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MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF *COLLETOTRICHUM* ISOLATES FROM *HEVEA BRASILIENSIS*

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The morphology of conidia and growth rate of isolates of the fungus causing raised, anthracnose and papery lesions of Colletotrichum leaf disease on rubber were compared. The isolates produced morphologically uniform conidia on potato dextrose agar (PDA). Conidial shape was more useful than size in differentiation of isolates. Growth rate of the isolates from raised lesions was significantly lower than that from anthracnose and papery lesions. The latter two appeared to be similar. In general, the isolates from raised lesions produced pink pigmentation on PDA while those from anthracnose and papery lesions produced grey pigments. The isolates from raised lesions were of *Colletotrichum acutatum*, distinct from anthracnose and papery lesion isolates which were of *C. gloeosporioides*.

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INTRODUCTION

Gloeosporium alborubrum (Petch.) is a serious pathogen of rubber (*Hevea brasiliensis*) widely distributed in India and causing significant damage in nurseries and young plantations (Deka *et al.*, 1996; Manju *et al.*, 1999). It is reported to cause reduction in rubber production by 7 to 45 per cent in Indonesia and 12 per cent in Sri Lanka (IRRI, 1994). The infection of *G. alborubrum* appears as minute, circular, brown lesions on the leaflets. Later the lesions develop a thick brown margin and become raised above the surface as conical projections. Leaves are finally shed. Anthracnose disease of rubber, characterized by concentric circular leaf spots is attributed to *Colletotrichum gloeosporioides* (Penz) Sacc. This disease is confined to nurseries and young plants in the fields and causes less damage (Ramakrishnan and Pillay, 1962). The same pathogen has been reported to cause papery lesions on the leaflets (Rajalakshmy and Joseph, 1988). Though several other species of *Colletotrichum* and *Gloeosporium* have been reported on rubber, all these species are closely akin to *C. gloeosporioides*. They represent the conidial stage of *Glomerella cingulata* (Stonem.) Spauld. and Schrenk (Carpenter and Stevenson, 1954).

There has been some uncertainty about the taxonomic status of these two genera. The difference between these was only the presence of setae in the acervuli of *Colletotrichum* and their absence in *Gloeosporium* (Arx, 1970). However, setae formation may be influenced by environmental factors (Frost, 1964; Baxter *et al.*, 1985) and is not a reliable character for separation of these two genera.

As the perfect stage of both the genera *Colletotrichum* and *Gloeosporium* is *Glomerella*, it was suggested that both these fungi can be recognized as *Colletotrichum gloeosporioides*. Saccas (1959) had suggested the name *C. gloeosporioides* f. sp. *heveae*. However, the differences in the symptoms, virulence and morphology of the pathogens have prompted the maintenance of their identity in many treatises on leaf diseases of rubber (Edathil *et al.*, 2000).

This investigation was aimed at comparing the morphological and cultural char-

acters of the different fungal isolates from the three types of symptoms with a view to place them appropriately.

MATERIALS AND METHODS

Collection and maintenance of isolates

Twenty nine infected leaf samples, which exhibited the three types of symptoms *i.e.*, raised, anthracnose and papery lesions, were collected from different rubber clones/seedlings during 1999 from various locations (Table 1). The pathogens were isolated on potato dextrose agar (PDA). The cultures were purified by single spore isolations and were transferred to and grown on PDA in petri dishes. One isolate from each type of symptom was sent to International Mycological Institute, Kew, London, for confirmation of the identification. Stock cultures were maintained on PDA at 4°C.

Table 1. **Source and designation of Colletotrichum isolates**

Lesion type and isolate no.	Clone	Location**
Raised lesion		
C1-1	BD 10	A
C1-2	BD 10	A
C2-1	GT 1	A
C2-2	GT 1	A
C – 3	RRII 208	A
C – 5	PB 280	A
C – 6	PB 310	A
C7 –1	PB 217	A
C7 – 2	PB 217	A
C-20	PB 5/51	A
C-4*	RRII 105	A
C-8	RRII 105	A
C23-1	RRII 105	B
C23-2	RRII 105	B
C-30	RRII 105	C
Anthracnose lesion		
C-9	Seedling	A
C-10	Seedling	A
C-11	Seedling	A
C-12*	Seedling	A
C-25	Seedling	A
C-27	Seedling	D
C-26	RRIM 701	A
C-28	PB 260	C
C-29	RRII 105	C
Papery lesion		
C-15	PR 255	A
C-16*	RRII 30	A
C-17	RRII 301	A
C-18	PB 235	A
C-31	RRII 105	C

* Isolates identified by IMI ** Location A = RRII; B = Cheruvally; C = Mundakayam; D = Kaliyar

Growth characters

All the monoconidal isolates were grown on PDA in petri dishes. Mycelial plugs (5 mm diameter) were removed from the margins of five-day-old cultures of each isolate and transferred to fresh PDA in petri dishes. The plates were incubated at room temperature (25-28°C) with 12 h alternations of light and dark. Three replicates of each isolate were maintained. The growth rate and the morphological and cultural characteristics were observed on sixth and eighth day after inoculation, respectively.

Conidial characteristics

Each isolate was grown on PDA in petri dishes for eight days under alternation of 12 h light and dark at room temperature (25-28°C). Conidial suspensions were prepared by floating cultures with sterile distilled water and lactophenol (1:1, v/v) and observed under a light microscope. Size and shape of conidia of each isolate was determined by examining 50 arbitrarily chosen conidia at 100x under microscope and categorized as (1) fusiform conidia, acute angled at both ends and (2) cylindrical, with at least one of the ends rounded.

Type of symptoms

Three isolates (one isolate each from raised, anthracnose and papery lesions) were selected and inoculated on five *Hevea* clones, *viz.*, RRII 105, GT 1, BD 10, PB 86 and PB 5/51. These isolates were grown on PDA in petri dishes for eight days at room temperature (25-28°C) under alternate 12 h light and dark. Conidia for inoculation were

washed from the surface of PDA and suspended in sterile distilled water containing one drop of Tween 80 in 100 ml. Inoculum was adjusted to 1.5 x 10^5 conidia per ml and sprayed on the leaves using an atomizer. Immediately after inoculation, all the inoculated twigs were covered with polythene bags to avoid external contamination and were maintained at near 100 per cent relative humidity. The control plants were sprayed with sterile water containing Tween 80 and covered with polythene bags. The observations on types of lesion development were recorded from the third day after inoculation up to the 10th day.

RESULTS

Cultural characteristics

Colony colour of most isolates from the raised lesions was white during the first few days of growth and later became pale orange or greyish white with pink tinge. The reverse side of the colony was generally pink. Isolates from anthracnose and papery lesions were light grey to dark grey. The reverse of the cultures of both these isolates was grey to dark grey and occasionally black or dark green in colour (Table 2).

The raised lesions were composed of aerial mycelia, which were dense and moderately elevated with the regular margins

Table 2. **Morphological and cultural characteristics of isolates**

Isolate No.	Growth characteristics	Colony colour		Radial growth (mm)
		Front	Reverse	
C1-1	Submerged, dense	Reddish yellow	Pink	55.00
C1-2	Submerged, felty, dense	Reddish brown	Pinkish white	54.33
C2-1	Submerged, dense	Pinkish grey	Pinkish grey	53.33
C2-2	Submerged, dense	Pinkish grey	Pinkish grey	55.00
C - 3	Submerged, felty, dense	Whitish pink	Pinkish white	54.00
C - 5	Submerged, felty, dense	Whitish pink	Pinkish white	50.67
C - 6	Submerged, felty, dense	Whitish pink	Pinkish white	55.00
C7 -1	Submerged, dense	Reddish yellow	Pink	55.00
C7 - 2	Submerged, felty, dense	Whitish pink	Pinkish white	55.00
C-20	Submerged, felty, dense	Whitish pink	Pinkish white	52.67
C-4*	Submerged, felty, dense	Whitish pink	Pinkish white	52.33
C-8	Submerged, felty, dense	Pale orange	Pinkish white	49.00
C23-1	Submerged, felty, dense	Pale orange	Pinkish white	55.33
C23-2	Submerged, felty, dense	Pale orange	Pinkish white	54.00
C-30	Submerged, dense	Greyish pink	Pink	32.67
C-9	Raised, woolly	Dark grey	Dark grey	90.00
C-10	Raised, woolly	Grey	Dark grey	82.00
C-11	Raised, woolly	Grey	Dark grey	79.33
C-12*	Raised, woolly	Dark grey	Dark grey	90.00
C-25	Raised, woolly	Black	Black	86.67
C-27	Raised, woolly	Light grey	Dark green	90.00
C-26	Raised, woolly	Grey	Dark grey	84.67
C-28	Raised, woolly	Grey	Dark grey	90.00
C-29	Raised, woolly	Creamy white	White	90.00
C-15	Raised, woolly	Dark grey	Dark grey	83.33
C-16*	Raised, woolly	Dark grey	Dark grey	83.33
C-17	Raised, woolly	Light grey	Light grey	85.00
C-18	Raised, woolly	Grey	Dark grey	80.00
C-31	Raised, woolly	Dark grey	Black	90.00
CD (P=0.05)				1.43

* Isolates identified by IMI

and distinct annulations. Isolates from anthracnose and papery lesions had coarse, raised and woolly, abundantly elevated mycelia.

Growth response

The isolates could be clearly separated into fast and slow growing groups based on growth. The mean growth rate of isolates from raised lesions was significantly less than that of anthracnose and papery lesions isolates. The growth rate of raised lesions isolates averaged 5 to 9 mm per day whereas that of anthracnose and papery lesions isolates averaged 13 to 15 mm per day. The slow growing group corresponded with the group showing acute conidia, while the fast growing group produced cylindrical conidia.

Conidial characteristics

Conidial masses of all the isolates were salmon pink to orange except isolate C-10, which was yellow. So this criterion could not be used for the differentiation of the isolates. The isolates were separated on the basis of conidial morphology into two groups namely cylindrical and fusiform. Isolates from raised spots had fusiform conidia while isolates from anthracnose and papery lesions had cylindrical ones (Table 3).

Conidia of all the raised lesions isolates were consistently fusiform. The shape of conidia agreed with the description of the conidia of *C. acutatum*, but their length, which ranged from 9 to 22 mm, varied slightly from that previously recorded (8-16 mm) for conidia of *C. acutatum* (Simmonds, 1965; Dyko and Mordue, 1979; Baxter *et al.*, 1983; Smith and Black, 1990). However, the range is well within the conidial length (12.5 – 22.5 mm) reported by Gunnel and Gubler (1992).

Conidia of anthracnose and papery lesion isolates were cylindrical (oblong with obtuse ends) and were generally shorter and broader than conidia of raised lesion isolates. The size and shape of conidia were within the range of measurements previously reported for isolates of *C. gloeosporioides* from a variety of hosts (Mordue, 1971; Baxter *et al.*, 1983). The mean lengths of the conidia from raised, anthracnose and papery lesions were 14.67, 12.91 and 12.61 mm and their breadths 3.67, 4.46 and 3.81 mm, respectively.

The isolate from raised spots was identified (IMI-383015) as *Colletotrichum acutatum* Simmond, the anthracnose lesion isolate (ISI-383016) as *Glomerella cingulata* (Stonem.) Spauld. & Schrenk (anamorph *C. gloeosporioides*) and papery lesion isolate (IMI 383017) as *G. cingulata* (anamorph *C. gloeosporioides*).

Types of symptoms

Three selected isolates (one each from raised, anthracnose and papery lesion types) produced different types of symptoms on five clones (Table 4) and re-isolation from lesions always yielded isolates with characteristics similar to the used inoculum. Isolates from raised spots produced typical raised spot symptoms (Ramakrishnan and Pillay, 1961) while anthracnose and papery lesions isolates either produced circular lesions as reported by Ramakrishnan and Pillay (1961) or brown to black pin point lesions which later turn into yellow spots. The isolates from both anthracnose and papery lesions produced almost identical symptoms. It was also noted that isolates of raised spots produced more number of lesions than the anthracnose and papery lesions isolates on the tested clones.

DISCUSSION

Among the criteria used in the present study for the identification of isolates pathogenic to rubber, conidial shape provided the best means. These results are in agreement

Table 3. **Conidial size shape and colour of isolates**

Isolate No.	Length (mm)		Width (mm)		Shape*	Colour**
	Range	Mean	Range	Mean		
Raised lesion						
C1-1	10-20	13.01 + 1.86	3-5	3.78 + 0.48	F	S
C1-2	9-18	13.88 + 2.10	3-5	4.05 + 0.47	F	S
C2-1	12-20	15.42 + 1.45	4-5	4.40 + 0.49	F	S
C2-2	12-20	15.73 + 1.57	3-5	4.13 + 0.39	F	S
C-3	9-18	14.06 + 1.35	3-5	4.07 + 0.35	F	S
C-5	12-20	15.82 + 1.19	3-5	3.24 + 0.43	F	S
C-6	10-20	16.19 + 1.25	3-4	3.25 + 0.43	F	S
C7-1	12-19	14.63 + 1.38	3-5	3.60 + 0.51	F	S
C7-2	10-18	14.18 + 1.30	3-4	3.08 + 0.27	F	S
C-20	12-22	16.28 + 1.29	3-5	3.02 + 0.13	F	S
C-4	12.5-20	14.22 + 1.49	3-5	3.70 + 0.52	F	S
C-8	10-18	14.03 + 1.65	3-5	3.93 + 0.29	F	S
C23-1	11-18	14.79 + 1.26	3-5	3.88 + 0.33	F	S
C23-2	13-18	15.93 + 1.46	3-4	3.15 + 0.36	F	S
C-30	10-15	12.03 + 1.01	4	4.00 + 0.00	F	S
Pooled		14.67		3.67		
Anthracnose						
C-9	10-16	12.99 + 1.34	4-5	4.95 + 0.22	C	S
C-10	10-14	10.92 + 0.85	4-5	4.96 + 0.20	C	Y
C-11	10-18	13.74 + 1.55	4-5	4.30 + 0.46	C	S
C-12	10-14	11.03 + 0.97	3-5	3.94 + 0.49	C	S
C-25	11-15	13.70 + 1.17	4-5	4.98 + 0.13	C	S
C-27	10-18	12.67 + 1.35	3-5	4.02 + 0.13	C	S
C-26	12-18	13.71 + 1.12	4-5	4.97 + 0.19	C	S
C-28	12-16	14.06 + 1.12	4-5	4.02 + 0.14	C	S
C-29	11-15	13.32 + 1.10	4	4.00 + 0.00	C	S
Pooled		12.91		4.46		
Papery lesion						
C-15	12-17	14.00 + 1.33	4	4.00 + 0.00	C	S
C-16	9-14	11.58 + 1.20	3-5	3.09 + 0.29	C	S
C-17	10-16	13.19 + 1.40	4	4.00 + 0.00	C	S
C-18	10-15	12.25 + 1.27	4-5	4.53 + 0.50	C	S
C-31	10-16	12.07 + 1.70	3-5	3.44 + 0.50	C	S
Pooled		12.61		3.81		

* F = Fusiform;C = Cylindrical ** S = Salmon pink; Y = Yellow

with previously reported data (Gunnel and Gubler, 1992; Denoyes and Baudry, 1995). Conidial shape has been used by several taxonomists for separating species of *Colletotrichum* (Gunnel and Gubler, 1992; Simmonds, 1965; Sutton, 1980).

Conidial morphology has been traditionally emphasized over other taxonomic criteria in the genus *Colletotrichum*, although their conidia are potentially variable. The importance of the media in determining conidial morphology is illustrated by the study of Smith and Black (1990) in which no significant differences were found between *C. fragarie* and *C. gloeosporioides* produced on oat meal agar. Growing the fungus on PDA in this study ensured production of uniform conidia within a species. The shape of conidia was more important than their size in separating *C. gloeosporioides* and

Table 4. Symptoms produced on *Hevea* clones

Clone	Isolate		
	Raised lesion (C-4)	Anthracnose (C-12)	Papery lesion (C-16)
RRII 105	Initially minute brown to black dots. Later turn into small circular lesion. Finally raised above the surface.	Initially small black flecks. Later turn into yellow spots. Finally become dark grey to back and circular.	Initially brown dots. Later turn into small circular lesions. Finally become papery.
GT 1	Initially minute brown to black dots. Later turn into small circular lesion. Finally raised above the surface.	Initially minute brown necrotic lesions. Later turn into yellow spots with black centre. Finally become circular.	Small brown circular and slightly raised lesions.
BD 10	Initially minute brown to black dots. Later turn into small circular lesion. Finally raised above the surface.	Small necrotic lesions	Small black circular dot. Slightly raised.
PB 86	Initially minute brown to black dots. Later turn into small circular lesion. Finally raised above the surface.	Small black necrotic lesions. Circular and slightly raised.	Initially black dots. Later turn yellow and become circular.
PB 5/51	Initially minute brown to black dots. Later turn into small circular lesion. Finally raised above the surface.	Initially small, black flecks. Later turn into necrotic lesions.	Initially small, black dots. Later become yellow spots.

C. acutatum, as conidial measurement of these species overlapped. Since wide variation is apparent, conidial size is of little value in distinguishing species (Gunnel and Gubler, 1992). In this study the shape of conidia was found to be a reliable character in the separation of the isolates.

The form genera *Gloeosporium* and *Colletotrichum* have been traditionally separated by the presence of setae in the acervuli of the latter. The *Gloeosporium* species reported from strawberry has since been renamed *Colletotrichum acutatum* Simmond. The use of the shape of setae produced on strawberry leaf medium, as a further morphological criterion for separating the species has been reported(Gunnel and Gubler, 1992). However, setae formation may be influenced by environmental factors (Arx, 1970; Baxter *et al.*, 1985; Frost, 1964).

Isolates from raised spots produced a pink pigment on PDA. Baxter *et al.* (1983) noted the formation of a red pigment by *C. acutatum*. Simmonds (1965) observed that several isolates of *C. acutatum* from papaw fruit (*Carica papaya* L.) were bright pink in colour. Hindorf (1970) described wine red cultures of *C. acutatum* isolated from coffee. Dingley and Gilmour (1972) described *C. acutatum* f. sp. *pinea* as producing a carmine pigment on PDA. Gorter (1962) named isolates from olive fruit that were identical to a red strain of *G. cingulata* studied by Andes and Keitt (1950), *Gloeosporium fructigenum* f. sp. *chromogenum*. Baxter *et al.* (1983) compared an isolate of *G. fructigenum* f. sp. *chromogenum* with an isolate of *C. acutatum* from strawberry and concluded that they were non-specific.

C. acutatum isolates can also easily be separated from *C. gloeosporioides* isolates by their slower growth rate on PDA in petri dish culture (Smith and Black, 1990) and on the basis of colony colour and conidial morphology (Dyko and Mordue, 1979; Jayasinghe *et al.*, 1997). Jayasinghe and Fernando (1998) could also observe differences in sensitivity of isolates to three fungicides and in their growth at a temperature range of 25 to 30°C.

All the isolates produced symptoms on tested clones, the number of spots varied

greatly and more lesions were produced by raised lesion isolates on tested clones than anthracnose and papery lesion isolates. The symptoms produced by isolates from anthracnose and papery lesions were almost similar and differed from isolates from raised spots. This might be due to higher virulence of isolates from raised spots than the other isolates from anthracnose and papery lesions. Manju *et al.* (1999) also observed that raised spots lesions cause more damage on rubber.

Morphological and cultural characteristics of all isolates from raised spots were similar to those of *C. acutatum*. This was confirmed by the IMI. Hence, this isolate should be recognized as *C. acutatum* distinct from the anthracnose and papery lesion isolates which are *Glomerella cingulata* (anamorph *Colletotrichum gloeosporioides*). On the basis of a study of morphology of 52 isolates, Jayasinghe *et al.* (1997) reported that *C. acutatum* is the main cause of Colletotrichum leaf disease of rubber in Sri Lanka. This appears to be true in India as well.

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