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SHORT COMMUNICATION

# *Deightoniella torulosa* (Syd.) M.B. Ellis, causing leaf spot in acclimatized *Grande naine* (Musa, AAA) plants

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**ABSTRACT:** In the present work, some features concerning the morphological and pathogenic characterization of the causal agent of the leaf spotted disease in *Grande naine* (Musa, AAA) cultivar were studied. Morphological characterization of conidia and conidiophores were done under a light microscope using fragments of spotted leaves. Pathogenecity tests were performed on three-month-acclimatized *Grande naine* plants. Conidiophores were straight or slightly curved obpyriform to obclavate and subhyaline to olive in colour. Conidia size varied from 35-70 µm in length to 13-25µm in width, with 3-6 pseudosepta. Obpyriform to ellipsoid conidia were observed in Grande naine spotted leaves. The first symptoms appeared 14 days post inoculation (dpi) as black necrotic spots (1-2 mm diameter). They became oval with black border at 45-56 days post infection (dpi). This is the first report of *Deightoniella torulosa* (Syd.) M. B. Ellis causing leaf spot in acclimatized *Grande naine* plants.

**Key words:** banana, pythopathogen.

## *Deightoniella torulosa* (Syd.) M.B. Ellis, agente causal de manchado de la hoja de plantas aclimatizadas de *Grande naine*

**RESUMEN:** En el presente trabajo, se evaluaron aspectos relacionados con la morfología y la caracterización patogénica del agente causal del manchado de las hojas en plantas del cultivar *Grande naine* (Musa, AAA). Los ensayos de patogenicidad se realizaron en plantas aclimatizadas de tres meses. Para la caracterización morfológica, se observaron conidios presentes en fragmentos de hojas necrosadas bajo un microscopio clínico de luz transmitida. Los conidióforos fueron rectos o ligeramente curvados, obpiriformes u obclavados, de colores subhialinos a oliváceos. El tamaño de los conidios varió de 35-70 µm de largo x 13-25 µm de ancho, con la presencia de 3-6 pseudoseptos. Los conidios mostraron formas desde obpiriformes a elipsoidales. Los primeros síntomas aparecieron a los 14 días posteriores a la inoculación (dpi) en forma de manchas necróticas (1-2 mm diámetro). Posteriormente, se convirtieron en manchas ovales con borde negro entre los 45-56 dpi. Este constituye el primer informe de *Deightoniella torulosa* (Syd.) M. B. Ellis causando el manchado de las hojas en plantas de *Grande naine* acclimatizadas. Finalmente, la identificación del agente causal del manchado de las hojas de plantas aclimatizadas de *Grande naine* es importante para implementar estrategias de control más efectivas y disminuir las pérdidas ocasionadas en las plantas de *Grande naine* micropropagadas.

**Palabras clave:** bananos, fitopatógeno.

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Micropropagation of banana and plantain is commonly used to produce great numbers of healthy plants. The principal limitations are found in the acclimatization phase, where abiotic and biotic factors may reduce survival. Fungal diseases are the main constraints in the acclimatization of micropropagated plants, particularly due to high humidity and the use of poor quality substrates. Black Sigatoka (*Mycosphaerella fijiensis* Morelet) and damping off, caused by *Pythium* spp., are frequently found in acclimatized *Musa* plants causing severe losses (1).

Banana fruit speckle and black tip are caused by *Deightoniella torulosa* (Syd.) M. B. Ellis, which is also known as swamp spot, black tip, or tip-end rot in field conditions (2, 3). This same fungus also causes *Deightoniella* leaf spot in old plantations where leaves are not removed. The disease is relatively a minor problem wherever banana is grown (4). *D. torulosa* is also known as a saprophytic colonizer of dead *Musa* leaves and flowers. It is also a weak parasite of older foliages of banana and has been reported on young leaves of *Musa* seedlings (5). The pathogen was recorded on both young and old leaves; it is distributed in tropical and subtropical areas of the world, being common in regions of Ethiopia (6).

This fungus may cause speckle-like spotting of the petiole and pin-spotting disease on preharvest fruits. *Deightoniella* leaf spot may be confused with the leaf spot caused by other pathogens (3). Nevertheless, the morphological and pathogenic characterizations are important aspects to differentiate *D. torulosa* from other *Musa* pathogenic or saprophytic fungi. Acclimatization of *Musa* micropropagated plants is affected by several fungal diseases, particularly *Mycosphaerella fijiensis* Morelet. The appearance of a new symptomatology in *Grande naine* micropropagated plants was the principal problem to solve in this work. The aim of this paper was to identify the causal agent of leaf spotting in acclimatized *Grande naine*.

***Fungal isolation***. Banana leaves with necrotic symptoms from acclimatized *Grande naine* plants were placed in a wet chamber for 48h at 28ºC in dark. Conidia from necrotic lesions were taken with a needle under an Olympus microscope (100x magnifications) and transferred to Petri dishes containing PDA (Fluka) medium. They were incubated for 20 days at 20ºC in dark. Mycelium fragments from the young colony surface were transferred to slant PDA tubes and incubated for 30 days at 20ºC in dark. All the tubes were stored at 4ºC.

**Cultural and morphological characterization**. The cultural characteristics of the colonies of the causal agent were determined by visual observation of the mycelium texture, the reverse color, the transpired liquid and the pigmentation of culture media.

Fragments of necrotic leaves from artificially inoculated *Grande naine* plants were used to determine conidiophore and conidia morphology under a clinic microscope (Carls Zeiss), using 200x magnification. Discs of leaves (1 cm diameter) with necrotic lesions were discoloured in a KOH 10% ($^{m}/_{v}$) solution during 24h. The samples were washed with destilled water three consecutive times. Finally, leaf discs were mounted on slides with lactophenol (phenol 20 g; lactic acid 20 g; glycerol 40 g; water 20 ml). Morphological characterization and identification of *D. torulosa* was done according to Subrumanian's (7) and Jones' (3) criteria.

***Pathogenicity test*.** Ten *Grande naine* (AAA) plants were inoculated with a mycelial suspension ($1{,}20x10^{5}$ mycelial fragments.$ml^{-1}$). The mycelia were obtained from a liquid culture medium of Potato Dextrose Broth (PDB, Fluka), inoculated with two disc (1 cm of diameter) from the edges of three day colonies. Plants were previously acclimatized for eight week in a greenhouse, and then inoculated by brushing the abaxial leaf surface. Control plants were treated with sterile distilled water containing one drop of Tween 20. After inoculation, the plants were incubated for 72 h at 90-100% relative humidity using a water spraying system. Neither John nor Albert was observed.

Characteristic of the colonies were dark at mycelia reverse and brown dark while superficial mycelia were brown dark. Neither liquid transpiration nor pigmentation from colonies was observed. The conidiophores were 35-70 µm in lenght x 13-25 µm in width; they were straight or slightly curved (Figure A). Obpyriform to ellipsoid conidia were produced in all the inoculated Grande naine leaves (Figure B). They were subhyaline to olive in colour with 3 to 6 pseudosepta. All these cultural and morphological characteristics matched with those reported to *Deightoniella torulosa*.

Leaf spotting appeared as small points on the leaf lamina (Figure C), which were yellowish at first, then became brownish and finally black, becoming oval in shape with a black border (Figure D). Lesions increased in size enlarging in both length and width. Blotches of various sizes were observed on inoculated leaves, which sometimes covered the whole length of the leaf border. Finally, the plants showed completely necrotic and holding down leaves (Figure E).

The results were different to those reported by Jones (3), where the lesions produced by *D. torulosa* were more prevalent along the edges of the leaf blade and on the older leaves of banana plants grown in the field. He

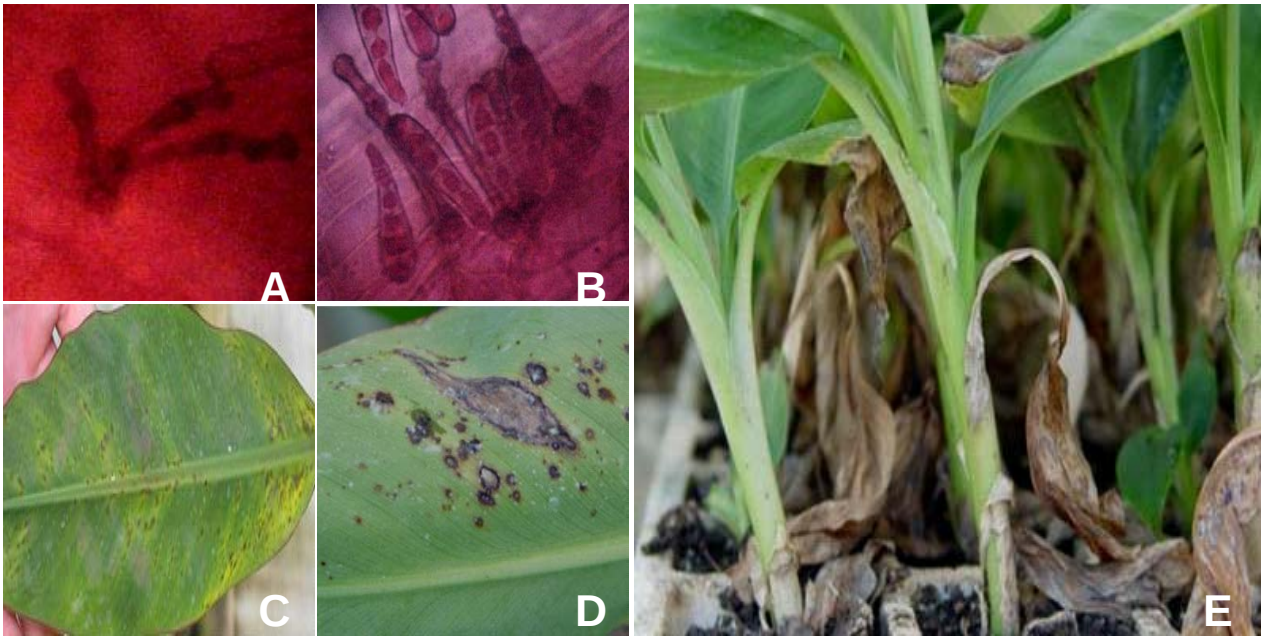


**FIGURE.** Morphology of conidiophores and conidia (A and B) of *Deightoniella torulosa* (Syd.) in artificially inoculated leaves of *Grande naine*. Symtomatology observed at 14 (C), 45 (D) and 63 days post inoculation./ *Morfología de los conidióforos y conidios (A y B) de* ***Deightoniella torulosa*** *(Syd.) en hojas de* ***Grande naine*** *inoculadas artificialmente. Sintomatología observada a los 14(C), 45 (D) y 63 días posteriores a la inoculación.*

also observed black necrotic spots between 1 and 2 mm in diameter which increased in size becoming oval with a black border. The mature spots in this study were shorter in comparison with those observed by Jones (4).

Similar leaf spotting symptoms were showed by Koné *et al*. (6). They demonstrated that *D. torulosa* induced necrosis in leaves of three cultivars of banana (Orishel, Figue Sucrée and Grande Naine), but they used conidia suspension and older banana plants for the artificial inoculation assays. Finally neither completely necrotic leaves nor holding down leaves were observed by them (6), and the progress of the disease varied according to each banana cultivars.

In nature, *D. torulosa* is commonly found in dead banana leaves and conidia are produced during alternative periods of rain and dryness. Spores are discharged from conidiophores when the humidity declines, and then they become airborne and dispersed (8). Presence of necrotic leaves and humidity changes may favour the incidence and dispersion of the fungus (9). During acclimatization of banana plants, humidity is maintained high.

Limited scientific papers about *Deightoniella torulosa* management are available and most of them are focused on the evaluation of synthetic fungicides and essential oils with potential for its control.

This is the first report of *D. torulosa* (Syd.) causing leaf spotting in acclimatized Grande naine plants. Similarly, cultural, morphological and pathogenic characterizations of this pathogen had not been described before in banana acclimatized plants, neither artificial inoculation using mycelia suspension has been developed.

The results from this paper could be important to support the artificial inoculation and the future analysis of *Musa- D. torulosa* interactions between this pathogen and different banana cultivars under controlled conditions. Finally, better control strategies could be implemented and designed to reduce leaf spotting in acclimatized Grande naine plants by using the possibilities offered by these results.

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