Twenty-Six New Pine Hosts of Fusiform Rust

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ABSTRACT

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Forty-five pine species and cultivars (*Pinus* spp.) were tested for susceptibility to fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*) by inoculation with basidiospores. Twenty-six species or cultivars were found to be susceptible. Eighteen other species were confirmed as susceptible.

Fusiform rust, caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedgc. & N. Hunt) Burdsall & G. Snow, is a major disease of some southern pines (*Pinus* spp.). Although believed to be native to and presently found only in the southeastern United States, the possibility of the introduction of this pathogen into other geographic areas has prompted host range studies in the United States (3) and in Italy (13). With increased global trade and movement of raw materials, there is a growing probability that viable spores or infected plant materials will inadvertently be introduced into parts of the world where susceptible pine and alternate hosts are growing together.

The purpose of this study was to screen a collection of pine species of worldwide distribution from various parts of the northern hemisphere for susceptibility to the fusiform rust fungus and to compare these susceptibilities to standards of known high and low resistance.

MATERIALS AND METHODS

Artificial inoculations were performed using the standardized procedures of the Resistance Screening Center, Asheville, North Carolina (1,6), to evaluate slash (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly (*P. taeda* L.) pine seedlings for resistance to the fusiform rust fungus, *C. q. fusiforme*.

Seeds of as many pine species as were commercially available were purchased, germinated, and grown as tubelings in a 5:3:1 mixture of vermiculite, peat moss, and perlite. The purpose was to screen a representative selection of species of differing phylogenetic origins; there were no commercial sources for some species, however, so some pine subsections are not represented. With the exception of *P. aristata* Engelm. and *P. lambertiana* Dougl., species of Haploxylon pines were specifically excluded. One susceptible and one resistant slash pine seed lot (susceptible Georgia slash and resistant FA-2) were included as standards (6). Two replicates of three 20-tree trays containing 8-wk-old seedlings were inoculated 1 day apart with basidiospores of *C. q. fusiforme*. Because of the varied growth patterns of the many different species, height at time of inoculation varied from 2.5 to 15.2 cm. Height at inoculation, however, was uniform within a species.

Basidiospores were obtained from telia on leaves of northern red oak (*Quercus rubra* L.) seedlings that had been inoculated with the aeciospore composite technique of Matthews and Rowan (7).

The concentration of inoculum was 20,000 basidiospores per milliliter. This concentration was selected based on the additive relationship found between inoculum density and infection levels on slash pines (8) and is recommended for separating levels of resistance to fusiform rust in slash pine selections. Loblolly pine is assessed at 50,000 spores per milliliter. The slash pine check seedlots and slash pine inoculum concentration were selected for this evaluation because it fit into a series of operation tests at the center. The inoculated seedlings were held in a chamber at 21 C and 100% relative humidity for 24 hr, then were moved to a head house for 24 hr and finally to a greenhouse, where they were maintained for 6 mo. Natural lighting was supplemented to maintain a 16-hr photoperiod.

The percentage of symptomatic seedlings per species was determined after 6 mo and was based on the presence of at least one visible gall or lesion per seedling. For some species, such as *P. montezumae* Shaw, on which typical fusoid stem galls did not form, a lower stem swelling was considered indicative of seedling infection. Proportions of galled seedlings per species were evaluated by ANOVA and Duncan's multiple range test. To verify symptoms as rust induced, samples were removed from galls and fixed in 2% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated, and then embedded in Spurr's epoxy resin. Semithin sections, 0.5–1.0 mm thick, were prepared and stained with methylene blue and azure A (1:1). After rinsing and drying, the mounted sections were examined by light microscopy. At least three galls per species, except for *P. pinaster* Ait. (two galls), were examined histologically for presence of rust hyphae and haustoria.

RESULTS AND DISCUSSION

With few exceptions, only one gall was formed per seedling. Gall morphology was relatively constant within each species but varied somewhat among species (Fig. 1). On most species, galls were fusoid with long, tapering ends (Fig. 1C–E). On some species (e.g. *P. attenuata* Lemmon and *P. rigida* Mill.; Fig. 1A and G), galls were visible only as slight swellings or lesions. The only species that regularly formed rounded galls or galls with abrupt ends were *P. contorta* Dougl. var. *murrayana*, and *P. thunbergii* Parl. Species that formed rather general swellings included *P. montezumae* (Fig. 1F), *P. longifolia* Roxb., and *P. hartwegii* Lindl. The "puffy" nature of these swellings on the latter two species was due largely to hyperplasia of cortical cells. The most variable gall development was observed on *P. engelmanii* Carr., which formed rather irregularly shaped swellings. Some of these swellings were long and tapered on both ends, while others were thickened and stubby (Fig. 1B).

Histological examinations revealed abundant hyphae and haustoria in all gall types of all species and cultivars, even in those with relatively low infection levels. A notable feature of infection was the increased accumulation of tanniniferous materials in host cortical cells in the immediate vicinity of rust hyphae and haustoria. Like Walkinshaw (16), we detected no apparent difference in tannin accumulations among pine species with either high or low levels of infection.

The percentage of inoculated seedlings that developed at least one visible gall ranged from a high of 96% for *P. attenuata* to 0% for *P. resinosa* Ait., the only species tested that developed no galls after inoculation (Table 1). Based on infection percentages, species were somewhat clustered into groupings of 8–14, but a clearcut delineation of relative susceptibility groupings was not possible. Adjacent Duncan groupings had considerable overlap. Thirteen families had more infection than the susceptible slash pine check, seven had values between the resistant and susceptible slash pine checks, and the remainder had fewer galls than the resistant check (Table 1).

Of pines native to the southeastern United States, *P. glabra* Walt. was one new host, with an infection level of 13%. Czabator (2) had previously concluded that this species was not susceptible. Powers (12) found *P. glabra* to be essentially immune with infection levels of only 0.2% in seedlings exposed to natural infection. The 13% infection level in the present study is probably largely due to the favorable inoculation and incubation conditions used at the Resistance Screening Center. *P. banksiana* Lamb. was confirmed as having a low level of susceptibility (5).

For pine species from the western United States, *P. contorta* var. *murrayana*, *P. c.* Dougl. var. *latifolia*, *P. muricata* D. Don, *P. ponderosa* Laws. var.
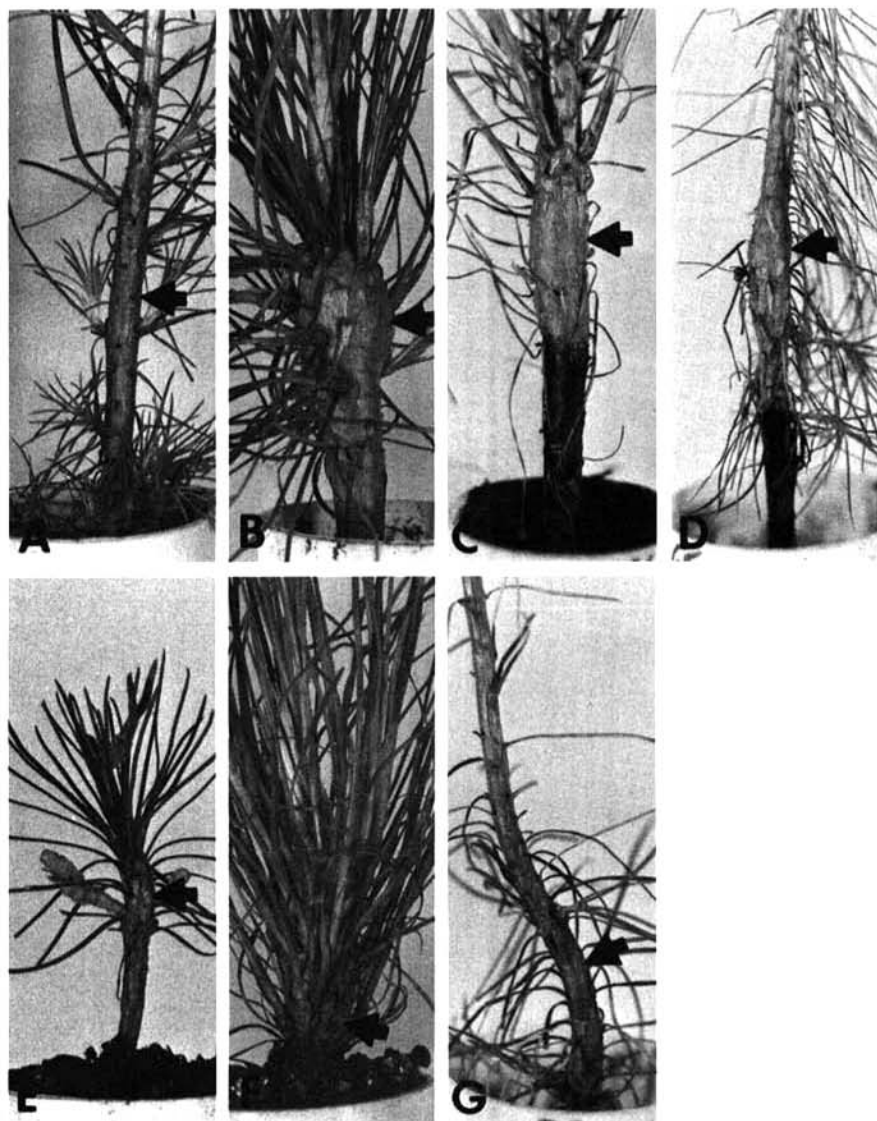

Fig. 1. Seedlings of (A) *Pinus attenuata*; (B) *P. engelmanii*; (C) *P. michoacana*; (D) *P. patula*; (E) *P. montana*; (F) *P. montezumae*, and (G) *P. rigida*, showing the variation in gall reaction (arrows) to infection by *Cronartium quercuum* f. sp. *fusiforme*.

scopularum, P. p. Laws. var. *colorado,* and *P. radiata* D. Don were confirmed as being susceptible. Large proportions of all of these species became infected. Newly identified susceptible western species were *P. attenuata, P. aristata,* and *P. lambertiana.* The latter two species are of special interest because they are Haploxylon pines. As far as we know, this is the first report of the infection of any Haploxylon pine species by *C. q. fusiforme.*

Ten additional species of Caribbean and Central American pines were found to be susceptible. *P. cubensis* Grisebach and *P. engelmanii* were highly susceptible. *P. greggii* Engelm., *P. rudis* Endl., *P. michoacana* Martinez f. *tumida,* and *P. oocarpa* Schiede were moderately susceptible. *P. patula* Schl. & Cham. was moderately low in susceptibility and *P. tenuifolia* Benth., *P. montezumae,* and *P. hartwegii* had low susceptibility. The susceptibilities of *P. caribaea* Morelet var. *hondurensis, P. caribaea* var. *caribaea,* and *P. pseudostrobus* Lindl. were confirmed (4).

Of Asian species, *P. eldarica* Medw., *P. roxburghii* Sarg., and *P. longifolia* were highly susceptible. *P. densiflora*

Sieb. & Zucc. and *P. massonniana* Lamb. had low susceptibility. The susceptibility of *P. thunbergii* was confirmed (2).

Susceptible pine species from Europe and the Mediterranean area included *P. pityusa* Steven and *P. brutia* Ten. Less susceptible species were *P. montana* Mill., *P. pinaster,* and *P. mesogeensis* Fieschi & Gaussen. Species confirmed as susceptible were *P. canariensis* Smith, *P. halepensis* Mill., *P. nigra* Arn. and *P. pinea* L. (2).

Infection levels reported here for *P. pinaster, P. nigra, P. mugo* Turra, and *P. pinea* are close to those reported by Raddi and Powers (13). Infection rates reported by Dwinell and Powers (3) for *P. radiata* and *P. ponderosa* were similar to values recorded by us for these same species. The low infection levels on *P. banksiana* observed by Kais and Snow (5) were similar to the level of 5% observed in the present study.

How well the levels of infection reported here reflect potential reaction of these pine species to natural inoculation in the field is unknown. Techniques used in the Resistance Screening Center favor the pathogen and probably do not reflect true field values. In one test, Miller and Powers (10) did find essentially identical rankings in artificially inoculated families of loblolly pine compared to those same families after 5 yr of growth in a high-hazard rust area.

There were no significant differences in rust infection levels among species grouped according to their phylogenetic origin based on the scheme of Millar and Kinloch (9) (Table 2). However, when the species were grouped according to their present natural geographic occurrence, they segregated into three quite distinct and separate rust infection groups (Table 3).

Snow (15) has concluded that the specialized forms of the pathogen and the considerable variation in levels of resistance in natural pine hosts are proof that a process of mutual selection has occurred during a long association between the hosts and the pathogen. Low

Table 1. Pine species and cultivars tested for susceptibility to *Cronartium quercuum* f. sp. *fusiforme*

Pinus species and cultivars	Subsection[u]	Geographic occurrence[v]	Percent infection[w,x]
P. attenuata (knobcone pine)	O	WUS	96 a
P. cubensis (Cuba pine)	A	CB	94 ab
P. contorta var. *latifolia* (lodgepole pine)[y]	CO	WUS	90 a-c
P. canariensis (Canary pine)[y]	CA	CI	90 a-d
P. contorta var. *murrayana* (lodgepole pine)[y]	CO	WUS	88 a-d
P. caribaea var. *hondurensis* (Caribbean pine)[y]	A	CB	88 a-d
P. caribaea var. *caribaea* (Caribbean pine)[y]	A	CB	87 a-d
P. muricata (bishop pine)[y]	O	WUS	86 a-e
P. ponderosa var. *scopularum* (ponderosa pine)[y]	PO	WUS	85 a-e
P. eldarica[z]	SY	A	84 a-e
P. radiata (Monterey pine)[y]	O	WUS	79 a-f
P. roxburghii (Chir pine)	CA	A	77 a-f
P. elliottii (Georgia slash-susceptible standard)			75 b-g
P. engelmanii (Apache pine)	PO	CB	73 c-g
P. pityusa (Pitzunda pine)	SY	M	70 d-h
P. brutia (Calabrian pine)	SY	M	67 e-i
P. halepensis (Aleppo pine)[y]	SY	M	65 f-j
P. lambertiana (sugar pine)	ST	WUS	62 f-j
P. greggii (Gregg pine)	O	CA	58 g-j
P. ponderosa var. *colorado* (ponderosa pine)	PO	WUS	56 g-j
P. elliottii (FA-2-resistant standard)			53 h-k
P. nigra austriaca (Austrian pine)	S	M	51 i-l
P. pinea (Italian stone pine)[y]	PI	M	50 i-l
P. rudis[z]	PO	CA	50 i-l
P. michoacana forma *tumida*[z]	PO	CA	48 i-m
P. oocarpa sebieda[z]	O	CA	48 i-m
P. nigra var. *pallasiana* (Austrian pine)	SY	M	46 j-m
P. thunbergii (Japanese black pine)[y]	SY	A	35 j-n
P. pseudostrobus (false Weimouth pine)[y]	PO	CA	33 k-o
P. nigra (black pine)[y]	SY	M	32 l-p
P. patula[z]	O	CA	30 m-q
P. mugo pumilo[y]	SY	M	30 m-q
P. montana (Swiss mountain pine)	SY	M	25 m-r
P. aristata (bristlecone pine)	B	WUS	22 n-s
P. tenuifolia[z]	PO	CA	17 n-t
P. montana var. *mughus* (mountain pine)	SY	M	15 o-t
P. mugo mughus (Swiss mountain pine)[y]	SY	M	14 p-t
P. glabra (spruce pine)	A	EUS	13 q-t
P. pinaster (French maritime pine)	SY	M	12 q-t
P. densiflora (Japanese red pine)	SY	A	8 r-t
P. montezumae (Montezuma pine)	PO	CA	8 r-t
P. massonniana (Masson pine)	ST	A	7 r-t
P. banksiana (jack pine)[y]	CO	EUS	5 st
P. mesogeensis[z]	SY	M	5 st
P. rigida (pitch pine)[y]	A	EUS	5 st
P. hartwegii (Hartweg pine)	PO	CA	4 st
P. resinosa (red pine)	SY	EUS	0 t

[u] According to phylogenetic origin: A, Australes; B, Balfourianae; CA, Canarienses; CO, Contortae; O, Oocarpae; PI, Pineae; PO, Ponderosae; ST, Strobi; and SY, Sylvestris.

[v] A, Asia; CA, Central America; CB, Caribbean; CI, Canary Islands; EUS, eastern United States; M, Mediterranean; WUS, western United States.

[w] Means of two runs, with two replications of 20 seedlings per run.

[x] Numbers followed by the same letter are not significantly different according to Duncan's multiple range test.

[y] Confirms previous reports of susceptibility.

[z] These species have no common name.

Table 2. Rust infection levels of pine species according to their phylogenetic origin

Subsection	N	Mean infection level (%)
Canarienses	2	83.4 a[z]
Contortae	4	67.0 a
Oocarpae	6	66.1 a
Strobi	1	62.0 a
Australes	5	57.2 a
Pineae	1	50.0 a
Ponderosae	9	41.6 a
Sylvestris	17	33.2 a
Balfourianae	1	22.0 a

[z] Numbers followed by the same letter are not significantly different according to Duncan's multiple range test.

Table 3. Rust infection levels of pine species according to their present geographic occurrence

Geographic occurrence	N	Mean infection level (%)
Canary Islands	6	89.5 a^z
Caribbean	18	89.4 a
Western United States	53	75.8 a
Asia	29	40.7 b
Central America	69	36.9 b
Mediterranean	60	36.9 b
Eastern United States	22	5.0 c

^{z}Numbers followed by the same letter are not significantly different according to Duncan's multiple range test.

levels of infection on some eastern United States pine species such as sand (*P. clausa* (Chapm.) Vasey), shortleaf (*P. echinata* Mill.) (2), and *P. glabra* suggest a long period of coexistence.

There is some evidence that the rust pathogen either originated in Mexico or Central America or at least survived there during the most recent Pleistocene glaciation (14). A mean infection level of 36.9% for the Central American species tested in the present study tends to support this supposition (Table 3). Species from the Caribbean, Canary Islands, and the western United States likely evolved in the absence of the rust, and this could explain their high levels of susceptibility (Table 3). What is difficult to explain from the information presented in Table 3 is the 40.7 and 36.9% levels of infection in Asian and Mediterranean species, respectively. These moderate susceptibilities may suggest an earlier interaction with *C. quercuum*, but such an interaction does not fit with the presently understood migration routes of the pines (11). This possible interaction, and the rather high infection levels on the Haploxylon species, are worthy of further investigation. General susceptibility of Haploxylon species might suggest that *C. quercuum* differentiated somewhat earlier than Millar and Kinloch (9) hypothesized.

The greatly expanded host range list

identifies several pine species with rather high relative susceptibilities to the fusiform rust fungus. Many of these species have co-evolved in their native habitats with species of *Quercus*, and their ranges may overlap with other potentially susceptible alternate host species. Dwinell and Powers (3) observed that infected leaves of California black oak (*Q. kelloggii* Newb.) produced large numbers of viable telial columns and cast basidiospores at rates comparable to similarly infected leaves of water oak (*Q. nigra* L.), the primary natural alternate host of *C. q. fusiforme*. Fifteen species and cultivars of oaks are common natural hosts of fusiform rust (2); in addition, 30 other species of *Castanea, Castanopsis, Lithocarpus,* and *Quercus* have been successfully inoculated with aeciospores or urediniospores. Clearly, the susceptibility of other oak species needs to be determined, especially where potential pine and oak hosts grow in close proximity, and where climatic conditions might be favorable for infection.

It is important to note that these data represent one seedlot selection or each of the species screened and, therefore, represent a limited examination of overall resistance or susceptibility. Additional seedlots need to be screened for each species to gain a better understanding of the variation within a species. However, these data do show that a large number of species are susceptible. It is also clear that even though some species may not be seriously impacted by fusiform rust they can serve as hosts.

Local climatic conditions would likely limit the potential destructiveness of *C. q. fusiforme* in some areas of the world. However, until these conditions are clearly identified, it seems prudent to avoid increasing the geographic range of this rust disease by restricting movement of potentially infected plant materials.

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