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Characterization of *Rhizoctonia* species associated with foliar necrosis and leaf scorch of clonally-propagated Eucalyptus in Brazil

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Abstract

The objective was to identify and characterize the causal agent of foliar necrosis and leaf scorch of *Eucalyptus* spp. in Brazil. Nineteen putative isolates of *Rhizoctonia* obtained from Eucalyptus plants during clonal propagation were compared with isolates from other hosts and with tester strains of anastomosis groups of *Rhizoctonia solani*. Features compared were morphological characteristics of anamorphs and teleomorphs, numbers of nuclei per cell in the vegetative hyphae, anastomosis of hyphae, and ability to produce necrotic lesions on cuttings and damping-off of *E. grandis* × *E. urophylla* hybrid seedlings. *Rhizoctonia solani* AG1 ('AG1-IB like') was the most frequent causal agent isolated from Eucalyptus plants and cuttings with symptoms of leaf scorch and foliar necrosis respectively. These isolates were highly virulent on Eucalyptus cuttings and presented naturally epiphytic growth on Eucalyptus shoots. Binucleate isolates and isolates of *R. solani* AG4 were also virulent on cuttings and were most virulent on Eucalyptus seedlings causing pre- and post-emergence damping-off. Virulence on Eucalyptus cuttings and seedlings was not restricted to a single species or anastomosis group of *Rhizoctonia*.

Introduction

In Brazil, since 1986, severe losses have occurred in clonally-propagated Eucalyptus (*Eucalyptus* spp.) in screenhouses of production nurseries and after outplanting to the field. Losses in screenhouses are chiefly associated with leaf necrosis and stem decay of cuttings during the initial phase of rooting, while in the field the main symptoms are leaf scorch, leaf spot and defoliation. The disease has been observed in the major areas where Eucalyptus is grown but it is assuming more importance in the north of the Minas Gerais State and in the tropical areas as the east coast of the country and in the north Amazon region in the State of Pará (Ferreira and Silveira, 1995; Ferreira, 1991). Causes of the diseases in screenhouses and in the field have been attributed to *Rhizoctonia* spp. (Alfenas et al., 1988) and to *R. solani* Kühn (Carvalho et al., 1989a, b; Ferreira, 1991; Silveira and Alfenas, 1993). Under highly humid conditions, sclerotial epiphytic strains of *R. solani* grow from the soil to the lower branches and, later, to the apical region of shoots and trees and produce leaf scorch and defoliation (Rezende and Ferreira, 1992). These strains also cause mortality of cuttings in nurseries when infected shoots are used for clonal multiplication (Ferreira, 1991). A binucleate isolate of *Rhizoctonia* sp. has been associated with leaf blight of *E. grandis* in highland areas of the State of São Paulo (Ferreira and Silveira, 1995). A more detailed study, especially of the taxonomic relationships of the causal agents is necessary whilst the etiologies of the diseases remain unclear.

The identification of *Rhizoctonia* spp. does not adequately characterize the pathogen because there are many groups that are differentiated primarily by hyphal anastomosis affinity which show distinctive biological characteristics, including pathogenicity, growth habit, and epidemiology (Ogoshi, 1985; 1987). Anastomosis groups of *Rhizoctonia* spp. and of *R. solani* are sometimes considered to constitute reproductively isolated populations or 'biological species' in nature (Anderson et al., 1982). This affirmative has been reinforced by biochemical and molecular data (Jabaji-Hare, 1996; Kuninaga, 1996; Cubeta et al., 1996).

The objective of the present study was to identify and characterize the causal agents of foliar necrosis and leaf scorch of *Eucalyptus* spp. in Brazil. Fungal isolates from Eucalyptus and from other hosts were examined for morphological features of the anamorph and teleomorph. Anastomosis tests were conducted with tester strains of *R. solani* anastomosis groups. Some Brazilian isolates and Japanese tester strains of representative groups of *R. solani* were tested for ability to produce necrotic lesions on cuttings, and damping-off of *E. grandis* × *E. urophylla* hybrid seedlings.

Materials and methods

Pathogen isolates

Putative *Rhizoctonia* isolates were obtained from Eucalyptus cuttings with symptoms of necrosis and from established Eucalyptus plants with leaf scorch symptoms. For isolation, sclerotia and diseased tissues from Eucalyptus were surface-sterilised by immersion in NaOCl (0.1% chlorine) for 20–30 s, washed in sterile water and placed directly on agar medium. After 24–36 h at 25 °C, colonies were examined with the aid of a dissecting microscope and hyphal tips were transferred to potato–dextrose–agar (PDA) medium. The isolates were maintained on PDA medium, stored at 14 °C and transferred at 6-month intervals. Some isolates from other hosts were also included for comparisons (Table 1).

Enumeration of the nuclei

Nuclei were counted in stained vegetative cells with safranine-O in 3% aqueous solution of KOH (Yamamoto and Uchida, 1982; Kronland and Stanghellini, 1988) or Giemsa–HCl (Furtado, 1968; Herr, 1979). The Giemsa–HCl method was improved by using 2-day-old colonies that were grown in a liquid Sucrose–Yeast–Asparagine medium (SYA) (sucrose = 10 g, yeast extract = 2 g, L-asparagine = 2 g, KH_2PO_4 = 1 g, $MgSO_4 \cdot 7H_2O$ = 0.1 g, $ZnSO_4 \cdot 7H_2O$ = 0.44 mg, $FeCl_3$ = 0.48 mg, $MnCl_2 \cdot H_2O$ = 0.36 mg, distilled-H_2O for 1 l final volume, pH adjusted to 5.3 with NaOH before sterilization at 120 °C 20 min).

Hyphal anastomosis tests

Each test isolate was paired with the following Japanese tester strains of *R. solani*: AG1-IA (CSKA, CSIW); AG1-IB (SHIBA2, B19); AG1-IC (F2, BV7); AG2-1 (PS4, F56), AG-2 IIIB (C96, C330), AG2-2 IV (65L.2, RI64); AG3 (ST9, ST-11-6); AG4-HG1 (AH1), AG4 (140); AG5 (CU 8904, GM 10); AG6-HG1 (OHT-1), AG7 (H0-1556) and AG-BI (TS-2-4) (Sneh et al., 1991; A. Ogoshi, pers. comm.). For each pairing, a 0.3 cm-diameter disk from 2- to 4-day-old colonies of the test isolate and the tester strain were positioned 3-cm apart on sterilized cellophane paper on a microscope slide (7.5 × 2.5 cm). Before pairings, the cellophane paper was soaked in distilled water to prevent drying. Slides with disks were kept in a saturated atmosphere in humidity chambers in the dark at 25 °C, and observed for anastomosis after 24, 30, 36, 42, and 48 h. Each pairings were replicated four all times. Positive controls consisted of pairings between tester strains of the same anastomosis group and subgroup. The isolates RH-3, RH-18, and RH-10 were also paired with the other isolates obtained in the study to confirm previous results of anastomosis and enumeration of the nuclei. Anastomosis was regarded as positive when hyphae grew together, made contact, and their walls fused, followed by death of adjacent cells ('killing reaction') (Parmeter et al., 1967; 1969). Hyphal anastomosis was observed with a compound microscope using safranine-O–KOH as a mounting stain (Yamamoto and Uchida, 1982).

Morphology of the anamorph

The color of mycelium, and the presence and morphology of sclerotia and monilioid cells were recorded for isolates grown on PDA for 10 days at 25 °C in the dark. Morphology of sclerotia produced on Eucalyptus sprouts that were naturally infected or artificially inoculated were also examined.

Table 1. Origin, nuclear condition and hyphal anastomosis groups of the isolates of *Rhizoctonia* studied

Isolate	Host	Symptoms	Origin/Date	Nuclear cell number of vegetative hyphae*	Hyphal anastomosis groups and subgroups
RH-1	*Eucalyptus sp.*	Leaf scorch	Ipatinga MG/1987	6–10–13	AG1-IB
RH-2	*Eucalyptus sp.*	Leaf scorch	Rio Jari, PA/1991	6–10–15	AG1-IB
RH-3	*Eucalyptus sp.*	Leaf scorch	Teixeira de Freitas BA/1991	6–10–15	AG1-IB
RH-4	*Eucalyptus sp.*	Leaf scorch	Teixeira de Freitas BA/1992	6–10–12	AG1-IB
RH-5	*Eucalyptus sp.*	Cutting rot	Aracruz, ES/1992	2–(3–4)	ND*
RH-6	*Eucalyptus* sp.	Leaf scorch on seedlings	Viçosa, Mg/1988	6–10–15	AG4
RH-7	*Gossypium* sp.	Damping -off	Viçosa, Mg/1988	6–10–15	AG4
RH-8	*Eucalyptus grandis* H.ex M.	Damping -off	Viçosa, Mg/1988	6–10–15	AG4
RH-10	*Eucalyptus* sp.	Cutting rot	T. de Freitas, BA/1988	2–(3–4)	ND
RH-11	*Eucalyptus* sp.	Cutting rot	Aracruz, ES/1991	6–10–15	AG4
RH-12	*Phaseolus vulgaris L.*	Damping -off	Venda Nova, ES/1992	6–10–15	AG4
RH-15	*Eucalyptus* sp.	Leaf scorch	T. de Freitas, BA/1992	6–10–15	AG1-IC
RH-16	*Eucalyptus* sp.	Cutting rot	Aracruz, ES/1992	6–10–12	AG1-IB
RH-17	*Eucalyptus* sp.	Cutting rot	Aracruz, ES/1992	6–10–15	AG1-IB
RH-18	*Eucalyptus* sp.	Cutting rot	Aracruz, ES/1992	6–10–15	AG1
RH-19	*Eucalyptus* sp.	Leaf scorch	Aracruz, ES/1992	6–10–15	AG1-IB
RH-20	*Eucalyptus* sp.	Leaf scorch	Eunápolis, BA/1993	6–10–15	AG1-IB
RH-21	*Phaseolus vulgaris*	Web-blight	Linhares, ES/1993	6–10–15	AG1-IB
RH-22	*Eucalyptus* sp.	Leaf scorch	T. de Freitas, BA/1993	6–10–15	AG1-IB
RH-23	*Caesalpinia echinata* LAM.	Leaf scorch	Viçosa, Mg/1993	2–(3–4)	ND
RH-24	*Thuja* sp.	Damping -off	Viçosa, Mg/1994	2–(3–4)	ND
RH-25	*Eucalyptus* sp.	Leaf scorch	Ipatinga, Mg/1994	6–10–15	AG1-IB
RH-26	*Phaseolus vulgaris*	Web-blight	Ponte Nova, Mg/1994	6–10–15	AG1-IC
RH-27	*Eucalyptus* sp.	Leaf scorch	Ipatinga, Mg/1995	6–10–15	AG1-IB
RH-28	*Eucalyptus* sp.	Leaf scorch	S. J. dos Campos, SP/1995	2–(3–4)	ND
RH-29	*Eucalyptus* sp.	Leaf scorch	Viçosa, Mg/1995	6–10–15	AG1-IB

* Values in parentheses were observed mostly in terminal cells of young branches of binucleate hyphae. The minor nuclear cell number verified in the multinucleate hyphae were commonly in terminal cells of the young branches and the major nuclear cell number were verified mostly in large thick-walled cells of the mycelium ('runner hyphae').
ND = anastomosis group was not determined because tester binucleate strains for pairings were lacking.

Teleomorph induction and morphology

To promote teleomorph formation, bunches of 10–15 detached Eucalyptus shoots were inoculated with a mycelial suspension of each test isolate of *Rhizoctonia*. The isolates were grown in SYA medium at 25 °C in darkness for 4 days, after which the mycelium was recovered in a Buchner funnel, suspended in tap water (0.05% w/v), and homogenized (Polytron, Brinkman Instruments®) for 1 min. The Eucalyptus shoots were surface sterilized, dried with paper towels, immersed in the mycelial suspensions, and positioned with their basal ends in tap water in 0.5 l containers. The inoculated shoots were kept in a glass chamber, under intermittent misting with aerated water (5 min on : 15 min off), with relative humidity oscillating between 80% and 100%, temperature of 25 °C and a 12-h photoperiod (600–2000 lux, cool-white fluorescent lights). Isolates that did not sporulate on Eucalyptus shoots were evaluated for teleomorph production by the following methods modified from previous authors: (i) transfer from a rich to a poor medium (Whitney and Parmeter, 1963), (ii) addition of aerated steamed soil over cultures on potato–yeast–dextrose agar (PYDA) medium on plates (Tu and Kimbrough, 1975) and (iii) divided plate, with growth from a rich

to a poor medium, under aerated wet to dry conditions (Adams and Butler, 1983a, b). The morphology of basidia, sterigmata, basidiospores, supporting hyphae of the basidium, and hyphal branching of the hymenium tissues were examined under light microscopy (400–1000× magnification), using lactophenol–cotton blue as a mounting stain.

Pathogenicity tests

Isolates that differed in origin, morphology and anastomosis grouping were selected to be compared for ability to produce necrotic lesions on leaves of cuttings and damping-off of young Eucalyptus plants. The substrate for cuttings and seeds was a mixture of composted Eucalyptus bark and vermiculite (7 : 3, v/v) amended with fertilizer Osmocote (NPK, 17 : 9 : 13) at 0.01% (w/v), autoclaved at 120 °C for 1 h and again after 24 h. After sterilization, each 300 g of the mixture was infested with 100 ml of a water suspension of comminuted mycelium (0.05% w/v). The mycelium was obtained as described for teleomorph induction. Each 300-g portion of the infested substrate was lightly compacted into a plastic chamber (Gerbox type).

As in routine use to induce adventitious rooting, the 8–10 cm long cuttings, taken from 45-day-old shoots of *E. grandis* × *E. urophylla* hybrid, were prepared with a pair of leaves each of which was trimmed to half length. Five cuttings were planted in each one of the three chambers per isolate (three replicates). The incubation conditions were as described for teleomorph induction. In the controls, only sterilized water was added to the substrate. After five days, disease severity was assessed on the half-leaves of cuttings, using the following scale: 0, no lesions; 1, lesions <1 cm in diameter; 2, lesions >1 cm in diameter to 50% of the leaf area; 3, lesions on >50% of the leaf area.

To test the ability of isolates to cause damping-off, 49 seeds of *E. grandis* × *E. urophylla* hybrid were sown 1 cm apart on infested substrate in each of a series of three Gerbox chambers, which were closed by lids and positioned in a germination chamber at 25 °C and with a 12-h photoperiod (2000 lux). After 48 h, when the seedlings began to emerge, lids of the Gerbox chambers were removed and the seedlings were irrigated daily with 20 ml sterilized water per unit. Incidence of seedling emergence and of post-emergence damping-off were determined daily from days 7–15 after sowing. Variance analyses of the data and Scott–Knott averages comparison were done on PC compatible software (SAEG – Genetics and Statistics Analyses System, Euclides,1983).

Results

Enumeration of nuclei

Of the six isolates of *Rhizoctonia* from Eucalyptus cuttings with necrosis on leaves, four were multinucleate and two binucleate (Table 1). Among twelve isolates from plants or shoots with symptoms of leaf scorch collected in the field, eleven were multinucleate and one binucleate.

Anastomosis groups

Three multinucleate isolates from plants with leaf scorch, and all multinucleate isolates from necrotic lesions on cuttings, anastomosed with isolates of group AG-1 of *R. solani* (Table 1). Multinucleate isolates RH-6, RH-8, and RH-11 from Eucalyptus, RH-7 from cotton and RH-12 from bean anastomosed with isolates from group AG-4. Binucleate isolates RH-5, RH-10, and RH-28 from Eucalyptus, RH-23 from *Caesalpinia echinata* and RH-24 from *Thuja* sp., did not anastomose with any of the *R. solani* tester strains. When the binucleate RH-10 was used as a tester anastomosis occurred with the binucleate isolates RH-5 and RH-24.

Anamorph morphology

On Eucalyptus shoots, the multinucleate Brazilian isolates RH-1 to 4, RH-17, RH-19, RH-20, RH-22, RH-25, and RH-27 produced small and irregular sclerotia (100–300 μm in length) that were whitish or greyish when young and light-brown when mature (Figure 1D). On PDA, the isolates did not form monilioid cells and their sclerotia were formed among entangled hyphae (Figure 1E). The mycelium was light-brown to yellowish-brown and, on PDA, formed flat sclerotial masses, 2–10 mm in diameter, that were whitish-grey when young and light-brown at maturity. Translucent drops of a dark-brown liquid were exuded in cavities in the surface of sclerotial masses. These isolates were thus morphologically similar to Japanese isolates B19 and SHIBA 2 of *R. solani* AG1-IB (Watanabe and Matsuda, 1966). The other three Brazilian AG1 isolates, RH-16, RH-21, and RH-29, were also similar to *R. solani* AG1-IB isolates on the host, but on

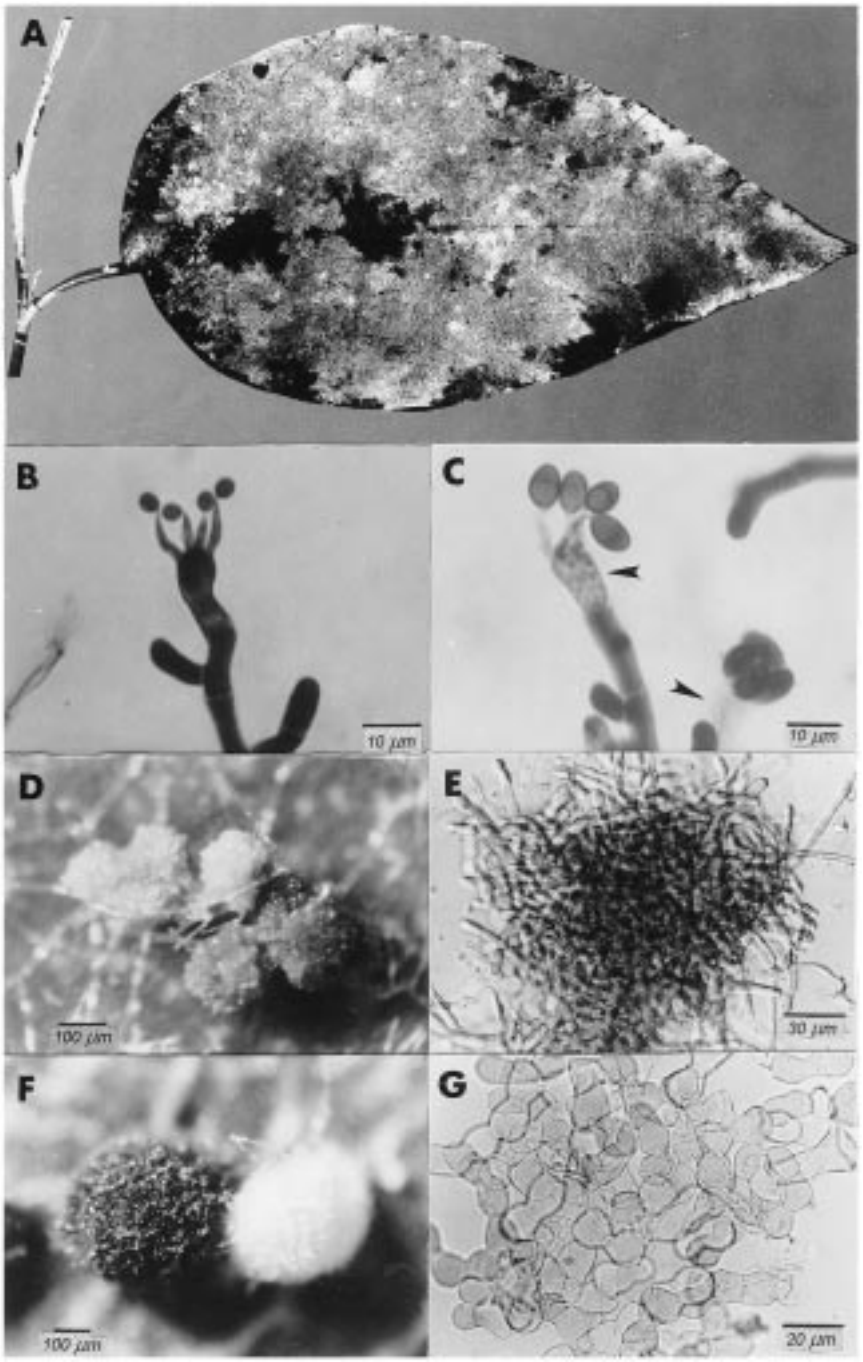

Figure 1. Morphological characteristics of Brazilian isolates of *Thanatephorus cucumeris* (*R. solani* of anastomosis group 1) similar to that of subgroup AG1-IB (A, B, C, D and E, isolate RH3) and to subgroup AG1-IC (F and G, isolate RH15). A – appearance of the hymenium on surface of eucalyptus leaf; B – basidium and young basidiospores; C – collapsed basidia (arrows) and mature basidiospores; D – 'microsclerotial' type sclerotia; E – 'microsclerotial' type of sclerotial formation among entangled hyphae; F – regularly spherical sclerotia, white when young and nearly black at maturity; G – forming sclerotia showing monilioid chlamydosporic cells.

PDA medium they produced dark-brown mycelium and barrel-shaped monilioid cells (10–20 × 20–35 μm) that were isolated or in loose aggregates on aerial mycelium. *In vitro*, sclerotia of these isolates were less than 500 μm in diameter and were formed by clusters of monilioid cells. On inoculated shoots these isolates produced abundant small irregular sclerotia similar to those produced by AG1-IB isolates. When on PDA, the Japanese AG1-IB isolates showed creamy to light-brown mycelium, sclerotial crusts with superficial drops of a dark-translucent exudate, and monilioid cells.

Cultural characteristics and sclerotial morphology of Brazilian isolates RH-15 and RH-26 were similar to those of Japanese isolates B19 and F2 of *R. solani* AG1-IC (Watanabe and Matsuda, 1966). On PDA, mycelium of RH-15 and of RH-26 was creamy to light-brown; the sclerotia were regularly spheroid, 300–1500 μm in diameter, initially white, and brown to black at maturity (Figure 1F). When immature, the sclerotia had oblong, hyaline monilioid cells, each 15–20 μm in major diameter (Figure 1G).

Isolate RH-18, a multinucleate form of *R. solani* AG-1 from Eucalyptus with scorch symptoms, produced light-cream to brownish-cream mycelium on PDA, and concentric zones of light aerial mycelium with barrel-shaped monilioid cells (7.5–15.0 × 15.0–25.0 μm) on aerial agglomerations of mycelium. On the host, RH-18 produced only dense epiphytic light-cream mycelium.

The binucleate isolates RH-5 and RH-10 were similar in morphology. On PDA each produced light-cream to greyish-cream mycelium, and formed sclerotial crusts that were brown and < 1 mm in major diameter at the base of the medium in petri dishes and culture tubes. Loose aggregations of monilioid cells, that were 10–15 × 15–30 μm in size and generally barrel-shaped, were produced on aerial mycelium and on sclerotia, which were rare. Neither isolates produced sclerotia when inoculated on Eucalyptus. Mycelial characteristics of binucleate isolates RH-23 and RH-24 were similar to those of RH-5 and RH-10, but RH-23 did not form monilioid cells and formed flat brown sclerotial mass in concentric zones on PDA medium. Similarly, RH-24 did not produce monilioid cells and its sclerotia were formed at the edge of colonies among entangling hyphae.

The binucleate isolate RH-28 that was isolated from Eucalyptus branches with leaf scorch symptoms formed distinctive sclerotia in the field. The sclerotia were elongated, 200–300 × 500–900 μm, and creamy-brown. Under field conditions, creamy-grey to greyish-brown mycelial strands up to 2 mm in diameter were superficially formed on the affected branches of the trees (Ferreira and Silveira, 1995). On PDA, colonies of RH-28 developed slowly at 25 °C, were creamy-yellow when young and, after 15 days, were dark-brown and velvety near the center and light-brown near the edges. Monilioid cells, sclerotia, and mycelial cords were not observed in culture.

Teleomorph induction and morphology

Teleomorphs were produced when host tissues were inoculated with the isolates RH-2, RH-3, RH-4, RH-15, RH-16, RH-17, and RH-20 from Eucalyptus, RH-21 from bean and RH-24 from *Thuja* sp., but not by other isolates or by other methods of induction tested. Except RH-24, all the other cited isolates produced basidial branching, basidia and basidiospores that confirmed that the teleomorph was *Thanatephorus cucumeris* Frank (Donk) (Talbot, 1970) (Figure 1B and 1C). All these isolates were multinucleate and belonged to anastomosis group AG-1 of *R. solani*. Binucleate isolate RH-24 formed a dense, smooth hymenium with sparse basidial branching and loose clusters of short basidia on Eucalyptus leaves. The basidia were obovoid to pyriform and two to three times greater in diameter than the basidiogenous hyphae. The basidiospores were subspherical, and exhibited repetitive germination, characteristic of *Ceratobasidium* Rogers. The size of the basidia (7, 5–10–13 × 6–7–8.5 μm) and basidiospores (4.0–6–7.0 × 3.2–5–6.2 μm) did not conform with those of species of *Ceratobasidium* (Sneh et al., 1991; Stalpers and Andersen, 1996).

Pathogenicity tests

All isolates except RH-28 caused some necrosis on Eucalyptus cuttings but the severity of symptoms varied widely among isolates (Figure 2A). Multinucleate isolates RH-2, RH-3, RH-6, RH-18, RH-21, and F2, and binucleate isolates RH-5, RH-10 and RH-24 produced severe disease at levels that did not differ significantly ($P < 0.05$). Multinucleate isolates RH-15, RH-16, and RH-29, and binucleate isolate RH-23 produced moderately severe disease. Isolate RH-8, a multinucleate form from Eucalyptus seedlings with damping-off, and Japanese isolate B19, produced mild symptoms.

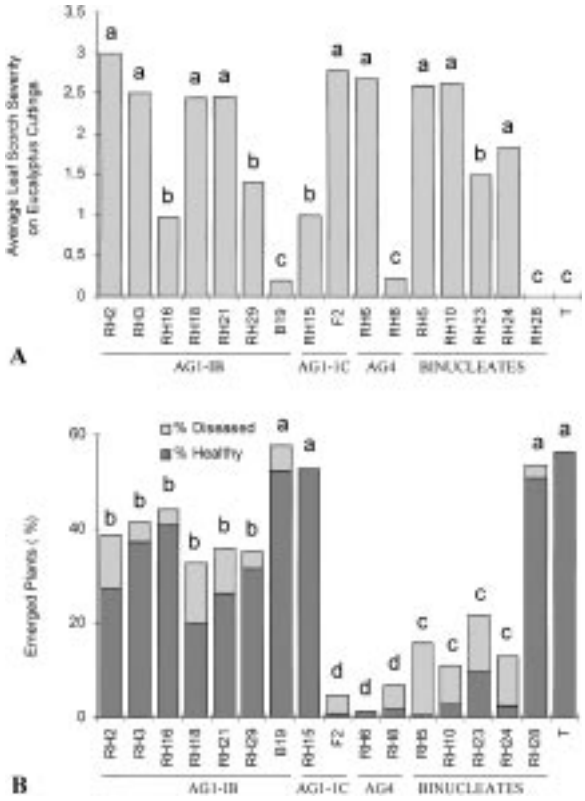

Figure 2. Virulence of *Rhizoctonia* isolates to *E. grandis* × *E. urophylla* hybrid cuttings and seedlings. A – Average severity of leaf scorch on leaves of eucalyptus cuttings five days after inoculation (CV = 25%); B – Percentage of emerged healthy and diseased eucalyptus seedlings (CV = 20%) fifteen days after sowing. The RH designation identifies Brazilian test isolates and B19 and F2 correspond to Japanese tester strains of *R. solani* AG1-IB and -IC, respectively. The control not inoculated is T. The bars with the same letter did not share significant different values at 5% probability by Skott-Knott test.

All isolates, except RH-15, RH-28, and B-19, reduced emergence of Eucalyptus seedlings, and isolates RH-19, and RH-28 caused post-emergence damping-off (Figure 2B). RH-6, RH-8, and F2 caused severe reduction in emergence. Binucleate isolates RH-5, RH-10, RH-23, and RH-24, but not RH-28, reduced seedling emergence and all caused post-emergence damping-off. Isolates of *R. solani* group AG-1 (RH-2, RH-3, RH-16, RH-18, RH-21, and RH-29) from aerial parts and cuttings of Eucalyptus caused pre-emergence damping-off but incidence of seedling emergence was greater than that for isolates of *R. solani* group AG4 from seedbeds (RH-6 and RH-8).

Discussion

At the International Symposium on *Rhizoctonia*, in Noordwijkerhout, The Netherlands, in 1995, it was proposed that *Rhizoctonia solani* Kühn be established as the new type species of the genus (Stalpers and Andersen, 1996). Thus, our paper follows the concept of the genus *Rhizoctonia* according to the redefinition of the species *R. solani*, based on that of Parmeter and Whitney (1970) and to redefinition of the genus, as was later amended by Ogoshi (1975).

In the present work, with the exception of one binucleate isolate (RH-28), the fungal isolates that were pathogenic to foliage and shoots of Eucalyptus were identified as *R. solani* and made hyphal anastomosis with AG1 tester strains. Production of the teleomorph *T. cucumeris* by most of the AG1 isolates confirmed that the anamorphs were those of *R. solani* (Parmeter and Whitney, 1970; Talbot, 1970). In Kerala, India, *R. solani* was also reported to be a principal pathogen of Eucalyptus in forest nurseries, where it caused damping-off of seedlings, and necrosis and leaf scorch of transplants, but these reports did not include any characterization at the subspecific level (Sharma, 1986; Sharma and Florence, 1984).

Among the Brazilian *R. solani* AG1 isolates from Eucalyptus, one belonged to subgroup AG1-IC (Ogoshi, 1987) AG1-type 3 (Sherwood, 1969) and twelve had cultural characteristics similar, but not identical, to Japanese isolates B19 and SHIBA2 of *R. solani* AG1-IB (Sneh, 1991). The differences were that some of the Brazilian cultures lacked monilioid cells and, differed in color on PDA. On the host, Brazilian isolates produced epiphytic growth and microsclerotia, similar to that produced by *R. solani* AG1-IB Japanese isolates. Because the three subgroups of *R. solani* AG1, -IA, -IB and -IC, were based primarily on pathogenicity and on sclerotial and cultural morphology, but not on hyphal anastomosis (Ogoshi, 1985), the present findings indicate that additional morphological sub-populations exist in the anastomosis group 1 of the *R. solani*. Isolates of those known subgroups IA, IB and IC constitute a portion of the diversity of the *R. solani* AG1. Liu and Sinclair (1993) compared 61 isolates of *R. solani* AG1 by means of RFLP analysis of spacer sequences of the nuclear rDNA, and revealed the existence of six intraspecific subgroups (ISG). According to Vilgalys and Gonzales (1990), variability of rDNA-RFLPs among isolates within each of the AG1 subgroups, IA, IB and IC, suggests

the existence of additional genetically divergent subpopulations. Detailed studies with a larger diversity of isolates are needed to find the relative importance of morphological features, such as the color of cultures, sclerotial formation and the presence and morphology of monilioid cells, and molecular data on subgrouping *R. solani* AGI. The subgroup AG1-IB, which was once classified as a different species, *Rhizoctonia microsclerotia* Matz, 1917 (Parmeter and Whitney, 1970), was also assigned to 'web-blight type' (Watanabe and Matsuda, 1966) and to anastomosis subgroups type 1 of *R. solani* (Sherwood, 1969). In this work, the AG1 'microesclerotial type' isolates from Eucalyptus were named *R. solani* 'AG1-IB like'.

Isolate RH-18 was included in AG1 on the basis of hyphal anastomosis, but showed cultural characteristics and sclerotia that differed from isolates of AG1-IA, AG1-IB and AG1-IC, and did not correspond with descriptions of other groups (Sneh et al., 1991; Ogoshi, 1987). Mycelium of RH-18 lacked brown pigmentation on PDA, which should exclude the isolate from *R. solani* (Parmeter and Whitney, 1970; Talbot, 1970). However RH-18 repeatedly anastomosed with Brazilian and Japanese isolates of *R. solani* AG1. In *R. solani*, hyphal anastomosis is considered strong evidence for identification of this species once this character is thought to be regulated by complex compatibility factors (Parmeter and Whitney, 1970). RH-18 appears to be an isolate which does not possess the diagnostic characteristics of any group or subgroup of the genus, in common with the 'overlapping isolates' of Sherwood (1969). This author observed the existence of isolates of the four main anastomosis groups of *R. solani* that share some characteristics such as lacking sclerotia and strong pigmentation. Additionally, there is no mention in the literature about hyphal anastomosis between isolates of *R. solani* AG1 and bridging isolates of other anastomosis groups or species (Carling, 1996).

Morphological observations and anastomosis data indicate that there was taxonomic diversity among the binucleate isolates. From the evidence, RH-5 and RH-10 from Eucalyptus belong to the same anamorphic species. Mycelial characteristics and production of sclerotia of RH-5 and RH-10 indicate that they are *R. fragariae* Husain and McKeen (Husain and McKeen, 1963; Sneh et al., 1991). RH-24 that causes damping-off in *Thuja* sp. produced subspherical to spherical basidiospores similar to, but larger than, those of the orchid symbionts *C. sphaerosporium* Warcup and Talbot and *C. globiosporum* Warcup and Talbot (Sneh et al., 1991). Basidia of RH-24 also did not match descriptions of the orchid symbionts. RH-24 differed from RH-5 and RH-10 in that it produced superficial sclerotia formed as entangled hyphae but not monilioid cells on PDA medium. RH-24 anastomosed with RH-5 and RH-10, however anastomosis does not clearly delimit anamorphs and teleomorphs of binucleate *Rhizoctonia* (Ogoshi, 1985; Oniki et al., 1986; Burpee et al., 1980). In the absence of available culture types, the binucleate *Rhizoctonia* RH-5, RH-10, RH-23 and RH-24 were not identified at species level.

The binucleate isolate RH-28 has morphological characteristics of *Rhizoctonia* (Ogoshi, 1975), except that it produced mycelial strands on host branches in the field, which could be considered as rhizomorphs (Ferreira and Silveira, 1995). The presence of rhizomorphs exclude all fungi from *Rhizoctonia* genus concept (Ogoshi, 1975). More detailed studies on the morphology of mycelial strands, sclerotia, septa and basidia are needed to identify RH-28 to genus and species levels. Inoculation of Eucalyptus branches with mycelium of this isolate caused a few lesions on some leaves, but failed to produce sexual structures, sclerotia and mycelial strands or anything like rhizomorphs (data not presented).

According to Baker (1970), isolates of *R. solani* that are ecologically adapted to aerial or underground parts of plants are relatively specialized, while those that are found on the soil surface are relatively unspecialized. *R. solani* AG-4 is one of the main examples of the relatively unspecialized form, and includes isolates that have broad host ranges, attack plants at the soil level, and cause pre- and post-emergence damping-off (Sneh et al., 1991). In the present study, isolates RH-6 and RH-8 of *R. solani* AG-4 caused necrotic lesions on foliage of Eucalyptus cuttings, but other isolates in this group did not attack the shoots. For these unspecialized isolates, as for the AG4 isolates and for binucleate isolates that occur in the soil surface, the rooting medium would be the main source of inoculum in production systems of Eucalyptus cuttings.

The *R. solani* 'AG1-IB-like' isolates that caused leaf scorch of Eucalyptus conformed with the ecological group 'true aerial forms' of Baker (1970). This author suggested that in regions with humid climates, these aerial forms have a fast epiphytic growth and produce abundant sclerotia on host, which could favor an aerial existence independent of the soil. Besides Eucalyptus, *R. solani* AG1-IB is a pathogen of aerial parts of plants

in various families, especially the Leguminosae (Sneh et al., 1991; Ogoshi, 1985). These characteristics must be considered for the control of disease in Eucalyptus nurseries and hedges when the diseased or symptomless shoots are sources of inoculum in cuttings used for propagation systems (Ferreira, 1991).

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