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SHORT COMMUNICATION

The role of *Colletotrichum* species on the *Colletotrichum* leaf disease of *Hevea brasiliensis* - a preliminary study

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Abstract: *Colletotrichum* Leaf Disease (CLD) is considered as one of the major causes for the decline in yields of rubber in the Asian continent. Since early 1900's the pathogen *Colletotrichum gloeosporioides* has been considered as the causative agent of this disease in all rubber-growing countries in the world. However, observations of a recent survey in Sri Lanka revealed that the fungus *Colletotrichum acutatum* also plays a significant role in the development of CLD in rubber plantations. Therefore, a complete understanding of the involvement of the two *Colletotrichum* species in causing CLD is necessary. In order to assess the effect of the two species separately and in combination, an *in-vitro* investigation was carried out using young rubber leaves. The results suggest that *C. acutatum* is the major pathogen causing larger lesions. The results also showed that the two fungi can be synergistic in causing CLD if *C. acutatum* is introduced before *C. gloeosporioides*.

Keywords: *Colletotrichum acutatum, Colletotrichum gloeosporioides*, pathogenesis

INTRODUCTION

Hevea brasiliensis (Willd. ex Juss.) Muell. Arg. (the rubber tree) is the major source of natural rubber (NR) in the world. The rubber tree, is subject to a wide range of foliar diseases, which cause yield losses of rubber latex. Among the foliar diseases, *Colletotrichum* Leaf Disease (CLD) is considered as one of the major causes of declining yields of rubber in Asia[1]. The fungus *C. gloeosporioides* (Penzing and Sacc.) was identified as the causative agent of CLD after studying diseased rubber leaves from Sri Lanka[2]. Thereafter, *C. gloeosporioides* has been proved to be the causative agent of CLD in many other parts of the world. However, a more recent survey in Sri Lanka revealed that the fungus *C. acutatum* (Simmonds ex Simmonds) also plays a significant role in the development of CLD in rubber plantations[3].

It is therefore essential to develop a quick, easy and accurate screening method for the pathogens to study and evaluate their effects. In this investigation a laboratory based *in-vitro* bioassay was developed and the effect of the two *Colletotrichum* species in causing CLD of rubber was assessed.

METHODS AND MATERIALS

Isolation of causative organisms of CLD: Diseased leaf pieces were surface sterilized by immersing in 20 ml of 0.1% $HgCl_2$ solution in a petridish for about 30 sec. The leaf pieces were thereafter washed with 3 serial washings in sterilized distilled water and dried on a sterilized filter paper. The sterilized leaf pieces were cut into small portions so as to include the margins of diseased area using a sterilized blade. These pieces were cultured on plates of Potato Dextrose Agar (PDA) supplemented with Streptomycin at a concentration of 0.12 g/L. The inoculated plates were incubated at room temperature. Two to three days later the fungal colonies that grew from the cultured leaf pieces were transferred to fresh PDA plates and pure cultures were obtained by using the hyphal tip method.

Identification of isolated fungi: Sticky tape method[4] was used to identify the isolated fungi. The morphological characteristics and identification keys[5] were used. The identifications were confirmed by using reference cultures at the Rubber Research Institute of Sri Lanka (RRISL), Agalawatta.

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Determination of pathogenicity of the Colletotrichum species: Conidial suspensions of test fungi were prepared by flooding 7 d old cultures with sterile distilled water. The concentration of conidia in the suspension was adjusted using sterilized distilled water to $10x10^4$ conidia/mL and 0.02 mL of each suspension was placed on the abaxial surface of copper brown leaves (obtained 14 d after bud break) of *H. brasiliensis* in the following manner:

Treatment 1- Spore suspension of *C. acutatum*
Treatment 2- Spore suspension of *C. gloeosporioides*
Treatment 3- Mixed spore suspension of *C. gloeosporioides* and *C. acutatum* (1:1)
Treatment 4- Control (Sterile distilled water)

For each treatment, 4 leaves were used and 4 drops of the relevant conidial suspension were placed on both sides of axis of each leaf. The inoculated leaves were incubated at room temperature in moist chambers for 4 d. Depending on the sizes of the lesions produced they were grouped into 4 categories as follows:

(a) - No lesions
(b) - Lesion diameter between 5 mm – 10 mm
(c) - Lesion diameter between 10 mm- 15 mm
(d) - Lesion diameter > 15 mm

Mean sizes of lesions were also determined for different spore concentrations ($1x10^4$, $2.5x10^4$, $5.0x10^4$, $1x10^5$, $2.5x10^5$, $5.0x10^5$ and $1x10^6$ conidia/mL) of the two *Colletotrichum* spp. on detached young leaves of *H. brasiliensis*.

The combined effect of the two *Colletotrichum* species was also investigated following the same procedure as described previously. The treatments were as follows:

Treatment 1 - *C. acutatum* (Initial inoculation) + *C. gloeosporioides* (after 3 days)
Treatment 2 - *C. gloeosporioides* (Initial inoculation) + *C. acutatum* (after 3 days)
Treatment 3 - *C. gloeosporioides* + *C. acutatum* (Added simultaneously)
Treatment 4 - Control (Sterile distilled water)

The lesions were also categorized as described earlier.

RESULTS

Isolation of causative organisms of CLD

Cultures of *C. acutatum* and *C. gloeosporioides* were isolated from infected *Hevea* leaves. Majority of the isolates were *C. acutatum.*

Pathogenicity of the two *Collectotrichum* species

C. acutatum produced significantly larger lesions than those produced by *C. gloeosporioides*. The lesions produced by *C. acutatum* were dark and moist while *C. gloeosporioides* produced colorless moist lesions. Lesions were not produced when mixed inocula were used (Table 1).

Effect of inoculum concentration on pathogenicity of the two *Colletotrichum* species

The ability to produce lesions by the 2 species varied with the spore concentrations. The ability to produce a lesion decreased significantly as the inoculum concentration increased beyond $10x10^4$ conidia/mL. The maximum size of lesions was produced at $10x10^4$ conidia/mL. Both

Table 1: Comparison of size of lesions produced by *C. acutatum* and *C. gloeosporioides* separately and in combination on young leaves of *H. brasiliensis*

No of replicate	Category based on the size of lesions[x] produced			
	C. acutatum	*C. gloeosporioides*	*C. gloeosporioides* and *C. acutatum*	Control
1	d	c	a	a
2	d	d	a	a
3	c	c	a	a
4	d	c	a	a

[x] The responses produced by each fungus separately and in combination on young leaves of *Hevea* after inoculation of leaves with $10x10^4$ conidia/mL inoculum drop and incubation for 4 days at room temperature (28 ± 2° C) are:

(a) - No lesion production
(b) - Lesion diameter between 5 mm – 10 mm
(c) - Lesion diameter between 10 mm- 15 mm
(d) - Lesion diameter > 15 mm

Table 2: Comparison of size of lesions produced by *C. acutatum* and *C. gloeosporioides* on young leaves of *Hevea* at different inoculum concentrations

Concentration (Conidia/mL^{-1})	Category based on the size of lesions[y] produced.			
	C. acutatum	*C. gloeosporioides*	*C. gloeosporioides* and *C. acutatum*	Control
$1x10^4$	b	a	a	a
$2.5x10^4$	b	b	a	a
$5.0x10^4$	c	b	a	a
$1x10^5$	d	c	a	a
$2.5x10^5$	b	a	a	a
$5.0x10^5$	a	a	a	a
$1x10^6$	a	a	a	a

[y] The responses produced by each fungus separately and in combination on young leaves of *Hevea* after inoculation of leaves with different inoculum concentrations ($1x10^4$ to $1x10^6$) and incubation for 4 days at room temperature (28 ± 2°C) are:

(a) - No lesion production
(b) - Lesion diameter between 5 mm – 10 mm
(c) - Lesion diameter between 10 mm – 15 mm
(d) - Lesion diameter > 15 mm

Table 3: Comparison of size of lesions produced by different treatments at two time intervals

Time intervals (days)	Category based on the size of lesions[z] produced			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
3 d after 1st inoculation	c	b	a	a
3 d after 2nd inoculation	d	b	a	a

[z] The responses produced by different treatments at two time intervals are:

(a)- No lesion production
(b)- Lesion diameter between 5 mm – 10 mm
(c)- Lesion diameter between 10 mm- 15 mm
(d)- Lesion diameter > 15 mm

C. acutatum and *C. gloeosporioides* did not produce lesions at high spore concentrations (Table 2).

Combined effect of the two *Colletotrichum* species

The inoculation of *C. acutatum* initially, followed by *C. gloeosporioides* 3 days later increased the size of the lesions produced by *C. acutatum.* But the size of the lesions produced by *C. gloeosporioides* remained unchanged when *C. acutatum* was inoculated 3days afterwards. Lesions were not produced when a mixed inoculum of the two fungi and sterile distilled water were applied (Table 3).

DISCUSSION

Rubber plants in all rubber growing countries are affected by the CLD, resulting in secondary leaf fall reducing yields significantly[6]. Considering the seriousness of this disease, it is essential to understand the effect of the two *Colletotrichum* species in causing CLD. However, pathogenesis by *Colletotrichum* has received very little attention except for few studies carried out on *Hevea* isolates and strawberry isolates[7].

The results of this investigation show that the two species of *Colletotrichum* behave differently on *Hevea* leaves. *C. acutatum* produced darker moist lesions and *C. gloeosporioides* produced colourless moist lesions. This may be due to the variability in pathogenicity between *C. acutatum* and *C. gloeosporioides*. These results are similar to the observations made by Brown and Soepena[8].

The conidia of both *C. acutatum* and *C. gloeosporioides* showed self-inhibition at high conidial concentrations leading to decreasing lesion development. This is a

well-known phenomenon among fungal pathogens and is considered to be present in all fungi as an adaptation to increase pathogenicity by enabling the dispersal of ungerminated conidia, which are more resistant to fluctuations of environment than germinated ones[9].

Lesions were not produced when a mixed inoculum was used. A likely reason for the inhibition of lesion production when a mixture of conidia was used for inoculation would be, the involvement of inhibitory substances present at the conidium surface as reported by Bailey *et al* [7]. Further, the inhibition of conidia germination, when a mixed inoculum is used would also be due to the competition between two *Colletotrichum* species for space and nutrients.

The inoculation of *C. acutatum* initially followed by *C. gloeosporioides* 3 days later resulted in enhanced lesion production. The reason for this could be that *C. acutatum* predisposes the leaves enabling easier infection by *C. gloeosporioides.* Therefore, *C. acutatum* can be considered as the main causative agent of CLD. The results of this study show that *C. acutatum and C. gloeosporioides* can act synergistically in causing CLD. However, this aspect has to be studied further to determine the mechanism of the synergistic effect.

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