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First report of bacterial wilt caused by *Ralstonia solanacearum* on eucalypts in South Africa

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Summary

Ralstonia solanacearum, the causal agent of bacterial wilt, has one of the widest host ranges of all phytopathogenic bacteria. This pathogen was first reported on *Eucalyptus* spp. in the late 1980s in Brazil. Since then, there have been reports of its occurrence on this host in Australia, China and Venezuela. Early in 1997, an 18-month-old clonally propagated *Eucalyptus grandis* × *Eucalyptus camaldulensis* (GC) hybrid in Zululand, KwaZulu/Natal, showed signs of wilting. The vascular tissue of infected trees was dicoloured and bacterial exudation was produced from cut surfaces. The bacterium was consistently isolated from diseased tissue, purified and identified as *R. solanacearum* biovar 3 race 1, using the BioLog bacterial identification system. Inoculation trials were conducted on three *E. grandis* × *E. camaldulensis* clones (GC515, GC550 and GC505). Clone GC550 displayed wilting after 3 days and all cuttings subsequently died. Clones GC515 and GC505 appeared to be less susceptible with cuttings not showing signs of disease until 7 days after inoculation. After 14 days, 90 and 80%, respectively, of cuttings of these two clones had died. This is the first report of bacterial wilt on *Eucalyptus* in South Africa.

1 Introduction

There are over 1.5 million ha of exotic forest plantations in South Africa (Department of Water Affairs and Forestry 1995). Approximately 50% of these plantations are *Eucalyptus* spp. In recent years, a number of fungal diseases have been found to cause severe damage to eucalypts in South Africa. These include, for example, cankers caused by *Cryphonectria cubensis* (Conradie et al. 1990) and *Coniothyrium zuluense* (Wingfield et al. 1997). No reports of phytopathogenic bacteria infecting this host have thus far been reported from South Africa. This is despite the fact that two diseases caused by bacteria have been reported on eucalypts, namely bacterial wilt (Dianese et al. 1990) and bacterial die-back (Truman 1974).

Early in 1997, a rapid wilt of young, clonally propagated *Eucalyptus grandis* × *Eucalyptus camaldulensis* (GC) hybrid trees was noted in a limited area in the KwaZulu/Natal province of South Africa. Symptoms included wilting, leaf drop, death of stems, reduced growth and dark discoloration of the wood. Infected trees usually died within 6 months. The aim of this study was to identify the causal agent of this disease. Three GC clones were also compared for their susceptibility to the pathogen.

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2 Materials and methods

2.1 Isolation procedures

The cut surfaces of the main stems of infected trees were surface sterilized in a 1 : 9 solution of commercial bleach (5.25% sodium hypochlorite), washed with sterile distilled water and then placed in 250 ml Erlenmeyer flasks containing sterile distilled water. After 15 min bacteria were seen streaming from the cut surfaces into the water. A bacterial loop was dipped into the suspension and streaked on to Nutrient Agar (16 g l^{-1} nutrient broth; Biolab Diagnostics Ltd, Halfway House, South Africa, 20 g l^{-1} agar) and incubated at 30°C for 3 days. Bacteria were consistently isolated from infected trees. No bacteria were isolated from healthy tissue.

2.2 Identification procedure

Pure cultures of the bacterium from wilted trees were obtained by streaking out the bacteria from single colonies on BioLog's Universal Growth Medium (BioLog Inc., Hayard, CA, USA). Once pure cultures had been obtained, characteristics such as cell morphology, Gram stain and colony morphology were determined. The bacterium was identified using BioLog's Gram negative microplate technique (GN Microplate®; BioLog), together with BioLog's MicroLog®1, MicroLog 2 or MicroLog 3 computer software program (BioLog).

The biovar was identified on the basis of the bacterium's ability to utilize and/or oxidize three hexose alcohols (mannitol, sorbitol and dulcitol) and three disaccharides (lactose, maltose and cellobiose). The method described by HAYWARD (1964) was used. The hexose alcohols and disaccharides were used as carbon sources in a basal medium containing 1 g of $NH_4H_2PO_4$, 0.2 g of KCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1 g of peptone in 1 l distilled water. Bromothymol blue was added to the media as the indicator dye. The pH was adjusted to 6.8–7.0 before autoclaving. Cultures were incubated at 30°C for 14 days.

The race was identified by inoculating four 12-week-old tobacco plants (cv. A23) with a bacterial suspension using a concentration of 10^9 colony forming units (CFU)/ml. Micropipette tips (P2) containing 100 μl of the suspension were inserted into axils of three leaves of each test plant. After 48 h the inoculum was taken up by the transpiration stream of the plant and the tips were removed. Four other tobacco plants were inoculated with sterile water to serve as the control. The plants were inspected daily and their reaction to infection by the bacterium was recorded.

2.3 Pathogenicity tests

Inoculation tests were conducted on three GC clones, namely, GC505, GC515 and GC550. These three clones had previously been planted commercially in Zululand and were known to be susceptible to the wilt pathogen. Twenty 6-month-old plants of each clone were inoculated with a bacterial suspension (2×10^6 CFU ml^{-1}). Control plants were inoculated with sterile water. Plants were carefully removed from the growth media, the root tips were clipped and the wounded roots dipped into the bacterial suspension or sterile water. The plants were inspected daily. Once symptoms were expressed, the bacterium was re-isolated from infected cuttings using the technique described previously.

3 Results

3.1 Symptoms

In early 1997, 18-month-old clonally propagated GC hybrid trees in Zululand, KwaZulu/Natal, were reported to be dying of an unknown cause. Typical symptoms included wilting and death of trees (Fig. 1A). Upon further investigation it was found that the vascular tissue

Fig. 1. Symptoms associated with bacterial wilt of eucalypts in South Africa. (A) Wilting of a GC clone in the field. (B) Discoloration of the vascular tissue of a wilted tree

of infected trees was discoloured (Fig. 1B) and bacterial exudation was produced from the cut surfaces of the stems.

3.2 Identification

The bacterium isolated from the wilted eucalypt trees was Gram negative, rod-shaped, 0.5–0.7 × 1.5–2.5 μm in size with a lophotrichous flagellar arrangement. Colonies on nutrient agar were cream in colour, fluid, smooth and elevated. The results from the BioLog system identified the bacterium as *Ralstonia solanacearum* (Smith) Yabuuchi et al. The bacterium was able to utilize mannitol, sorbitol and dulcitol and oxidise lactose, maltose and cellobiose. On this basis, the biovar was identified as biovar 3.

Three days after inoculation, the tobacco plants developed typical symptoms of bacterial wilt. Leaf veins turned brown to black, wilted and black lesions developed on the stems of the tobacco plants. None of these symptoms developed on the control plants. Because tobacco is a host only to race 1 (BUDDENHAGEN et al. 1962), these results indicate that this isolate belongs to race 1.

3.3 Pathogenicity tests

In the case of GC550, wilting was evident 3 days after inoculation. After 10 days, leaf drop occurred and by the 14th day all 20 cuttings of this clone had died. Clones GC505 and

GC515 appeared to be slightly less susceptible, with fewer plants showing signs of disease after 14 days. Of the 20 cuttings inoculated, 16 and 18 cuttings of GC505 and GC515, respectively, wilted and subsequently died 14 days after inoculation. *Ralstonia solanacearum* was re-isolated using the method described previously from all inoculated plants. Control plants showed no symptoms.

4 Discussion

In this study, we report, for the first time, on the occurrence of bacterial wilt, caused by *Ralstonia solanacearum*, on eucalypts in South Africa. Its sudden appearance in a single plantation was surprising. This plantation had previously been planted with *Eucalyptus maculata*, which showed no symptoms, thus eliminating the possibility that the pathogen had survived in soil or plant debris from a previous agricultural crop and then subsequently infected the young eucalypts. The compartment is in close proximity to a rural community and cattle were often seen to move through the area (B. ESLER, Mondi Forests, personal communication). It is, therefore, likely that the bacteria were transmitted from the rural farmland where subsistence crops are grown, to the plantation via contaminated soil attached to the hooves of cattle.

Ralstonia solanacearum was first reported on *Eucalyptus* spp. in the early 1980s in Brazil (SUDO et al. 1983 cited by DIANESE et al. 1990). Since then, there have been reports of its occurrence on this host in China (WU and LIANG 1988a), Taiwan (WANG 1992), Australia (ASKIEW et al. 1994) and Venezuela (CIESLA et al. 1996). *Eucalyptus* spp. differ in their susceptibility to this pathogen. In China, the most susceptible species have been found to be *Eucalyptus tereticornis*, *Eucalyptus urophylla* and *E. camaldulensis* (WU and LIANG 1988b). In addition CIESLA et al. (1996) lists *E. grandis, Eucalyptus leizhou, Eucalyptus pellita, Eucalyptus propinqua, Eucalyptus saligna* and *E. grandis* × *E. urophylla* hybrids as being susceptible to *R. solanacearum* under natural conditions. In South Africa, this pathogen was only found to be infecting GC hybrid trees.

Thus far, control of bacterial wilt of various hosts has been ineffective. Breeding for disease resistance has not been very successful because of the extensive variability of bacterial strains and the interactions of a wide range of biotic and abiotic factors (JAVIER 1994). In the case of eucalypts, WU and LIANG (1988a) have found that certain provenances of *E. grandis* × *E. urophylla, E. saligna, Eucalyptus citrodora* and *Eucalyptus exserta* in China are the most resistant to infection, whereas *E. propinqua* and *E. grandis* are moderately susceptible.

Ralstonia solanacearum has been separated into biovars (HAYWARD 1964), which is primarily of application in the context of epidemiology, rather than taxonomy. Some biovars (3 and 4) are more nutritionally versatile than others (1 and 2) (HAYWARD 1994). Biovars 3 and 4 have a broader host range than, for example, biovar 2-A which is specific to potato. The nutritional versatility, for example, of biovar 3 makes it a better competitor in soil where the ability to utilize plant exudates may be advantageous. In Australia and China biovar 3 was found to be infecting eucalypts, whereas in Brazil biovar 1 was reported. The South African isolates are thus similar to those from Australia and China.

Isolates of *R. solanacearum* can be separated from one another into five races differing in host ranges (BUDDENHAGEN et al. 1962; QUIMIO 1976; HE et al. 1983). Race 1 affects tobacco, tomato, many solanaceous hosts and certain diploid bananas. Race 2 causes Moko disease of bananas and bacterial wilt of *Heliconia.* Race 3 affects potatoes and tomatoes, race 4 infects ginger and race 5 infects mulberry. Race 1 of *R. solanacearum* has been recorded on eucalypts from all countries where the wilt of this plant has thus far been found.

The appearance of bacterial wilt in South Africa is of considerable concern to the South African Forestry Industry. Under optimal disease conditions, *R. solanacearum* can

cause extensive damage. Management strategies to reduce the impact of this pathogen in eucalypt plantations are now a priority. A rapid screening technique to detect this bacterium is needed, and commercially important clones should be tested to determine their level of tolerance to bacterial wilt.

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Résumé

Première mention du flétrissement bactérien des eucalyptus à Ralstonia solanacearum, en Afrique du Sud

L'agent de flétrissement *Ralstonia solanacearum*, possède un des plus larges spectres d'hôtes parmi les bactéries phytopathogènes. La bactérie a été mentionnée pour la première fois sur *Eucalyptus* spp. à la fin des années 80, au Brésil. Depuis, elle a été mentionnée sur ce groupe d'hôtes en Australie, Chine et Vénézuéla. Début 1997, un hybride, âgé de 18 mois propagé de façon clonale, *E. grandis* × *E. camaldulensis* (GC), a montré des signes de flétrissement au Zululand, KwaZulu/Natal. Les tissus vasculaires des arbres infectés étaient colorés et une exsudation bactérienne avait lieu à partir des surfaces coupées. La bactérie a été régulièrement isolée des tissus malades, purifiée et identifiée comme *R. solanacearum*, avec le système BioLog. La *R. solanacearum* des eucalyptus appartenait au biovar 3, race 1. Des inoculations ont été réalisées sur trois clones de *E. grandis* × *E. camaldulensis* (GC515, GC550, et GC505). Le clone GC550 a montré un flétrissement après 3 jours et toutes les boutures sont mortes. Les clones GC515 et GC505 sont apparus moins sensibles, les boutures ne présentant des symptômes qu'après 7 jours. Après 14 jours, 90% et 80% des boutures de ces deux clones respectifs sont mortes. Ceci est la première mention du flétrissement bactérien de l'eucalyptus en Afrique du Sud.

Zusammenfassung

Erstnachweis einer durch Ralstonia solanacearum verursachten Bakterienwelke an Eukalyptus in Südafrika

Das Bakterium *Ralstonia solanacearum* verursacht eine Welkekrankheit und hat einen der grössten Wirtskreise aller phytopathogenen Bakterien. Es wurde erstmals Ende der 1980er Jahre an *Eucalyptus* spp. in Brasilien nachgewiesen, später auch in Australien, China und Venezuela. Anfang 1997 zeigten 18 Monate alte klonal vermehrte *Eucalyptus grandis* × *Eucalyptus camaldulensis* (GC)-Hybriden in Zululand (Kwa Zulu, Natal), Welkesymptome. Das Leitgewebe infizierter Bäume war verfärbt und auf frischen Schnittflächen traten Bakterienexsudate aus. Das Bakterium wurde regelmässig aus dem erkrankten Gewebe isoliert und mit Hilfe des BioLog Bacterial Identification Systems als *R. solanacearum*, Biovar 3, Rasse 1 identifiziert. Bei Inokulationsversuchen mit drei *E. grandis* × *E. camaldulensis*-Klonen (GC 515, GC 550 und GC 505) zeigte der Klon 550 nach drei Tagen Welkeerscheinungen und alle Stecklinge starben anschliessend ab. Die Klone 515 und 505 schienen etwas weniger anfällig zu sein und zeigten erst nach sieben Tagen Symptome, nach 14 Tagen waren aber 90 bzw. 80% der Stecklinge abgestorben. Damit wurde diese Bakterienwelke erstmals an Eukalyptus in Südafrika nachgewiesen.

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