppression of various families. The fastest growing (stand volume) families in this study did not exhibit increased infection often correlated with faster growth. These are also the families that exhibited dominant ideotypes (Adams and Land 2006). This domination caused the suppression and decreased growth rates of the more susceptible families. The lack of rapid growth in susceptible families may afford some protection from infection. Still, alternative combinations of families are needed before one or both of these causes can be accepted. Alternative combinations are also needed to test if a difference would exist at all if mixed-family deployment included families that all had greater rust resistance.

CONCLUSIONS

Loblolly pine genetic improvement for stand growth does not preclude breeding toward fusiform resistance. Families with high individual-tree growth rates were associated with greater infection rates, while faster growing families on a stand level in this study did not suffer from increased prevalence of rust at age 17. Decreased density due to spacing did result in more infection among those trees living at age 17. Thus, selection of phenotypes based on individual-tree traits may be adverse for rust resistant selections and losses in quality must be weighed against gain in volume when using wider spacing. While significant, the relatively small decrease in infection using a denser spacing does not warrant alteration of spacing strategies when, alternatively, emphasis can be placed on deployment of fast-growing, resistant genotypes in conjunction with continued promotion of fast growth with silvicultural practices.

Deployment of several families has generally been recommended to ensure adequate genetic diversity so that resistance in not overcome by the fungi. This study supports use of multiple families and highlights that mixed family deployment, in lieu of planting several families in blocks, is directly beneficial for the decrease of rust incidences at age 17. Still, mechanisms that are causing superiority of the mixed deployment are unknown. Spatial relationships among the trees or stand domination/suppression dynamics may be at work.

Impact of family, spacing, and deployment on rust prevalence by this analysis reflect the effect on living stems at age 17. This represents a time at which a stand could have a merchantable harvest. However, variations of both RAM and density related mortality among these factors early in stand history may be masking variation effects. Families or spacings with high early mortality may cause age 17 infection percentages to be lower than the actual susceptibility rate for that treatment. Further studies across stand history will be needed to investigate how age/mortality impacts the final outcome which is presented here.

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