

SOOTY MOULD-PLANTHOPPER ASSOCIATION ON LEAVES OF THE BLACK MANGROVE *AVICENNIA GERMINANS* (L.) STEARN IN SOUTHWESTERN PUERTO RICO

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Summary

Recent attention has been given to terrestrial and marine manglicolous fungi because of mangrove tree mortalities. However, this mycobiota in many Caribbean Islands is practically unknown. The halotolerant fungus *Asteridiella sepulta* (Pat.) Hansf. (Ascomycota, Meliolaceae), which is one of many species that form sooty mould, has been isolated from leaves surfaces of *Avicennia germinans* (L.) Stearn in southwestern Puerto Rico. In this study of *A. germinans*, we found the planthopper *Petrusa marginata* (Brunnich) (Homoptera, Flatidae) excretes a sugary honeydew upon which the dematiaceous mycelium of *A. sepulta* grows. Although *A. sepulta* has been previously collected on *A. germinans*, the association of the fungus and the planthopper in black mangrove was not previously reported. *Asteridiella sepulta* produces a flat colony with a spongy subiculum on surfaces of leaves, twigs, and small branches of *A. germinans*.

Keywords: sooty mould • *Asteridiella sepulta* • manglicolous fungi • phytopathology • *Petrusa marginata* • planthopper • Puerto Rico • West Indies

Introduction

A mangrove forest is a dynamic ecotone, or a transition zone, between the terrestrial and marine habitats. In its simplest sense, a mangrove is used as a generic term referring to a group of woody, halophylic plant formations that

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grow along sheltered tropical and subtropical coastlines (TOMLISON, 1986). The mangroves are derived from a variety of plant taxa and they vary in their dependence upon littoral habitats (LUGO and SNEDAKER, 1974).

The black mangrove, *Avicennia germinans* (L.) Stearn (Avicenniaceae), is a small tree or shrub (about three to 12-m high) that grows in lagoons and coastal swamps in paleotropics and neotropics. It has been recorded in continental tropical America, Bermuda, The Bahamas, United States of America (from Florida to Texas), throughout the West Indies (except Dominica), including Trinidad, Tobago, and Curaçao (LITTLE and WADSWORTH, 1964; MARTORELL, 1976). In Puerto Rico, *A. germinans* has been reported from the main island, Vieques, and Culebra (LITTLE and WADSWORTH, 1964; MARTORELL, 1976). *Avicennia germinans* is very widely distributed along tropical and subtropical protected silty seashores and forming mangals in brackish water at mouths of rivers, usually with other mangrove species, but rarely forming monotypic stands (LITTLE and WADSWORTH, 1964; JIMÉNEZ and LUGO, 1985a; TOMLISON, 1986). Except for *A. germinans*, the other mangrove species that occur in Puerto Rico are the red mangrove (*Rhizophora mangle* L.) (Rhizophoraceae), the white mangrove [*Laguncularia racemosa* (L.) Gaerth. f.], and the buttonwood (*Conocarpus erectus* L.) (Combretaceae) (LITTLE and WADSWORTH, 1964).

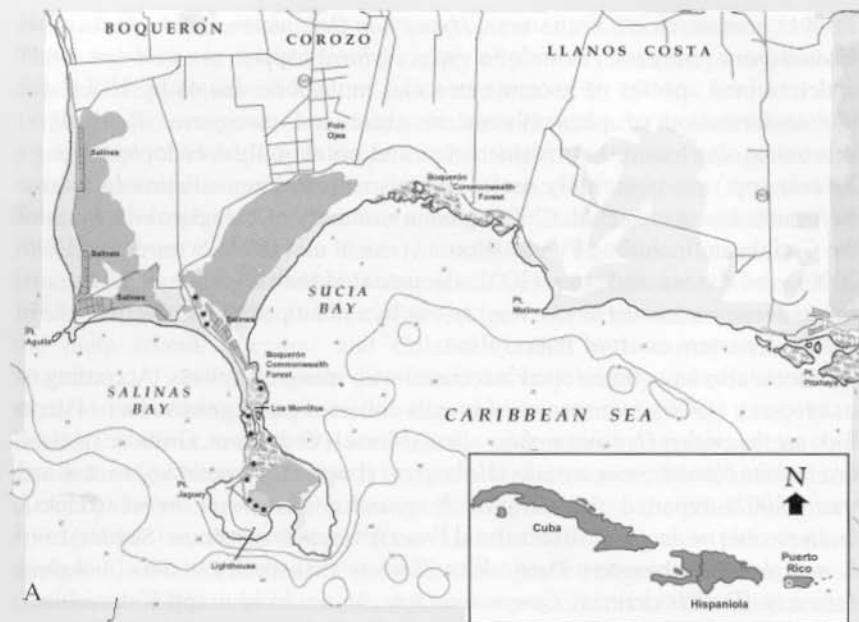
Recent attention has been given to mangrove tree mortalities, which are caused by anthropogenic misuse and unfavorable environmental conditions (ANDERSON and LEE, 1995) as well as by fungal diseases (JIMÉNEZ and LUGO, 1985a, 1985b; WIER et al., 2000), among other biotic and abiotic factors. Although terrestrial and marine manglicolous fungi have been extensively studied in various parts of the world (JOHNSON and SPARROW, 1961; KOHLMAYER and KOHLMAYER, 1979; ROLLET, 1981; HYDE and LEE, 1995), in many Caribbean Islands these fungi are poorly known.

Previous mangrove fungal collections in Puerto Rico are summarized in STEVENSON (1975), ACEVEDO (1987), NIEVES-RIVERA et al. (1998), CALZADA (1999), TATTAR et al. (1994), WIER et al. (1996, 2000), and ACEVEDO (2001), MINTER et al. (2001), TATTAR and WIER (2002). STEVENSON (1975) gave a summary of manglicolous fungi of Puerto Rico and the U.S. Virgin Islands. ACEVEDO (1987) reported 18 species of manglicolous fungi (ascomycetes and mitosporic fungi) from *R. mangle* in La Parguera. NIEVES-RIVERA et al. (1998) reported *Schizophyllum commune* Fr.: Fr. and *Hypoxylon* Sect. *Hypoxylon* in *Avicennia nitida* Jacq. (*A. germinans*) and *Rhizophora mangle*, respectively, from Boquerón Wildlife Refuge. CALZADA (1999) studied three phytopathogenic fungi [*Pestalotiopsis disseminata* (Thuem.) Stey., *Phoma eupyrena* Sacc., *Pterosporidium rhizophorae* (Vizioli) W.H. Ho et K.D. Hyde] causing foliar diseases in *R. mangle* of La Parguera (Phosphorescent Bay and La Parguera Channels). ACEVEDO (2001) assayed marine fungi (e.g., *Didymosphaeria Rhizophorae* J. KOHLMAYER et E. KOHLMAYER, *Hydronectria*

Tethys J. KOHLMAYER *et* E. KOHLMAYER, *Hypoxylon Oceanicum*, Schatz, *Lulworthia Grandispora* Meyers, *Pestalotia* sp., *Xylaria* spp., as well as other undetermined species of ascomycetes and mitosporic fungi) by HPLC for biotransformation of phenanthrene in algae and mangrove (*R. mangle*) substrates; she found that marine fungi and notably algal endophytes (e.g., *Xylaria* spp.) are potentially useful organisms for bioremediation in marine environments. MINTER *et* al. (2001) gave a summary of manglicolous fungi of the Caribbean, including Puerto Rico. TATTAR *et* al. (1994), WIER *et* al. (1996, 2000), and TATTAR and WIER (2002) documented the incidence of *Cytospora rhizophorae* J. KOHLMAYER *et* E. KOHLMAYER as a plant pathogen in *R. mangle* in the southwestern coast of Puerto Rico.

Insects also have been found associated with mangrove plants. According to MARTTORELL (1976), two species of insects collected on *A. germinans* in Puerto Rico are the cricket *Hygronemobius alleni* (Morse) (Orthoptera, Grillidae) and the tree termite *Nasutitermes costalis* (Holmgren) (Isoptera, Termitidae). TATTAR and WIER (2002) reported the termites *N. costalis*, *Neotermes mona* (Banks), *Incisitermes nr. incisus* (Silvestri) and *Procryptotermes corniceps* (Snyder) from *R. mangle* in southwestern Puerto Rico. Twenty-two species of ants [including *Azteca* sp. (Dolichoderinae), *Camponotus* spp., *Myrmelachista* spp. (Formicidae), *Crematogaster* spp., *Hylomyrma* sp., *Pheidole* spp., *Solenopsis* spp. (Myrmicinae), *Pachycondyla villosa* (Fabricius) (Ponerinae), *Pseudomyrmex gracilis* (Fabricius), *Pseudomyrmex* sp. (Pseudomyrmecinae)] have been recorded from the Brazilian mangrove plants *Avicennia schaueriana* Stapf *et* Leechman: Moldenke, *L. racemosa*, and *R. mangle* (CORTES-LOPES and DOS SANTOS, 1996). However, the recent reports of the gall midges *Actilasioptera* spp. (GAGNÉ and LAW, 1998) and *Meunieriella avicenniae* (Cook) (Diptera, Cecidomyiidae) (GAGNÉ and ETIENNE, 1998), the causative agents of the leaf gall in black mangroves, show that mangrove insect fauna is rather poorly known. Therefore, there has been no assessment on the effects insects have on these plants.

In February 5, 2001, while collecting manglicolous fungi, the senior author observed a coating on the leaves of the black mangrove (*A. germinans*), with a black soot caused by mycelia giving the false impression of pollution caused by passing vehicles. After a careful examination under the dissecting microscope, the soot was found to be a mycelium produced by a fungus. This fungus was *Asteridiella sepulta* (Pat.) Hansf. (Ascomycota, Meliolaceae). The type collection for *A. sepulta* is contained in voucher 6416, collected from *A. nitida* (*A. germinans*) from Cataño, Puerto Rico, by A.A. Heller (STEVENSON, 1975). The purpose of the present study is to record details of the sooty mould-plant hopper association on leaves of the black mangrove in southwestern Puerto Rico.



B

FIGURE 1A. Map of south-western Puerto Rico, showing sites mentioned in text.

B. View of Los Morrillos, Boquerón Commonwealth Forest, Cabo Rojo, Puerto Rico.

Materials and Methods

Locality

Leaves of *A. germinans* were collected at Los Morrillos (LM (Coordinates: 17°57.215'N, 67°11.867'W)), which is part of the Boquerón Commonwealth Forest, Road 301, km 11.4, Barrio El Corozo, Cabo Rojo, Puerto Rico (Figures 1A, B). This forest is approximately 0 to 30 m above sea level and the general environment of the region is classified as a subtropical dry forest (EWEL and WHITMORE, 1973). The rainy season in Puerto Rico ranges from May

to June and August to September, with two rainfall peaks. Annual average rainfalls in the weather station located at Lajas Agricultural Experiment Station of the University of Puerto Rico-Mayagüez, are 1016 to 1270 mm (RAVALO *et al.*, 1986).

According to 'CNN.com/weather', the annual average climatic conditions for Boquerón, Cabo Rojo, Puerto Rico from 2000 to 2001) were: air temperature, 28.0 °C; relative humidity, 74.0 per cent; wind, variable (mostly from ENE or SSE at < 5 to 30 km/h; sunrise, 06:05 h, sunset, 18:49 h. In 'Atmos Carib-Caribbean Atmospheric Research Center' at University of Puerto Rico-Mayagüez Campus, the annual average climatic conditions for Magueyes Island Marine Laboratories (MIML) in La Parguera, Lajas, Puerto Rico from 2000 to 2001 were: air temperature, 26.6 °C; atmospheric pressure, 1013.9 mb. GLYNN (1973) conducted early meteorological observations in MIML. Therefore MIML was selected because it has the same xeric conditions and its proximity to the study site. The geologic formations of the study site are: Holocene's mangrove swamps (Qm) and beach deposits (Qb) (VOLCKMANN, 1984; TORRES-FIGUEROA, 1993). Soils of LM are classified as tidal flats (Tf), tidal swamps (Ts), coastal beaches (Co), and limestone rock lands (Lr) (USDA, 1993).

Isolation of Sooty Mould

The senior author collected individual leaves of *A. germinans* August 3, 2001. Most of the leaves of *A. germinans* were collected close to roads and trails. These were photographed and the identity of the sooty mould was confirmed with STEVENS (1916, 1917, 1927), CIFERRI (1954), and HUGHES (1976). Leaves were placed in wet chambers in Petri-dishes, following CALZADA (1999). Distilled water was used in the wet chambers. The paraffin-sealed wet chambers were placed in light/dark at room temperature. Microscopic observations were made with a light microscope (Nikon Labophoto-2 Microscope). Drawings were made with a camera lucida. All voucher specimens are placed at the Center for Forest Mycology Research, in the process of curation before being deposited in the Herbarium of the Department of Natural Sciences, University of Puerto Rico at Río Piedras (UPRRP).

Insect Collection

The insect specimens collected in *Avicennia germinans* were preserved in 70 per cent ethanol and identified by various specialists (Drs. Arístides Armstrong, Ángel L. González, and Silverio Medina-Gaud of the Department of Crop Protection, University of Puerto Rico, Mayagüez). Planthopper identification was provided by Dr. Stuart H. McKamey (Smithsonian Institution in Washington, D.C.).



FIGURE 2. Growth *in situ* of sooty mould (*Asteridiella sepulta*) on an upper surface of leaf of the black mangrove *Avicennia germinans*.

Results

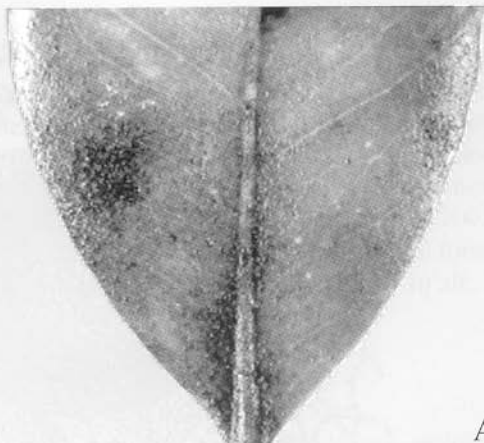
Asteridiella sepulta was found on the phylloplane (surface of living leaves, *sensu* HUGHES, 1976) (Figure 2) producing a flat, spongy subiculum colony of sooty mould on the front and back of the leaves, twigs, and small branches of *A. germinans* (Figures 3A–B). This sooty mould was found throughout the year on the leaves of *A. germinans*. It must be a halotolerant fungus to be able to grow on black mangrove leaves, which are often covered with salt crystals. *Asteridiella sepulta* also grew in the margins of the leaves and the stems, apparently following the path of running water after a rain, moist, dew, or by condensation (Figure 3C).

Diagnostic

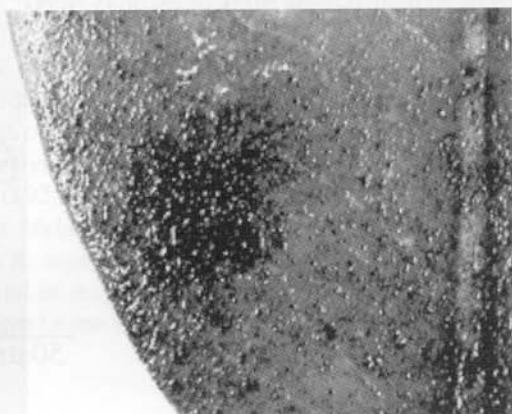
Colonies circular, 1–7 mm in diameter, amphigenous, dense, easily secedent, well defined, sub-epiphyllous spots, single or confluent, black. Hyphae brown, sinuous to tortuous, branching alternate or irregular, not opposite, at acute angles, forming a mat, densely reticulate and becoming almost solid (Figure 4A). Capitate hyphopodia alternate, more or less antrorse, usually

FIGURE 3A-C.

A-B. Growth of *Asteridiella sepulta* on the lower surface of the front A and (magnified) back B of the same leaf of *A. germinans*.

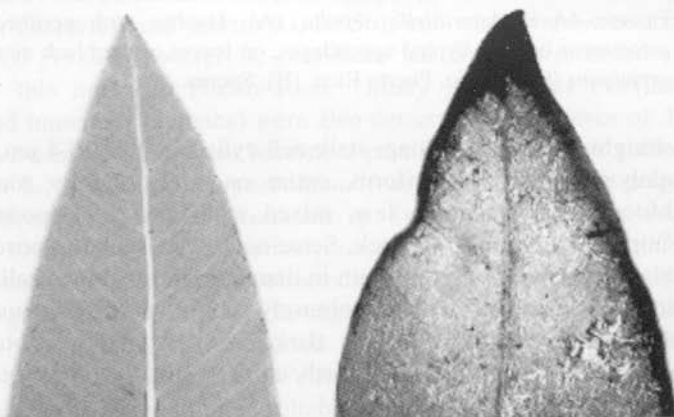


A

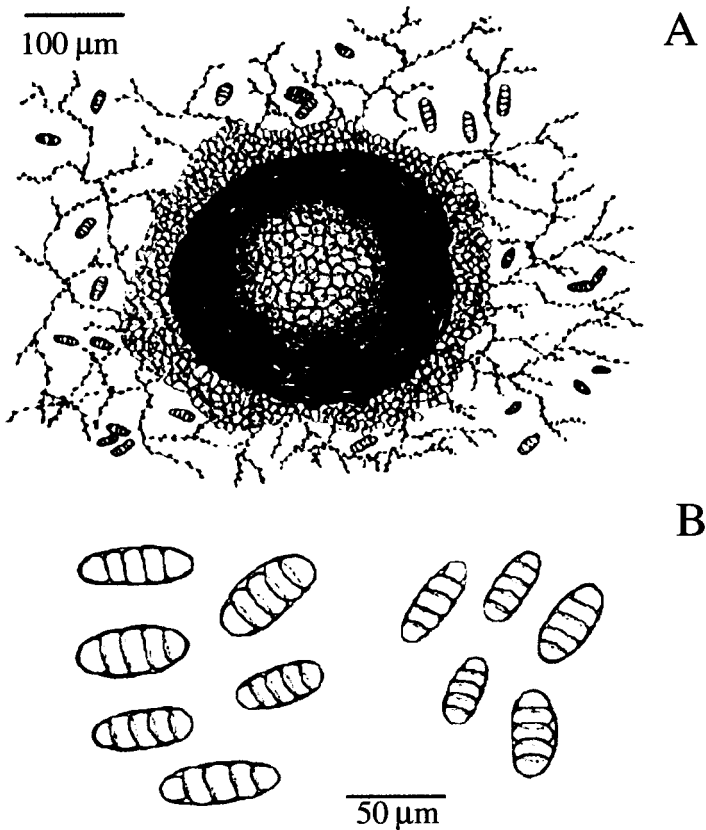


B

C Leaves of black mangrove *Avicennia germinans*, with a clean surface (left) and *Asteridiella sepulta* infested surface (right).



C



FIGURES 4A–B. *Asteridiella sepulta*. (A). Hyphae with perithecium and young ascostroma bearing hyphal appendages, on leaves of the black mangrove *Avicennia germinans* (Cabo Rojo, Puerto Rico). (B). Spores.

straight, 23–24.3 μm long; stalk cell cylindrical, 0.9–10.4 μm long; head cell globose to widely pyriform, entire or rarely slightly rounded-angulose. Mucronate hyphopodia few, mixed with capitate, opposite or alternate, ampulliform with short neck. Setae none. Perithecia in loose central group, black, globose, rough, 165 μm in diameter; most surface cells are prolonged into translucent dark brown, obtusely conoid outgrowths, not striate (Figure 4A). Asci not seen. Spores dark brown, cylindrical, obtuse, 4 septate, constricted, ends obtuse, smooth, thin-walled, 51.1–52.6 × 18.2–19.8 μm (Figure 4B).

Material Studied

Los Morrillos, Boquerón Commonwealth Forest, in coastal forest next to Parador Las Salinas, on living leaves of *A. germinans*, 1.5 m alt., 3 August 2001, Á.M. Nieves-Rivera, PR-935, 936, 937 (UPRRP); Los Morrillos, Boquerón Commonwealth Forest, in the shores of a hypersaline lagoon, next to old bridge, on living leaves of *A. germinans*, 0.5 m alt., 3 August 2001, Á.M. Nieves-Rivera, PR-938 (UPRRP); Guánica Dry Forest, in coastal forest next to Parador Copa Marina, on living leaves of *A. germinans*, 1.5 m alt., 7 August 2001, Á. M. Nieves-Rivera, PR-939, 940 (UPRRP).

Distribution

Sooty mould mycelia develops in the front and back of the leaves, petioles, twigs, and branches (REYNOLDS, 1976). The range of the area covered by sooty moulds extends about 2.0×1.5 km in *A. germinans* of LM. However, the fungus dissemination seems to depend on the planthopper *Petrusa marginata* (Brunnich) (Homoptera, Flatidae) and *A. germinans* distribution along the coastline. Previous collections of *A. sepulta* in Puerto Rico were reported by STEVENS (1916, 1917, 1927) as *Irenina (Meliola) sepulta* Pat., CHARDÓN (1920) as *Meliola sepulta* Pat., TORO (1925) as *Irene sepulta* (Pat.), in the Dominican Republic by CIFERRI (1954) as *Meliola (Irenina) sepulta* (Pat.) Stevens, and Trinidad by DENNIS (1970) as *A. sepulta*. STEVENSON (1975) summarized *A. sepulta* distribution to be found in *A. germinans* forests of Puerto Rico, the Dominican Republic, and Sierra Leone (Africa).

Insects

The planthopper *P. marginata* has been previously reported in Puerto Rico by OSBORN (1935), CALDWELL and MARTORELL (1950), and MALDONADO-CAPRILES and MEDINA-GAUD (1985); however, *A. germinans* has not been recorded as host plant for this insect in Puerto Rico. Many planthopper exuviae, exocuticles, and immature (nymphs) were also detected on the leaves of *A. germinans*. *Petrusa marginata* has become a pest in plantations of coffee *Coffea arabica* L., coco-plum *Chrysobalanus icaco* L., and the sea grape *Coccoloba uvifera* (L.) jasmine *Jasminum* sp. (MALDONADO-CAPRILES and MEDINA-GAUD, 1985) (Figure 5). *Petrusa marginata* [*Oremis (Petrusina) marginata* of OSBORN, 1935] has been collected on *Lantana* sp. and *Cordia* sp. at Ensenada, Aguirre, and other points throughout the island (OSBORN, 1935). CALDWELL and MARTORELL (1950) reported *P. marginata* from Monserrat, B.W.I., to Mona Island, Puerto Rico. *Petrusa marginata* is very common along coastal areas and also present in suitable habitat up to 762 m especially along the south coast of Puerto Rico (CALDWELL and MARTORELL, loc. cit.). During our study, few other insects were collected by the senior author on *A.*

germinans, including the common bee *Apis mellifera* L. (Hymenoptera, Apidae), the green lacewing *Chrysopa* sp. (Neuroptera, Chrysopidae), the leaf gall midge *M. avicenniae*, the ants *Solenopsis* spp. (Hymenoptera, Formicidae), the cricket *H. alleni*, the tree termite *N. costalis*, and three spiders of the Group Aranae.

Discussion

Sooty moulds are usually associated with the liquid excrement of sucking insects, known as 'honeydew' is a common occurrence on many trees (AUCLAIR, 1963). Undigested sucrose in honeydew makes an excellent growth medium for dark-spored fungi (TATTAR, 1989). However, sooty moulds also have been found on plants not infested with insects which produce honeydew, living and dead vegetation, on the surface of rocks, and the forest floor (HUGHES, 1976).

In Puerto Rico sooty moulds caused by *Capnodium* spp. and other undetermined fungal species are found in honeydew excretions of aphids and scale droppings on Tea (*Camellia sinensis* (L.) O. Kuntze), Sour orange (*Citrus aurantium* L.), Orange (*C. sinensis* (L.) Osbeck), Coffee (*C. arabica*), Mango (*Mangifera indica* L.) sea grape (*Coccoloba uvifera*) and white mangrove, or ornamental plants (*Anthurium* sp., *C. icaco*, *Gardenia* sp., *Ixora* sp., *Jasminum* spp.). The mycelial mats of these fungi are easily removed by peeling off the surface from the leaf where they are found, usually revealing a clean, intact plant surface (REYNOLDS, 1976). MALDONADO-CAPRILES and MEDINA-GAUD (1985) refer to sooty mould in Spanish as 'hongo de hollín' *L. racemosa*, or 'fumagina'.

The presence of saprophytic sooty mould *A. sepulta* does not initially infect the leaves of *A. germinans*, but covers the leaf surfaces only after the honeydew of *P. marginata*. Planthoppers, like aphids, feed on the leaves of both deciduous hardwoods and evergreens (TATTAR, 1989). They excrete excess sucrose in a honeydew excrement (AUCLAIR, 1963). We suspect this fungus-leaf covering does not adversely affect photosynthesis because of the healthiness in leaves examined (leaves were green, robust, and intact), and its similarity to cases of myxomycete-grass associations (NIEVES-RIVERA, 2000). However, a heavy accumulation of sooty mould can prevent photosynthesis (TATTAR, 1989).

The covering of the leaves by the fungus looked like 'soot,' giving the false impression of pollution caused by passing vehicles. The 'soot' was found to be fungal mycelia which had expanded over the foliar surface to cover, in some cases, 25 to 98 per cent of the leaf, similar to the percentages reported in pecan leaves by TEDDERS and SMITH (1976), and WOOD et al. (1988). Coating by *A. sepulta* was not detected on other mangrove species, such as *R. mangle* and *C. erectus*. In his study of the genus *Meliola* in Puerto Rico, STEVENS (1917) reported *Meliola lagunculariae* Earle and *M. nigra* Stevens on the white

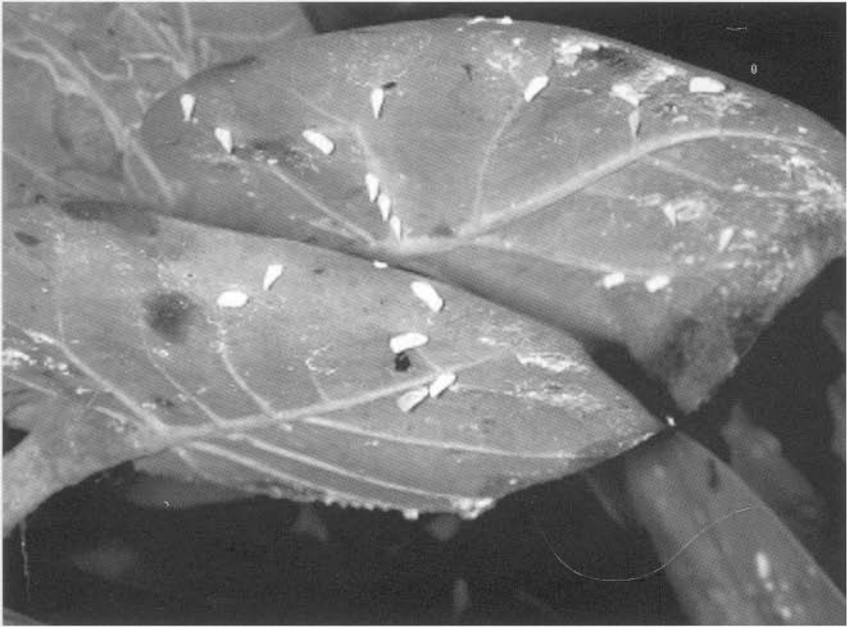


FIGURE 5. Sea grape *Coccoloba uviera*, with an infestation of *Petrusa marginata* on lower surface. Photograph taken at Playa Jobos, Isabela, Puerto Rico.

mangrove *L. racemosa* in Puerto Rico. However, other black mangrove populations in different locations around Puerto Rico have been observed with sooty moulds, for example, the mangals located in Magueyes Island, La Parguera mangrove channels, Bahía de Jobos estuary in Salinas, Las Cabezas De San Juan natural reserve in Fajardo Caño Corazones in Mayagüez, Guayanilla and Ponce coasts (NIEVES-RIVERA, unpubl. data).

Sooty moulds, like myxomycetes (NIEVES-RIVERA, 2000) are saprobes and their fruiting bodies may cover portions of the plant, but apparently do not infect them. The plasmodium (in the case of myxomycetes) does not affect the leaf by reducing its photosynthesis or respiration as true fungi do (for example, powdery mildews (MIGNUCCI and BOYER, 1979)) (NIEVES-RIVERA, 2000). Although sooty moulds are not considered of economic importance, MALDONADO-CAPRILES and MEDINA-GAUD (1985) recommended the use of Diazinon AG-500, Cygon 2.67 or Endosulfan 50 PH to control *P. marginata*, thus controlling the sooty mould. However, it is our concern in eluding the use of commercial pesticides and insecticides to avoid the resultant pollution and turn our attention to the use of entomopathogenic fungi for planthopper and leafhopper biocontrol (SOPER, 1985).

In conclusion, sooty mould-planthopper occurrence on black mangrove

leaves is another example of fungus/insect interaction, that does not appear to be detrimental. However, if a black mangrove forest were to be stressed from changes in climate, attack by borers in high incidence or other negative anthropogenic impact, a heavy incidence of sooty mould could exacerbate the stress and lead to decline. Therefore, the continued study of sooty mould on *A. germinans* is merited.

Acknowledgements

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Resumen

Se le ha dado una reciente atención a los hongos manglícolas terrestres y marinos a causa de las mortalidades de árboles de mangle. Sin embargo, en muchas islas caribeñas esta micobiota es prácticamente desconocida. El hongo halotolerante *Asteridiella sepulta* (Pat.) Hansf. (Ascomycota, Meliolaceae), considerado como una de las especies que forman la fumagina, ha sido aislado de la superficie de las hojas de *Avicennia germinans* (L.) Stearn en el suroeste de Puerto Rico. En este estudio de *A. germinans*, encontramos que el saltahoja *Petrusa marginata* (Brunnich) (Homoptera, Flatidae) excreta una secreción azucarada sobre la cual crece el micelio dematiáceo de *A. sepulta*. Aunque *A. sepulta* ha sido previamente colectado en *A. germinans*, la asociación del hongo con los saltahoja en el mangle negro es poco conocida. *Asteridiella sepulta* produce una colonia plana con un subículo esponjoso sobre la superficie de las hojas, vástagos, y pequeñas ramas de *A. germinans*.

Résumé

L'attention s'est récemment portée sur les champignons marins et terrestres attaquant la manglier du fait du taux de mortalité des arbres dans les mangroves. Toutefois cette mycobiote est pratiquement inconnue dans de nombreuses îles des Caraïbes. Le champignon halotolérant *Asteridiella sepulta* (Pat.) Hansf. (Ascomycota, Meliolaceae), une des nombreuses espèces formant la fumagine, a été isolé de la surface des feuilles d'*Avicennia germinans* (L.) Stearn dans le sud-ouest de Porto Rico. Dans cette étude de *A. germinans*, nous avons trouvé que l'homoptère *Petrusa marginata* (Brunnich) (Homoptera, Flatidae) excrete un miellat riche en sucre sur lequel croît le mycelium dématié de *A. sepulta*. Bien que l'on ait auparavant recueilli *A. sepulta* sur *A. germinans*, l'association de ce champignon et de cet homoptère chez la manglier noir n'avait encore jamais été signalée. *Asteridiella sepulta* produit une colonie plate avec un subiculum spongieux sur la surface des feuilles, les rameaux et petites branches de *A. germinans*.