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CYLINDROCLADIUM QUINQUESEPTATUM: CULTURAL CHARACTERISTICS AND REPRODUCTIVE MORPHOLOGY OF THE CLOVE ISOLATE IMI 342173 FROM SRI LANKA

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ABSTRACT

Cylindrocladium quinqueseptatum Boedijn and Reitsma, is a destructive pathogen of *Eugenia caryophyllata* (clove) and is identified as a potential pathogen of *Hevea brasiliensis* in Sri Lanka. Further, the fungus infects a number of ornamental and forest trees.

We report using photomicrographs the cultural characteristics and the morphology of the reproductive structures; conidia, vesicle, stipe, phialides, chlamydospores and microsclerotia of clove isolate IMI 342173. Our investigations demonstrates that the isolate IMI 342173 from Sri Lanka resembles the isolates of *C. quinqueseptatum* obtained from different hosts in other parts of the world.

Key words: *Cylindrocladium quinqueseptatum, Eugenia caryophyllata, Hevea brasiliensis*, morphology.

INTRODUCTION

Cylindrocladium quinqueseptatum Boedijn and Reitsma (telemorph: *Calonectria quinqueseptata* Figueiredo & Namekata) causes damping off, seedling blight, leaf spot, foliage and shoot blight extensive defoliation and dieback on a wide variety of hosts including *Acacia* spp., *Annona squamosa* (Figueiredo & Namekata, 1967), *Camellia sinensis* (Peerally, 1974 c), *Eucalyptus* spp. (Figueiredo & Namekata, 1967; Pitkethley, 1976; Sharma & Mohanan, 1982, Ivory *et al.*, 1993) and *Eugenia carryophyllata* (Reitsma & Sloof, 1950; Sarma & Nambiar, 1978).

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This pathogen is widely distributed in the humid tropics and diseases reach epidemic propotions when high humid environmental conditions are present. (Reitsma & Sloof, 1950; Sharma & Mohanan, 1982; Bolland *et al.*, 1985; Jayasinghe & Wijesundera, 1994).

In Sri Lanka the fungus *C. quinqueseptatum* was first recorded as a pathogen in 1982 on *Eugenia carryophyllata* (Jayasinghe & Liyanage, 1982) from Ratnapura district. The fungus was reported to cause considerable mortality of *E. carryophyllata* seedlings and severe defoliation in young plants especially during the South-West monsoon period (May - September). Subsequently its pathogenicity has been proved. However, no detailed studies of the fungus in Sri Lanka was carried out till 1990 when *Hevea brasiliensis* clone RRIC 36 (a clone bred and released by the Rubber Research Institute of Sri Lanka under the Multilateral Clone Exchange Programme) was severely affected with *C. quinqueseptatum* in Malaysian budwood nurseries (Anon, 1990).

Leaf spots caused by *C. quinqueseptatum* on *Hevea brasiliensis* was first reported in Malaysia in the year 1972 (Anon, 1972). However, it remained insignificant until 1990, when it caused defoliation of several clones in budwood nurseries and plantations. The most susceptible *Hevea* clones in Malaysia are PB 86, RRIM 607, RRIC 36, PC 111 and PC 121 (Anon, 1990). Under favourable weather conditions young leaves shrivel, blacken and fall. Leaf spots appear on mature leaves as numerous chlorotic pin heads (Fig.1b) and leaves become purplish brown, surrounded by a prominent yellow halo.

On cloves initial symptoms on leaves begin as minute chocolate brown spots which enlarge to form spots of irregular shapes (Fig.1a) under continuous wet weather. Lesions can also appear on the petioles and on the stem of seedlings. Diseased leaves abscise and if persistant wet weather occurs, seedlings are killed due to extensive leaf fall and dieback.

In the original description of Boedijn & Reitsma (1950), a short account of cultural characteristics and reproductive morphology of the clove isolate is provided. Subsequently Peerally (1974 c) described the species *C. quinqueseptatum* together with host range and geographical distribution. Crous and Wingfield (1994) in their review on the genus *Cylindrocladium* provides elaborative descriptions and illustrations for the *Eucalyptus* (IMI 201927) and *Scolopendrium* isolates (IMI 114953). We report here in detail the cultural and reproductive morphology of the clove isolate (IMI 342173) of *C. quinqueseptatum* using photomicrographs.

METHODS AND MATERIALS

IMI 342173 was obtained from a diseased clove seedling in Ratnapura District, Sri Lanka and a monconidial culture was maintained on Czapek Dox Agar (CDA) medium. Slide cultures were prepared using Bacto Agar when necessary and either a commercial preparation of lactophenol cotton blue (Difco) or 0.1% erythrosin in 10% ammonia was used for staining purposes. Cultures were incubated at room temperature (28 ± 2°C) under normal light and dark regimes. Culture characteristics were studied using 14-day old cultures grown on either CDA or Lima Bean Agar (LBA). Photomicrographs were taken after 7th and 20th day incubation on conidial morphology and microsclerotia respectively.

RESULTS AND DISCUSSION

Cultural characteristics

On CDA, the cultures were about 80mm in diameter with submerged mycelial growth (Fig 1d). Centre (25 mm) of the colony was whitish brown and slightly cottony. Periphery (15 mm) was dark reddish brown, geletenous, falattened with submerged profusely branched radially growing mycelial strands with numerous chlamydospores in chains (Fig. 1e). Margins of colonies were smooth and no distinct concentric zones were observed. The lower surface of the medium took a reddish brown colour.

On LBA, the colonies were of the same size as on CDA (Fig. 1c). Centre (7 mm) was slightly brown, slightly cottony, more or less flattened. Then next 15mm were pale brown, had radially growing thin flattened mycelial zone. The Periphery

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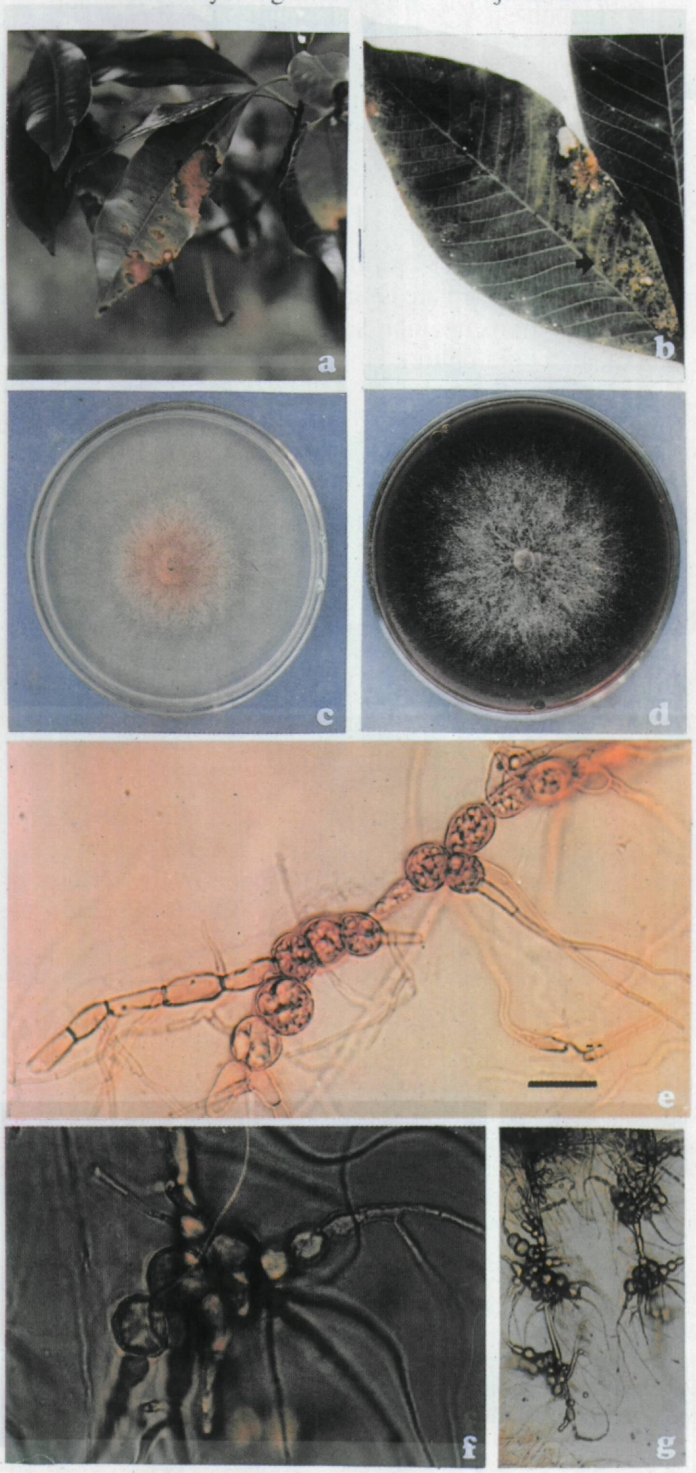
Fig. 1 Disease symptoms produced by *Cylindrocladium quinqueseptatum* and cultural characteristics of clove isolate IMI 342173
(a) Irregular brownish lesions on *Eugenia carryophyllata* leaves,
(b) Chlorotic spots on a leaf of *Hevea brasiliensis* (Courtesy - IRRDB),
(c) Fourteen day old culture on LBA and (d) CDA,
(e) Chlamydospores arranged in chains,
(f) Aggregation of chlamydospores giving rise to microsclerotia,
(g) Microsclerotia arranged in raws on mycelial strands (periphery of a 20-day old culture grown on CDA).
Fig. 1e is stained with 0.1% erythrosin in 10% ammonia.
Fig. 1f is stained with lactophenol-cotton blue. Scale bar in Fig. 1e represents 25 μm in figs. 1e, 1f and 100 μm in Fig. 1g.


a
b
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e
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(20 mm) was whitish, and had very thin, profusely branching mycelial strands. No chlamydospores or distinct concentric zones were present. Only the central portion of the medium (20 mm) had a different colour (reddish brown) on the under surface.

Reproductive morphology

Conidiophore (Figs. 2a,e) hyaline, dichotomously branched near the apex, primary branches non septate (Fig. 2a) or 1-2 (Fig. 2e) septate, 10 - 32 x 4 - 7 μm, secondary branches non septate (Fig. 2e), 8 - 16 x 3 - 7 μm. The main axis of the conidiophore elongates into a stipe.

Stipe (Figs. 2a-d) hyaline, septate (Fig. 2c), 150-250 μm long and terminates as a vesicle.

Vesicle (Figs. 2a,c) hyaline, thin walled (Fig. 2c) characteristictly clavate (Fig. 2a), 3-6 μm in diameter.

Phialides hyaline, allantoid (Fig. 2f) or cylindrical (Fig. 2g), 9-18 x 4-6 μm.

Conidia hyaline, cylindrical, 3-8 septa (Figs. 2h - k) mostly with 5- septate, rounded at both ends, 70-110 x 5-8 μm, arranged in a cylindrical matrix (Figs. 2b,d) if undisturbed.

Chlamidospores produced on CDA in chains (Fig. 1e), pigmented, 16-28 x 10-18 μm, sometimes aggregated to form microsclerotia (Fig. 1f), 36-130 x 21-36 μm and these are also mostly arranged in rows (Fig. 1g).

Fig. 2 Conidial and conidiophore morphology of the clove isolate of *Cylindrocladium quinqueseptatum* (IMI 342173)

(a) Penicillately branched conidiophore (Cp) with the stipe (S), an elongation of the main axis of conidiophore and attached conidia (C). Note the characteristic clavate vesicle (V) at the apex of the stipe.

(b) and (d) conidiophore showing position of undisturbed conidia arranged in a cylindrical manner. Note a droplet of water covering terminating visicle of the stipe in b.

(c) stipe to show the septation (Sep) and the origin of vesicle. Note the thin walled vesicle compared to wall of the stipe.

(e) Primary (Pr) and secondary (Se) conidiophore with numerous phialides (P) at apices.

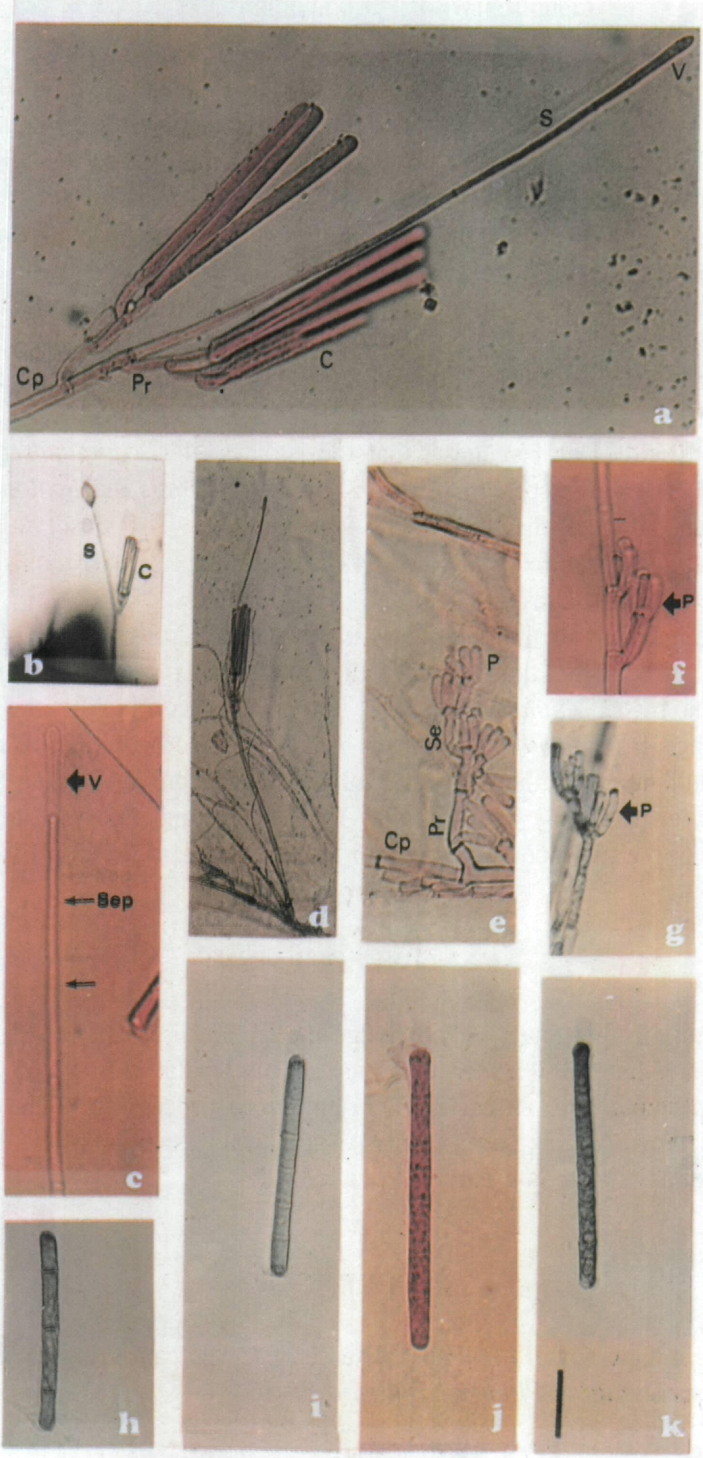
(f) Allantoid phialides

(g) Cylindrical phialides

(h-k) Conidia of varying sizes and different numbers (3-8) of septa. Note the rounded ends of conidia.

Figs. 2a,c,d,e,f and g are stained with 0.1% erythrosin in 10% ammonia.

Figs. g,h,i and k are stained with lactophenol cotton blue. Scale bar in Fig. 2k represents 25 μm in Figs 2a,c,e,f,g,h,i,j,k and 100 μm in figs 1b and d


V
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Morphology of Cylindrocladium quinqueseptatum

Above observations show that the dimensions of conidia, phialides and vesicle agree with the original description of Boedijn & Reitsma (1950). However, a comparison cannot be made with regard to the measurements of stipe, chlamidospores and microsclerotia as these are not fully described in the original description.

The description of the morphology of phialides and vesicles, structures considered to be reliable characters in species concept of the genus *Cylindrocladium* (Sobers & Seymour, 1967; Sobers & Alfieri, 1972; Peerally, 1974 a - j, 1991; Crous & Wingfield, 1994) are given in this account according to the recent chart provided by Crous & Wingfield (1994).

The available literature on reproductive morphology documented by Boedijn & Reitsma (1950), Figueiredo & Namekata (1967), Peerally (1974 c, 1991) and Crous & Wingfield (1994) suggests that the isolates of *C. quinqueseptatum* from various hosts *viz Annona* sp., *Eucalyptus* spp., *Eugenia* sp. and *Scolopendrium* sp. from different parts of the world are very similar. The observations of this investigation suggest that the isolate IMI 342173 obtained from Sri Lanka also closely resembles the above isolates.

In their review of the genus *Cylindrocladium*, Crous and Wingfield (1994) showed, for the first time, that microconidia can also be produced in addition to the typical conidia by *C. quinqueseptatum*. They were working on the isolate IMI 114953 obtained from *Scolopendrium* sp. in USA. Microconidia are described as cylindrical and hyaline similar to typical conidia (macroconidia) but they have obtuse ends, shorter lengths (40-) 46 (-50) x (4-) 7 (-8) μm and one septum. We were unable to detect the microconidial stage in the clove isolate IMI 342173.

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