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Susceptibility of Elite *Acacia mearnsii* Families to Ceratocystis Wilt in South Africa

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Ceratocystis albofundus is a recently described pathogen infecting *Acacia mearnsii* in South Africa, and it causes a disease known as Ceratocystis wilt. Symptoms of the disease include die-back, gummosis and wilting of infected trees. In order to select trees tolerant to this fungus, susceptibility tests were conducted on trees representing fourteen families of *A. mearnsii*. A virulent isolate of *C. albofundus* was selected and inoculated into the stems of twelve-month-old plants in a plantation. Lesion lengths, in the bark, and disease development were assessed after 6 weeks. All fourteen families of *A. mearnsii* were susceptible to infection by *C. albofundus*. Considerable variation was, however, noticed between individual trees within the same family and the incorporation of disease tolerant trees into breeding programmes is proposed.

Key words: *Acacia mearnsii*, *Ceratocystis albofundus*, disease tolerance

Acacia mearnsii de Wild. (black wattle) is an important exotic plantation species in South Africa and provides a valuable source of export income and employment. The wood is used for the production of high quality paper, viscose, mining timber, poles, charcoal, and firewood. Bark derivatives, such as tannin, are utilized in the manufacture of adhesives used in chipboard, plywood and corrugated cardboard containers (Acland, 1971; Rusk *et al*., 1990; Anonymous, 1993, 1994).

Ceratocystis albofundus De Beer, Wingfield and Morris is a recently described fungal pathogen of *A. mearnsii* in South Africa (De Beer, 1994; Wingfield *et al*., 1996). This fungus was first reported from *A. mearnsii* in the KwaZulu-Natal Midlands of South Africa in 1990 after trees were observed dying of an unknown cause (Morris *et al*., 1993; De Beer, 1994). The pathogen was first tentatively identified as *Ceratocystis fimbriata* Ellis et Halsted, but after comprehensive morphological and molecular comparisons, it was shown to represent a new species of *Ceratocystis* (Wingfield *et al*., 1996). Distinguishing characteristics of this fungus include light perithecial bases with dark necks and divergent ostiolar hyphae (De Beer 1994; Wingfield *et al*., 1996). The disease caused by *C. albofundus* is known as Ceratocystis wilt and it is characterized by wilt and die-back of trees, canker formation on stems, gummosis and wood discolouration (Morris *et al*., 1993; De Beer, 1994).

Acacia mearnsii, like other plantation species in South Africa, is planted in a monoculture with a relatively uniform genetic base (Wingfield, 1984; Wingfield and Swart, 1994). This greatly increases the risks associated with diseases of these trees. No effective control measures currently exists for the control of Ceratocystis wilt, or any other fungal pathogen, of *A. mearnsii*. Clonal forestry with *Eucalyptus* spp. have, however, proven to be very effective in the management of fungal diseases, such as Cryphonectria canker (Wingfield *et al*., 1997). The aim of this study was to screen fourteen commercial families of *A. mearnsii* for their relative susceptibility to Ceratocystis wilt. It is hoped that this will provide the industry with disease tolerant families and so reduce the impact of diseases.

Materials and Methods

Susceptibility tests, using *C. albofundus* isolate CMW4850 were conducted on twelve month old *A. mearnsii* trees in the field. The test isolate was obtained from a single ascospore transfer from an isolate collected from a diseased *A. mearnsii* tree during December 1994. The isolate is maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), Tree Pathology Co-operative Programme (TPCP), University of Pretoria, South Africa. The experiment was conducted on the Bloemendal Experimental farm (29° 32' 93"S, 30° 27' 33"E) near Pietermaritzburg in the KwaZulu/Natal Midlands. Fourteen commercial *A. mearnsii* families were supplied by the Institute for Commercial Forestry Research (ICFR) for screening purposes. With the exception of family 9–71, these families were all obtained from controlled crosses, made from trees that had been evaluated on growth, form and visual disease appearance. Trees were planted from seed as five randomly replicated five tree plots, for each of the families. Inoculations were conducted in December 1995 and the results evaluated in January 1996.

Inoculations were done by removing a piece of bark with a 9 mm diameter cork borer, thus exposing the sapwood. A piece of agar overgrown with *C. albofundus* was placed into each wound on the stem, approximately 1.5 m above soil level, and covered with masking tape to prevent desiccation of the wound and fungus. The relative susceptibility of each tree was determined after 6 weeks by measuring the size of the lesion produced by *C. albofundus*. The test fungus was re-isolated from the inoculated trees at the completion of the experiment. Lesion lengths were measured in the outer bark of inoculated trees. The data were analyzed using the SAS for PC programme (6.04) [SAS Institute 1991, CARY, NC]. The

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analysis of variance procedure, using Tukey's studentized range test (HSD), was used to determine the significance of differences between families. Heritibility assessments were also applied to the results (Falconer, 1960; Patterson and Thompson, 1971).

Results

Ceratocystis albofundus was highly virulent to trees in all fourteen families of *A. mearnsii* tested. Inoculations resulted in the formation of longitudinal lesions (Fig. 1a) originating from the inoculation points. On the most susceptible trees, lesions were also formed on the remainder of the stem, mostly above the inoculation wound. These lesions were also caused by *C. albofundus*, which had spread upwards in the vascular tissue of the tree. Many trees also had blister-like lesions (swollen gum pockets) [Fig. 1b] under the bark of the main stem, and the outer bark was discoloured black (Fig. 1c). After 6 weeks, 17.7 % of the total number of trees in the trial had been killed by the fungus (Table 2; Fig. 1d) and many more were showing signs of wilt and die-back. After removal of the bark, brown discoloured areas were found in the wood. Many of the trees were also observed to be exuding gum. Lesion lengths in the bark and sapwood were found to be of the same length.

Individual trees within the fourteen families varied from highly susceptible (dead) to relatively tolerant (very small lesions). The only significant differences ($p = 0.05$) in susceptibility between individual families, however, based on lesion lengths, were between families 15–71, 3–71, 13–71, 10–71, and 16–71. The remaining families did not vary significantly from each other in their susceptibility to *C. albofundus* (Table 1). The most disease tolerant family was 15–71, followed by 3–71 and 13–71, with families 16–71 and 19–71 being the most susceptible. Of the 25 trees inoculated for each family, four had died after 6 weeks in family 15–71, while 10 trees had died in family 16–71. The lowest amount of mortalities were observed in families 13–71 (4 %) and 3–71 (4 %) and the highest amount in families 16–71 (40 %) and 19–71 (44 %) (Table 2).

For the heritibility calculations, the lesion lengths in both the wood and bark were expressed as a proportion of the maximum score and converted by an arcsin transformation to achieve stability of variance and approximate normality. These measurements were then analysed using a mixed model with the random effects being family, plots within blocks and trees within plots, having variance components σ^2_f, σ^2_p, and σ^2_t, respectively. These estimates of the variance components were calculated by restricted maximum likelihood REML and found to be $\sigma^2_f = 0.0284$, $\sigma^2_p = 0.0297$, and $\sigma^2_t = 0.3809$ for the bark and $\sigma^2_f = 0.0263$, $\sigma^2_p = 0.0272$, and $\sigma^2_t = 0.3615$ for the wood. Hence, the heritibility, h^2 was calculated and found to be 0.065 for bark and 0.063 for wood. These small values imply that the genetic component is very small relative to the environmental variation.

Table 1 Lesions produced on 14 families of *A. mearnsii* after inoculation with *C. albofundus* during December 1995.

Family number[1]	Average lesion length in bark(mm)[2,3]
F 15-71	210.20^{a}
F 3-71	233.80^{a}
F 13-71	241.55^{a}
F 10-71	246.20^{a}
F 8-71	298.30^{ab}
F 9-71	305.30^{ab}
F 5-71	347.60^{ab}
F 17-71	383.30^{ab}
F 6-71	419.70^{ab}
F 7-71	449.60^{ab}
F 18-71	468.65^{ab}
F 4-71	544.85^{ab}
F 19-71	629.75^{ab}
F 16-71	727.75^{b}
Mean	393.33

[1]All families originate from full-sib crosses, except for family 9–71. Family numbers refer to numbers of controlled crosses made by the ICFR, Pietermaritzburg. [2]Each value is a mean of 25 measurements. [3]Values followed by different letters differ significantly in the degree of disease tolerance at $p = 0.05$.

Table 2 Percentage mortality experienced within the 14 families of *A. mearnsii* 6 weeks after inoculation with *C. albofundus*.

Famiry number	% Mortality[a]
F 3–71	4
F 13–71	4
F 8–71	12
F 9–71	12
F 10–71	12
F 4–71	16
F 5–71	16
F 6–71	16
F 7–71	16
F 15–71	16
F 17–71	16
F 18–71	24
F 16–71	40
F 19–71	44

[a]Number of trees dead out of total of 25 planted for each family.

Discussion

Results of the susceptibility trials reported in this study confirm that *C. albofundus* is highly pathogenic to *A. mearnsii*. This pathogen is capable of causing death of susceptible trees within a relatively short time period. The economic value of many of the trees that do not die is reduced considerably due to the formation of gum pockets under the bark and discolouration (mottling) of the bark.

Ceratocystis albofundus is a newly described species and is currently known only from South Africa (Morris *et al.*, 1993; Wingfield *et al.*, 1996). The first report of this species was as *C. fimbriata* from a *Protea* sp. (Gorter, 1977). The occurrence of this fungus on *Protea*, which is endemic to South Africa, and the fact that it has not been found elsewhere in the world, suggests that it may have its origin in South Africa. It has, to date, been reported from the KwaZulu/Natal, South Eastern Mpumalanga and Eastern Cape Provinces of South Africa. It thus occurs not only in the major *A. mearnsii* growing areas of

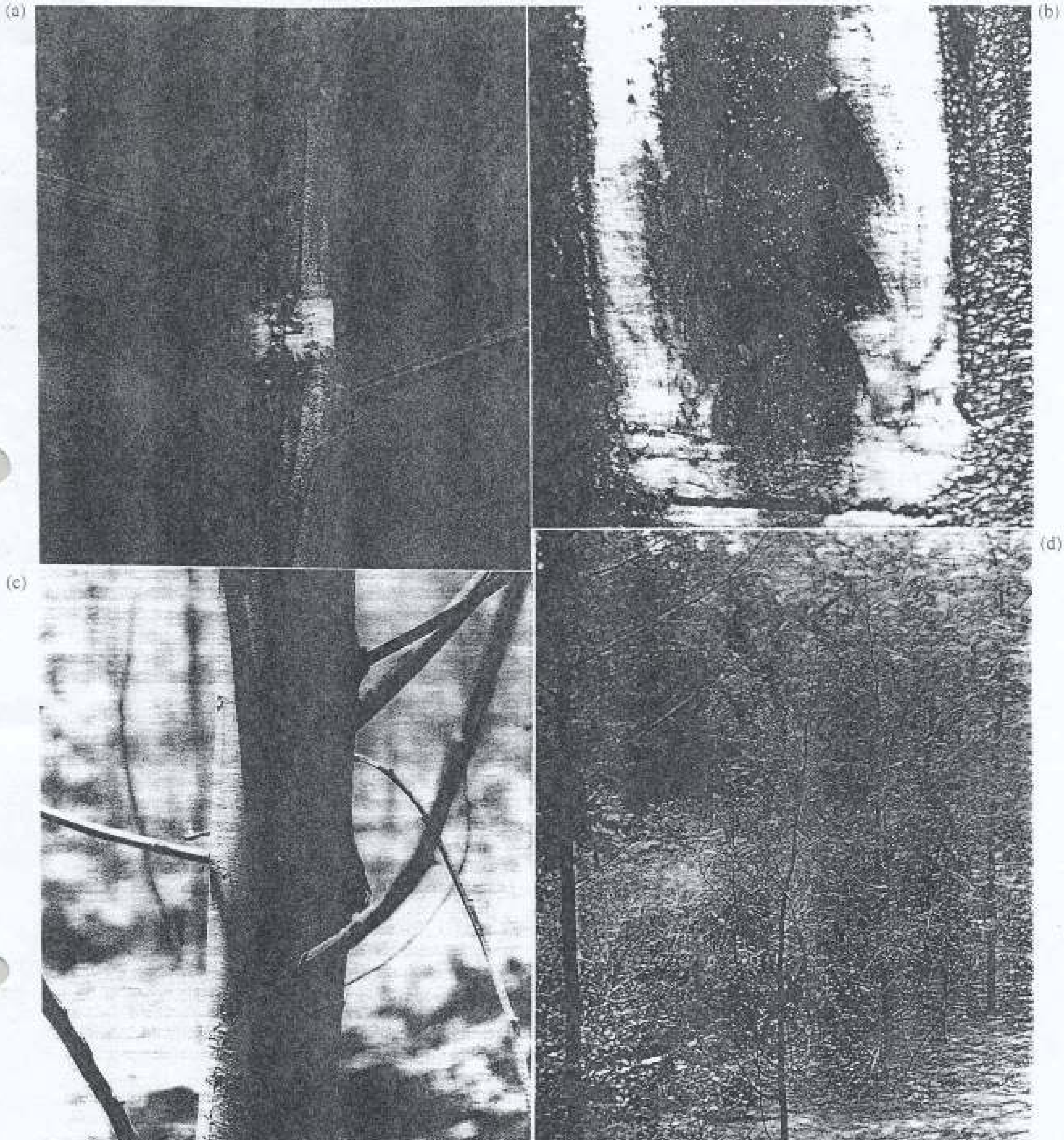

Fig. 1 Symptoms of Ceratocystis wilt on *Acacia mearnsii*, after artificial inoculation with *Ceratocystis albofundus*. (a) Inoculation site, showing the development of a canker from the point of inoculation. (b) Blister-like lesion produced on the stem of an inoculated tree. (c) Black mottle-like lesion developing along the length of the stem. These lesions develop above the point of inoculation. (d) Highly susceptible *A. mearnsii* tree 6 weeks after inoculation. Initial symptoms include the wilting of the tree, whereafter death follows within a few days.

the country, but also in jungle stands outside the commercial growing areas (Roux and Wingfield, 1997).

Ceratocystis spp. are well-known pathogens of various plant species and are the cause of diseases such as black rot of sweet potato (*Ipomoea batatas* [L.] Lam.) (Halsted, 1890), poplar canker (Gremmen and De Kam, 1977), blue stain of conifers (Davidson, 1935; Perry, 1991; Seifert, 1993) cankers of prune, apricot and peach (De Vay *et al.*, 1968; Leather, 1966). It is thus not strange that *C. albofundus*, which is very similar to *C. fimbriata*, is currently the most devastating pathogen of *A. mearnsii* in South Africa.

The wattle industry in South Africa consists of many medium to small scale farmers (70 %) who rely heavily on income from wattle propagation (Anonymous, 1994). The rest of the industry consists of large private companies (Anonymous, 1994). *Acacia mearnsii* is grown both for wood and bark, both being used commercially (Anonymous 1994, 1995; Sherry, 1971). Any cankers or lesions on the bark, which spread into the wood, such as those caused by *C. albofundus*, affect the quality of the bark and increases the labour involved in the removal of the bark from the trees. Bark is harvested manually from felled trees and sold per ton weight. Diseases affecting the bark also lead to a general reduction in yield (Moffet and Nixon, 1963; Sherry, 1971; Haigh, 1993).

It is important to the wattle industry of South Africa to minimize the effects of diseases. Currently the most effective method to accomplish this goal is by planting disease tolerant trees. This paper reports on the first step in selecting *A. mearnsii* trees tolerant to Ceratocystis wilt. The results obtained from this evaluation are, however, of a preliminary nature. The data generated show little evidence for the presence of disease tolerant families. The differences in genetic make-up of family progeny varies considerably and this probably accounts for the lack of clear differences between different families. Despite the susceptibility of all families tested in this experiment, we are encouraged by the presence of disease tolerant individuals within these families. We believe that these individuals will form an important basis for controlled pollinations and a breeding programme that will lead to disease tolerant clones. Vegetative propagation of these plants is also a goal that is being actively pursued. This will allow confirmation of their disease tolerance, by screening their progeny, and form the basis of future clonal propagation of *A. mearnsii* in South Africa.

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