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Susceptibility of Eucalyptus grandis to Cryphonectria cubensis

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Abstract

Artificial inoculation of rooted *Eucalyptus grandis* cuttings with *Cryphonectria cubensis* in the glasshouse showed that significant differences exist in the susceptibility of various *Eucalyptus* clones to the pathogen. A 61.3 % difference in susceptibility was observed between the most resistant and susceptible group of clones. Results appeared, however, to have been influenced by genotype × environment interaction.

1 Introduction

The forest industry in South Africa has in recent years established extensive plantations consisting of selected *Eucalyptus* clones. Misgivings have, however, arisen that clonal forestry will give rise to large, genetically uniform stands that will be at risk from pests and diseases. Discovery in South Africa of *Eucalyptus* canker caused by *Cryphonectria cubensis* (Bruner) Hodges (Wingfield et al. 1989), has thus necessitated the initiation of a strategy to ensure that clones susceptible to this important disease are not widely planted.

Clonal selection is traditionally done in the field and is based on the phenotypic appearance of ortets (Leakey et al. 1982; Zobel and Talbert 1984; Leakey 1987). Trials to test the disease resistance of clonal populations on specific field sites may, however, take many years to complete. A method which could provide an earlier assessment of resistance or even a preliminary ranking would, therefore, be extremely useful.

Considerable inter- and intraspecific variation in resistance to *Cryphonectria* canker is known to exist within the genus *Eucalyptus* (Alfenas et al. 1983). Vegetative propagation could, therefore, provide an opportunity to screen clones for resistance to certain diseases. In Brazil, canker caused by *C. cubensis* was minimised by the selection of *E. grandis* trees that displayed some measure of resistance to the pathogen in the forest and the subsequent establishment of clonal plantations (Campinhos and Ikemori 1983). In South Africa, we have no knowledge as to the susceptibility of large commercial plantations of *E. grandis* clones to *Cryphonectria* canker. The limited occurrence of the disease in this country precludes field selections for disease resistant trees. The alternative is to resort to artificial screening techniques.

Artificial inoculations in the forest are generally very time consuming and labour intensive. Therefore, in this preliminary study, the feasibility of screening rooted cuttings of commercially important *E. grandis* clones for resistance to *C. cubensis* was tested in the glasshouse. Owing to the relatively small size of cuttings, a primary aim of this study was to establish whether measurable variation amongst clones can be observed on rooted cuttings after artificial inoculation. Repeatability of results over a short space of time was also considered.

2 Methods

Inoculum of *C. cubensis* was prepared by culturing the fungus in petri dishes on potato-dextrose agar (PDA) overlaid with sterile gauze strips (10 × 50 mm). Thirty 6-month-old ramets of each of twenty *E. grandis* clones were wounded by lightly scraping off a length of bark (3 × 10 mm) from the stem of each plant. Fifteen ramets of each clone were then inoculated by wrapping a strip of gauze colonized by *C. cubensis* around each wound. Sterile gauze strips were used for fifteen control plants of each clone. Parafilm was then wrapped around all gauze-covered wounds. After 10 days, bark surrounding inoculation points on one side of each stem was removed with a scalpel, and extent of cambial discolouration along the length of the stem was measured. Isolations were made from the discoloured cambium on each plant on PDA to confirm the presence of *C. cubensis*. The trial was arranged as a completely randomized design and a one-way analysis of variance (ANOVA) was performed on the lengths of cambial lesions resulting from inoculations. A Scott-Knott test (GATES and BILBRO 1978) was used to separate treatment means into discrete, non-overlapping groups or clusters.

A second trial was conducted six months later under similar environmental conditions in the glasshouse with six clones which, in the first trial, displayed either high or low levels of resistance. Inoculations were performed as described previously and isolations were made from the discoloured cambium to confirm the presence of *C. cubensis*. The trial was arranged as a completely randomized design with 15 seedlings per treatment. A two factorial variance analysis was performed on corresponding data of both trials to determine the degree of variation between trials. The Scott-Knott test (GATES and BILBRO 1978) was used to separate treatment means. A Spearman correlation coefficient was calculated to investigate similarity in ranking order between the two trials.

3 Results

All plants inoculated with *C. cubensis* in the first trial displayed cambial lesions 10 days after inoculation. *C. cubensis* was re-isolated from the advancing edge of cambial lesions on all inoculated plants. Control plants showed no sign of cambial discolouration. Two weeks after inoculation with *C. cubensis*, foliage above inoculation points on all plants had died due to ring-girdling of the stem.

There were significant differences ($P < 0.01$) in lesion lengths between different clones (Fig. 1). The 20 clones were separated into four distinct groups (A–D) according to mean lesion length by means of the Scott-Knott test. There was a 61.3% difference in mean lesion length between the most resistant and susceptible group of clones. Some degree of

Table 1. **Average responses of ramets of 6 clones of Eucalyptus grandis to artificial inoculations in the glasshouse with Cryphonectria cubensis**

Clone	Mean lesion length (mm)[1]			
	Trial 1	Rank	Trial 2	Rank
GC15	19.9[a]	1	20.7[a]	1
TAG27	24.5[b]	2	24.4[b]	2
ZG14	25.3[b]	2	32.5[c]	3
GC10	26.1[b]	2	24.8[b]	2
TAG73	28.9[c]	3	26.9[b]	2
TAG28	31.3[c]	3	25.6[b]	2

[1] Means followed by the same letter are significantly similar ($P < 0.05$) according to the Scott-Knott procedure. S. E. = 6.4.

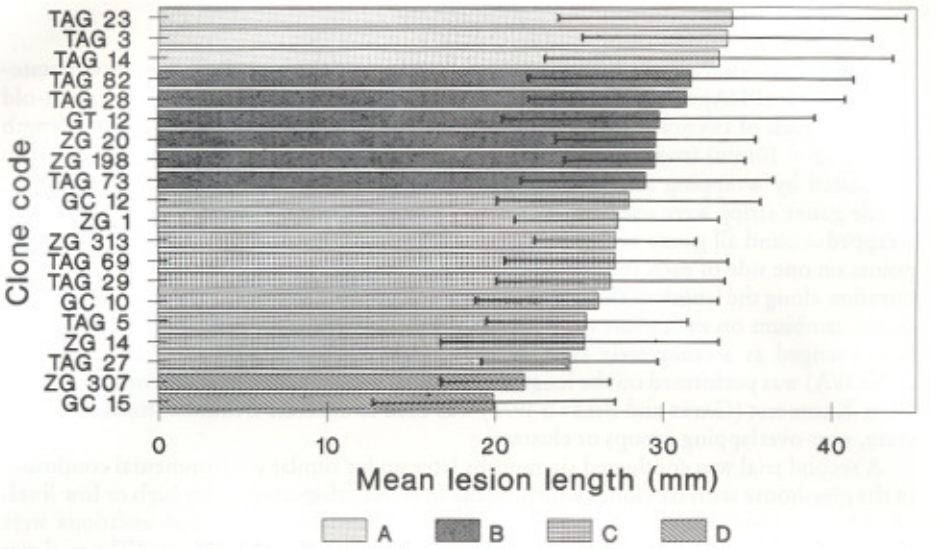

Fig. 1. Mean lesion lengths of 20 *Eucalyptus grandis* clones 10 days after inoculation with *Cryphonectria cubensis* in the glasshouse. Clones denoted by the same uppercase letter are not significantly different ($P < 0.01$) according to the Scott-Knott test. Thin black bars indicate standard deviation for each clone. S. E. = 3.3

variation did exist among ramets within a particular clone; standard deviations for the 20 clones varied from 4.73 to 10.28 (Fig. 1).

In the second trial, there were significant differences ($P = 0.05$) in lesion length between the six clones (Table 1). There was a significant clone × trial interaction ($P < 0.01$) which was evident from the ranking order of particular clones which differed significantly between trials ($P < 0.05$). This difference was most apparent for two clones; lesions caused on TAG28 and ZG14 respectively, were 22.4% shorter and 28% longer in the second trial. The ranking of three clones, GC15, TAG27, and GC10, remained the same for both trials (Table 1).

4 Discussion

In Brazil, positive results have been obtained in the selection of *E. grandis* trees resistant to *C. cubensis* (Campinhos and Ikemori 1983). Artificial inoculations of 1.5-year-old seedlings in the glasshouse with *C. cubensis* indicate that lesion length is an effective parameter for measuring resistance among *Eucalyptus* species and that results can be extrapolated to the field (Alfenas et al. 1983). The present study indicates that artificial inoculations are also effective in glasshouse assessments of clonal resistance to *C. cubensis*.

Although the present study indicated some general trends, the discrepancy between the two trials in terms of the ranking order indicates a need for caution when evaluating artificial screening trials for resistance to diseases. A special concern in tree improvement and genetic testing relates to the genotype × environment interaction (Zobel and Talbert 1984). Environment in the context of this principle, may result in pseudoresistance complicating the interpretation of true genetic or inherent resistance of plants. Pseudoresistance refers to the apparent resistance of potentially susceptible plants that may be caused by age, environment, cultural conditions, or other factors. Change of rank is an important indication of genotype × environment interaction. It is therefore, crucial to distinguish between pseudoresistance and genetic resistance if a program to select for resistance to *C. cubensis* is to be successful.

It is important to develop resistance that is effective over several, if not all, developmental stages of the host. The age difference of six months between plants used in the two trials probably resulted in the interaction observed between trials. Thus it would seem prudent to repeat glasshouse trials a number of times with rooted cuttings of varying age-groups before drawing any conclusions as to the relative resistance of *Eucalyptus* clones to *C. cubensis*.

The primary emphasis of this study was to evaluate the feasibility of inoculating rooted cuttings in the glasshouse and measuring differences in resistance between clones of *E. grandis*. While this appears to be possible, difficulties with genotype × environment interaction are likely to be experienced. Furtermore, it is important that the degree of correlation between glasshouse and field trials is determined in future investigations. Only then recommendations based on the extrapolation of glasshouse data to the field can be made.

Résumé

Sensibilité de Eucalyptus grandis à Cryphonectria cubensis

L'inoculation artificielle en serre de boutures racinées de *E. grandis* par *C. cubensis*, a montré que des différences significatives de sensibilité existent entre divers clones d'*Eucalyptus*. Une différence de 61,3 % à été observée entre le groupe le plus résistant et le moins résistant. Les résultats apparaissent cependant avoir été influencés par l'interaction génotype × environnement.

Zusammenfassung

Anfälligkeit von Eucalyptus grandis gegenüber Cryphonectria cubensis

Künstliche Inokulationen von *Eucalyptus grandis* Stecklingen mit *Cryphonectria cubensis* im Gewächshaus zeigten signifikante Unterschiede in der Anfälligkeit verschiedener *Eucalyptus* Klone gegenüber dem Pathogen. Zwischen dem anfälligsten und dem resistentesten Klon wurde ein Unterschied von 61,3 % in der Anfälligkeit festgestellt. Die Resultate lassen zudem vermuten, daß der Grad der Anfälligkeit auch von der Wechselwirkung zwischen Genotyp und Umwelt abhing.

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