A Rapid Technique for Determining Resistance of Slash Pine to Fusiform Rust

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ABSTRACT

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Slash pine seedlings of 14 half-sib families were tested for resistance to fusiform rust by artificial inoculations with extremely heavy inoculum densities. After 14 days some seedlings within each family had developed red stem lesions. Both the number of lesions per seedling and number of seedlings with lesions were determined for each family and found to be inversely correlated to the proportion of seedlings developing stem galls 9 mo after standard screening inoculations with much lower inoculum densities. These results indicate that the relative resistance of slash pine to fusiform rust can be determined within 14 days by using extremely high inoculum densities.

Additional key words: Cronartium quercuum f. sp. *fusiforme, Pinus elliottii* var. *elliottii, Cronartium fusiforme,* early selection.

Fusiform rust of southern pines (caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) is one of the most destructive of the native forest tree diseases in the United States. Since host resistance is presently the only economical means of control in the field, research workers have concentrated on developing methods of screening pine selections for resistance. Several screening methods have evolved from this work and many of these are reviewed by Schmidt (8). More recently, Matthews and Rowan (4) developed a technique called the concentrated basidiospore spray system (CBS), in which an electronic particle counter is used to prepare known concentrations of basidiospores that are uniformly applied by passing flats of seedlings beneath a spray of inoculum delivered from a fixed nozzle. After 9 mo, resistance of the inoculated seedlings is evaluated by inspecting them for the presence or absence of stem galls.

The CBS system is now used by the Southeastern Area State and Private Forestry Branch of the U.S. Forest Service at the Fusiform Resistance Screening Center, Asheville, NC, where thousands of seedlings are tested for rust resistance annually. Although the CBS system is effective, recent studies suggest that improved predictions can be based on symptoms other than galls alone. Walkinshaw et al (9) measured various responses of infected seedlings at 6 mo and based on these symptoms developed a predictive model which corresponds more closely to field performance than predictions based on galls alone. This model offers a possibly significant improvement over procedures currently used, but evaluations would be significantly improved if the testing times were shortened. In a detailed study of the early symptomatology of fusiform rust, Lundquist and Luttrell (2) present evidence that seedling stem pigmentation developed after exposure to extremely heavy inoculum density is positively correlated with resistance. This paper reports further investigation of that relationship.

MATERIALS AND METHODS

Seeds of 14 half-sib families of slash pine (*Pinus elliottii* (Englem.) var. *elliottii*) with levels of resistance previously determined by the CBS technique and in some cases by field tests, were planted in sterile soil. Two weeks after planting, seedlings were transplanted into polypropylene tubes (17 × 100 mm) containing medium-grain vermiculite and placed in test tube racks (40 seedlings per rack). Modified Hoagland's nutrient solution (7) containing 100 μg N/ml, 75 μg P/ml, and 100 μg K/ml was applied at a rate of 25 ml per seedling, twice weekly for the duration of the experiment. Six-week-old seedlings were inoculated on the same day with a suspension of basidiospores containing 2×10^6 spores per milliliter (determined by a Coulter Counter®, Coulter Electronics, Inc., Hialeah, FL 33010), applied with a chromatography sprayer at a rate of 10 ml per seedling. Immediately after inoculation, the seedlings were placed in a mist chamber at 20 C for 24 hr. They were kept in an air-conditioned headhouse at 20–25 C for an additional 24 hr. Subsequently, seedlings were examined daily for symptom development. This is called the high-inoculum technique (HIT).

RESULTS

The first macroscopic symptom of infection resulting from the HIT was red pigmentation on the needles and stems of many seedlings. The pigment appeared within 14 days after inoculation; in some seedlings it developed on both the needles and the stem, while on others it developed only on the needles or only on the stem. Because preliminary observations suggested that there was no correlation between needle pigmentation and resistance, and because needle pigmentation was more difficult to analyze thoroughly owing to the greater surface area over which pigment could appear, the observations reported here refer only to seedling stem pigmentation.

The pigment appeared in various patterns over the stem surface. On some seedlings, it showed as small distinct spots ranging from <1 mm to 2 mm in maximum diameter. On others, it appeared as large red areas 3–5 mm or more in maximum diameter. When pigmented seedlings within each family were counted, both distinct spots and large areas were scored equally as lesions, and seedlings showing either or both of these patterns were scored as pigmented.

The proportion of pigmented seedlings varied greatly between families, ranging from a high of 87% for FA-2 to a low of 13% for S-37 (Table 1). When these values were compared to previously obtained estimates of resistance determined by the CBS method, a significant inverse correlation ($R = -0.86$) was found (Fig. 1). Families previously classified as resistant by using the CBS system had high proportions of seedlings with red pigment at 14 days and those shown to be susceptible had low proportions of pigmented seedlings.

The number of stem lesions that developed on seedlings within each family is shown in Table 1. Average number of spots per seedling ranged from 2.7 for 76-56 to 19 for FA-2. Families ranked

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TABLE 1. Comparison of responses of half-sib families of slash pine to *Cronartium quercuum* f. sp. *fusiforme* at three different times after inoculation according to the high-inoculum technique (HIT) and the concentrated-basidiospore system (CBS)

Family	Seedlings (%)		Mean number of spots/seedling (HIT) 23 days
	Pigmented (HIT) 14 days	Galled (CBS) 9 mo	
FA-2	87	33	19.0
2907-5	68	19	16.0
Jones 14	65	39	13.8
2797-10	64	41	14.0
3032-5	55	40	8.5
2792-14	53	35	12.9
Jones 18	52	33	11.3
3016-1	48	35	8.5
2981-3	38	65	9.3
3720-19	22	51	4.8
3355-5	21	64	6.9
S-118	15	87	2.9
76-56	14	87	2.7
S-37	13	87	5.1

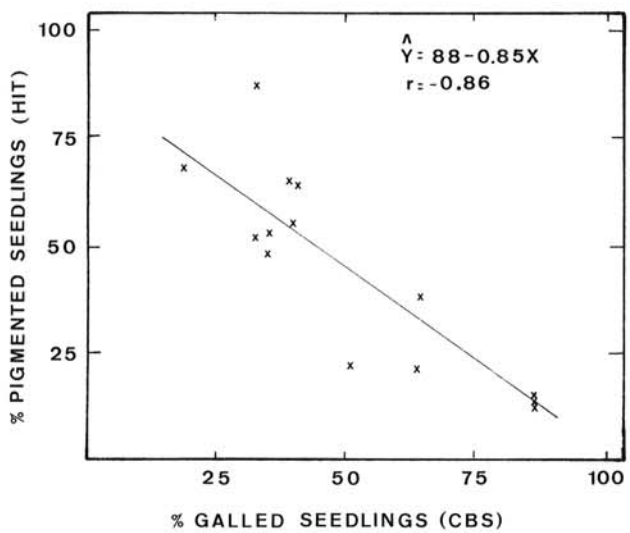

Fig. 1. Comparison of methods for testing the resistance of slash pine seedlings to fusiform rust. Relationship between the percentage of seedlings developing red stem pigmentation 14 days after inoculation according to the high-inoculum technique (HIT) and the percentage of seedlings developing galls 9 mo after inoculation according to the concentrated-basidiospore system (CBS).

on the basis of number of spots per seedling were in roughly the same order as that of rankings based on proportion of pigmented seedlings. Resistant families had relatively greater numbers of spots than did susceptible families.

The percentages of seedlings that remained ungalled 9 mo after inoculation with the HIT are shown in Table 2. These values are compared with estimates of the percentages of seedlings remaining ungalled after 9 mo when the CBS system was used in previous tests. The percentage of change (resistance breakdown), due to the use of extremely heavy inoculum densities, varied greatly among the families, ranging from 3% for 3032-5 to 93% for FA-2.

DISCUSSION

The HIT incorporates three major modifications of the CBS: first, a much higher inoculum density is used (2×10^6 spores per milliliter in the HIT versus $25–50 \times 10^3$ spores per milliliter in the CBS); second, test seedlings in the HIT are grown under more uniform conditions in vermiculite-filled tubes to which controlled

TABLE 2. Breakdown in resistance of slash pine families caused by high inoculum densities as measured by a comparison of the proportion of seedlings remaining ungalled 9 mo after inoculation according to the high-inoculum technique (HIT) or according to the concentrated-basidiospore system (CBS)

Family	Ungalled seedlings (%)			Resistance breakdown (%)
	CBS	HIT	(CBS − HIT)	([CBS − HIT]/CBS)
FA-2	67	5	62	93
2907-5	81	57	24	30
Jones 14	61	52	9	15
2797-10	59	30	29	49
3032-5	60	58	2	3
2792-14	65	28	37	57
Jones 18	67	50	17	25
3016-1	65	20	45	69
2981-3	35	23	12	34
3720-19	49	13	36	74
3355-5	36	4	32	89
S-118	13	3	10	77
76-56	13	8	5	39

amounts of nutrients are added; and third, while the CBS system is based on gall development in susceptible seedlings after 9 mo, the HIT is based on pigmentation developed in pine seedling stems after 14 days.

Some previously published studies (1,3,4,6) deal with the influence of inoculum level on disease development and show that resistance, as measured by the presence of galls, can be overcome by relatively high inoculum densities. This research confirms that greater proportions of seedlings within certain resistant families develop galls when subjected to high inoculum densities, but it also shows that under these conditions parameters other than galls can be used to estimate resistance. With the HIT, both the number of pigmented spots that develop on seedlings and the proportion of seedlings that become pigmented are correlated with resistance. On the basis of these parameters, resistant families of slash pine can be identified much more quickly than with the CBS system.

This study also demonstrates that the breakdown of resistance is not determined solely by the inoculum density, but is influenced also by the host genotype, and that some pine families are more prone to breakdown than others. Family FA-2 has previously been considered one of the most resistant of all slash pine selections. In fact, it is used as a resistant control by the Resistance Screening Center. But under the HIT, it shows the greatest loss of resistance (Table 2). On the other hand, other families such as 3032-5 normally maintained a relatively high proportion of their originally determined level of resistance. This type of distinction suggests that there may exist two types of resistance: one that can withstand heavy inoculum loads and another that cannot.

A survey of fusiform rust across the southeastern United States (5) has shown that the incidence of disease in certain areas is much greater than in others. In such high-hazard areas, where the inoculum load is naturally very high, it might be advantageous to plant pine families that have a high resistance maintenance. In areas of moderate hazard, where the natural inoculum load is less, it might be safe to plant families that are less stable.

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