Colletotrichum gloeosporioides, a Possible Biological Control Agent for *Clidemia hirta* in Hawaiian Forests

EDUARDO E. TRUJILLO, Professor, Department of Plant Pathology, University of Hawaii, 3190 Maile Way, Honolulu 96822, and FRANCES M. LATTERELL, Research Plant Pathologist, and ALBERT E. ROSSI, Biologist, USDA, ARS, Foreign Disease-Weed Science Research Unit, Fort Detrick, Bldg. 1301, Frederick, MD 21701

ABSTRACT

Trujillo, E. E., Latterell, F. M., and Rossi, A. E. 1986. *Colletotrichum gloeosporioides*, a possible biological control agent for *Clidemia hirta* in Hawaiian forests. Plant Disease 70:974-976.

An isolate of *Colletotrichum gloeosporioides* recovered from diseased leaves of *Clidemia hirta* collected in Panama was shown to be a highly aggressive pathogen of *C. hirta* cultivars from Hawaii. Disease symptoms on leaves appeared 4 days after inoculation as small brown spots that developed a week later into large angular spots with yellow halos. Severe premature defoliation and tip dieback followed. This pathogen shows promise as an agent for biological control of this weed in Hawaiian forests.

Additional key words: anthracnose, Melastomataceae, noxious weeds

Clidemia hirta (L.) D. Don, a member of the Melastomataceae, is a major Hawaiian weed of tropical American origin. It was introduced accidentally in 1941 and was spread rapidly by fruit-eating birds and man (12). Today, it is considered the most important noxious weed in rain forest areas of Oahu, where more than 40,000 ha are infested. In the last 10 yr, this weed has spread to forests of Hawaii, Maui, Molokai, and Kauai, with the greatest infestation occurring in the Puna and Waiakea forests of the island of Hawaii (Victor Tanimoto, Hawaii Department of Land and Natural Resources, *personal communication*).

The first author made two trips to Central America during October and November 1984 and September 1985 to study and collect pathogens of Hawaiian weeds of tropical American origin. Two *C. hirta* sites were identified in 1984: one in Costa Rica, 2 km north of Quezada City, and the other in Panama at Gariche River. In 1985, the Costa Rican *Clidemia* plants were disease-free, whereas at the Panama site, the leaves were covered with anthracnoselike lesions. The lesions were dark brown, irregular, angular, and large (nearly 1 cm in diameter). No defoliation was noted. Acervuli with black setae, characteristic of *Colletotrichum* spp., were seen on the undersides of the lesions. A number of spotted *Clidemia* leaves were collected at this site, pressed in newspaper, air-dried for 3 days, and brought under a quarantine permit to the USDA Foreign Disease-Weed Science Research Unit at Fort Detrick, Frederick, MD, for pathogen isolation and pathogenicity tests. Microscopic examination confirmed that the pathogen was a species of *Colletotrichum*.

MATERIALS AND METHODS

Isolation and culture of pathogen. Standard isolation procedures were used to recover the pathogen from diseased leaves. Spores were also removed with glass microneedles from sporulating acervuli on the undersurfaces of diseased leaves and streaked onto 2% water agar (WA) plates for germination and hyphal growth. Bacteria-free hyphal tips from single spores were transferred from WA to 10% V-8 juice agar (10% V-8) (10) 3 days later. Cultures were grown also on oatmeal agar (OA) (10), and autoclaved corn-leaf-piece substrate (CLP) (4). Cultures were incubated at 26 C under continuous illumination (20W daylight fluorescent).

Pathogenicity and host range studies. Ten-month-old plants of *C. hirta* from Oahu, grown at Frederick from branch cuttings shipped from Honolulu, were used in the initial tests to determine pathogenicity of *Colletotrichum* isolates from the Panama specimens. In two tests, 20 plants were sprayed with spore suspensions (10^5/ml) made from 10-day-old single-spore isolates grown on 10% V-8. Inoculated plants were incubated in a dew chamber (5) at 28 C for 24 hr, then transferred to a plant growth room and incubated at 28 C and 75% relative humidity for 7 days. In a host range study, susceptibility of *C. hirta* was compared with that of other members of the Melastomataceae: *Melastoma malabathricum* L., *Dissotis rotundifolia* (J. E. Smith) Triana, *Arthrostemma latifolium* D. Don, *Miconia calvescens* DC., *Topobea maurofernandeziana* Cogn., *Tibuchina semidecandra* (Schlecht. & Mart.) Ogn., *Medinilla heterophylla* Gray, *M. magnifica* Lindl., and three other unidentified species of *Medinilla*, all of which have been introduced as ornamentals to Hawaii over a number of years. Cuttings of these species collected in Hawaii were hand-carried to Frederick, recut for insertion in fresh water, and placed for inoculation and incubation in 50-cm-diameter spherical clear plastic terraria (model TR-345, Lawnware Products, Inc., Morton Grove, IL). Relative humidity inside the terraria was kept at 100% for the first 24 hr of incubation and at 95% thereafter. The floor of the terrarium was constructed according to manufacturer's instructions: a layer of gravel at the bottom, a layer of charcoal, and soil mix on top. Sufficient water was added to wet the floor, and the walls were atomized with distilled water when the plants were placed inside. Relative humidity was maintained at 100% by keeping the terrarium vent closed. In the ambient laboratory temperature of 24–26 C, temperature within the terraria did not exceed 28 C with the vent closed (monitored by probes of a YSI Telethermometer). A 3-cm vent opening to allow air exchange lowered the relative humidity to 95% in an ambient relative humidity of 50%. A battery-operated Psychro-Dyne psychrometer (Environmental Tectonics Corporation, available from Cole-Parmer, Chicago, IL) was used to measure relative humidity.

Similar inoculations were made in Hawaii on rooted cuttings of these same cultivars after permission was granted on 3 January 1986 by the Hawaii Agricultural Quarantine Section to conduct a limited host range study.

In each terrarium, spore suspensions (10^5/ml) were applied with an atomizer to the leaf surfaces of four plants or cuttings of each species including *C. hirta*.

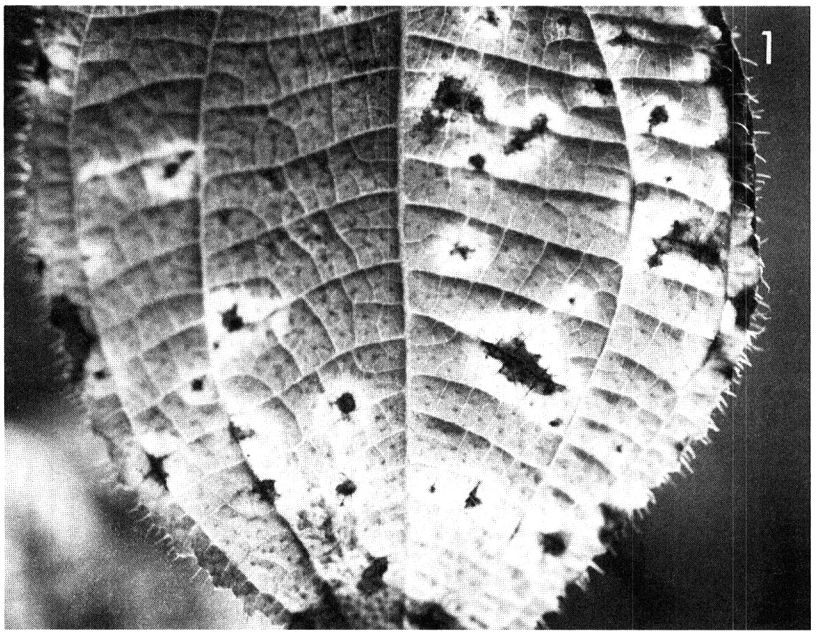
Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

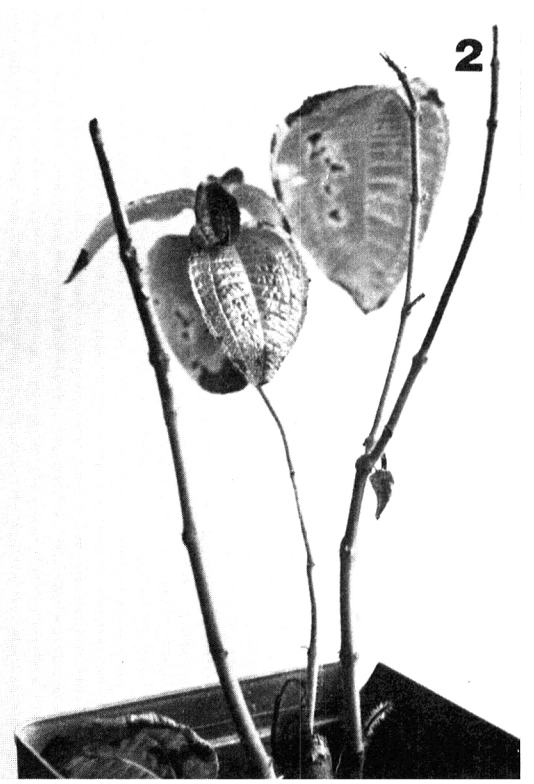
Journal Series paper 3008 of Hawaii Institute of Tropical Agriculture and Human Resources.

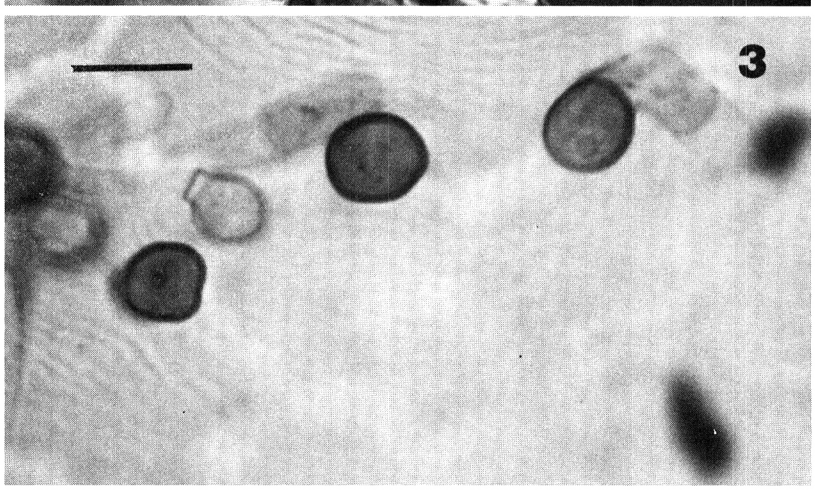
Accepted for publication 30 April 1986 (submitted for electronic processing).

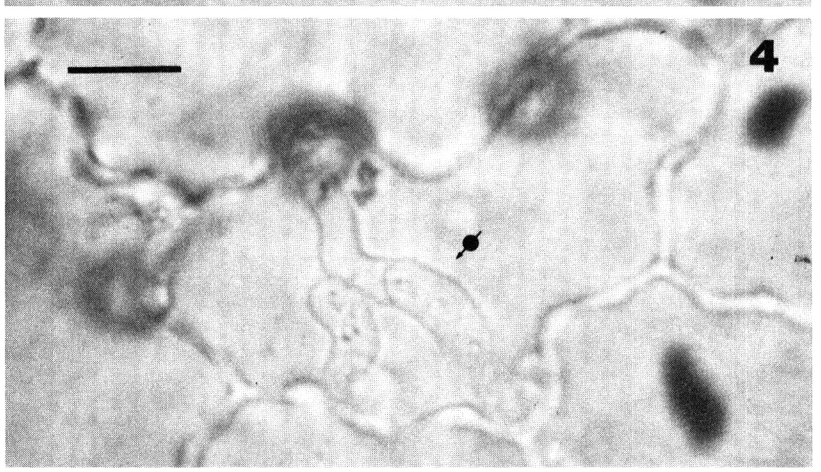
The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

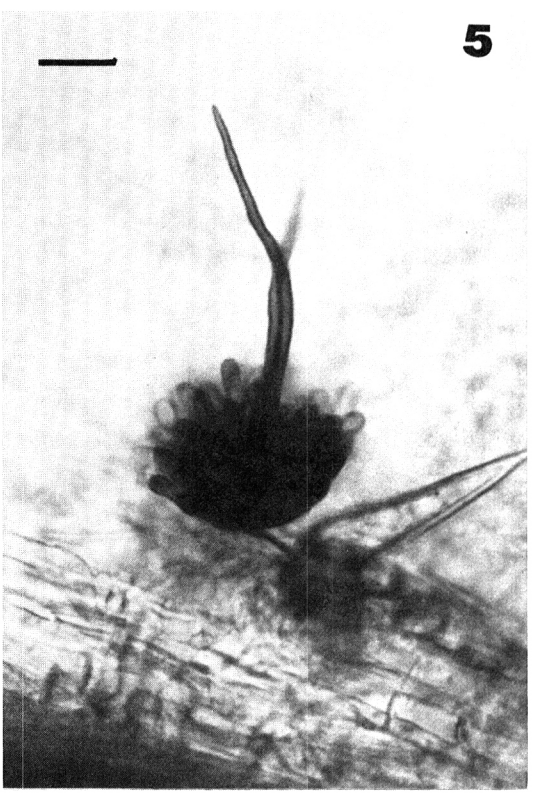
Histological studies. Infection and disease development in tissues was observed on whole mounts of artificially infected leaves stained in 0.01% acid fuchsin in lactic acid (9) 24, 48, and 96 hr after inoculation. To clear the tissues and stain the pathogen, the whole mounts were boiled in the stain for 1 min or until no chlorophyll was seen. The tissues were immersed in pure lactic acid for 5 min to remove excess stain, then mounted in pure lactic acid. Free-hand cross sections











Figs. 1–5. Anthracnose of *Clidemia hirta* caused by *Colletotrichum gloeosporioides*. **(1)** Leaf from Oahu *Clidemia* cultivar showing anthracnose lesions 10 days after inoculation with spore suspension of *C. gloeosporioides* (10^5 / ml) isolated from diseased *Clidemia* leaves collected in Panama. **(2)** Oahu *Clidemia* cultivar showing defoliation and tip dieback 10 days after inoculation. **(3)** Acid fuchsin/lactic acid-stained and cleared whole mount of a *Clidemia* leaf 72 hr after inoculation and incubation at 25 C and 100% relative humidity, showing germinated spores and appressoria on the leaf epidermis. Scale bar = 10 μm. **(4)** Same whole mount as in 3 but at a different focal plane, showing penetration hyphae within the epidermal cell immediately beneath the appressorium of *C. gloeosporioides*. Scale bar = 10 μm. **(5)** Acid fuchsin/lactic acid-stained and cleared whole mount of a diseased *Clidemia* leaf 5 days after inoculation, showing sporulating acervulus with black setae on the veins and laminae. Scale bar = 28 μm.

of infected leaves and petioles were prepared.

RESULTS AND DISCUSSION

Fungal and cultural characteristics. Cultures of the pathogen isolated from *Clidemia* leaves collected in Panama and from lesions on artificially inoculated *Clidemia* cultivars from Oahu had identical characteristics and were typical of *Colletotrichum gloeosporioides* (Penz.) Sacc. When the pathogen was grown on 10% V-8 or CLP, orange masses of cylindrical spores were produced in acervuli with black setae. The nonseptate hyaline spores averaged 17.5 (12.5–18.7) μm long and 5.5 (5.0–7.5) μm wide. Cultures grown on OA produced similar fruiting structures but with greater mycelial development than on the other media.

Pathogenicity tests. The Oahu *Clidemia* plants inoculated in Frederick with spore suspensions and incubated in a dew chamber developed small light brown angular leaf lesions 5 days after incubation. Lesions became circular to irregular with dark brown centers and light yellow halos 7 days after inoculation (Fig. 1). They often coalesced into large brown areas that caused the leaves to abscise, resulting in premature defoliation (Fig. 2). Many small infections were detected on leaf axils. Plants of Oahu *Clidemia* cultivars inoculated in Frederick and Hawaii and kept in terraria for 24 hr at high relative humidity and 28 C showed symptoms on leaves (light brown specks with yellow halos) within 5 days. Under microscopic examination, infection was apparent on the large multicellular trichomes covering the leaves and stems. These trichomes shriveled and became brown, with acervuli of the pathogen appearing on their surfaces within 5 days. Eight days after inoculation, infection extended to the cortical tissues of the stem, causing brown discoloration of the cortex and death of the apical portions of the plant (Fig. 2). Acervuli became visible on the abaxial sides of leaves, mostly on leaf veins and at leaf axils, 5–8 days after inoculation. These were produced also on the adaxial leaf surface when inoculated plants were kept in a humid environment for more than 5 days.

Histological pathology. Acid fuchsin/lactic acid-stained and cleared whole mounts of *Clidemia* leaves made 18 hr after inoculation and incubation showed spore germination and appressorial formation on the leaf epidermis (Fig. 3) and multicellular trichomes. Penetration of the leaf epidermis and stem trichomes was evident 48 hr after inoculation. Fungal hyphae within the epidermal cells immediately beneath the appressoria were observed frequently (Fig. 4). Seventy-two hours after inoculation, subepidermal hyphal cushions of developing acervuli were visible below the appressoria that had produced infection pegs, which penetrated the leaf epidermis. Stained free-hand cross sections of the leaf mesophyll and infected petioles showed the pathogen to be intracellular, forming subepidermal erumpent acervuli with black setae and producing abundant spores 5 days after inoculation. Stained and cleared whole mounts showed formation of acervuli mostly on the prominent veins of the lower leaf surface (Fig. 5) and multicellular trichomes of the stem. However, acervuli were also present on both surfaces of the laminae not directly associated with either of these structures.

In host range studies, *C. gloeosporioides* formed appressoria on the leaf epidermis of all species inoculated. Appressoria were observed 24 hr after inoculation on all whole mounts of leaves from the different species tested. After 48 hr, penetration hyphae were observed only on leaves of *Clidemia hirta*, in which intracellular penetration of epidermal cells also was observed. Ninety-eight hours after inoculation, whole mounts of *C. hirta* showed well-developed hyphal cushions below the appressoria. None of the other species tested was invaded by the pathogen, even though plants and cuttings were maintained under favorable conditions for infection for 10 days. All the cuttings remained in good vigor, whereas all *Clidemia* plants had large brown leaf lesions and were completely defoliated after this period.

We have shown *Clidemia hirta* to be a new host of *Colletotrichum gloeosporioides*, a cosmopolitan pathogen with many highly specialized forms (11). We believe the potential for severe damage by this pathogen to *Clidemia* in Hawaii is considerable because of its demonstrated ability to defoliate test plants under very high and moderately humid conditions both within terraria and in ambient growth room humidity. The infested area of Oahu receives 7 m of rainfall annually, and relative humidity is consistently high, usually 100% from 6 P.M. to 7 A.M. and seldom lower than 75% during the day (8).

The ability of this pathogen to grow and produce spores on most common media and in tank culture makes it an ideal candidate for manipulation as a biological control agent for weeds. The successful use of *C. gloeosporioides* f. sp. *aeschynomene* as a bioherbicide for northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.) in paddy rice (*Oryza sativa* L.) (1–3,6,7) is evidence of the potential bioherbicidal value of host-specific forms of this species. An extensive host range determination is essential before this pathogen, which we tentatively designate *C. gloeosporioides* f. sp. *clidemiae*, can be approved for use as a biological control agent of *Clidemia hirta* in Hawaii.

ACKNOWLEDGMENTS

Supported in part by Section 406, Grant No. 84-CSRS-2-2454 and CSRS special grant for biological control No. 82-CSRS-2-2029. We appreciate the invaluable cooperation of W. M. Dowler, Research Leader, USDA, Foreign Disease-Weed Science Research Unit, Fort Detrick, Frederick, MD.

LITERATURE CITED

1. Bowers, R. C. 1982. Commercialization of microbial biological control agents. Pages 157-173 in: Biological Control of Weeds with Plant Pathogens. R. Charudattan and H. L. Walker, eds. John Wiley & Sons, New York.
2. Boyette, C. D., Templeton, G. E., and Smith, R. J., Jr. 1979. Control of winged water primrose (*Jussiaea decurrens*) and northern jointvetch (*Aeschynomene virginica*) with fungal pathogens. Weed Sci. 27:497-501.
3. Daniel, J. T., Templeton, G. E., Smith, R. J., Jr., and Fox, W. T. 1973. Biological control of northern jointvetch in rice with an endemic fungal disease. Weed Sci. 21:303-307.
4. Latterell, F. M., and Rossi, A. E. 1984. A *Marasmiellus* disease of maize in Latin America. Phytopathology 69:728-731.
5. Mitchell, J. E., and Cherry, E. 1954. A variable temperature incubation chamber permitting controlled deposition of dew. (Abstr.) Phytopathology 44:498.
6. TeBeest, D. O., and Templeton, G. E. 1985. Mycoherbicides: Progress in the biological control of weeds. Plant Dis. 69:6-10.
7. Templeton, G. E. 1975. EPA issues first experimental use permit for biological control fungus. Rice J. 78(7):30.
8. Trujillo, E. E. 1965. The effects of humidity and temperature on Phytophthora blight of taro. Phytopathology 55:183-188.
9. Trujillo, E. E., and Snyder, W. C. 1963. Uneven distribution of *Fusarium oxysporum* f. sp. *cubense* in Honduran soils. Phytopathology 53:167-170.
10. Tuite, J. 1969. Plant Pathological Methods. Burgess Publishing, Minneapolis, MN.
11. von Arx, J. A. 1957. Die Arten der Gattung *Colletotrichum* Cda. Phytopathol. Z. 29:413-468.
12. Wester, L. L., and Wood, H. B. 1977. Koster's curse (*Clidemia hirta*), a weed pest in Hawaiian forests. Environ. Conserv. 4:35-41.