

VARIATION IN CULTURAL AND MORPHOLOGICAL CHARACTERS WITHIN COCOA ISOLATES OF *COLLETOTRICHUM GLOEOSPORIOIDES*

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SUMMARY

Isolates of *Colletotrichum gloeosporioides* Penz. causing infection on leaves and pods of cocoa were collected from various cocoa gardens in the southern states of India. The isolates were grouped into three groups based on the symptoms produced on cocoa leaves, viz., leaf blight, shot hole and irregular spot. Cultural and morphological characters of 299 isolates causing leaf blight (210 isolates), shot hole (77 isolates), and irregular spot (12 isolates) symptoms were studied. When the isolates were grown on potato dextrose agar medium for 10 days, they showed great variability in colour and consistency of the mycelium, growth rate, degree of sporulation and size of conidia indicating that there is considerable amount of genetic heterogeneity. The isolates from each of the three symptom groups were classified into white, dark and light types based on the colour of the colony. The light type isolates showed the highest degree of sporulation. Rate of growth was the highest in white and dark types, whereas sporulation was the lowest in these groups. Out of a total of 299 isolates collected from various symptom groups, 249 isolates were of light type indicating the predominance of this group among the population of *C. gloeosporioides*.

There was significant negative correlation between rate of growth and degree of sporulation among the isolates.

The white, dark and light types could be further classified into groups and subgroups based on the marked variation in the rate of growth, sporulation, presence of acervuli and conidia size. Of the 299 isolates, 28 produced acervuli on 10-day-old cultures of which 26 belonged to light type. There was marked variation in the size of the conidia. Seven isolates of the light type culture group produced perithecia with asci ascospores in 10 to 20-day-old cultures.

Isolates belonging to light type have been reported as more pathogenic than the other two groups. There appeared to be marked interrelationship between colony colour, rate of growth, sporulation and virulence. The higher rate of sporulation coupled with more pathogenic nature of the light types appears to help them to perpetuate and predominate under natural condition.

VARIATION DES CARACTERISTIQUES CULTURALES ET MORPHOLOGIQUES DES ISOLATS DE *COLLETOTRICHUM GLOEOSPORIOIDES* DU CACAoyer

RESUME

Des isolats de *Colletotrichum gloeosporioides* Penz., provoquant l'infection des feuilles et des cabosses de cacaoyers, ont été collectés dans diverses cacaoyères, dans les états du sud de l'Inde. Les isolats étaient réunis en trois groupes, selon les symptômes produits sur les feuilles du cacaoyer, c'est-à-dire flétrissement des feuilles, trous des tiges et taches irrégulières. Les caractéristiques culturelles et morphologiques de 299 isolats de *C. gloeosporioides*, provoquant le flétrissement des feuilles (210), les trous des tiges (77) et les taches irrégulières (12) ont été étudiées. A cette fin, les isolats ont été cultivés sur un milieu d'agar à la dextrose de pomme de terre pendant 10 jours. Ils ont révélé une grande variabilité de couleur et de compacité du mycélium, du taux de croissance, du degré de sporulation et de la dimension des conidies, indiquant qu'il existe une quantité considérable d'hétérogénéité génétique. Les isolats des trois groupes de symptômes ont été classés en types blanc, noir et pâle, d'après la couleur de la colonie des 299 isolats étudiés: 249 (83,23%) étaient des types peu colorés. Les isolats de type pâle révélaient le degré le plus fort de sporulation. Le taux de croissance était le plus élevé dans les types blanc et noir, tandis que la sporulation était la plus faible dans ces groupes. Donc, les isolats du type peu coloré étaient prédominants dans la population de *C. gloeosporioides*.

Le degré de sporulation diminuait avec l'augmentation des taux de croissance. Il existait une corrélation négative significative entre le taux de croissance et le degré de sporulation.

Les types blanc, noir et de couleur légère, pouvaient être classés davantage en groupes et en sous-groupes basés sur la variation marquée du taux de croissance, de sporulation, la présence d'acervuli et la dimension des conidies. Sur les 299 isolats, 28 produisaient des acervulis dans des

cultures de 10 jours, dont 26 appartenaient au type peu coloré. La taille des conidies variait beaucoup. Sept isolats du groupe de cultures à type peu coloré produisaient des périthécies avec ascis et ascospores dans des cultures de 10 à 20 jours.

L'on a signalé que les isolats appartenant au type peu coloré étaient plus pathogènes que les deux autres groupes. Il semble donc y avoir une interrelation marquée entre la couleur de la colonie, le taux de croissance, la sporulation et la virulence. Le taux plus élevé de sporulation, joint à la nature plus pathogène des types peu colorés, pourrait les aider à se perpétuer et à prédominer dans les conditions naturelles.

VARIAÇÃO DE CARACTERÍSTICAS CULTURAIS E MORFOLÓGICAS EM ISOLADOS DE *COLLETOTRICHUM GLOEOSPORIOIDES* NO CACAU

RESUMO

Colheram-se isolados de *Colletotrichum gloeosporioides* Penz., causadores de infecção em folhas e frutos do cacau, em diversas plantações de cacau nos estados do sul da Índia. Os isolados foram agrupados em três grupos, com base nos sintomas causados às folhas, p. ex. doença da folha, buracos e manchas irregulares. Estudaram-se as características culturais e morfológicas de 299 isolados de *C. gloeosporioides* causadores de doença da folha (210), buracos (77) e mancha irregular (12). Para tal, os isolados foram cultivados em meio de agar de dextrose de batata durante 10 dias. Demonstraram grande variabilidade em cor e consistência do micélio, taxa de crescimento, grau de esporulação e dimensão de conídios, indicando haver considerável heterogeneidade genética. Os isolados dos três grupos sintomáticos foram classificados em brancos, escuros e claros, de acordo com a cor da colônia. Dos 299 isolados estudados, 249 (83,23%) foram de cor clara. Os isolados claros mostraram o mais alto grau de esporulação. A taxa de crescimento foi maior nos tipos branco e escuro, sendo a esporulação mais baixa nesses grupos. Os isolados claros, portanto, foram predominantes na população de *C. gloeosporioides*.

O grau de esporulação diminuiu à medida que aumentou a taxa de crescimento. Houve uma correlação negativa significativa entre taxa de crescimento e grau de esporulação.

Os tipos branco, escuro e claro podem-se classificar ainda em grupos e sub-grupos, segundo sua marcante variação em taxa de crescimento, presença de acérvulos e tamanho dos conídios. Dos 299 isolados, 28 produziram acérvulos em culturas aos 10 dias, 26 dos quais eram do tipo claro. Houve uma grande variação no tamanho dos conídios. Sete isolados do tipo claro produziram peritécios dotados de ascos e ascospores em culturas de 10 e 20 dias.

Os isolados do tipo claro têm-se demonstrado mais patogênicos do que os dos outros dois grupos. Parece haver, portanto, uma forte correlação entre a cor da colônia, taxa de crescimento, esporulação e virulência. Um mais alto índice de esporulação, juntamente com a natureza mais patogênica dos tipos claros talvez lhes permita perpetuarem-se e predominar sob condições naturais.

VARIACION EN CARACTERISTICAS CULTURALES Y MORFOLOGICAS EN LOS AISLADOS DE CACAO DE *COLLETOTRICHUM GLOEOSPORIOIDES*

RESUMEN

Se recogieron aislados de *Colletotrichum gloeosporioides* Penz., que causan una infección de las hojas y mazorcas del cacao, de varios plantíos en los estados del Sur de la India. Los aislados fueron agrupados en tres grupos basados en los síntomas producidos sobre las hojas del cacao como roya de la hoja, agujeros en los brotes y manchas irregulares. Se estudiaron las características culturales y morfológicas de 299 aislados de *C. gloeosporioides* que causan roya de la hoja (210), agujeros en los brotes (77) y manchas irregulares (12). Para ello se cultivaron los aislados en un medio de agar con dextrosa de papa durante 10 días. Mostraron gran variabilidad en color y consistencia del micelio, tasa de crecimiento, grado de esporulación y dimensiones de conidia, indicando que hay una heterogeneidad genética considerable. Los aislados de los tres grupos de síntomas se clasificaron en tipos blanco, oscuro y claro, sobre la base del color de la colonia. De los 299 aislados estudiados, 249 (83,23%) eran del tipo claro. Los aislados del tipo claro mostraron el mayor grado de esporulación. La tasa de crecimiento fue la más alta en los tipos claro y oscuro. Así se encontró que los aislados de tipo claro eran los predominantes en la población de *C. gloeosporioides*.

El grado de esporulación disminuyó con el aumento en la tasa de crecimiento. Hubo una correlación negativa significativa entre la tasa de crecimiento y el grado de esporulación.

Los tipos blanco, oscuro y claro fueron además clasificados en grupos y sub-grupos basados en la marcada variación en la tasa de crecimiento, esporulación, presencia de acérvulos y dimensiones de conidia. De los 299 aislados, 28 produjeron acérvulos en cultivos de 10 días, y de ellos 26 pertenecían al tipo claro. Hubo una marcada variación en la dimensión de la conidia. Siete aislados del grupo de cultivo del tipo claro produjeron peritecia con ascites y ascosporas en cultivos de 10 a 20 días.

Se ha visto que los aislados pertenecientes al tipo claro son más patógenos que los de los otros dos grupos. Así parecería haber una interrelación señalada entre color de la colonia, tasa de crecimiento, esporulación y virulencia. La tasa más alta de esporulación unida a la naturaleza más patógena de los tipos claros puede ayudarles a perpetuarse y a predominar en condiciones naturales.

INTRODUCTION

A number of species of *Colletotrichum* have been reported to infect cocoa from various countries (Briton-Jones, 1934; Sanchez, 1957; Corbett, 1964; Ram *et al.*, 1972; Ram *et al.*, 1973; Turner, 1974; Thorold, 1975; Sarma & Nambiar, 1976; Reddy & Chandra Mohanan, 1976; Dakwa & Danquah, 1978). Interspecific variations have been worked out in detail in certain species like *C. sakazumii* on sugarcane (Abbott, 1938; Chona and Srivastava, 1960), *C. lini* (Zarzycka, 1976) and *C. gloeosporioides* on coffee (Muthappa, 1974; Hindorf, 1975; Feitosa *et al.*, 1977). *C. gloeosporioides* has been reported to cause pod rot and foliar infection of cocoa in the cocoa growing areas of southern states of India

(Chandra Mohanan & Kaveriappa, 1983). The isolates of *C. gloeosporioides* collected from various cocoa growing areas in South India exhibited marked variation in their virulence (Chandra Mohanan & Kaveriappa, 1984). However detailed studies have not been conducted so far on the cultural and morphological variations among the isolates of *C. gloeosporioides* pathogenic on cocoa. Hence the present study was taken up to fill this lacuna.

MATERIALS AND METHODS

A total of 299 isolates of *C. gloeosporioides* pathogenic on cocoa leaves and pods were collected from different

localities in Kerala, Karnataka and Tamil Nadu states of South India. Of these 210 isolates produced leaf blight symptoms and 77 and 12 isolates produced shot hole and irregular spot symptoms respectively when inoculated on cocoa seedlings (Chandra Mohanan & Kaveriappa, 1984). Cultural and morphological characters of all these isolates were studied. Potato dextrose agar medium (PDA) was used for growing the fungus and studying various characters. Since fungal growth and sporulation were reported to vary with varying composition in media (Leroux *et al.*, 1977), PDA prepared from a single lot of fresh potato was used throughout the study. The cultural and morphological characters of each isolate of the three symptom groups were separately recorded.

Colony characters

Mycelial disc of 5 mm diameter from 5-day-old cultures maintained on PDA was placed upside down in the centre of a 9 cm diameter petri plate containing 15 ml PDA. The plates were then incubated at $27 \pm 2^\circ\text{C}$. Three replicates were maintained for each isolate. The colony diameter was measured at an interval of 2, 4, 6, 8 and 10 days after inoculation. From this the rate of growth of each isolate was determined.

Ten-day-old colonies of the isolates maintained on PDA were examined for the characters such as colour, production of aerial mycelium and texture of the colony. The sizes of acervuli, setae, perithecia, asci and ascospores, whenever present were also recorded. The cultures which did not produce perithecia on the 10th day were incubated under room temperature for 30 days and examined frequently for the production of perithecia.

Degree of sporulation

To assess the degree of sporulation 5 mm diameter mycelial discs, three each from the periphery, middle and centre of the colony were cut with a sterile cork borer. The discs were transferred to a test tube containing 5 ml sterile distilled water and shaken well for 5 minutes on a mechanical shaker. From this 0.2 ml suspension was transferred to a slide and three such slides were prepared for each replication. The number of conidia per microscopic field (6.3×40 magnification) was recorded. The average number of conidia from 15 microscopic fields (5 from each slide) was considered as the degree of sporulation for each plate.

Size of conidia

The length (L) and breadth (B) of 150 conidia, 50 from each replication, were measured for each isolate. The L/B ratio was calculated.

Relationship between rate of growth and sporulation

To determine the relationship between rate of growth and sporulation the isolates were grouped into five classes based on the degree of sporulation as below:

Sporulation class	Degree of sporulation (average number of conidia/microscopic field (6.3×40))
I Poor	0-1
II Average	2-5
III Good	5-15
IV Very Good	16-25
V Abundant	More than 25

Twenty per cent of the isolates randomly selected from each of Groups I to IV and all the seven isolates of the Group V were included for the study. A correlation was worked out between the degree of sporulation and the rate of growth of the selected isolates.

RESULTS

The studies revealed marked variations among the isolates of each symptom group in their cultural and morphological characters. On the basis of colony colour the isolates of the three symptom groups were broadly classified into white, dark and light type.

White type: Colonies of this type appeared white from above and white to ivory from below. They were characterised by abundant mycelial growth and poor sporulation.

Dark type: The colonies of this type appeared olive gray from above and olive gray to black from below. In this type also there was abundant mycelial growth.

Light type: The colony appeared light gray to olive gray from both the surfaces. There was much variation in the production of aerial mycelium.

Some of the isolates showed diurnal zonation of dense and sparse mycelial growth. Some of the isolates exhibited saltation.

Rate of growth

There was marked variation in the rate of growth of the isolates. Radial growth rates of the isolates of leaf blight group varied from 4.8 mm to 10.83 mm per day (average 8.72 mm/day). Whereas in shot hole group it was 2.69 mm to 9.45 mm per day (average 6.53 mm/day), in the leaf blight group the average rate of growth was maximum (9.77 mm/day) in white type, followed by dark type (8.41 mm/day).

In the shot hole group also the average rate of daily growth was maximum in white type (7.66 mm) followed by dark type (6.67 mm). In the shot hole group the range in daily radial growth was 7.19-9.41 mm, in white type, 5.59 to 9.45 mm in dark type and 2.69 to 9.29 mm in light type. Thus, in general, the rate of growth was fast in white and dark types, and slow in light type. In the irregular spot group the radial growth rate varied from 6.25 mm to 9.12 mm per day (average 7.34 mm/day). Thus the rate of growth between white, dark and light type of leaf blight and shot hole groups varied considerably.

Production of acervuli and perithecia: Of the 299 isolates studied only 28 produced acervuli after 10 days incubation on PDA of which 26 belonged to the light type of leaf blight group and two isolates belonged to dark type of shot hole group.

The acervuli were round to elongate and 70-300 μm (mean 175 μm) in diameter. Setae were sparse to profuse, dark brown to black, straight or slightly curved, 2-5 septate, swollen at the base and tapering towards the apex (base 6.33 μm , middle 4.22 μm and apex 2.11 μm) and measured 40-160 μm length.

All the isolates which produced perithecia belonged to the light type and none of the dark or white type isolates produced perithecia. Of the 299 isolates two light type isolates of the shot hole group produced perithecia with asci and ascospores after 10 days of incubation. Five

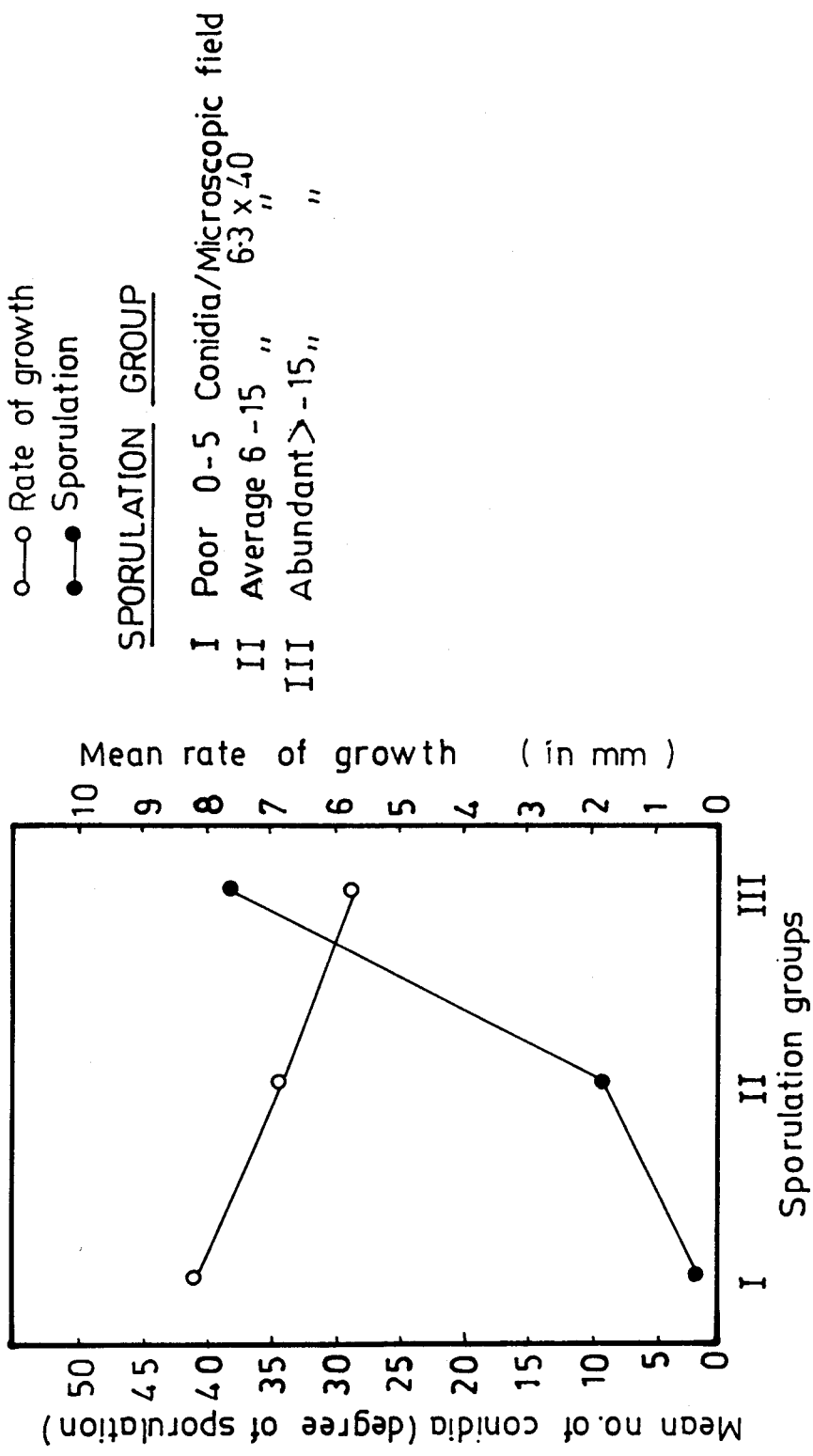


FIG. 2. Relationship between rate of growth and degree of sporulation of *C. gloeosporioides* isolates.

light type isolates of the leaf blight group produced perithecia with asci and ascospores on 15 to 20-day-old cultures on PDA.

The perithecia were globose to obpyriform, ostiolate and measured 104–280 μm (average 187 μm) in length and 58–210 μm (average 150 μm) in breadth. The asci were hyaline, clavate, with an ostiole at the apex and were 47–80 $\mu\text{m} \times 8$ –15 μm in size (average 63 \times 13 μm). The ascospores were straight to slightly curved with both ends rounded, light brown, single celled and measured 13–22 $\mu\text{m} \times 5$ –8 μm (average 18 \times 6 μm).

The isolates under each symptom group were classified on the basis of colour of the colony, production of acervuli, rate of growth and degree of sporulation, since the apparent differences among them were mainly due to these characters (Fig. 1). Of the 299 isolates studied, 249 (83.23%) were light types, 25 (8.36%) each dark types and white types. In leaf blight group 83.33% and in shot hole group 80.52% belonged to light type whereas in irregular spot group all the 12 isolates studied were light types. Within each major group also there was variation in colony colour. The white, dark and light types were again divided into sub-groups on the basis of production of acervuli, growth rate and degree of sporulation in that order. Isolates with rate of growth less than 5 mm/day were considered as slow growing, 6–8 mm/day as moderately growing and 9–10 mm/day as fast growing. The isolates with 0–5 conidia per microscopic field (6.3 \times 40) were grouped as poorly sporulating, those with 6–20 conidia per microscopic field as moderately sporulating and those with more than 20 conidia per microscopic field as abundantly sporulating.

In the leaf blight group all the white type isolates were fast growing with poor sporulation. In the shot hole group white type isolates were fast to moderately growing with poor sporulation. The dark type isolates of leaf blight group also were fast to moderately growing with poor sporulation. In both leaf blight and shot hole groups the fast growing isolates were mostly poorly sporulating. In general white types were the least sporulating followed by dark type. In all the cases light types were highly sporulating.

Size of conidia

Measurements of conidia of the 299 isolates showed marked differences in their length and breadth. Among the leaf blight isolates the biggest conidia size (21.31 \times 6.99 μm) was observed in light type isolate. In the shot hole group also the biggest conidial size (16.03 \times 5.24 μm) was observed in light type isolate. The maximum size of conidia of irregular spot isolate was 19.63 \times 9.93 μm . The conidial sizes of isolates under each group varied. The L/B ratio varied between the groups and within the groups. L/B ratio of conidia of white type isolates varied considerably from the other two groups.

Relationship between rate of growth and degree of sporulation

Other than colony colour, the rate of growth and degree of sporulation were found to be important characters exhibiting marked variation. Studies on the

relationship between rate of growth and sporulation of the isolates revealed that degree of sporulation decreases with increase in the rate of growth (Fig. 2). In most of the cases the isolates with more radial growth were found to be poorly sporulating when grouped on PDA. There was significant negative correlation between rate of growth and degree of sporulation ($r = -0.55^{**}$ at $P = 0.05$ level; $y = 50.69 - 5.42x$, where $x =$ rate of growth and $y =$ sporulation).

DISCUSSION

The isolates of *C. gloeosporioides* studied in the present work have shown great variability in colour and consistency of the mycelium, growth rate, degree of sporulation, size of conidia and virulence, indicating that there is considerable amount of genetic heterogeneity. The classification of the isolates of *Colletotrichum* sp. pathogenic on other crops was also mainly based on the variation in the colour of the colony, rate of growth, sporulation and conidial size (Chona and Srivastava, 1960; Hindorf, 1973; Muthappa, 1974; Feitosa *et al.*, 1977). Isolates of *C. gloeosporioides* causing leaf blight of cocoa in Ghana were grouped into four types based on the colour of the colony, shape of conidia, and consistency of aerial mycelium of the cultures (Dakwa and Danquah, 1978). The *C. falcatum* isolates were also classified into light and dark types based on the cultural characters (Chona and Srivastava, 1960). The existence of light and dark types of *C. falcatum* was also reported from Louisiana (Abbott, 1938 and 1946). The isolates of *Colletotrichum* sp. causing coffee berry disease were divided into four groups based on cultural characteristics (Gibbs, 1969). In the present investigation the light type isolates were found to be predominant in the population of *C. gloeosporioides*. In general white and dark type isolates showed higher rate of growth than the light type. But white types were the least sporulating and less pathogenic followed by dark type, whereas the light type isolates showed highest degree of sporulation and were more pathogenic (Chandra Mohanan & Kaveriappa, 1984). The poor sporulation coupled with less virulent nature of the isolates seems to be the reason for the less frequency of white and dark types in nature. In *C. lini* also the less frequent white types were reported to be poorly sporulating and less pathogenic (Zarzycka, 1976). Studies on different isolates of *C. falcatum* also showed that the colour of the mycelium and degree of sporulation were interrelated and the light types were highly sporulating and predominant over the dark types. Thus there appeared to be marked interrelationship between colony colour, rate of growth, sporulation and virulence.

Perhaps higher rate of sporulation and more pathogenic nature of the light type of isolates help them to perpetuate and predominate under natural conditions. Out of 299 pathogenic isolates studied 83% were light types. It is therefore necessary to give more importance to this group of isolates while screening cocoa cultivars for resistance to *Colletotrichum* disease in S. India.

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