A Simple Method to Obtain Single Conidium Isolates Directly from Banana (*Musa* sp.) Leaves Infected with *Mycosphaerella fijiensis* Morelet

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Abstract. A simple methodology to isolate conidia of *Mycosphaerella fijiensis* from infected banana leaves was implemented. A method reported previously was laborious and required skilled trained personnel. Here we describe a sampling method that consumes short periods of time and low costs and that can be carried out and reproduced by personnel with limited lab experience or expertise. It is practical and useful for extensive studies of foliar pathogen population and distribution.

Additional keywords: Black leaf streak, black sigatoka, conidia, asexual reproduction.

Resumen. Se desarrolló una metodología sencilla para aislar conidios de *Mycosphaerella fijiensis* a partir de hojas de banano infectadas. El método que había sido reportado previamente es laborioso y requiere ser llevado a cabo por personal capacitado y entrenado. Se describe un método de muestreo que consume poco tiempo y es de bajo costo, y que puede ser llevado a cabo y reproducido por personal con poca experiencia. Es práctico y útil para ser aplicado en estudios poblacionales extensos y de distribución de patógenos foliares.

Palabras clave adicionales: Enfermedad de la roya negra, sigatoka negra, conidios, reproducción asexual.

Black leaf streak disease or black Sigatoka, caused by *Mycosphaerella fijiensis* Morelet (Mycosphaerellaceae, Ascomycetes), is considered the most devastating disease of banana and plantain (*Musa* spp. AAA, AAB, AA, and ABB groups). It is spread through ascospores (sexual) and conidia (asexual). Analyses of single ascospore-derived isolates have been used to study the genetic structure and diversity of populations (Carlier *et al*., 1994, 1996). Revised methods to trigger ascospore discharge have been reported by several authors (González, 1999; Johanson, 1997; Molina-Tirado and Castaño-Zapata, 2003; Stover, 1969, 1976). The only method to isolate conidia from *M. fijiensis* directly from the infected leaves, has been reported by Fullerton and Tracey (1984). However, this method is laborious and time consuming, requiring the excision of the epidermis of the infected leaves. Recently, Aguirre *et al*. (2003) picked conidia from leaves, using an agar-filled syringe containing a dye, to identify *Mycosphaerella* species. Therefore, an experiment was conducted using either surface-sterilized or non-sterile plant materials, and a collection of fungal isolates was successfully obtained from both methods. Infected banana and plantain leaves were collected from fungicide untreated fields, in Yucatan and Tabasco, Mexico. Portions of the leaves with symptoms from Fouré stages 2-5 (Fouré, 1982) were selected and cut in 4 × 4 cm squares. For the first treatment, leaves were surface sterilized with 20% commercial sodium hypochlorite bleach plus Tween 20 (500 µL/L) for 10 min, dried with sterile paper towels, placed on top of sterile filter paper soaked with sterile distilled water, and sealed inside a Petri dish with wet filter paper. Plates were incubated at 20°C for up to 5 days as this temperature has been reported to induce conidiation (Jacome and Schuh, 1993). Every 24 h conidia were isolated from the leaves as described below. The second method consisted of using pieces of the leaves directly from the field, followed by isolation of conidia as follows: The abaxial surface of the infected leaves was pressed against a water-agar (3%) plate, and observed under a stereoscopic microscope. Using the needle of a subdermal syringe, single conidia were picked, and incubated on potato-dextrose-agar (PDA) plates containing 200 mg/mL Amoxicillin. *M. fijiensis* single-spore isolates were grown at 26°C with continuous white light. After 2-3 weeks, *M. fijiensis* colonies

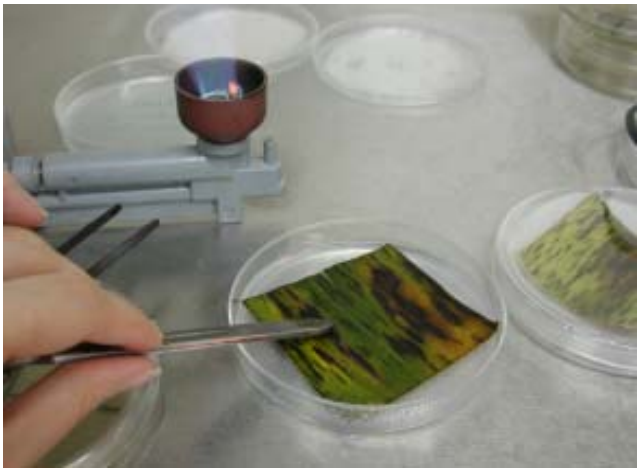

Fig. 1. Pressing a piece of infected leaf on agar plates for isolation of conidia of *Mycosphaerella fijiensis*. The infected leaves were obtained from susceptible cultivars Grand Naine and False Horn Plantain.

were identified by their morphology and growth rate, and re-plated on PDA (Fig. 1). With the first method, conidia were not observed until the 3rd and 4th days of incubation. On these days, single conidia were found distributed over the plate (Fig. 2). Few spores from other fungi were observed. On the 5th day, most of the leaves were already senescent, and in general, conidia that were observed had germinated. With respect to the second method, using non-sterilized plant material, conidia were found localized in packed groups, presumably each corresponding to a sporodochium, and numerous spores from other fungi were observed, but were avoided when *M. fijiensis* conidia were transferred to PDA. After subculture, a total of 134 isolates were obtained with the first treatment and 131 by the second one; thus, demonstrating the efficiency of both methods. A collection

Fig. 2. *Mycosphaerella fijiensis* isolates obtained from infected banana (*Musa* sp.) leaves showing typical growth on potato-dextrose-agar plates.

of 265 isolates was generated, and is being used for population studies. In these experiments, *M. fijiensis* produced conidia within 3 days after the disinfection process, but not on senescent leaves. The use of non-sterile material from the field was simple and rapid, although some disadvantages such as the presence of different spores on the surface of the leaf should be considered. We would recommend using the infected material directly from the field the same day or the day following the sampling, and surface sterilization of leaves if many samples are required and it is not possible to process all the material at once. The main advantages of this method are i) conidia are more readily available than ascospores, ii) the method is much simpler than other methods reported, iii) the infected material can be processed on the same day of sampling or incubated for up to three more days, iv) no special equipment or potent microscopes are needed, and v) it may be used for preliminary diagnosis of other fungi.

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LITERATURE CITED

Aguirre, M.C., Castaño-Zapata, J. y Zuluaga, L.E. 2003. Método rápido de diagnóstico de *Mycosphaerella musicola* Leach y *M. fijiensis* Morelet, agentes causantes de las sigatokas amarilla y negra. Revista de la Academia Colombiana de Ciencias XXVII:619-623.

Carlier, J., Lebrun, M.H., Zapater, M., Dubois, C., and Mourichon, X. 1996. Genetic structure of the global population of banana black leaf streak fungus *Mycosphaerella fijiensis*. Molecular Ecology 5:499-510.

Carlier, J., Mourichon, X., González de León, D., Zapater, M., and Lebrun, M.H. 1994. DNA restriction fragment length polymorphisms in *Mycosphaerella* species that cause banana leaf spot diseases. Phytopathology 84:751-756.

Fouré, E. 1982. Les cercosporioses du bananier et leurs traitements. Comportement des variétés.1: Incubation et évolution de la maladie. Fruits 37:749-766.

Fullerton, R.A., and Tracey, G.M. 1984. Tolerance of *Myosphaerella fijiensis* to benomyl and carbendazim in the Pacific Islands. Tropical Agriculture (Trinidad) 61:134-136.

González, M. 1999. Metodología para la manipulación y cultivo *in vitro* de *Mycosphaerella fijiensis*. Hoja Técnica No. 30. Manejo Integrado de Plagas (Costa Rica) 53:i-iv.

Jacome, L.H., and Schuh, W. 1993. Effect of temperature on growth and conidial production *in vitro*, and comparison of infection and aggressiveness *in vivo* among isolates of *Mycosphaerella fijiensis* var *difformis*. Tropical Agriculture (Trinidad) 70:51-59.

Johanson, A. 1997. Detection of Sigatoka Leaf Spot Pathogens of Banana by the Polymerase Chain Reaction. Natural Resources Institute. Chatham, UK. 38 p.

Molina-Tirado, O.V. y Castaño-Zapata, J. 2003. Análisis de algunos componentes de resistencia en los híbridos de

banano y plátano FHIA 01, FHIA 17 y FHIA 21 a las Sigatokas negra (*Mycosphaerella fijiensis* Morelet) y amarilla (*M. musicola* Leach). Revista de la Academia Colombiana de Ciencias 27:181-189.

Stover, R.H. 1969. The *Mycosphaerella* sp. associated with banana leaf spots. Tropical Agriculture (Trinidad) 46:325-332.

Stover, R.H. 1976. Distribution and cultural characteristics of the pathogens causing banana leaf spot. Tropical Agriculture (Trinidad) 53:111-114.