Relative Pathogenicity of *Cryphonectria cubensis* on *Eucalyptus* Clones Differing in Their Resistance to *C. cubensis*

S. W. van Heerden, Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; **H. V. Amerson,** Department of Forestry, North Carolina State University, Raleigh; and **O. Preisig, B. D. Wingfield,** and **M. J. Wingfield,** Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria

ABSTRACT

van Heerden, S. W., Amerson, H. V., Preisig, O., Wingfield, B. D., and Wingfield, M. J. 2005. Relative pathogenicity of *Cryphonectria cubensis* on *Eucalyptus* clones differing in their resistance to *C. cubensis.* Plant Dis. 89:659-662.

Cryphonectria cubensis causes a destructive canker disease of *Eucalyptus* species. Management of this disease is primarily through breeding and selection of disease resistant trees. One means of selecting such trees is by artificial inoculation with the pathogen. In routine screening trials in South Africa, an isolate of *C. cubensis*, considered to be highly pathogenic, has been used for such inoculations. Although the most resistant clones under natural conditions are the same as those detected in inoculation trials, a question has arisen whether all clones respond similarly to different *C. cubensis* isolates. Thus, a trial consisting of five clones, known to differ in susceptibility to infection by *C. cubensis*, was established. These trees were inoculated with nine South African *C. cubensis* isolates previously shown to differ in pathogenicity. Inoculations showed a significant isolate × clone interaction as well as an "apparent immunity" for one clone × isolate interaction, providing evidence highly suggestive of a vertical resistance component in the pathosystem. Disease screening in this pathosystem has traditionally relied on a single pathogen isolate; however, considering data presented here, future reliance on a single isolate may be inadequate.

Additional keywords: gene-for-gene resistance, host–pathogen interaction

Cryphonectria canker, induced by *Cryphonectria cubensis* (Bruner) C.S. Hodges, is a serious canker disease on *Eucalyptus* species in many tropical and subtropical areas of the world (2,5–8). Cryphonectria canker was first reported in South Africa in 1989 (23). The South African form of this disease is characterized by swollen basal stem cankers and is also favored by high rainfall (2,000 to 2,400 mm/year) and temperatures above 23°C (5,12,23). Since *Eucalyptus* is one of the major plantation trees in the country, it has been important to develop effective management to ensure minimal losses due to Cryphonectria canker.

Various options exist to reduce the impact of Cryphonectria canker. Chemical control has been considered, but due to the low value of individual *Eucalyptus* trees, this is not economically viable (12). Biological control using hypovirulent strains of the pathogen is also attractive but is a very long term option (16). Currently, the most feasible approach is to breed and select disease resistant *Eucalyptus* trees (1,22).

Deployment of naturally selected disease resistant *Eucalyptus* spp. has reduced losses in plantations due to *C. cubensis* (3,4). Monoclonal plantations are attractive to forestry companies because of the uniformity of selected clones and their higher productivity over shorter periods of time. However, the combination of favorable environmental conditions and the genetic uniformity of these plantations might lead to substantial losses due to *C. cubensis* if clones with poor resistance are inadvertently planted. Virtually nothing is known regarding the genetics of susceptibility of *Eucalyptus* spp. to infection by *C. cubensis*. However, it has been assumed that resistance to this pathogen is a quantitative trait. This is due to the broad range of susceptibility displayed in inoculation trials by progeny resulting from a cross between a resistant and susceptible *Eucalyptus* clone (S. W. van Heerden, *unpublished data*). One means to screen trees for disease resistance is through artificial inoculation, which reduces confusion related to disease escape in natural infection trials. Thus, artificial inoculation has been effective in screening *Eucalyptus* trees for resistance to Cryphonectria canker (1,18).

Routine screening of *Eucalyptus grandis* clones and hybrids with *C. cubensis* to identify disease resistant planting stock has been conducted in South Africa for several years. Associated trials such as those assessing the capacity of *Eucalyptus* clones to heal wounds after mechanical damage have shown a positive correlation with resistance to disease caused by *C. cubensis* (19). Likewise, a strong genotype-by-environment effect has been shown using inoculation trials with the fungus in different areas of South Africa (18). These trials have all been conducted using a single genotype of *C. cubensis*, which was selected from a large collection of isolates to represent an isolate with a high level of pathogenicity. However, the question has arisen as to whether *Eucalyptus* clones might show differential resistance to infection by different isolates of *C. cubensis*. The aim of this study was to address that question by inoculating a selection of clones with a collection of isolates having different levels of pathogenicity.

MATERIALS AND METHODS

Isolates. Nine South African *C. cubensis* isolates were selected for this study. In a previous trial (17), eight of these isolates were shown to differ in pathogenicity. Four isolates with low levels of pathogenicity and four highly pathogenic isolates were specifically selected. All isolates had also previously been shown to belong to different vegetative compatibility groups (VCGs) of *C. cubensis* (17). As a positive control, *C. cubensis* isolate CMW2113, which has been considered to be highly pathogenic (17) and has been used in annual disease screening trials, was included as the ninth isolate. All the isolates used in this study are stored in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

***Eucalyptus* clones.** Five *Eucalyptus* clones selected for this experiment were planted in a field trial. These clones were selected based on differences in their level of disease resistance when challenged with the *C. cubensis* isolate CMW2113 (Table 1), as determined in a previous inoculation trial (M. J. Wingfield, *unpublished data*). Each of the *Eucalyptus* clones was vegetatively propagated by making cuttings from parent hedge plants. These cuttings were rooted and hardened before being planted in a randomized complete block design.

Corresponding author: S. W. van Heerden
E-mail: schalk.vanheerden@sakata-eu.com

Accepted for publication 2 November 2004.

DOI: 10.1094/PD-89-0659

Table 1. *Eucalyptus* clones selected from previous field inoculations with *Cryphonectria cubensis* isolate CMW2113 and used in the current study

Clone no.	Clone	Disease susceptibility
ZG14	*E. grandis*	Highly susceptible
TAG5	*E. grandis*	Moderately resistant
GU21	*E. grandis* × *E. urophylla*	Resistant
GC121	*E. grandis* × *E. camaldulensis*	Resistant
GT529	*E. grandis* × *E teretericornis*	Moderately resistant

The trial was established in 1998 in the Canewoods plantation, Kwambonambi area, Kwazulu-Natal, South Africa (28°38′ S; 32°06′ E). The trial consisted of 20 rows. In each row, the five clones were planted in blocks of 10 trees each. These blocks were randomized between the rows to ensure that the same clone did not occur in one area of the trial. Thus, a total of 200 trees were planted per clone. The trees were planted with a spacing of 3 × 2.5 m, and the trial was surrounded by buffer rows of *E. grandis* trees and allowed to grow for 24 months before treatment.

Inoculation procedure and evaluation. The inoculum was prepared by growing each of the nine *C. cubensis* isolates on 90-mm-diameter petri dishes containing 2% malt extract agar (MEA) (Biolab, Johannesburg, South Africa). Prior to inoculation, the plates were incubated at 25°C for 7 days with an alternating 12-h day and night period. The trees were inoculated by removing a cambial disk about 140 cm from the ground with a 20-mm-diameter cork borer. A corresponding 20-mm disk, taken from an actively growing culture of one of the nine fungal isolates, was placed in the wound with the mycelium side facing the cambium. The wounds were sealed with masking tape to reduce desiccation. All trees were inoculated on the southern side. The trial was inoculated so that there were 20 replicates for each of the nine isolates on all five clones. As a control, 20 additional trees from each clone were inoculated in a similar manner with a sterile MEA plug. Thus, a total of 1,000 trees were tested in this experiment.

Lesion length, width, and the circumference of the tree at the point of inoculation were measured 6 months after inoculation. Differences in lesion width among tree genotypes and isolates were analyzed using a two-way ANOVA (SAS/STAT Users Guide Version 8, SAS Institute, Cary, NC), with lesion width as the dependent and the clones and isolates as the trial factors. Tree circumference was included as a covariate. A simple effects analysis was done with data for the nine different isolates to determine the individual effects of the isolates on the clones. The data were also reanalyzed with the exclusion of the most disease susceptible *E. grandis* clone, ZG14, and that of a single "apparently immune" host-by-pathogen interaction (GC121 and CMW11335). This was done to ensure that the disease responses observed were not unjustifiably influenced by one clone or one particular interaction.

Table 2. Two-way analysis of variance table with all clones, without clone ZG14, and without the clone GU121 × isolate CMW11335 combination

	SS	df	Mean square	*F* ratio	*P*
All clones					
Isolates	467,165.6	9	51,907.3	13.7	0.0001
Clones	1,130,832	4	282,708	74.64	0.0001
Isolate × clone	392,093.6	36	10,891.5	2.88	0.0001
Circumference	52,863.8	1	52,863.8	13.96	0.0002
Error	3,454,158.8	912	3,787.5		
Without clone ZG14					
Isolates	158,523.2	9	17,613.7	9.39	0.0001
Clones	64,837.3	3	21,612.4	11.53	0.0001
Isolate × clone	84,505.3	27	3,129.8	1.67	0.0184
Circumference	23,775	1	23,775	12.68	0.0004
Error	1,370,475	731	1,872		
Without clone GU121 × isolate CMW11335 combination					
Isolates	466,557.1	9	51,839.7	13.42	0.0001
Clones	1,114,136	4	278,534	72.11	0.0001
Isolate × clone	389,021.4	35	11,114.9	2.88	0.0001
Circumference	53,868.3	1	53,868.3	13.95	0.0002
Error	3,453,154.3	894	3,862.58		

RESULTS

Six months after inoculation, most of the inoculated trees had developed obvious cankers in the cambium. No lesion development was associated with the control inoculations, which were grown over by callus tissue. The trees used in this inoculation study tended to have relatively small lesions (mean lesion width 60.8 mm) and thus exhibited a high level of resistance to infection. Lesion widths differed significantly among the isolates ($F = 13.7$; df = 9; $P < 0.001$) and among the clones ($F = 74.6$; df = 4; $P < 0.001$) (Table 2). Since the clones used in this study were known to differ in resistance to *C. cubensis* and the isolates to differ in pathogenicity, this result was expected. There was also a significant isolate × clone interaction (Table 2), indicating that not all clones responded in the same way to all isolates. One interesting combination, isolate CMW11335 × clone GC121, showed no lesion development (Table 3). There was also a significant difference observed for tree circumference, which was used as a covariant in the analysis of variance (Table 2).

Simple effects analysis on the inoculation data for *C. cubensis* isolate CMW2113 showed that clone ZG14 was the least resistant and that lesions on this clone were significantly larger than those on the other clones (Table 3). Inoculations with isolate CMW2113 also showed that clone TAG5 had larger lesions than those on clones GT529, GC121, and GU21, although they were not significantly different from each other (Table 3).

In all the inoculations, clone ZG14 was the most diseased, followed by clone TAG5, while clones GT529, GC121, and GU21 fluctuated slightly in disease severity from isolate to isolate. The only significant difference was between clone ZG14 and the other clones. This was true for inoculation with all isolates except CMW11346, for which clones ZG14 and TAG5 did not differ significantly from each other (Table 3).

Exclusion of ZG14 from the data and subsequent analysis of variance also indicated that lesion widths differed significantly among isolates ($F = 9.39$; df = 9; $P < 0.001$) and among clones ($F = 11.53$; df = 3; $P < 0.001$) (Table 2). A significant isolate × clone interaction was still observed with ZG14 excluded; however, significance was at the 95% confidence level (Table 2) rather than the 99% level observed with inclusion of ZG14 (Table 2). There was also a significant difference observed for the circumference, which was used as a covariant in the analysis of variance as previously observed (Table 2). Exclusion of the "apparently immune" combination, isolate CMW1135 × clone GC121, and subsequent analysis also showed that interaction effects persisted (Table 2). Hence, interaction effects were not solely dependent on that combination.

DISCUSSION

The current breeding strategy for *Eucalyptus* in South Africa involves screening of possible planting stock in the field for resistance to canker disease caused by *C. cubensis*. Until the present time, all inoculations for disease screening have been done with a single *C. cubensis* isolate (CMW2113) that was considered to be highly pathogenic. Results from this study show that this isolate produced intermediate disease levels in all clones where significant differences existed, and probably was a very good choice for previous screenings using only one isolate (Table

Table 3. Mean lesion width (mm) ± SEM caused by South African *Cryphonectria cubensis* isolates after inoculation on five different *Eucalyptus* clones

Isolates	Eucalyptus clones															Mean[w]
	GC121			GT529			GU21			TAG5			ZG14			
CONTROL	20 ± 0		a[x]	20 ± 0		a	20 ± 0		a	20 ± 0		a	20 ± 0		a	20
CMW2113	34.2 ± 6.7	ab[y]	a	23.9 ± 3.9	ab	a	24.5 ± 2.3	a	a	72.9 ± 11.4	ab	a	144.7 ± 30.5	abc	b	60.04
CMW11345	58.9 ± 10.6	b	a	61.3 ± 7	b	a	52.8 ± 15.0	a	a	90.5 ± 10.2	b	a	228.9 ± 27.4	c	b	98.48
CMW11344	31.9 ± 6.2	ab	a	23.3 ± 3.2	ab	a	35 ± 13.9	a	a	37 ± 9.3	a	a	144.7 ± 28.9	abc	b	54.38
CMW11319	37.5 ± 9.1	ab	a	48.3 ± 16.1	ab	a	61.8 ± 18.6	a	a	107.6 ± 17.9	b	a	210.3 ± 23.7	bc	b	93.10
CMW11326	28.2 ± 5.3	a	a	21.0 ± 1.0	a	a	38.2 ± 11.9	a	a	37.2 ± 8.1	a	a	125.8 ± 29.0	ab	b	50.08
CMW11339	21 ± 1.0	a	a	23.5 ± 3.5	ab	a	38 ± 13.0	a	a	36.6 ± 7.9	a	a	94 ± 22.8	a	b	42.62
CMW11335	20 ± 0	a	a	38.4 ± 18.4	ab	a	54.2 ± 20.3	a	a	30.5 ± 5.3	a	a	151.3 ± 25.7	abc	b	58.88
CMW11346	35.5 ± 3.1	ab	a	37.3 ± 4.4	ab	a	45.5 ± 13.7	a	a	71.7 ± 10.1	ab	ab	119.3 ± 22.9	abc	b	61.86
CMW11318	41.8 ± 5.8	ab	a	32.9 ± 5.1	ab	a	36.1 ± 13.1	a	a	75.5 ± 10.8	ab	a	185.3 ± 25.8	abc	b	74.32
Mean[z]	32.90			32.99			40.61			57.95			142.43			

[w] Mean lesion width (mm) within the isolates.
[x] Significant differences between the different *Eucalyptus* clones within a single *C. cubensis* isolate. Clones with the same letter do not differ significantly from each other according to Tukey's test ($P = 0.05$).
[y] Significant differences between the different *C. cubensis* isolates within a single *Eucalyptus* clone. Isolates with the same letter do not differ significantly from each other according to Tukey's test ($P = 0.05$).
[z] Mean lesion width (mm) within the clones.

3). Indeed, if it is necessary to rely on a single isolate, CMW2113 would be the isolate of preference. Nevertheless, current results have also shown existence of a significant clone × isolate interaction among the samples tested as well as apparent disease immunity when isolate CMW11335 × clone GC121 was tested. These data argue against reliance on a single fungal isolate for disease screening, and the use of a single isolate in future screening protocols for disease resistance in *Eucalyptus* may be inadequate.

van der Plank (15) indicated that virulence and vertical resistance are demonstrated by an interaction in the analysis of variance. He further suggested that aggressiveness and horizontal resistance are indicated by main effects between pathogen, isolates, and host varieties. The overall conclusion here was that both types of host–pathogen response can be present in a host–pathogen system (15). Results of the present study have shown a significant isolate × clone interaction and apparent immunity for one clone × isolate interaction. We can, therefore, speculate that disease resistance to Cryphonectria canker of *Eucalyptus* in South Africa seemingly involves a vertical resistance component.

It is suggested that polygenic disease resistance (horizontal resistance) is durable resistance, whereas gene-for-gene resistance (vertical resistance) represents temporary resistance (11). The inheritance of disease resistance in forest trees has mostly been explained by polygenic models (20). However, several studies (9,10,13,14) provide strong evidence for a possible gene-for-gene model to explain resistance to fusiform rust disease, caused by *Cronartium quercuum* (Berk.) Miyabe ex. Shirai f. sp. *fusiforme*, in *Pinus elliottii* Engelm. var. *ellottii* (slash pine). Another study has indicated that long-term resistance to fusiform rust disease was not exclusively polygenic and could be obtained from a single qualitative resistance gene in loblolly pine (21). When there is evidence of vertical resistance inheritance, as in the present study or as noted above for fusiform rust disease, it is important that breeding models take these specific host–pathogen interactions into consideration.

Results of this study provide further support for the reliability of the artificial inoculation protocol used to screen trees for resistance toward Cryphonectria canker. van Zyl et al. (19) showed that the capacity of *Eucalyptus* clones to heal wounds caused by mechanical damage can be directly correlated with tree susceptibility. Molecular markers might also be used in the near future to select disease resistant trees. However, for an effective disease screening strategy that will select clones of *Eucalyptus* having durable resistance, it is important to understand the genetics of plant–pathogen interactions. Our preliminary data suggesting a vertical resistance component will need to be confirmed using a detailed genetic analysis of the *Eucalyptus–C. cubensis* pathosystem.

ACKNOWLEDGMENTS

We acknowledge the financial support of the South African forestry industry to the Tree Pathology Co-operative Programme (TPCP), the THRIP initiative of the Department of Trade and Industry (DTI), and the National Research Foundation (NRF), South Africa. We also acknowledge Mondi Forests South Africa for providing a trial site as well as the maintenance of this site before and during this experiment.

LITERATURE CITED

1. Alfenas, A. C., Jeng, R., and Hubbes, M. 1983. Virulence of *Cryphonectria cubensis* on *Eucalyptus* species differing in resistance. Eur. J. For. Pathol. 13:179-205.
2. Boerboom, J. H. A., and Maas, P. W. T. 1970. Canker of *Eucalyptus grandis* and *E. saligna* in Surinam caused by *Endothia havanensis*. Turialba 20:94-99.
3. Campinhos, E., and Ikemori, Y. K. 1983. Mass production of *Eucalyptus* spp. by rooting cuttings. Silvicultura 8:770-775.
4. Conradie, E., Swart, W. J., and Wingfield, M. J. 1992. Susceptibility of *Eucalyptus grandis* to *Cryphonectria cubensis*. Eur. J. For. Pathol. 22:312-315.
5. Florence, E. J. M., Sharma, J. K., and Mohanan, C. 1986. Stem canker disease of *Eucalyptus* caused by *Cryphonectria cubensis* in Kerala. Kerala For. Res. Inst. Sci. Pap. 66:384-387.
6. Gibson, I. A. S. 1981. A canker disease new to Africa. FAO, For. Genet. Resour. Inform. 10:23-24.
7. Hodges, C. S., Geary, T. F., and Cordell, C. E. 1979. The occurrence of *Diaporthe cubensis* on *Eucalyptus* in Florida, Hawaii and Puerto Rico. Plant Dis. Rep. 63:216-220.
8. Hodges, C. S., and Reis, M. S. 1974. Identificacao do fungo causador de cancro de *Eucalyptus* spp. no Brazil. Braz. Florestal 5:19.
9. Kinloch, B. B., and Walkinshaw, C. H. 1991. Resistance to fusiform rust in Southern Pines: How is it inherited? Pages 219-228 in: Proc. IUFRO Rusts Pine Working Party Conf. 1989. Y. Hiratsuka, J. K. Samoil, P. V. Blenis, P. E. Crane, and B. L. Lainshley, eds.
10. Nelson, C. D., Doudrick, R. L., Nance, W. L., Hamaker, J. M., and Capo, B. 1993. Specificity of host:pathogen genetic interaction for fusiform rust disease on slash pine. Pages 403-410 in: Proc. South For. Tree. Imp. Conf. 22nd.
11. Robinson, R. A. 1996. Return to resistance: Breeding crops to reduce pesticide dependence. agAccess, Davis, CA.
12. Sharma, J. K., Mohanan, C., and Florence, E. J. M. 1985. Occurrence of Cryphonectria canker disease of *Eucalyptus* in Kerala, India. Ann. Appl. Biol. 106:265-276.
13. Stelzer, H. E., and Doudrick, R. L. 1997. Derivation for host and pathogen genotypes in the fusiform rust pathosystem on slash pine using a complementary genetics model and diallel data. Pages 320-330 in: Proc. South For. Tree. Imp. Conf. 24th.
14. Stelzer, H. E., Doudrick, R. L., Kubisiak, T. L., and Nelson, C. D. 1999. Prescreening slash pine and *Cronartium* pedigrees for evaluation of complementary gene action in fusiform rust disease. Plant Dis. 83:385-389.
15. van der Plank, J. E. 1984. Host-Pathogen interactions in plant disease. Academic Press, New York.
16. van Heerden, S. W., Geletka, L. M., Preisig, O., Nuss, D. L., Wingfield, B. D., and Wingfield, M. J. 2001. Characterization of South African *Cryphonectria cubensis* isolates infected with a *C. parasitica* hypovirus. Phytopathology 91:628-632.
17. van Heerden, S. W., and Wingfield, M. J. 2001. Genetic diversity of *Cryphonectria cubensis*

isolates in South Africa. Mycol. Res. 105:94-99.
18. van Heerden, S. W., and Wingfield, M. J. 2002. Effect of environment on the response of *Eucalyptus* clones to inoculation with *Cryphonectria cubensis*. For. Pathol. 32:395-402.
19. van Zyl, L. M., and Wingfield, M. J. 1999. Wound response of *Eucalyptus* clones after inoculation with *Cryphonectria cubensis*. Eur. J. For. Pathol. 29:161-167.
20. von Weissenberg, K. 1990. Värd-parasitförhållanden i skogliga ecosystem en litteraturstudie. Silva Fenn. 24:129-139.
21. Wilcox, P. L., Amerson, H. V., Kuhlman, E. G., Liu, B.-H., O'Malley, D. M., and Sederoff, R. R. 1996. Detection of a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping. PNAS USA 93:3859-3864.
22. Wingfield, M. J. 1990. Current status and future prospects of forest pathology in South Africa. South Afr. J. Sci. 86:60-62.
23. Wingfield, M. J., Swart, W. J., and Abear, B. J. 1989. First record of Cryphonectria canker of *Eucalyptus* in South Africa. Phytophylactica 21:311-313.