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ISOLATION, MORPHOLOGICAL IDENTIFICATION AND PATHOGENICITY OF *CYLINDROCLADIUM SCOPARIUM* AND *C. CLAVATUM* ISOLATES OBTAINED FROM PLANTS RHIZOSPHERE CULTIVATED IN PERNAMBUCO STATE

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SHORT COMMUNICATION

ABSTRACT

Twelve isolates of *Cylindrocladium scoparium* and 4 isolates of *C. clavatum* were obtained from the rhizosphere of various species of plants by baiting with *Ricinus communis* leaves. The isolates of *C. scoparium* developed conidia of 32-(45)-60 x 3 -(4)-5 µm, and pyriform to ellipsoidal vesicles. *C. clavatum* showed conidia of 36-(44)-49 x 2-(4)-6 µm and clavate vesicles. All isolates induced necrosis on leaves and hypocotyls of eucalypt seedlings, with varying expression of symptoms.

Key words: *Ricinus* leaf bait, morphological markers, eucalyptus pathogenicity

The genus *Cylindrocladium* Morgan (teleomorph: *Calonectria* De Not) comprises species considered soil inhabitants that are saprophytes and facultative parasites, widespread in different environments all over the world (1). Typical symptoms caused by the pathogenic species are root rot, damping-off, wilt, leaf spotting, or necrotic lesions on fruits (7).

The purpose of the present study was to isolate species of *Cylindrocladium* from the rhizosphere of various plants by a baiting method, to identify them by morphological criteria and to check their pathogenicity on eucalypt seedlings under greenhouse conditions.

Small pieces of *Ricinus communis* L. leaves were disinfected (4), and placed upon soil samples of 20 g in sterile Petri dishes, collected from the rhizosphere of various plants (Table 1) and moistened with sterile distilled water (3). The baits were incubated for two days under alternating light at a temperature of 25ºC and mycelia grown on the leaf segments were transferred to Petri dishes with PDA (Potato-Dextrose-Agar) to obtain pure cultures. For morphological identification, segments of disinfected *Ricinus* leaves were inoculated with plugs of 5mm diameter from a young mold colony grown on PDA and leaves were kept in a Petri dish on a cotton swab moistened with sterile distilled water as above, until the formation of fruiting bodies. These were stained with Amann's blue (4) on microscope slides and observed under a light microscope. Of each isolate, average length and width of 20 conidia were determined with an ocular micrometer. Shape and septation of conidia were also considered, as well the shape of the vesicle (1,2,7). Seedlings of eucalypt (*Eucalyptus citriodora*), 40 days after germination, were inoculated on both hypocotyls and leaves. Plugs as above were placed upon a slight incision in hypocotyls and fixed with Scotch tape. With leaves, the plug was put on the upper surface without wounding. Following inoculation, the plants were incubated for 48 h in a moist chamber at a temperature of 25ºC. The experiment was completely randomized using three replications in each treatment. The size of the lesions was measured four days after the inoculation. These data were subjected to Duncan's test, comparing means at a 5% level of significance.

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Table 1. Isolates, hosts, size of conidia and lesions (mm) induced by *Cylindrocladium scoparium* (Cs) and *Cylindrocladium clavatum* (Cc) on hypocotyls and on leaves of eucalypt (*Eucalyptus citriodora* Hook) plants, two days after to inoculation.

Isolates/ host	Size of conidia (µm)[1]		Pathogenicity[2]	
	Length	Width	Hypocotyl lesion	Leaf lesion
C. scoparium (Cs)				
Cs-Ara.1/ *Annona crassiflora* Mart.	33-(45)-59	3-(4)-5	19.66a	17.61ab
Cs-Ara.2/ *A. crassiflora*	33-(47)-60	3-(4)-5	18.30a	24.67a
Cs-Hel.3/ *Heliconia* sp.	32-(44)-57	3-(4)-4	18.16a	9.97bc
Cs-Pim.4/ *Capsicum annuum* L.	37-(45)-58	3-(4)-5	17.15a	14.49abc
Cs-Euc.5/ *Eucalyptus citriodora* Hook	32-(46)-59	3-(4)-5	16.92ab	29.32a
Cs-Euc.6/ *E. citriodora*	32-(44)-59	3-(4)-4	16.92ab	25.36a
Cs-Euc.7/ *E. citriodora*	32-(48)-57	3-(4)-5	15.72ab	9.02bc
Cs-Euc.8/ *E. citriodora*	34-(45)-60	3-(4)-5	15.62ab	13.28abc
Cs-Pin.10/ *Pinus caribaea* var. *hondurensis* Barret & Golfari	33-(46)-59	3-(4)-5	15.30ab	14.77abc
Cs-Pin.11/ *Pinus caribaea* var. *hondurensis*	33-(45)-58	3-(4)-4	14.93ab	16.28abc
Cs-Pin.12/ *Pinus caribaea* var. *hondurensis*	33-(46)-58	3-(4)-4	13.63ab	26.22a
Cs-Pin.13/ *Pinus caribaea* var. *hondurensis*	33-(45)-58	3-(4)-5	12.52ab	24.96a
C. clavatum (Cc)				
Cc-Euc.9/ *E. citriodora*	38-(45)-48	3-(4)-6	17.94a	7.57bc
Cc-Pin.14/ *Pinus caribaea* var. *hondurensis*	37-(45)-47	3-(4)-5	14.55ab	6.69c
Cc-Cel.15/ *Pennisetum purpureum* Schumacher	37-(44)-49	2-(3)-6	10.62b	7.66bc
Cc-Uru.16/ *Bixa orellana* L.	36-(43)-48	3-(4)-6	10.62b	6.88c
C.V.(%)			8.03	15.66

[1] Means ($\overline{\chi}$) of 20 conidia from each isolate cultivated on castor bean leaves. (Min.) Minimum and (Max.) maximum values are also shown;
[2] Date are log (x+1) tranforms. Means of three replications;
Means followed by the same letter (vertical) are not signicantly different Ducan's test at 5%.

Baits of *Ricinus* leaves as described permitted to obtain in high yield, 16 isolates of *Cylindrocladium*. This method was introduced by Orrego Fuente *et al.* (6), who used three different baits, but *Ricinus* leaves gave the best results.

From 16 isolates obtained, 12 were identified as *Cylindrocladium scoparium* (Cs), and four as *C. clavatum* (Cc). Conidial lengths of *C. scoparium* varied between extremes of 32-60 µm, and *C. clavatum* 37-49 µm (Table 1). Crous and Wingfield (1) found a higher range (40-66 µm) for *C. scoparium* cultured on CLA (Carnation Leaf Agar), and Moreira *et al.* (5) reported a lower range (24.5-43.5 µm) for this specie grown on *Ricinus* leaves. For *C. clavatum*, there were also small differences between the sizes of conidia observed and those cited by other researchers (1,7). However, the data of Hodges and May (2) were identical to the values described here. The width of conidia was much alike in both species and the mean of 4 µm was equal for all isolates except Cc-Cel.15 that seemed slightly slimmer. A similar value was reported by others (1,2,5).

The species *C. scoparium* displayed hyaline and septate hyphae, conidiophores with di- or trichotomic branches composing phialides which vary between doliiform and reniform, and a septate stipe with a pyriform or ellipsoidal vesicle in the apical end; the conidia were cylindrical with rounded ends, hyaline and bicelular. The colony grown on PDA was typically light brown with a white border and a slightly irregular margin (Fig. 1A). *C. clavatum* showed the same features of hyphae, conidiophores and conidia as the other species; but had a clavate vesicle on an also hyaline septate stipe. *C. scoparium* formed on PDA a colony with a dark brown center and an irregular white border (Fig. 1B). Both species developed also microsclerotia, but the production of these resistant structures and the spatial arrangement of the cells in their interior are inadequate for taxonomy (1).

With eucalypt seedlings inoculated on hypocotyls or on leaves, all isolates of *Cylindrocladium* were pathogenic (Figs. 1C, D), but not in the same degree. Cs-Ara.1 caused wilt as well as collapse of the three target plants tested 48 h after their inoculation and could thus be considered as the most aggressive isolate, although its hypocotyl lesions did not look different from the other isolates (Table 1). Patent pathology was seen at least in two of three replications of the other isolates, after 96 h of incubation, with exception of Cc-Pin.14 and Cc-Uru.16, which

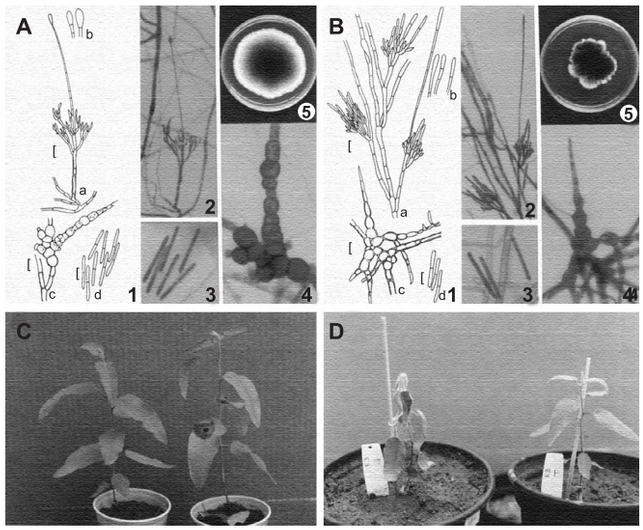

Figure 1. A - *Cylindrocladium scoparium*. 1-Schematic structures: (a) conidiophore; (b) vesicles; (c) microsclerotia; (d) conidia; length marker, 5 µm; 2, 3, 4-Microscopic aspects. 5-Colony. **B** - *Cylindrocladium clavatum*. 1-Schematic structures: (a) conidiophore; (b) vesicles; (c) microsclerotia; (d) conidia; length marker, 5 µm; 2, 3, 4-Microscopic aspects. 5-Colony. Aspect of lesions symptoms on leaves (**C**), and wilt symptom (**D**) on *Eucalyptus citriodora* Hook plants induced by *C. scoparium* after 72 hours.

induced only modest necrosis in the hypocotyl without any collapse of plants. Reproductive structures of the pathogen could be detected in only one of the lesions produced by Cc-Pin.14 and Cc-Uru.16, respectively. The isolates of *C. clavatum* were generally less aggressive to eucalypt plants than *C. scoparium*, although Cc-Euc. 9 came from a eucalypt rhizosphere.

C. scoparium was reported to cause wilt, collapse and death in all plants of *Eucalyptus camandulensis* Denh., six days after inoculation (6). Hodges and May (2) found *C. clavatum* preferentially on *Pinus* sp., in spite of its many other possible host plants.

RESUMO

Isolamento, identificação morfológica e patogenicidade de isolados de *Cylindrocladium scoparium* e *C. clavatum* da rizosfera de plantas cultivadas no Estado de Pernambuco

Doze isolados de *Cylindrocladium scoparium* e 4 isolados de *C. clavatum* foram obtidos da rizosfera de diversas plantas usando folhas de *Ricinus communis* como isca. A primeira espécie mostrou conídios com dimensões de 32-(45)-60 x 3 -(4)-5 µm e vesícula piriforme a elipsoidal. Os conídios de *C. clavatum* apresentaram dimensões entre 36-(44)-49 x 2-(4)-6 µm e vesícula clavada. Em plântulas de eucalipto todos os isolados induziram necrose em folhas e hipocótilos, variando apenas na expressão dos sintomas.

Palavras-chave: isca de *Ricinus,*marcadores morfológicos, patogenicidade em eucalipto

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