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Eur. J. For. Path. 29 (1999) 161–167
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ISSN 0300–1237

Wound response of *Eucalyptus* clones after inoculation with *Cryphonectria cubensis*

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Summary

Twenty-five different *Eucalyptus grandis* clones were artificially wounded and inoculated with a virulent isolate of *Cryphonectria cubensis*. The capacity of wounds to close through callus production was correlated with the relative susceptibility of these clones to infection by *C. cubensis*. Clones with the greatest capacity to close wounds were those that were also most tolerant to *C. cubensis* infection. Those with a lower capacity to close wounds were most susceptible to Cryphonectria canker.

1 Introduction

The forestry industry in South Africa depends almost exclusively on exotic species of *Eucalyptus* and *Pinus*. Eucalypts and pines are planted in more or less equal proportions and, together, they cover an area of approximately 1.5 million ha (Anonymous 1990). *Eucalyptus* forestry in South Africa is commonly based on a monoculture system, using a relatively small number of selected clones, and this could lead to large, genetically uniform stands that are at risk from pests and diseases (Wingfield et al. 1991). Strategies to ensure the planting of only disease tolerant clones of *Eucalyptus* are thus of considerable importance.

Cryphonectria cubensis (Bruner) Hodges causes a serious canker disease of *Eucalyptus* spp. planted in many tropical areas of the world (Bruner 1916; Boerboom and Maas 1970; Sharma et al. 1984; Wingfield et al. 1989). *Cryphonectria cubensis* has limited the planting of susceptible *Eucalyptus* spp. in Brazil, in areas where climatic conditions favour the growth and spread of the pathogen (Alfenas et al. 1982). The relatively recent discovery of the disease in South Africa, has prompted concern that this disease could impact negatively on the planting of *Eucalyptus* clones in the country (Wingfield et al. 1989).

Alfenas et al. (1983) showed that considerable inter- and intraspecific variation in resistance to Cryphonectria canker exists within the genus *Eucalyptus*. In Brazil, cankers caused by *C. cubensis* were thus minimized by the selection of *E. grandis* clones, displaying tolerance to the pathogen (Campinhos and Ikemori 1983). In South Africa, a programme has been established to select for tolerance of *E. grandis* clones to Cryphonectria canker (van der Westhuizen et al. 1992). However, trials to test disease tolerance of clones are time consuming, and cannot keep pace with the demand for new and improved clones. A method that could provide an earlier assessment of disease tolerance would therefore be useful.

Received: 17.7.1997; accepted: 24.8.1998; editor: G. A. Kile

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Cryphonectria cubensis initiates disease by infecting wounds or growth cracks in the bark, and cankers expand until the trees are girdled (Hodges et al. 1979; Boerboom and Maas 1970; Sharma et al. 1985). It is proposed that Cryphonectria canker of *Eucalyptus* can potentially be managed by incorporating host tolerance, related to the development of structural barriers to pathogen ingress. A rapid, reliable and economically efficient method to detect tolerance to pathogen ingress would expedite the exploitation of new *Eucalyptus* spp. or clones with tolerance to *C. cubensis*. An understanding of the processes through which new lateral cambial tissues (both phellogen and vascular cambium) are generated, could lead to a better understanding of disease tolerance. The objective of this study was to determine whether a relationship exists between the capacity of trees to close wounds and the relative susceptibility of different *E. grandis* clones to infection by *C. cubensis*.

2 Material and methods

2.1 Trees for inoculation

Two identical plots including 25 *E. grandis* clones ranging from highly susceptible to highly tolerant to infection by *C. cubensis* (M. J. Wingfield, unpublished data) were planted in October 1992 in Zululand, KwaZulu-Natal, South Africa. One plot was used to screen for tolerance to infection by *C. cubensis*, using artificial inoculation. The second plot was used to wound trees mechanically. Plots were designed with 40 trees in each of 25 rows, and were surrounded by two border rows of untreated trees. Clones were completely randomized in the plots. The trees were maintained using standard silvicultural procedures.

2.2 Pathogenicity tests

One-year-old trees were inoculated during October of 1993, when trees are most susceptible to infection. Trees were inoculated 20 cm above the soil line, using a cork borer (10 mm in diam.). This was done to remove the bark and expose the cambium. Care was taken not to injure the xylem deeper than the cambium. Inoculum of *C. cubensis* was prepared by culturing the fungus in Petri dishes on 2% w/v Potato Dextrose Agar (PDA) at 25°C for 7 days. A 10 mm disc of PDA, overgrown with an isolate of *C. cubensis* (D21–13 from Kwambonambi, KwaZulu-Natal, South Africa) known to be virulent in previous tests, was used to inoculate 20 trees for each clone. A sterile 10 mm diameter disc of PDA was used to inoculate the remaining 20 trees for each clone that represented the controls. All wounds were covered with masking-tape to limit contamination and the inoculum from drying out. Six weeks after inoculation, the masking-tape was removed and the canker widths on each tree was measured. Data, were then expressed as mean lesion width.

2.3 Wounding study

This experiment was conducted at the same time as the pathogenicity study. All the trees were mechanically wounded, 20 cm above the soil line, using a sharpened, oval-shaped, 25 mm × 45 mm wounding instrument (Fig. 1). This wounding instrument was specifically designed to provide a relatively large wound that would not be rapidly closed by callus. Bark was removed to expose the cambium, with care being taken to avoid injury to the

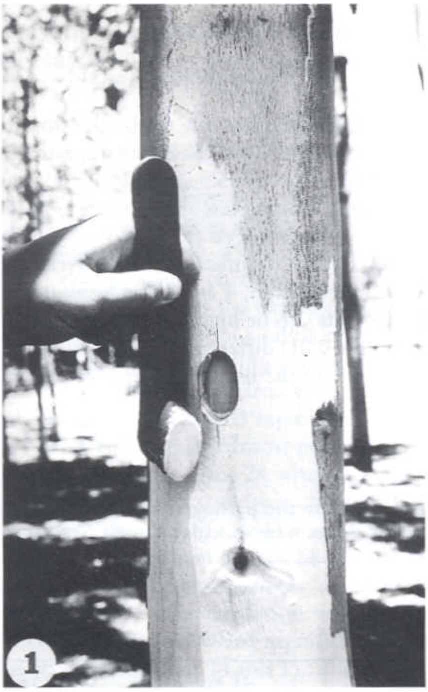

Fig. 1. Wounding tool used to remove an oval-shaped bark disc from trees

vascular cambium. *Cryphonectria cubensis* cultures were prepared by growing a virulent isolate of the fungus in Petri dishes on PDA. A 25 mm × 45 mm disc of colonized agar (similar in shape to the wounding tool), was used to inoculate 20 trees of each clone. Sterile PDA discs were used to inoculate the remaining 20 trees per clone, that served as controls. All wounds were covered with masking-tape to limit contamination and the inoculum from drying out. Six weeks after inoculation, the masking-tape covering the wounds was removed and the width of the exposed wound area between the callus tissue was measured. The circumference of each tree was measured at the time of inoculation/wounding and 6 weeks thereafter. This was done to indicate the average increase in tree diameter over a 6-week period.

2.4 Statistical analysis

The data obtained from this study were statistically analysed using a two factorial analysis of variance to assess differences in susceptibility towards Cryphonectria infection and the ability of *E. grandis* clones to close wounds. Means were tested for significance according to Tukey's procedure (Steel and Torrie 1980). Virulence of the pathogen on the various clones was correlated using Spearman's rank correlation coefficient (r_s) with the ability of these clones to close wounds (Steel and Torrie 1960). Spearman's rank correlation coefficient was also used to show the correlation between relative increase in diameter of trees and their ability to close wounds (Steel and Torrie 1960).

3 Results

3.1 Pathogenicity tests

Inoculation of clones with *C. cubensis* gave rise to the development of cankers of variable size. No Cryphonectria cankers occurred in the control treatment. Mean lesion width was greatest on clones 25 (226.7 mm) and 24 (185.5 mm) (Table 1). This confirmed the results of a previous study, using the same clones inoculated with *C. cubensis*, showing that these clones are highly susceptible to the pathogen (M. J. Wingfield unpublished data). Clones 22 and 23 were also highly susceptible to infection, but differed significantly (p = 0.01) from clones 25 and 24 (Table 1). Clones 1, 2, 3, 4 and 5 are known, from past field inoculations (M. J. Wingfield unpublished data), to be highly tolerant to infection by *C. cubensis*. These clones showed no significant (p = 0.01) differences in susceptibility amongst each other, but differed significantly (p = 0.01) from the highly susceptible clones (Table 1). The remaining

Table 1. **Lesion and wound widths for the pathogenicity and wounding study, 6 weeks after 25 *Eucalyptus grandis* clones were inoculated with *Cryphonectria cubensis*[1]**

		Pathogenicity study[2]	Wounding study[2]	
Clone No.	Stem diameter increase after 6 weeks (mm)	Lesion width (mm)	Width inoculated (mm)	Width control (mm)
1	42.54	41.3a	10.1a	6.9a
2	43.87	48.8ab	11.7ab	9.0abc
3	68.00	50.5abc	11.9ab	7.1ab
4	25.56	51.2abc	12.2ab	10.4abcd
5	53.76	51.4abc	12.8ab	10.9abcde
6	32.01	52.3abc	13.5abc	11.7abcde
7	59.34	52.3abc	13.3abc	11.4abcde
8	76.31	52.6abc	13.9abc	12.0abcde
9	68.90	53.9abc	14.4abc	13.7abcdef
10	42.89	56.7bcd	14.6abc	13.4abcdef
11	39.23	57.1bcd	15.0abc	13.9abcdef
12	46.57	57.4bcd	15.8abcd	14.5bcdef
13	57.82	57.7bcd	15.6abcd	14.6bcdef
14	66.00	57.9bcd	16.2abcde	14.9cdef
15	51.26	59.5bcde	16.9abcde	16.0cdef
16	35.76	62.5cde	16.8abcde	15.8cdef
17	35.08	65.2cdef	16.7abcde	15.7cdef
18	76.93	66.1cdef	17.2abcde	15.9cdef
19	23.56	70.8cdef	17.6abcde	16.1cdef
20	65.34	78.5defg	17.9bcde	16.8def
21	19.35	84.7efgh	18.3bcde	17.0def
22	36.00	95.6fgh	19.1bcde	17.5def
23	49.03	101.3gh	20.8ede	18.3ef
24	64.46	185.5h	23.7de	20.6f
25	68.94	226.7i	22.9e	20.1f

[1] Each value represents the average of 20 replications. Values in each column followed by different letters, differ significantly at p = 0.01 according to Tukey's procedure for comparison of means.

[2] Two identical plots of 25 *E. grandis* clones were used for the pathogenicity and wounding study. For the pathogenicity study, trees were inoculated by inserting inoculum into a wound made with a 10 mm cork borer. For the wounding study, a 25 mm wide ellipsoidal wound was made on stems, and half of these wounds were filled with inoculum. Results show widths of exposed wounds at the termination of the experiment.