

GENOTYPE X MINERAL NUTRIENT INTERACTIONS  
WITH FUSIFORM RUST RESISTANCE IN SLASH PINE

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Abstract.--The relationship of N, P and K availability to fusiform rust incidence in a resistant and a susceptible family of slash pine was examined in greenhouse pot cultures. Mean rust incidence for all treatments of the resistant family was 23.3% and was not significantly altered by application of N, P or K. A mean rust incidence of 53.8% was observed for all treatments of the susceptible family, with the addition of both P and K significantly increasing rust incidence. The P effect accounted for about 40% of the total variation in rust incidence due to treatment. Nutrient supply did not alter the relative resistance ranking of either family. Tissue nutrient concentrations in both families varied significantly with treatment. However, changes in rust incidence were not related to tissue nutrient content.

Additional keywords: Pinus elliottii, Cronartium fusiforme, forest diseases.

INTRODUCTION

Studies on mineral nutrient relationships with fusiform rust in southern pines have indicated that increased N and/or P supply generally resulted in increased rust incidence, especially if a nutrient deficiency existed prior to fertilization (Hollis et al., 1975; Rowan and Steinbeck, 1977). These studies were conducted on bulked seed lots from open pollinated pines and did not consider the host genotype as a source of variation in response to treatment.

Tree improvement programs have identified a substantial number of rust resistant families, some of which are likely to be planted in areas where fertilization practices are operational. This study was conducted to determine the effects of mineral nutrition on the infection of resistant and susceptible families of slash pine (Pinus elliottii Englem.) with fusiform rust (Cronartium fusiforme Hedgc.); and specifically to determine if resistant families would be predisposed by fertilization to fusiform rust.

METHODS AND MATERIALS

Seeds from two half-sib families of slash pine--one resistant and one susceptible--were obtained from the Florida Cooperative Tree Improvement Program. The seed was germinated in moist vermiculite and four seedlings were transplanted into 6 X 10 cm well drained plastic pots containing acid washed pure quartz sand. The seedlings were watered twice weekly with deionized water for four weeks prior to the initiation of nutrient solution application.

The effects of N, P and K fertilization and genotype were tested in a replicated 3 X 2 X 2 factorial experiment. Each family X nutrient treatment contained a total of 48 seedlings.

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The three elements were supplied in two concentrations, which were considered to be minimal and optimal concentrations for each. Nitrogen was supplied as:  $\text{NHO}/\text{NH}_4\text{NO}_3$  (80  $\text{NH}_4$ :20  $\text{NO}_3$  w/w) at 50  $\mu\text{g}/\text{ml}$  or 100  $\mu\text{g}/\text{ml}$ ; phosphorus as  $\text{NaH}_2\text{PO}_4$  at 5  $\mu\text{g}/\text{ml}$  or 25  $\mu\text{g}/\text{ml}$ ; and, potassium as  $\text{KCl}$  at 50  $\mu\text{g}/\text{ml}$  or 150  $\mu\text{g}/\text{ml}$ . The basic nutrient solution applied to all seedlings contained: 0.5  $\mu\text{g}/\text{ml}$  Mn as  $\text{MnSO}_4$ , 10  $\mu\text{g}/\text{ml}$  Mg as  $\text{MgSO}_4$ , 7  $\mu\text{g}/\text{ml}$  Ca as  $\text{Ca}(\text{NO}_3)_2$ , 2.5  $\mu\text{g}/\text{ml}$  Fe as Fe-EDTA, 0.2 g/ml B as  $\text{H}_3\text{BO}_3$ , 0.03  $\mu\text{g}/\text{ml}$  Cu as  $\text{CuSO}_4$ , 0.03  $\mu\text{g}^2/\text{ml}$  Zn as  $\text{ZnSO}_4$ , and 0.007 g/ml Mo as  $\text{NaMoO}_4$ . Nutrient solutions were applied twice weekly in 50 ml aliquots. The day prior to treatment each pot was flushed with an excess of deionized water.

Five weeks following the initiation of nutrient applications, two seedlings were harvested from each pot for tissue analysis. The remaining seedlings which were of uniform height (about 12 cm) were inoculated with a spray suspension of basidiospores at about 50,000 spores per ml (Matthews and Rowan, 1972). Nutrient treatment was continued on a twice weekly basis.

Six months following inoculation, rust incidence was assessed and the seedlings were harvested. Healthy and diseased seedlings were separated and pooled within treatments and families for analysis. Foliar contents of N, P and K were determined as previously reported (Hollis et al., 1975).

Statistical significance was determined by analysis of variance and paired comparisons.

#### RESULTS AND DISCUSSION

The influence of mineral nutrition on fusiform rust incidence in susceptible and resistant families of slash pine is presented in Table 1. Mean rust incidence for the resistant and susceptible families was 23.3% and 53.8%, respectively. The susceptible family was infected to a significantly greater extent than the resistant family in all treatments and no change in the relative resistance ranking of either family was observed.

Table 1.--Percents of slash pine seedlings infected with fusiform rust as a function of family and N, P, K application

| Element and Rate (PPM) | Resistant Family <sup>a/</sup> | % | Susceptible Family |
|------------------------|--------------------------------|---|--------------------|
| N - 50                 | 20.2 $\pm$ 4.1 A               |   | 51.8 $\pm$ 3.1 C   |
| N - 100                | 26.4 $\pm$ 3.5 A               |   | 55.8 $\pm$ 5.1 C   |
| P - 5                  | 21.4 $\pm$ 3.8 A               |   | 47.4 $\pm$ 4.1 C   |
| P - 25                 | 25.2 $\pm$ 4.1 A               |   | 60.1 $\pm$ 3.0 E   |
| K - 50                 | 26.8 $\pm$ 3.7 A               |   | 49.9 $\pm$ 4.0 C   |
| K - 150                | 19.9 $\pm$ 3.9 A               |   | 57.6 $\pm$ 4.1 D   |

<sup>a/</sup> Test means statistical significance: within family, same letter = NS; one letter difference,  $P = .05$ ; two letters different,  $P = .01$ ; between family means different at  $P \geq .01$ .

Significant increases in rust incidence due to the application of high amounts of P and K were observed for the susceptible family; whereas, rust incidence in the resistant family was not significantly affected by nutrient application. No significant change in rust incidence in response to N addition was observed in either family. Previous reports (Rowan and Steinbeck, 1977; Schmidt et al., 1972) indicated that application of high amounts of N and/or P generally resulted in a significant increase in rust incidence. However, such studies used open pollinated families representing a broad spectrum of genotypes. Where specific families are used a more sensitive test of response to treatment should be expected due to the reduction in genetic variability.

Approximately 40% of the total variation in rust incidence in the susceptible family due to treatment was accounted for by the addition of P (Table 2). This substantiates previous reports that P availability has a profound influence on the incidence of rust in susceptible families (Schmidt et al., 1972; Hollis et al., 1975). The results of this study also indicate a probable cause for concern regarding the use of P fertilizers on planting stock, not selected for rust resistance, in areas where the potential for increasing rust incidence is great, i.e. where susceptible oaks are abundant.

Table 2.--Percentage of total variation in rust incidence accounted for by each significant source of variation. a/

| Source of Variation | Resistant Family  | Susceptible Family |
|---------------------|-------------------|--------------------|
| N                   | N.S. <sup>b</sup> | N.S                |
| P                   | N.S               | 39.92 **           |
| K                   | N.S               | 14.73 *            |
| N X P               | N.S               | N.S                |
| N X K               | N.S               | N.S                |
| P X K               | N.S               | N.S                |
| <sub>2</sub> NXPXK  | N.S               | 32.18 **           |
| R - Treatment       | 0.571 N.S         | 0.862 **           |

a/

Percentage of total variation for each significant source calculated by dividing source mean square with sum of treatment mean squares. The quantity then being multiplied by 100.

Significance determined by ANOVA: N.S. = nonsignificant; \*, P = 0.05; \*\*, P = 0.01.

The addition of K produced contrasting results (Table 1). A significant increase in rust incidence in the susceptible family accounted for about 15% of the total variation due to treatment (Table 2). Such a response has not been previously reported for southern pines. In contrast **a decrease in**

rust incidence, albeit not significant, was observed in the resistant family due to K application. The wide range of variability in response to K between families expressed here points up the need for further efforts using genetic controls in studies of rust response to mineral nutrition.

Shoot nutrient content was examined at time of inoculation, and in healthy and diseased seedlings 6 months after inoculation. This was an attempt to determine whether shoot nutrient content at time of inoculation, or after symptom expression, had any direct relationship with the magnitude or direction or rust incidence response. Total shoot nutrient content at time of inoculation, for all three elements, significantly increased with rate of application for both families (Tables 3, 4, 5). However, only the addition of P and K to the susceptible family produced a significant increase in rust. This agreed with the findings of Rowan and Steinbeck (1977) that tissue nutrient content cannot be correlated with susceptibility to rust. The small differences between families in initial tissue concentration of the three elements also indicated that tissue susceptibility was not directly related to the absolute quantity of inorganic nutrients present.

Table 3.--Effect of N application on shoot nutrient content in seedlings of resistant and susceptible, half-sib, families of slash pine. a/

| Seedling Condition <sup>b/</sup> | Resistant Family         |             | Susceptible Family |             |
|----------------------------------|--------------------------|-------------|--------------------|-------------|
|                                  | PPM Applied              |             |                    |             |
|                                  | 50                       | 100         | 50                 | 100         |
| Initial                          | 22.8±0.8 A <sup>c/</sup> | 26.8±0.6 B  | 22.1±0.5 A         | 26.0±0.4 C  |
| Healthy                          | 7.6±0.4 AA               | 10.5±0.6 CA | 9.9±0.4 AA         | 10.7±0.4 AA |
| Rust Infected                    | 9.4±0.8 AB               | 10.6±1.3 AA | 11.1±1.2 AA        | 8.4±0.5 BC  |

a/ Nutrient content of shoot tissue expressed as  $\mu\text{g N/g dry weight} \times 10^{-3}$ .

b/ Seedlings within a family and treatment were pooled at time of harvest. Seedlings harvested 6 mos. after inoculation were segregated into healthy and rust infected groups.

c/ Statistical significance: first letter is a comparison between rates within each family and condition, second letter is a comparison between conditions within each rate and family; same letter, NS; one letter different,  $P = .05$ ; two letters different,  $P = 0.01$ . Significance determined by paired-t comparisons.

Table 4.--Effect of P application on shoot nutrient content in seedlings of resistant and susceptible, half-sib, families of slash pine.

| Seedling Condition <sup>b/</sup> | Resistant Family               |                   | Susceptible Family |                   |
|----------------------------------|--------------------------------|-------------------|--------------------|-------------------|
|                                  | PPM Applied                    |                   |                    |                   |
|                                  | 5                              | 25                | 5                  | 25                |
| Initial                          | 18.5 $\pm$ 0.4 A <sup>c/</sup> | 34.8 $\pm$ 1.0 A  | 19.6 $\pm$ 0.5 A   | 38.6 $\pm$ 1.0 C  |
| Healthy                          | 12.9 $\pm$ 1.1 AA              | 26.2 $\pm$ 1.4 CA | 10.4 $\pm$ 0.4 AA  | 24.6 $\pm$ 1.6 CA |
| Rust Infected                    | 10.5 $\pm$ 0.4 AB              | 25.8 $\pm$ 1.1 CA | 13.2 $\pm$ 1.4 AC  | 27.4 $\pm$ 1.6 CB |

<sup>a/</sup> Nutrient content of shoot tissue expressed as  $\mu\text{g P/g dry weight} \times 10^{-2}$ .

<sup>b/</sup> Seedlings within a family and treatment were pooled at time of harvest. Seedlings harvested 6 mos. after inoculation were segregated into healthy and rust infected groups.

<sup>c/</sup> Statistical significance: first letter is a comparison between rates within each family and condition, second letter is a comparison between conditions within each rate and family; same letter, NS; one letter different,  $P = 0.5$ ; two letters different,  $P = 0.01$ . Significance determined by paired-t comparisons.

Table 5.--Effect of K application on shoot nutrient content in seedlings of resistant and susceptible, half-sib, families of slash pine. <sup>a/</sup>

| Seedling Condition <sup>b/</sup> | Resistant Family               |                   | Susceptible Family |                   |
|----------------------------------|--------------------------------|-------------------|--------------------|-------------------|
|                                  | PPM Applied                    |                   |                    |                   |
|                                  | 50                             | 150               | 50                 | 150               |
| Initial                          | 11.3 $\pm$ 0.2 A <sup>c/</sup> | 14.4 $\pm$ 0.7 B  | 13.3 $\pm$ 0.3 A   | 17.2 $\pm$ 1.6 B  |
| Healthy                          | 9.5 $\pm$ 0.5 AA               | 11.0 $\pm$ 0.9 AA | 11.4 $\pm$ 0.8 AA  | 12.8 $\pm$ 0.8 AA |
| Rust Infected                    | 8.6 $\pm$ 0.4 AA               | 10.8 $\pm$ 0.7 CA | 9.9 $\pm$ 0.6 AB   | 12.9 $\pm$ 0.5 CA |

<sup>a/</sup> Nutrient content of shoot tissue expressed as  $\mu\text{g K/g dry weight} \times 10^{-3}$ .

<sup>b/</sup> Seedlings within a family and treatment were pooled at time of harvest. Seedlings harvested 6 mos. after inoculation were segregated into healthy and rust infected groups.

<sup>c/</sup> Statistical significance: first letter is a comparison between rates within each family and condition, second letter is a comparison between conditions within each rate and family; same letter, NS; one letter different,  $P = .05$ ; two letters different,  $P = 0.01$ . Significance determined by paired-t comparisons.

A comparison of the distribution of nutrients between healthy and diseased tissue should provide an insight into some of the nutritional requirements of obligate parasites, since tissue infected with an obligate parasite has often been considered to be a metabolic sink (Shaw, 1963). Such a comparison showed (Tables 3, 4, 5) that no pattern could be established for differences in elemental concentrations between healthy and diseased seedlings either within or among families or treatments. This contrasts the results of Martin (1972) that N and K concentrations were lower and P concentrations higher in blister rust infected tissue of western white pine. Perhaps a segregation of infected versus non-infected tissue within diseased seedlings would have provided more definite results.

The results of this study suggest that mineral nutrient applications do not predispose resistant families of slash pine to rust infection. Whether a broad range of nutrient application rates using genetic controls with varying degrees of resistance would produce substantially different results than this study remains to be tested in both the greenhouse and the field.

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