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# Cryphonectria cubensis, a potential pathogen of Psidium guajava in South Africa

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## Abstract

The pathogenicity of an isolate of *Cryphonectria cubensis* was tested in artificial inoculations on *Eucalyptus grandis, Psidium guajava* and *Syzygium cordatum.* The isolate was able to colonize host tissue of all three species following inoculations of branch sections. It rapidly colonized the tissue of potted guava plants of the important commercial cultivar, Fan Retief and resulted in the death of a number of inoculated branches. Pathogenicity was also proven on mature guava trees in the field. An important implication of these findings is that the cultivation of guavas in South Africa could be confronted with a potentially severe disease problem in the future.

## 1 Introduction

*Cryphonectria cubensis* (Bruner) Hodges is an important pathogen of *Eucalyptus* spp. in many countries throughout the world (Bruner 1916; Boerboom and Maas 1970; Hodges et al. 1979; Hodges 1980; Gibson 1981; Davidson and Tay 1983; Sharma et al. 1985 a and b; Florence et al. 1986; Hodges et al. 1986; Old et al. 1986; Wingfield et al. 1989). *C. cubensis* is also reported to occur naturally on myrtaceous hosts other than *Eucalyptus* spp. (Hodges et al. 1986; Hodges 1980). *Endothia eugeniae* (Nutman and Roberts) Reid and Booth, conspecific to *C. cubensis* (Alfenas et al. 1984; Hodges et al. 1986; Micales and Stipes 1984), has been associated with dieback of clove [*Syzygium aromaticum* (L.) Merr. and Perry] in Brazil and Indonesia (Hodges et al. 1986).

Since *C. cubensis* was recently discovered in South Africa (Wingfield et al. 1989), there has been speculation that the pathogen could spread to, or have originated, on other species of *Myrtaceae* in this country. Hodges et al. (1986) found that *C. cubensis* could infect numerous species of *Syzygium* following artificial inoculations. Three genera of indigenous *Myrtaceae* occur in South Africa viz., *Eugenia* L., *Syzygium* Gaertn., and *Metrosideros* Banks ex Gaertn. of which *Syzygium* is the most prominent and represented by *Syzygium cordatum* Hochst., *Syzygium gerrardii* (Harv. ex Hook. f.) Burt Davy., and *Syzygium guineense* (Willd.) DC. Exotic species such as guava (*Psidium guajava* L.) are planted commercially in S. A. on a large scale (Palmer and Pitman 1973). The total guava production for this country is approximately 35000 tons per year of which the cultivar, Fan Retief delivers 98% of total crop (Bolt 1984b; Du Preez 1986). The ability of *C. cubensis* to infect myrtaceous hosts other than *Eucalyptus* spp. could thus pose a potential threat to guava cultivation in this country.

The main purpose of this study was to establish whether local isolates of *C. cubensis* could become a threat to other exotic or indigenous myrtaceous hosts in this country. The relative virulence of a local eucalyptus isolate of *C. cubensis* was therefore tested on *E. grandis, P. guajava* and *S. cordatum.*

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## 2 Methods

### 2.1 Inoculation of branch sections

Freshly cut branch sections (2–4 cm in diameter and 15 cm in length) of *P. guajava*, *E. grandis* and *S. cordatum* were artificially inoculated under laboratory conditions with a single-spore isolate of *C. cubensis* obtained from diseased *E. grandis* trees in Natal, South Africa. Prior to inoculation, sections were surface-disinfected by swabbing with 0,5 % NaOCl and the ends coated with melted paraffin-wax. Each section was inoculated by removing a 10 mm diameter cambial disc with a cork borer and placing a disc of equivalent size from a 5-day-old Potato-dextrose agar (PDA) culture of *C. cubensis* in the wound. Sterile PDA discs were placed in wounds which served as controls. Each inoculated wound was covered with masking-tape. Inoculated branch sections were incubated at 25 °C and the length of the cambial discolouration was measured after 3 weeks. Isolations were made from the advancing edge of each lesion to verify the presence of *C. cubensis*. Ten replicates of each treatment were made. A two-way analysis of variance was performed on the data and Tukey's HSD procedure was used to separate means.

### 2.2 Glasshouse inoculations

Inoculum of *C. cubensis* was prepared by culturing the fungus in petri dishes on PDA overlaid with sterile gauze strips (10 × 50 mm). Twenty 2-year-old guava plants grown in pots under greenhouse conditions were wounded by lightly scraping off a length of bark (3 × 10 mm) from the stem of each plant. Fifteen plants were then inoculated by wrapping gauze colonized by *C. cubensis* around each wound. Sterile gauze strips were use for control plants. Parafilm (American National Can) was then wrapped around all gauze-covered wounds. After 6 weaks the gauze strips and surrounding bark were removed and the length of cambial discolouration measured. Isolations were made from discoloured tissue to verify the presence of *C. cubensis*. The experiment was conducted twice and a two factorial variance analysis was performed on the data of the two trials.

### 2.3 Field inoculation

Five-year-old guava trees were wounded 1.5 m above soil level, by removing a cambial disc with a 12 mm cork borer from each of two lateral branches having the same diameter. A 12 mm disc from a 5-year-old PDA culture of *C. cubensis* was placed in one wound and a sterile PDA disc in the other. All wounds were covered with masking-tape to prevent contamination and the inoculum from drying out. After 6 weeks the masking-tape and bark surrounding wounds was removed and the length of cambial discolouration measured. Isolations were made from the advancing edge of each cambial lesion to verify the presence of *C. cubensis*.

## 3 Results

### 3.1 Inoculation of branch sections

Cambial lesions were significantly shorter ($P < 0.05$) on *Syzygium* than on *Psidium* and *Eucalyptus* (Table 1, Fig. 1). Pycnidia had formed on the bark surrounding lesions of all three species. The mean length of cambial discolouration associated with the three species was significantly greater ($P < 0.05$) than that associated with controls (Fig. 2). Isolations from all lesions resulting from inoculation with *C. cubensis* revealed the presence of the pathogen.

*Table 1.* **Lesion lengths on excised stems of three species of Myrtaceae after inoculation with Cryphonectria cubensis**

| Species | Lesion length (mm)[1] | |
|---|---|---|
| | Inoculation | Control |
| *Psidium guajava* | $87.16^{c}$ | $10.41^{a}$ |
| *Eucalyptus grandis* | $71.92^{c}$ | $11.66^{a}$ |
| *Syzygium cordatum* | $44.50^{b}$ | $10.06^{a}$ |

[1] Values are means of ten Replicates. Values followed by the same letters within and between columns are not significantly different at $P < 0.05$. S. E. = 9.0

### 3.2 Glasshouse inoculations

Six weeks after inoculation with *C. cubensis*, the mean length of cambial discolouration was significantly greater ($P < 0.01$) than that for the controls (Table 2). The foliage above inoculation points of five plants had died due to ring-girdling (Fig. 3) and pycnidia were visible on the bark surrounding the inoculated lesions. The pathogen was isolated from all lesions that had resulted from inoculation with *C. cubensis*.

*Table 2.* **Lesion lengths on stems of Psidium guajava after field and glasshouse inoculations with Cryphonectria cubensis**

| | Lesion length (mm)[1] | |
|---|---|---|
| | Inoculation | Control |
| *Glasshouse inoculation* | | |
| Trial 1. | $32.66^{a}$ | $10^{b}$ |
| Trial 2. | $29.17^{a}$ | $10^{b}$ |
| *Field inoculation* | $88.04^{a}$ | $12^{b}$ |

[1] Values are means of fifteen replicates. Values followed by different letters between columns are significantly different at $P < 0.01$. Glasshouse inoculation S. E. = 14.82; Field inoculation S. E. = 9.63

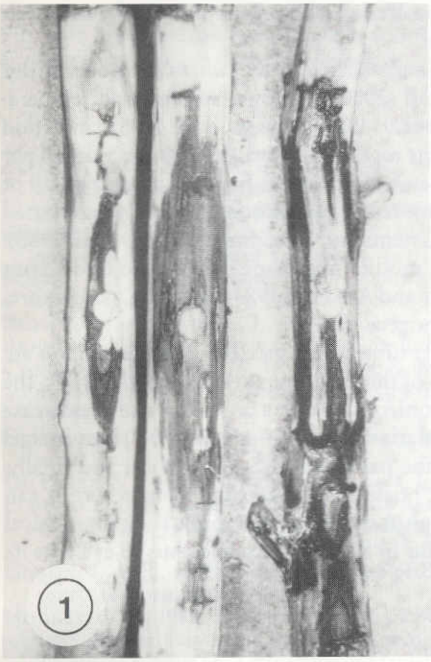
### 3.3 Field inoculations

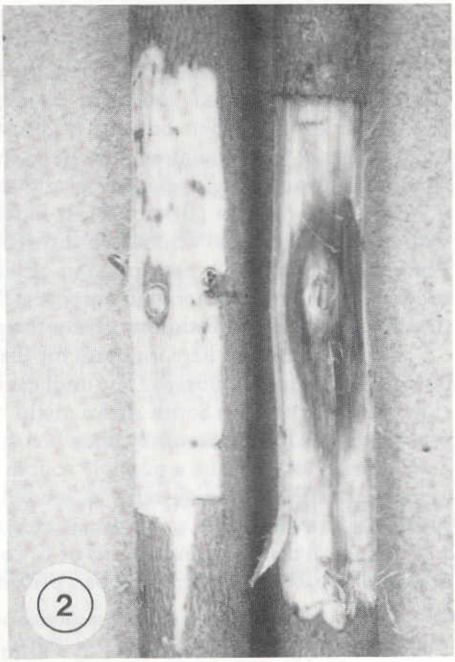
All branches inoculated with *C. cubensis* displayed cambial lesions (Fig. 4), and pycnidia were observed on the surrounding bark. Control plants showed no sign of cambial discolouration and callus formation was far more advanced than in plants inoculated with *C. cubensis* (Table 2). The pathogen was re-isolated from all lesions that had resulted from inoculation with *C. cubensis*.

## 4 Discussion

The present study demonstrated that an isolate of *C. cubensis*, from cankers on *E. grandis* was able to colonize host tissue of *S. cordatum* and *P. guajava* following artificial inoculations of branch sections. HODGES et al. (1986) tested the pathogenicity of an isolate of *C. cubensis* originating from *E. saligna* Sm. in Hawaii on branch sections of 13 species of Myrtaceae including *P. guajava*. The pathogen grèw on only four of species tested, excluding *P. guajava*. The disparity in the results of the two studies can probably be ascribed to differences between the isolate or cultivar used in each case.

It is significant that the isolate of *C. cubensis* used in the present study was able to cause lesions of approximately equivalent length on excised branches of mature *E. grandis* and *P. guajava* plants. The isolate, moreover, was able to colonize the tissue of potted guava





*Figs. 1–4.* Inoculation of three *Myrtaceae* genera with *Cryphonectria cubensis*. – Fig. 1. Lesions caused by *C. cubensis* on *Syzygium*, *Eucalyptus* and *Psidium*, from left to right. – Fig. 2. Control inoculation (l) and inoculation with *C. cubensis* (r) on branch section. – Fig. 3. Death of foliage above inoculation point 6 weeks after inoculation. – Fig. 4. Cambial lesion on branches of *P. guajava* following field inoculation (r) and control (l)