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SHORT SCIENTIFIC COMMUNICATION

A rapid, apple-based test for virulence in *Cryphonectria cubensis* isolates

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Summary

The presence of the *Eucalyptus* canker pathogen *Cryphonectria cubensis* in South Africa is of concern to the local forestry industry. Quantification of the virulence of this fungus through the inoculation of trees, is both time consuming, and expensive. In this study, the potential to use apples in screening for virulence of isolates of *C. cubensis*, was tested using different apple cultivars and inoculation procedures. The best indication of virulence was given on Golden Delicious apples incubated at 25°C for 14 days. Here, the lesion size associated with inoculations of *C. cubensis*, was found to correlate significantly with the virulence of the isolates, as determined by inoculations on trees.

1 Introduction

Cryphonectria cubensis (Bruner) Hodges, causes a serious canker disease of *Eucalyptus* spp. (Bruner 1916; Alfenas et al. 1983; Hodges et al. 1986; Wingfield et al. 1989). It was reported for the first time in South Africa in 1989 (Wingfield et al. 1989). Current disease management strategies rely on the planting of tolerant or resistant clones in affected areas (Alfenas et al. 1983; Conradie et al. 1990). Virulence tests, for *C. cubensis*, usually involve inoculating trees, either in the greenhouse or in plantations, and measuring the resultant lesions. Conradie et al. (1992) considered the potential for evaluating the virulence in greenhouse trials, and found this to be a reasonable approach. The efficacy of this approach has, however, been questioned by Van Der Westhuizen et al. (1992) who noted that the age of trees and consistency of experimental conditions can lead to variable results. Trees are also expensive, and a more controlled system for virulence screening, not requiring trees, would be useful. In this study, the potential of using apples in screening isolates of *C. cubensis* for their relative virulence was considered. Optimal conditions were also established for determining the relative virulence of *C. cubensis* using apples.

2 Materials and methods

Two South African *C. cubensis* isolates, one hypovirulent (D 13 – 3) (Van Der Westhuizen et al. 1994), and one virulent (CMW 2113), were used in this study. *Cryphonectria parasitica* strains EP 713 (ATCC 52571, hypovirulent), and EP 155 (ATCC 38755, virulent), obtained from the American Type Culture Collection, were used for comparative purposes. The apples (Golden Delicious, Starking and Granny Smith) were surface disinfected with ethanol, and single, 9 mm diameter holes were punched into the sides of the apples to a depth

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of approximately 15 mm. Each apple was inoculated with an agar (MEA) plug bearing fungal mycelium, and covered using the apple plugs which had been removed from the inoculation site. The inoculated apples were incubated in apple boxes sealed in black plastic bags. Control inoculations with sterile agar were included in all trials. The apple cultivar best suited for virulence screening, the optimum incubation temperature (20, 25, 30°C), and the optimal incubation period were determined by measuring the lesion sizes in two directions at 180° to each other, using an electronic calliper. These experiments included at least 12 replicate apples, and each experiment was repeated once. The mean lesion diameters for the virulent and the hypovirulent isolates of the test fungi were compared with each other using Student's *t*-test (SNEDECOR and COCHRAN 1980). Lesions were regarded as significantly different from each other at $p < 0.05$.

3 Results and discussion

In this study, it was possible to rapidly assess the relative virulence of *C. cubensis* isolates using the apple inoculation technique. The apple cultivar best suited for this was Golden Delicious, and the optimal condition for incubation was 2 weeks at 25°C. These results are consistent with those for *C. parasitica* (FULBRIGHT 1984; ELLISTON 1985; ENEBACK et al. 1994), although an incubation period of 3 weeks was used in the latter system.

Dark brown sunken lesions developed around the inoculation sites on apples (Fig. 1). The differences in mean lesion size between the virulent and hypovirulent *C. cubensis* isolates were significant in both Golden Delicious and Starking apples with the most significant difference between virulent and hypovirulent isolates being on Golden Delicious apples (Fig. 2). The use of Golden Delicious is consistent with published studies for *C. parasitica*, where the same cultivar was used (FULBRIGHT 1984; ELLISTON 1985; ENEBACK et al. 1994).

It was possible to visually discriminate between virulent and hypovirulent isolates of *C. parasitica* at all three temperatures considered. However, for *C. cubensis* this was possible

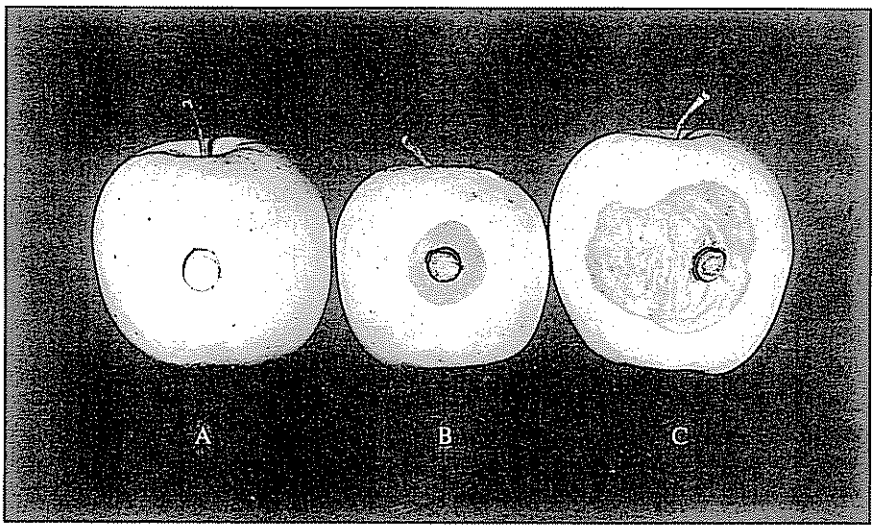

Fig. 1. Lesions associated with inoculation of *Cryphonectria cubensis* on Golden Delicious apples. Each apple cultivar was inoculated with (A) a sterile agar control, (B) a hypovirulent isolate (D 13-3) of *C. cubensis* and (C) a virulent isolate (CMW 2113) of *C. cubensis*

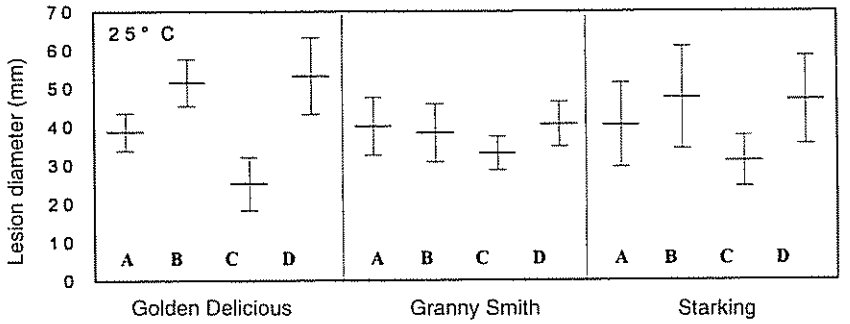

Fig. 2. Mean lesion diameter (with standard deviation) on three different apple cultivars after inoculation with *Cryphonectria cubensis* and *C. parasitica*, incubated at 25°C for 2 weeks. The resultant mean lesion diameters and standard deviations are indicated for each isolate tested with (A) a hypovirulent *C. cubensis* isolate (D 13–3), (B) a virulent *C. cubensis* isolate (CMW 2113), (C) a hypovirulent *C. parasitica* isolate (EP 713) and (D) a virulent *C. parasitica* isolate (EP 155)

only at 25°C. It is believed that this is also due to smaller differences in virulence between the virulent and hypovirulent isolates of *C. cubensis*, as compared with *C. parasitica*.

The mean lesion diameter associated with virulent and hypovirulent *C. cubensis* isolates differed significantly ($p < 0.05$) from the seventh day to the 14th day after inoculation (Fig. 3). From day 15, some of the lesions covered more than 50% of the apple surface, and measurement was discontinued. The optimal incubation period of apples inoculated with *C. cubensis* was 2 weeks. This is considerably shorter than the time necessary for greenhouse trials using trees, which run for a minimum of 4–6 weeks. In addition, the establishment of trees representing a uniform genetic base can also take more than 1 year. Assuming that appropriate apples are available throughout the year, this technique would be most useful.

Using apples to test the relative virulence of fungal isolates has many advantages. One of the greatest of these is that apples are less expensive than trees. The technique is versatile in

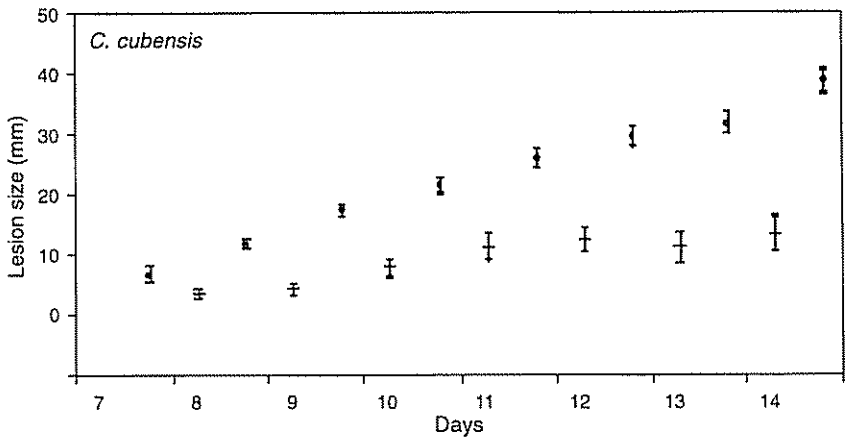

Fig. 3. Lesion diameters on Golden Delicious apples inoculated with *C. cubensis*, measured daily from day 7–14. (—) average lesion size for the hypovirulent isolate; (●) average lesion size for the virulent isolate

that it can be used anywhere, even for research groups not equipped with greenhouses or land on which to establish trees. It is also possible to screen isolates from other parts of the world under conditions that would not pose dangers in terms of quarantine. It is also well suited to screening large numbers of isolates, especially where a quick assessment of virulence is needed.

Acknowledgements

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Résumé

Un test rapide sur pomme pour évaluer la virulence d'isolats de Cryphonectria cubensis

La présence du *C. cubensis* en Afrique du sud, agent de chancre sur Eucalyptus, inquiète l'industrie forestière locale. Quantifier la virulence du champignon par inoculation d'arbres est long et coûteux. Dans la présente étude, on a utilisé différentes variétés de pommes pour tenter de trier des isolats de *C. cubensis* pour la virulence; plusieurs conditions d'inoculation on été testées. Le meilleur résultat a été obtenu avec des Golden Delicious, incubées à 25°C pendant 14 jours.

Zusammenfassung

Ein Schnelltest mit Äpfeln zur Ermittlung der Virulenz von Cryphonectria cubensis

Der Eucalyptus-Krebserreger *Cryphonectria cubensis* ist in Südafrika von erheblicher wirtschaftlicher Bedeutung. Die Quantifizierung der Virulenz dieses Pilzes durch die Inokulation von Bäumen ist zeitaufwendig und teuer. In der vorliegenden Studie wurde die Eignung von Äpfeln für Virurenztests mit Isolaten von *C. cubensis* untersucht. Dabei wurden verschiedene Apfelsorten und Inokulationsmethoden getestet. Der beste Hinweis auf die Virulenz ergab sich auf Golden Delicious-Äpfeln, die bei 25°C für 14 Tage inkubiert wurden. Hier korrelierte die Grösse der von den Inokulationen ausgehenden Nekrosen signifikant mit der Virulenz der Isolate, welche durch Inokulationen von Bäumen bestimmt worden war.

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