Original Article

# Speckle by *Corynespora torulosa* (Syd.) Crous: a pre-harvest fruit disease of *Musa* spp. in Cuba

## Punteado por *Corynespora torulosa* (Syd.) Crous: una enfermedad de precosecha de los frutos de *Musa* spp. en Cuba

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**ABSTRACT:** It was studied the etiology of a fruit disease spots in form of a speckling of Cavendish banana fruits that causes fruit quality deterioration and commercial rejection. For this purpose, peel spots were placed in humid chambers to incite fungal development, and isolations were carried out on water agar + streptomycin. To fulfill Koch postulates, CEMSA $^3/_4$ and Burro CEMSA fruits were inoculated with a spore suspension of $1x10^5$ conidia/ml and incubated at room temperature under high relative humidity. Fruits of Cavendish and Gros Michel (AAA), CEMSA $^3/_4$ and Pisang ceylan (AAB), Pisang awak and Burro CEMSA (ABB) were inoculated as described. The fungus *Corynespora torulosa* (Syd) Crous, comb. new (syn. *Deightoniella torulosa* (Syd.), M.B. Ellis) was uniformly recovered from the humid chambers and isolations. The artificial inoculations of Cavendish fruits with the isolates obtained reproduced the speckling symptoms, and *C. torulosa* was confirmed as the causal agent of speckling. All varieties inoculated showed to be susceptible to the pathogen. It is the first time that *C. torulosa* is described in Cuba as the causal agent of fruit speckling previously attributed to other causes. *C. torulosa* is a colonizer of senescent leaves, fruit flowers, and bracts of *Musa* plants and becomes airborne during appropriate environmental conditions. It is discussed that sanitation of senescent leaves and fruits organs are essential to reduce incidence of the disease.

**Keywords:** *Deightoniella torulosa*, fruit wastages, sanitation, resistance of cultivars.

**RESUMEN:** Se estudió la etiología de una enfermedad de los frutos en forma de punteado en frutos de bananos Cavendish que causa deterioro y rechazo comercial de frutos. Se colocaron manchas de la piel de los frutos en cámara húmeda para incitar el desarrollo de hongos y se realizaron aislamientos en agar agua + estreptomicina. Para cumplimentar los postulados de Koch, se inocularon frutos de CEMSA $^3/_4$ y Burro CEMSA con una suspensión de $1x10^5$ conidios/ml y se incubaron a temperatura ambiente y alta humedad relativa. Se inocularon frutos de Cavendish y Gros Michel (AAA), CEMSA – y Pisang ceylan (AAB), Pisang awak y Burro CEMSA (ABB) de la forma ya descrita. De las cámaras húmedas y aislamientos se recobró uniformemente el hongo *Corynespora torulosa* (Syd) Crous, comb. new (syn. *Deightoniella torulosa* (Syd.), M.B. Ellis). Las inoculaciones artificiales de los frutos de CEMSA $^3/_4$ y Burro CEMSA con los aislamientos obtenidos, reprodujeron el punteado de los síntomas y se confirmó que *C. torulosa* es el agente causal del punteado de los frutos. Todas las variedades inoculadas mostraron ser susceptibles al patógeno. Esta es la primera confirmación en Cuba de que *C. torulosa* es el agente causal del punteado de los frutos, antes atribuido a otras causas. *C. torulosa* es un colonizador de hojas senescentes, flores y brácteas de plantas de *Musa* spp. Se discute que el saneamiento de hojas viejas, y órganos de los racimos es esencial para reducir la incidencia de la enfermedad.

**Palabras clave:** *Deightoniella torulosa*, daños a frutos, saneamiento, resistencia de los cultivares.

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## INTRODUCTION

Banana and plantain are important crops in economic, food sustainability and social terms in Latin America and Caribbean countries (LA & C; 1,2,3,4). In Cuba, banana and plantain are also important due to the daily consumption and as cash crops that every week render benefits to the growers. Banana and plantain cultivated surface and production in Cuba in 2017 were (5): banana 24,046 ha and 295,526 t. and plantain 64,103 ha and 719,039 t. Diseases are important constrains to banana and plantain production and pre- and postharvest diseases reduce commercial yield, revenue, and profitability. Banana and plantain pre- and postharvest fruit diseases have been exhaustively studied in Cuba (6,7,8).

In the present paper, descriptions are made of the symptoms and etiology of a pre-harvest fruit spot found on Cavendish fruits in Artemisa province of Cuba in April 2019, as well as of the pathogen morphology and its pathogenicity on banana and plantain. The epidemiology and management practices are discussed.

## MATERIAL AND METHODS

Fruits of Cavendish banana plants with a speckling symptom were collected and taken to the plant pathology laboratory of INISAV to describe the symptoms and isolate and describe the pathogen.

Symptoms were described and photographed. To determine etiology, fruit peel with spots were washed under tap water, disinfected with alcohol 70 % for 1 min, washed three times with sterile distilled water and placed inside of sterile 100 mm Petri plates on sterile humid filter paper. Afterward, the plates were incubated at room temperature in moist chamber (100n % relative humidity) and observed daily until a fungal structure developed.

Pathogen isolation was carried out from the disinfected peel spots by placing 2 - 3 mm peel pieces from the border of the spots on water agar (16 g agar Biocen/L distilled water) + streptomycin 50 µg/ml. The plates were incubated at 29ºC and observed daily for fungal growth. Once noticed the fungal growth, it was transferred to Petri plates with PDA and incubated in the dark at 29ºC. The morphology of single spore colonies, the pigmentation, development of conidiophores and conidia, were described, and 50 conidia and conidiophores were measured at 400x magnification under a Carl Zeiss microscope and compared with CMI pathogenic fungal sheets descriptions. In order to fulfill Koch postulates, five individual fruit fingers of CEMSA $^3/_4$ (subgroup Plantain, AAB) and Burro CEMSA $^3/_4$ (subgroup Bluggoe, ABB) cultivars of approximately 40-50 days old were inoculated with a conidia suspension of $1x10^5$ conidia/ml in sterile water, prepared from a ten-day-old single spore colony of the fungus grown on PDA (20 g Oxoid agar / L of water) and incubated in humid chambers prepared in trays with humid filter papers at room temperature as above described to induce symptom development and sporulation.

Pathogenicity tests to different banana and plantain genotypes were carried out with Grand nain (subgroup Cavendish, AAA), Gros Michel (subgroup Gros Michel, AAA), CEMSA $^3/_4$ (subgroup Plantain, AAB), Pisang ceylan (subgroup Pisang ceylan, AAB) Manzano vietnamita (subgroup Pisang awak, ABB) and Burro CEMSA (subgroup Bluggoe, ABB). Conidial suspensions were prepared from seven-dayold single spore cultures grown on PDA and incubated as described for ten days. Fruit fingers of each cultivar were surface disinfected with 70 % ethylic alcohol, and three squares of 1 $cm^2$ were marked on each fruit with a felt pen and delimited with fused paraffin. Two of the squares were inoculated by depositing a drop (0.2 µl) of a spore suspension ($1x10^5$ conidia/ml) inside them, and one sterile water drop were deposited in the third square as control. Three replications with two fruits of each cultivar were used in a completely randomized design assay. Fruits were incubated in trays covered with humid filter papers and observed daily until symptom development.

## RESULTS

The symptoms found in the field consisted of numerous superficial tiny spots on the peel that did not affect pericarp of the fruit. Individually, the spots were round and firstly developed as a watery halo on the peel that became reddish

brown and later necrotic, more frequently with a 2-3 mm diameter that could reach 4 mm and surrounded by an external halo (Figure 1). Symptoms were more abundant close to the neck or peduncle of the fruit and on the inner side than on the external side of the fruit.

On the spots incubated in humid chambers, conidia developed at the extreme of long conspicuous brown conidiophores after 48 hours of incubation. From the spot tissues on agar, a single fungus developed that, after being isolated and incubated on PDA, produced initially clear gray colonies that turned dark gray and black with the presence of abundant characteristic brown conidiophores identical to those developed in humid chambers. At the extreme of hyphae, 14.64 - 43.92 x 2.44 - 4.88 µm pale brown conidiophores, swollen at the apex, developed single or in small groups with successive proliferations (Figure 2). Conidia developed singly at the ends of the conidiophores and their successive proliferations. Conidia were 45-75 (X= 57,7) x 10-20 (X=16.6) µm straight or slightly curved, obpyriform to obclavate, hyaline to gray olive more intense pigmented close to the scar, 1-5 pseudoseptate (more frequently 3 pseudosepta).

Artificially inoculated fruits develop typical speckling symptoms in 4 days (see Figure 3)

The fungus morphology and symptoms on fruits agreed with descriptions of fruit speckling disease (7,8) and its causal agent *Corynespora torulosa* (Syd.) Crous, comb. new (9) previously named *Deightoniella torulosa* (Syd.) M.B. Ellis (10).

In the pathogenicity tests with different banana and plantain genotypes, all inoculated fruits of cultivars developed typical symptoms four to five days after inoculation, without differences among them. No differences of incubation period among the cultivars were determined (data not showed).

## DISCUSSION

The symptom description and fungus morphology agreed with the descriptions of speckling made by Meredith (9) and with the fungus *C. torulosa* (syn. *D. torulosa;* 10,11). The first description of the disease belongs to Meredith in Jamaica in1963 (9), who studied the pathogen extensively. Fruit speckling by *C. torulosa* is widely distributed in LA & C, but it had not been previously confirmed in Cuba. Similar speckling on Cavendish fruits in Cuba and banana producing countries was frequently associated and confused with phytotoxicity lesions on fruits caused by the mixtures of oil and morpholine fungicide applications used to control black Sigatoka (12). Speckling is reported as the

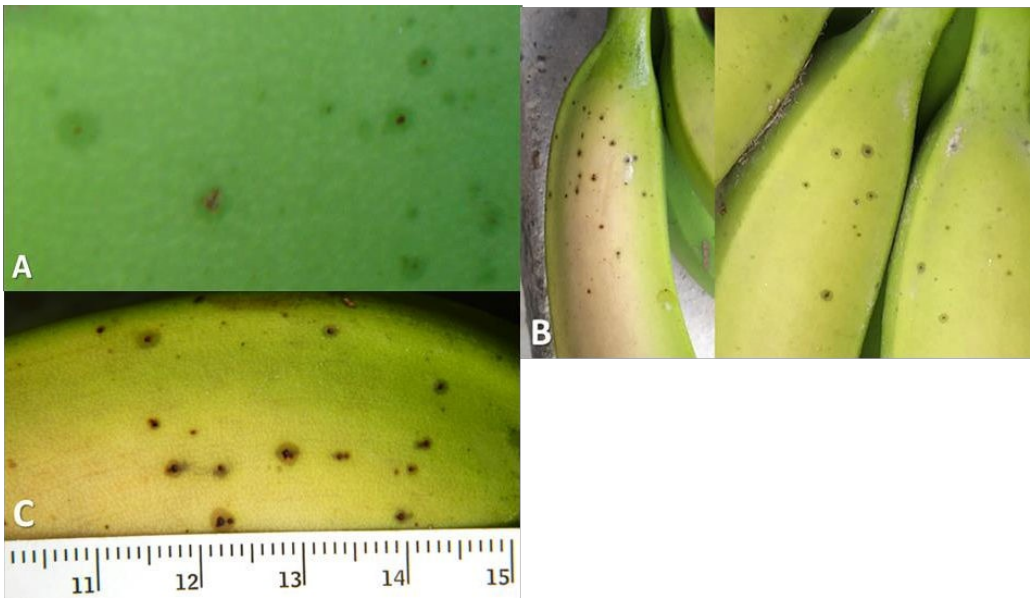


**Figure 1.** Symptoms of speckling on Cavendish banana fruits; A) first symptoms as a halo; b) typical spots as specks surrounded by a halo: C) presence of mature spots of different ages and size. / Síntomas de punteadura en frutos de bananos Cavendish: A) primeros síntomas en forma de halo; B) manchas típicas de pecas rodeadas de un halo; C) presencia de manchas maduras de diferentes edades y tamaños.

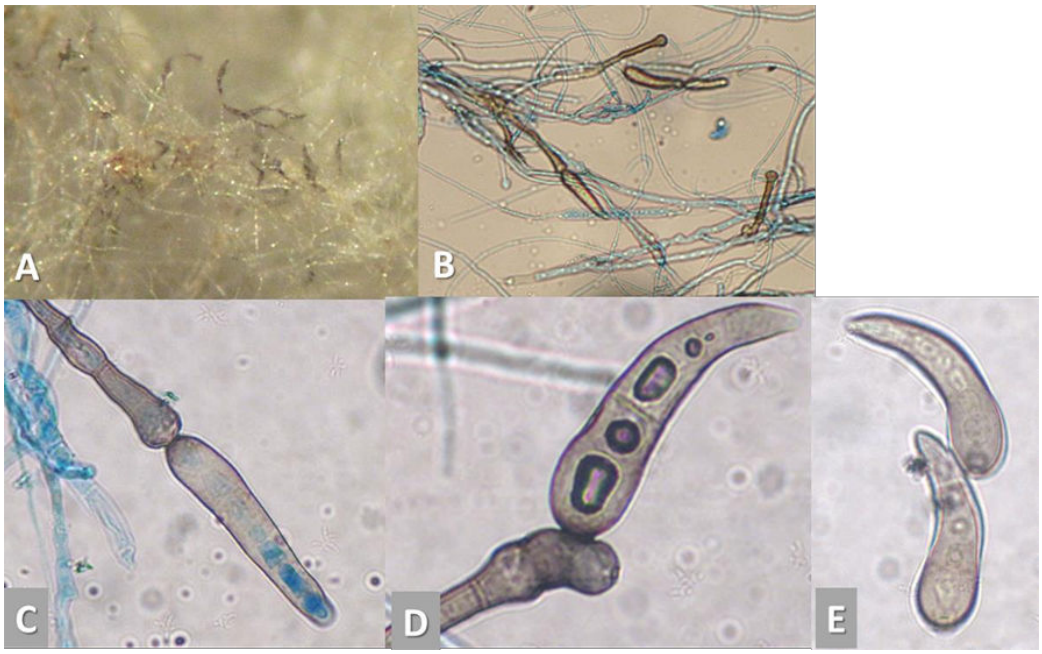


**Figure 2.** Conidiophore and conidia of *C. torulosa*: A) superior view at the binocular stereoscope of brown conidiophores and conidia developed at the extreme of the hypha from cultures on PDA (40X); B) almost straight brown conidiophores and curved obclavate conidia developed at the extreme of vegetative hyphae of colonies (200X); conidiogenic cell at the extreme of conidiophore with C) almost straight young conidia and D) mature obclavate curved conidia with pseudo septum (400X); E) obclavate curved young conidia (400X) / Conidióforos y conidios de *C. torulosa*. A) vista superior al estereoscopio binocular de conidióforos pardos y conidios desarrollados en el extremo de la hifa de cultivos en PDA (40X); B) conidióforos pardos, casi recto y conidios obclavados, curvos, desarrollados en los extremos de hifas vegetativas de colonias (200 x); C) conidios jóvenes casi rectos; D) conidios jóvenes obclavados casi rectos con pseudosepto (400 X); E) conidio joven obclavado curvo (400X)

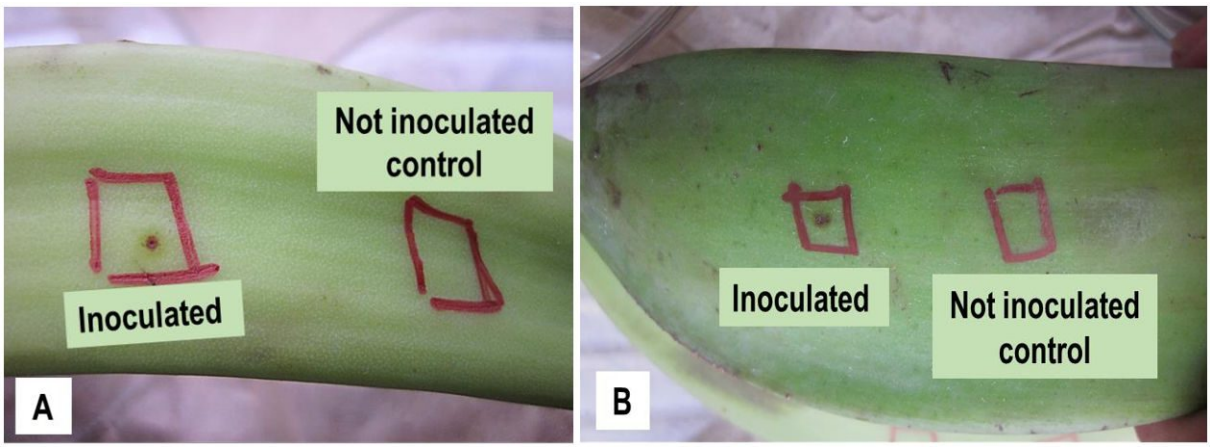


**Figure 3.** Pathogenicity test in fruits inoculated with a conidial suspension of *C. torulosa* to confirm Koch postulates. Note symptoms development on the left squares. The right square is the control with sterile water: A) In a young undeveloped CEMSA – - fruit (subgroup Plantain, AAB), and B) in Burro CEMSA fruit (Bluggoe subgroup, ABB). / Ensayo de patogenicidad en frutos inoculados con suspensión de conidios de *C. torulosa* para confirmar postulados de Koch. Notar el desarrollo de los síntomas en los cuadros izquierdos. El cuadro derecho es el control con agua destilada estéril: A) en frutos jóvenes en desarrollo de CEMSA – (subgrupo Plantain, AAB) y B) en frutos de Burro CEMSA subgrupo Bluggoe, ABB)

cause of important marketing rejection of fruits in heavy rainfall areas of Jamaica and Costa Rica (where there were reports of up to 70 % of bunches rejected; 13,14). *C. torulosa* is also the cause of a blemish of banana and plantain fruits, named black tip disease (6,7), and is sometimes associated with the crown rot complex of banana fruits (8). It has also been found causing leaf spots on leaves of banana and plantain plants (6), as well as causing a leaf blight of tissue culture plants in humid overcrowded nurseries (15). A further study with cross inoculation and a molecular phylogenetic analysis of isolates of the fungus associated with different types of symptoms would provide a better knowledge on the pathogenic variability and phylogenetic relationships of populations of the fungi associated with different syndromes. All banana and plantain cultivars of different subgroups developed freckle symptoms with the artificial inoculations with C. *torulosa*. It confirms previous information by Stover in 1972 (16).

Fruit speckling is more frequent and intense in the rainy season as *C. torulosa* sporulates profusely during high humidity conditions (7,14,16). The fungus can be found as saprophytic microbiota on the old senescent leaves of plants, on finger flowers and bunch bracts (7,8). When relative humidity falls, the spores are violently discharged from these organs and become airborne (7). It is for this that the disease is more prevalent in poorly culturally managed plantations (7,13,14). Elimination of old leaves and bunch bracts is crucial to eliminate available inoculum in the plantation and the reduction of incidence on fruits in commercialization.

## CONCLUSIONS

1. Speckling symptoms of banana fruits in Cuba is caused by *C. torulosa,* which is a widely spread disease in LA & C. This is the first report of the presence of the fungus causing fruit speckling on banana and plantain in Cuba.
2. The fungus can affect all cultivars belonging to different *Musa* taxonomic subgroups.

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