Hansfordia sp.: A Parasitic Pathogen of Dematiaceous Plant Pathogenic Fungi in Puerto Rico[1]

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ABSTRACT

***Hansfordia* sp. was found parasitizing papaya black rust and tomato leaf mold in the field in Puerto Rico and on *Cladosporium cladosporioides* in the laboratory. The fungus was first detected on the margins of lesions spreading towards the lesions' centers. Black rust and leaf mold colonies became completely covered and decolorized by the hyperparasite. Growth of *Hansfordia* sp. was greatest on black rust and least on *C. cladosporioides*. The fungus did not grow on corn meal agar but grew and sporulated on malt yeast and potato dextrose agars. Although *Hansfordia* did not grow on corn meal agar alone, it did grow sparingly on *C. cladosporioides* growing on corn meal agar. *Hansfordia* sp. reduced radial growth of *C. cladosporioides* 25, 10, and 0% on potato dextrose, corn meal and malt yeast agars, respectively. On potato dextrose agar, parasitism of *Hansfordia* sp. on *C. cladosporioides* reduced production of viable conidia by over 90%. Besides having potential importance in the biological control of leaf blights by dematiaceous fungi, this *Hansfordia* sp. may be an important tool in studying fungal melanin degradation.**

INTRODUCTION

Potentially parasitic relationships where both the host and parasite are fungi (mycoparasitism) can be exploited for controlling certain fungal diseases of plants (2). Successful use of fungal hyperparasites would be an effective means of reducing reliance on fungicides for plant disease control. Fungicides, like other pesticides, can cause damage to populations of nontarget and beneficial organisms (13). Routine longterm repeated use of fungicides and other pesticides could lead to accumulation of residues in soil, water, and food. Multiple applications of pesticides is commonplace in intensive fruit and vegetable production throughout the world.

In fungi, hyperparasitism is common and obligate parasitic plant pathogenic fungi are often targets of fungal hyperparasites (4). Examples of common hyperparasitic relationships include *Darluca* Cast. species on rust, *Ampelomyces* (Ehrenb.) Cess species on powdery mildews, and *Spiropes* Ciferri species on black mildews (5). Although all of these fungi are common in the tropics, few workers have reported on their biology and potential use under tropical conditions.

Hyperparasitic fungi of facultative parasitic plant pathogenic fungi are

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less studied than fungal hyperparasites of obligate plant parasitic fungi. In subtropical and tropical areas, *Hansfordia pulvinata* (Berk. & Curt.) Hughes has been reported overgrowing *Cercospora* Fres. leafspots (8). Barnett and Hunter (5), authorities on fungal hyperparasitism, described *Hansfordia* species as leaf saprophytes. Taber and Petit (14), however, recently suggested a species of *Hansfordia* for potential biological control of *Cercosporidium* Earle leafspot on peanuts in Texas.

The genus *Hansfordia* was erected by Hughes in 1951 and is believed to have 10 valid species (1). Of the four species described by Ellis (8, 9) only *H. pulvinata* appears naturally growing over other fungi. There are few studies on the biology of *Hansfordia* and no modern monographic study of the genus.

Hansfordia was not mentioned in Baker and Cook's (3) review of biological control of plant pathogens nor in Barnett and Binder's (4) review on host-parasite relationships between fungi. Considering their potential importance as fungal hyperparasites and the sketchy information on them, species of Hansfordia deserve more careful study.

MATERIALS AND METHODS

Studies were conducted at the Finca Laboratory and the Isabela Research and Development Center of the College of Agricultural Sciences of the University of Puerto Rico in Mayagüez. Hyperparasite life cycle and host-hyperparasite interactions were observed on field specimens from Isabela and in vitro after artificial inoculations. The hyperparasite was collected on papaya (*Carica papaya* L.) and tomato (*Lycopersicon esculentum* Mill.) overgrowing block rust (*Asperisporium caricae* (Speg.) Maubl.) and leaf mold (*Fulvia fulva* (Cooke) Ciferri), respectively. The hyperparasite was found on *Cladosporium cladosporioides* (Fresen.) de Vries, under laboratory conditions. Fungus-mycoparasite relations were observed using tape impressions and whole mounts under bright field microscopy. Diverse stages of overgrowth were sampled for each fungus-hyperparasite combination.

Hyperparasite morphology and measurements were observed for laboratory pure cultures and for parasite combinations. Pure cultures were isolated using potato dextrose agar (PDA, Difco, Detroit, MI) by affixing infected material on the top of sterile plastic (9 cm) petri plates with tape or vaseline and allowing the settlement of hyperparasite conidia on the culture media below. Growth and morphology of the fungus was observed and measured on corn meal (CMA, Difco), malt (MA, Difco), PDA, and the same agars with 0.5% yeast amendment (Y, Difco).

The effect of the hyperparasite on secondary inoculum was studied using mixed cultures with *C. cladosporioides* incubated 10 days at 25° C. Lower culture plates with PDA were inoculated by suspending mixed and pure plates of the test fungi and gently tapping the "seed" plates. Colonies of *C. cladosporioides* and the hyperparasite were identified and counted after 3 days at 25° C.

RESULTS

The hyperparasite on papaya black rust was first noted as a faint white growth on the fringes of the black rust pustule (fig. 1A and 1B). Growth and sporulation of both black rust and the hyperparasite were only noted on the lower leaf surface. The upper surface of the black rust lesion appeared as a light colored necrosis surrounded by a definite reddish brown margin (fig. 1C).

Figure 2 shows the comparative size and morphology of *F. fulva* and the hyperparasite. Hyperparasite appearance and development appeared similar on both *Fulvia fulva* and *C. cladosporioides*. Hyperparasite mycelium was thread-like (0.5–3.0 μ wide, $\bar{x}=1.8$ μ) and subhyaline. It was easily distinguished from the broad (3 to 6 μ) dark mycelium of its hosts. Conidia of the hyperparasite were smooth, globose and unicellular with 3.5 to 5.3 μ diameter ($\bar{x} = 4.5$ μ). They possessed thick walls (0.25–0.50 μ) and a hilum-like protusion where the conidiophore was attached. Conidia arose from denticulate projections of the conidiophore. Growth and sporulation of the hyperparasite were greatest on papaya black rust, intermediate on tomato leaf mold, and least on *C. cladosporioides*. Isolates from various fungal hosts were alike in pure culture.

Pure cultures of the hyperparasite formed a limited white colony which changed to grey at 5 to 6 days at 25° C. Colonies were 2 cm in diameter with a felt-like texture and appearance.

The hyperparasite showed no growth on CMA and limited growth on CMYA. Growth was good on PDA, PDYA, and MYA. Optimal sporulation was found on PYA and MYA. Although the hyperparasite did not grow on CMA alone, limited growth was found on *C. cladosporioides* on corn meal agar. On all media tested *C. cladosporioides* grew and sporulated. Its best growth was on MYA and the worst on CMA. The hyperparasite reduced radial growth of *C. cladosporioides* by 25 and 10% on PDA and CMA, respectively, after 8 days at 25° C. The hyperparasite did not affect radial expansion of *C. cladosporioides* on MYA.

Mean numbers of *C. cladosporioides* colonies on PDA after 8 days at 25° C were 23.0 per 9-cm plate when pure seed cultures and 1.3 per 9-cm plate for hyperparasitized cultures were used.

DISCUSSION

During the study the hyperparasite showed notable ability to bleach dematiaceous fungi. Fungal melanin is usually considered extremely resistant to chemical and biological degradation (6, 7, 11, 12). We have

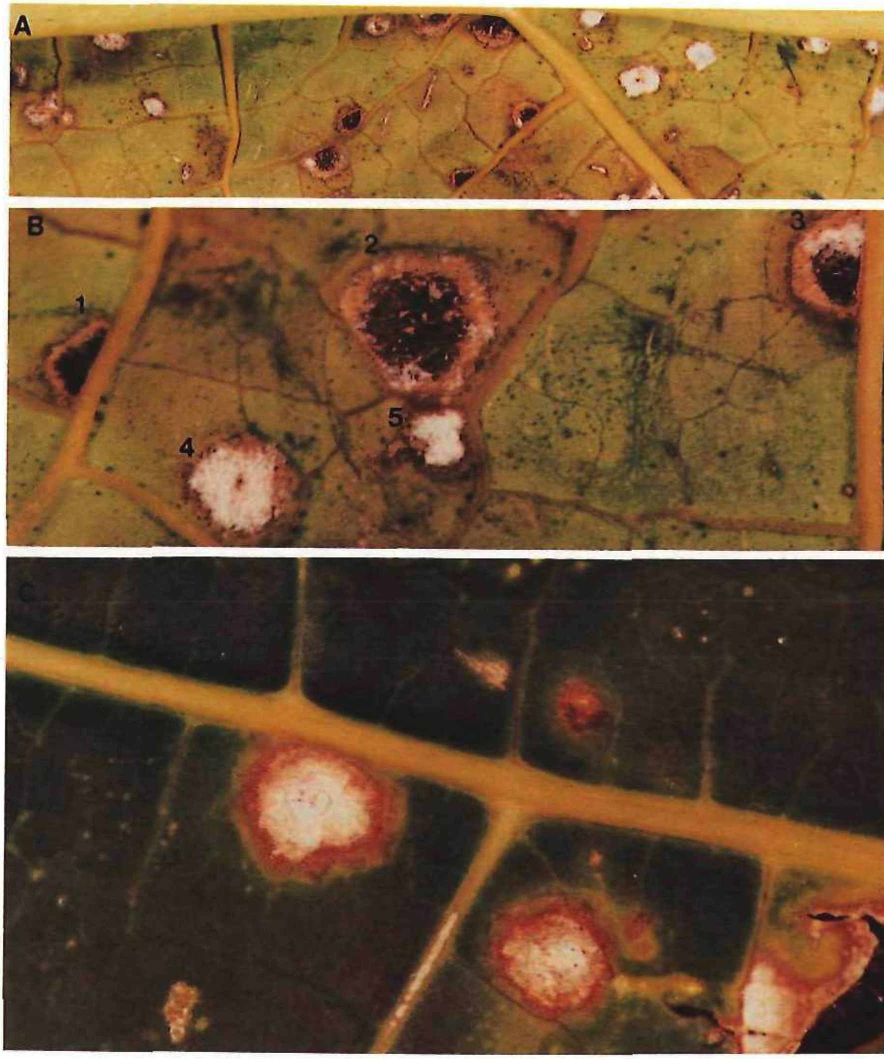

FIG. 1.—A. Lower surface of a papaya leaf showing the black growth of *Aerisporium caricae*, the black rust pathogen of papaya. Note various stages of overgrowth by a white powdery fungus, *Hansfordia* sp. (0.75 ×). B. A close-up view of hyperparasitized black rust. Note the stages of overgrowth classed in a 1 to 5 scale with 1 no hyperparasitic overgrowth visible, and 5 complete overgrowth (3 ×). C. The upper surface of a papaya leaf showing black rust lesions (reddish brown margins with whitish necrotic centers). Note the complete absence of signs of both the black rust pathogen and its hyperparasite, *Hansfordia* sp. (3 ×).

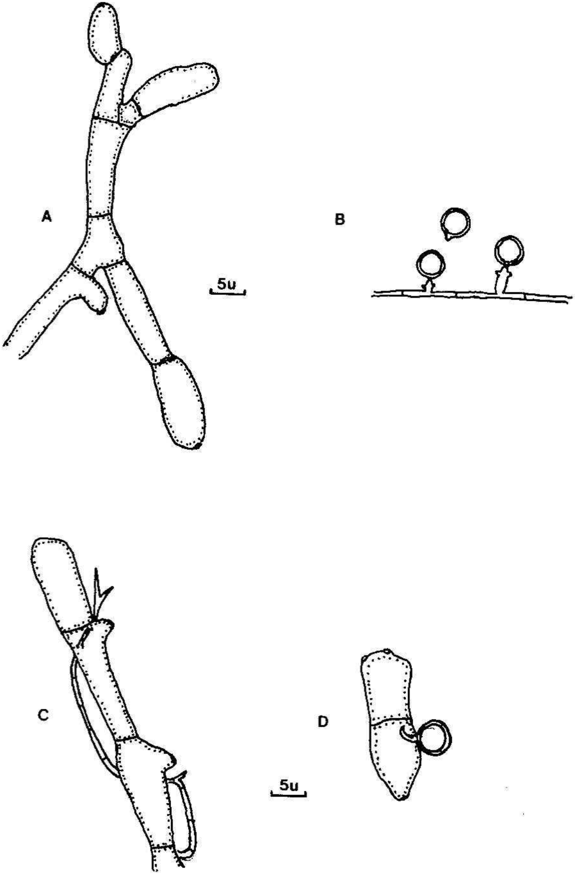

FIG. 2.—A. *Fulvia fulva* mycelium and conidia. B. Comparative size and morphology of the tomato leaf mold hyperparasite, *Hansfordia* sp. C. The association of *F. fulva* with *Hansfordia* sp.; note the perforation of the *F. fulva* hypha. D. The penetration of a *F. fulva* conidium by a germinated conidium of *Hansfordia* sp., note that the *Hansfordia* sp. conidium appeared to germinate from the point of attachment to the conidiophore.

not found this fungus on hyaline fungi or plant substrates which may suggest specialized adaptation for fungal melanin utilization.

Melanin bleaching was not the only indication of adaptation as a fungal parasite. The hyperparasite, unlike leaf saprophytes, showed limited growth on general laboratory media and appeared to need external sources of B-vitamins for normal growth and sporulation. Although Barnett and Hunter (5) consider *Hansfordia* species as leaf saprophytes, the fungus under study did not fit this concept.

Hyperparasites are potential biological controls of economically important plant diseases. Success of biological controls depends on favorable environments for hyperparasite development (3). Variable field conditions limit the use of hyperparasites. Under greenhouse conditions, environments can be modified to favor biological control by hyperparasites.

The brightest prospect for practical use of the hyperparasite studied is as a biological control of tomato leaf mold under greenhouse culture. Tomato leaf mold is a major disease of greenhouse tomatoes (10). Breeding for tomatoes resistant to leaf mold has not been successful based on the numerous pathological races of the pathogen. Resistance of leaf mold to hyperparasites is not known and further studies in biological control of leaf mold appear attractive.

Besides possible roles in studies of fungal melanin degradation and biological control, the species of hyperparasitic *Hansfordia* can be used for demonstrating host-parasite relationships. It displays parasitism and host reactions with less space and requiring less time than traditional plant host-fungal parasite systems. Life science laboratories can, therefore, demonstrate these concepts more economically.

RESUMEN

El hongo *Hansfordia* sp. se ha encontrado parasitando al hongo *Cladosporium cladosporioides* en el laboratorio, a la roya negra de la papaya y al moho de la hoja del tomate en Puerto Rico. El hongo fue detectado primeramente en los márgenes de las lesiones, extendiéndose con el tiempo hacia el centro de las lesiones. Las colonias de la roya y del moho de la hoja estaban completamente cubiertas y descoloridas por el hiperparásito. El mayor crecimiento de *Hansfordia* sp. fue sobre la roya negra y el menor sobre *C. cladosporioides*. El hongo no creció sobre el agar de harina de maíz, pero sí creció y esporuló en agar de malta y levadura y en agar de papa y dextrosa. A pesar de que *Hansfordia* no creció en agar de harina de maíz solo, creció, aunque escasamente, sobre *C. cladosporioides* cultivado en agar de harina de maíz. *Hansfordia* sp. disminuyó el crecimiento radial de *C. cladosporioides* un 25, 10 y 0% sobre agar de papa y dextrosa, agar de harina de maíz y agar de malta y levadura, respectiva-

mente. El parasitismo de *Hansfordia* sp. sobre *C. cladosporioides* disminuyó la producción de conidios viables en más de un 90% en agar de papa y dextrosa. Además de su gran potencial para el control biológico de tizones de las hojas causados por hongos oscuros, esta especie de *Hansfordia* podría usarse como modelo para estudiar la degradación de la melanina en hongos.

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