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Resistance of *Eucalyptus* Clones to *Ceratocystis fimbriata*

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ABSTRACT

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Ceratocystis fimbriata, the inciting agent of wilt, canker, and dieback in eucalyptus plantations, was first reported in Brazil in 1998. There is no information regarding the resistance of *Eucalyptus* spp. to this pathogen. We determined the reaction of 18 Brazilian, commercial clones of the hybrid *Eucalyptus grandis* × *E. urophylla* to inoculation by two isolates of the pathogen in two experiments. Container-grown, 8-month-old rooted cuttings of each clone were wound-inoculated with a conidial suspension (2.5×10^6 /ml). Plants similarly injected with sterile distilled water served as controls. The plants were evaluated after 30 days for length of xylem discoloration. There was a significant isolate by clone interaction, but most clones reacted similarly to the two isolates. Six clones, including one observed to be highly susceptible under field conditions, were highly susceptible (>20 cm discoloration) to one or both isolates of the pathogen. Four clones showed no more discoloration with either isolate than with the control inoculations, and the other eight clones were intermediate in susceptibility. Thus, highly resistant clones are available to manage this disease.

Additional keywords: clonal propagation

Ceratocystis fimbriata Ellis & Halst. is widely distributed in the world, especially in the Americas (3,7). The fungus generally infects through wounds and moves through xylem tissues in a wide range of hosts, but considerable host-specialization within *C. fimbriata* has been demonstrated based on xylem discoloration in wound-inoculated plants (1). The pathogen causes rots in storage roots of *Ipomoea batatas* and corms of *Colocasia esculenta*, wilt of *Crotalaria juncea*, and wilt, canker, and crown dieback in coffee (*Coffea arabica*), cacao (*Theobroma cacao*), mango (*Mangifera indica*), sycamore (*Platanus* spp.), *Prunus* spp., *Gmelina arborea*, *Populus* spp., wattle (*Acacia* spp.), and rubber tree (*Hevea brasiliensis*) (3). Wilt and canker caused by *C. fimbriata* on eucalyptus was first reported in 1998 in plantations of a *Eucalyptus grandis* × *E. urophylla* hybrid in southern Bahia, Brazil (6). Among Brazilian isolates of *C. fimbriata*, only eucalyptus isolates appeared pathogenic to eucalyptus (1). The disease has recently been reported in *E. urophylla* × *E. pellita* and *E. tereticornis* × *E. grandis* from the Republic of Congo (13) and in *E. grandis* from Uganda (12) and Uruguay (2). Congolese isolates were pathogenic to an *E. grandis* × *E.*

camaldulensis clone in an inoculation experiment (13). The strains from Africa and Uruguay have internal transcribed spacer (ITS)-rDNA sequences (2,12,13) very similar to those of Bahian isolates of *C. fimbriata* from *E. grandis* × *E. urophylla* (1), and the pathogen may be spreading from Bahia via infected cuttings. In both Africa and South America, substantial mortality can result in some plantations, and this emerging disease is of great concern.

Eucalyptus species are the most economically important forest plantation species in Brazil, where they are cultivated on 3 million hectares, representing about 22% of the world's afforested areas under eucalyptus (11). Eucalyptus is a major source of raw material for cellulose, paper, coke for the steel industry, furniture, railway sleepers, fence stakes, and posts. Clonal propagation permits the annual production of millions of uniformly fast-growing genotypes with homogeneous fiber characteristics and also facilitates development and deployment of disease resistance, if resistant clones can be identified. Because *Eucalyptus* spp. are native to Australia and the eucalyptus form of *C. fimbriata* likely is not (1,7), there was some doubt if suitable resistance could be found. This study was done to evaluate resistance to *C. fimbriata* in currently available eucalyptus clones used for planting and in breeding programs in Brazil.

MATERIALS AND METHODS

Two eucalyptus isolates (UFV 182 = C1345 and UFV 247 = C1442) of *C. fim-*

briata were used. Both isolates were from diseased eucalyptus near Teixeira de Freitas, Bahia, Brazil, and were shown to be pathogenic to eucalyptus in a separate study (1). The isolates were used in two inoculation trials (experiments) in December 2000 and March 2001 in a nursery at the Federal University of Viçosa, Minas Gerais, Brazil. Daily high temperatures during these periods were $25 \pm 3^\circ\text{C}$. In each trial, three rooted cuttings of each clone were inoculated with each isolate, and three rooted cuttings served as controls. For preparation of the conidial suspension, the fungus was grown in petri dishes (9 cm diameter) containing malt yeast extract agar (MYEA; 2% malt extract, 0.2% yeast extract, and 2% agar) for 8 days at 28°C with a photoperiod of 12 h. Ten milliliters of sterile deionized water was added to the surface of the plates, and the mycelium was scraped with a sterile spatula. The conidial suspension was filtered through sterile cheesecloth and the concentration adjusted after counting with a hemacytometer.

The eucalyptus clones were selected from commercially available hybrids of *E. grandis* × *E. urophylla*. The hybrids were collected from an orchard in which *E. grandis* females were open pollinated by *E. urophylla* males. Many progeny were cloned and proven to be superior for form and fast growth at a number of sites in Brazil. Cuttings from 18 of these elite clones were grown in a soil/manure mixture (3:1 vol/vol) in 2-liter containers under nursery conditions. The 8-month-old plants of 1.5 to 2.0 cm diameter were inoculated as described by Laia et al. (9) by injecting a small, vertical slit in the bark of the lower stem (about 3 cm above soil surface) with 1 ml of a conidial suspension (2.5×10^6 /ml) using a sterile syringe (needle gauge 21). The control plants were similarly injected with sterile distilled water. The injection site was wrapped with Parafilm.

The plants were distributed randomly on nursery benches and watered by sprinkler irrigation three times a day or as needed. The plants were evaluated at 30 days for the length of xylem discoloration by cutting lengthwise into the stems as described previously (1). A multi-factorial analysis of variance (ANOVA) and Fisher's protected LSD test (data from the two experiments combined) were conducted using SAS statistical software (SAS Institute, Cary, NC).

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Table 1. Analysis of variance of the length of xylem discoloration in clones of *Eucalyptus grandis* × *E. urophylla* in response to inoculation with two isolates of *Ceratocystis fimbriata*

| Source | df | MS | F value | P > F |
|------------------------------|----|----------|---------|--------|
| Clone | 17 | 1,148.00 | 95.08 | 0.0001 |
| Isolate | 1 | 884.12 | 73.22 | 0.0001 |
| Experiment | 1 | 17.34 | 1.44 | 0.2327 |
| Clone × isolate | 17 | 136.56 | 11.31 | 0.0001 |
| Clone × experiment | 17 | 5.20 | 0.43 | 0.9760 |
| Isolate × experiment | 1 | 4.39 | 0.36 | 0.5474 |
| Clone × isolate × experiment | 17 | 3.98 | 0.33 | 0.9945 |

RESULTS

There were no wilt symptoms or mortality within the 30-day period. However, there was substantial variation in the length of xylem discoloration among the clones and between the two isolates, and there was significant clone by isolate interaction (Table 1). There was no significant difference between the two experiments (Table 1), however, and the results of the two experiments were combined (Table 2).

The clones varied from highly resistant (almost immune) to highly susceptible based on the length of xylem discoloration. The length of discoloration in clones C15, C16, C17, and C18 inoculated with either isolate was no greater than that found in the noninoculated controls. Clones C13 and C14 were moderately resistant to both isolates. Generally, more discoloration was found with isolate C1442 than with C1345, and six clones inoculated with isolate C1442 had greater than 20 cm discoloration. Clone C1 was highly susceptible to both isolates. The remaining six clones had between 4 and 20 cm discoloration with both isolates and could be considered intermediate in susceptibility (Table 2).

DISCUSSION

This is the first study reporting the resistance in eucalyptus to *C. fimbriata*, a new and potentially important pathogen of this highly valuable crop. Resistance to *C. fimbriata* has also been found in *Theobroma cacao* and *Coffea arabica*, and this resistance has proven valuable in disease management (4,5,8,10,14). Thus far, the pathogen has caused substantial mortality in only a few clones of *E. grandis* × *E. urophylla* in Brazil. Among the clones we inoculated, only the susceptible clone C4 has been widely planted in Bahia, and the disease has been especially severe in this clone. However, other clones proved to be highly resistant or nearly immune, and these clones are currently being evaluated in the field.

The differences in aggressiveness of the two isolates to eucalyptus can be attributed to the genetic variability in *C. fimbriata* (1,7). However, the two isolates we used caused similar amounts of discoloration in the various clones and did not appear to differ greatly in aggressiveness. Baker et al. (1) inoculated rooted cuttings of clone C4 with the same eucalyptus isolates (C1345 and C1442) that we used and

found substantial discoloration, but a third eucalyptus isolate apparently lost pathogenicity and caused no more discoloration than the controls or the isolates from other hosts (cacao, mango, and *Gmelina arborea*). Isolates C1345 and C1442 were not pathogenic to cacao or *Gmelina arborea* but were pathogenic to mango (1). More work is needed on the host range and variation in aggressiveness among Brazilian isolates of *C. fimbriata*.

Work is also needed to determine the ideal age to evaluate resistance in the *C. fimbriata*/*Eucalyptus* pathosystem, but xylem discoloration, rather than mortality, appears to be a more replicable parameter for evaluating susceptibility and resistance (1). Lack of wilt or mortality in our trials may be related to plant age, and young plants under optimum growth conditions may die more quickly than older plants. In another test (unpublished data), 4-month-old plants of clone C4 wilted and died within 12 days of inoculation with an isolate of *C. fimbriata* from eucalyptus.

Clones with known susceptibility, such as C1 and C4, should not be planted in areas where the pathogen is known to be present. However, clone C4 has many superior characteristics, such as high cellulose yield, low levels of extractives, fast growth, precocious shedding of branches, and resistance to canker caused by *Cryphonectria cubensis*. Progeny from controlled crosses between elite *E. grandis* × *E. urophylla* genotypes, including clone C4, were planted in November 2002 to January 2003 in an area of South Bahia with a history of mortality caused by *C. fimbriata*. The results are preliminary, but segregation for susceptibility/resistance has been observed in the progeny from all the crossings involving clone C4 and in progeny from the C4 selfings (unpublished data). Artificial inoculations will be made to confirm the inheritance of susceptibility, and further efforts will be made to breed for resistance.

Besides avoidance of susceptible clones and use of resistant clones, management of the disease might include removal and destruction of infected plants, minimization of wounding (infection courts), and rotation with nonhost plants (3). The pathogen also appears to be spreading to new areas in infected cuttings (1,7), and care should be taken not to vegetatively propagate from diseased plants. At present,

Table 2. Length of xylem discoloration (cm) in clones of *Eucalyptus grandis* × *E. urophylla* in response to inoculation with two isolates of *Ceratocystis fimbriata*

| Clone | Isolate C1442 | Isolate C1345 | Control |
|-------|---------------------|---------------------|---------|
| C1 | 48.7 a ^z | 31.0 a ^z | 1.7 |
| C7 | 28.2 b | 11.3 c | 1.7 |
| C9 | 24.4 bc | 9.0 c | 1.6 |
| C4 | 22.4 c | 19.0 b | 1.8 |
| C3 | 21.6 c | 19.2 b | 1.8 |
| C6 | 20.5 c | 11.7 c | 1.8 |
| C10 | 16.1 d | 8.9 c | 1.9 |
| C5 | 15.0 de | 17.5 b | 1.8 |
| C2 | 13.7 def | 19.3 b | 1.7 |
| C11 | 12.0 ef | 8.8 c | 1.9 |
| C12 | 10.5 f | 4.3 d | 1.7 |
| C8 | 9.7 f | 10.0 c | 1.8 |
| C14 | 4.3 g | 4.0 d | 1.7 |
| C13 | 4.0 g | 4.0 d | 1.7 |
| C15 | 1.9 g | 1.9 d | 1.7 |
| C16 | 1.8 g | 1.9 d | 1.7 |
| C17 | 1.8 g | 1.8 d | 1.6 |
| C18 | 1.8 g | 1.7 d | 1.6 |

^z Means followed by the same letter in a column do not differ significantly (Fisher's protected LSD test, $P < 0.05$).

however, the disease in eucalyptus appears to be limited to only certain geographic areas of Brazil, where the pathogen may be soilborne (9), and to a limited number of clones. Avoidance of the most susceptible clones and planting highly resistant clones on infested sites appears to be the most effective management option.

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