Septoria Leaf Spot of Lantana from Ecuador: A Potential Biological Control for Bush Lantana in Forests of Hawaii

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ABSTRACT

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Pathogenicity tests with a *Septoria* sp. isolated from *Lantana camara* from Ecuador showed aggressiveness to four *L. camara* selections from Hawaii forests. The initial symptoms of the disease on inoculated leaves were chlorotic spots that appear within 2 weeks, becoming distinctly angular necrotic lesions in 4 weeks, inducing leaf chlorosis and defoliation 6 weeks after inoculation. Inoculum applied at 1×10^6 conidia per ml in 2% sucrose–0.5% gelatin solution produced an average of 87 lesions per leaf. Ornamental hybrids of *L. camara* × *L. montevidensis* also were susceptible, but these plants had significantly fewer lesions. All selections of *L. montevidensis* tested were immune. This *Septoria* sp. from Ecuador is the first pathogen found to be virulent on bush lantana, the most serious ecological threat to Kauai's forest, and its release is presumed to be an effective biocontrol agent for this weed.

Additional keyword: bioherbicide

Lantana camara L., bush lantana, is a native shrub of tropical and subtropical America (2) that was first introduced into Hawaii in 1858 as an ornamental (6). Fruit-eating birds distributed lantana seeds widely (10), and in a few years the plant became naturalized in Hawaii, forming thickets on waste, pasture, and forest lands. Weedy lantana is a spiny bush with densely pubescent leaves and pink-yellow or orange-red showy flowers. The worst infestation of *L. camara* is present in the Kokee forest on Kauai, where more than 10,000 ha are affected. Hurricane Iniki, which passed over Kauai in 1992, destroyed most of the native Kokee forest vegetation, suppressing competition and allowing the bush lantana population to explode.

Lantana is a serious threat to the survival of the native Hawaiian flora in the Kokee forest. This weed is an ecological disaster that interferes with reforestation and drastically reduces the use of hiking trails. Chemical control of weeds in Hawaii's forests and watersheds is undesirable and costly, whereas biological control of introduced weeds with fungal pathogens has proved environmentally sound and cost-effective (7). In late 1986, funding to study pathogens of lantana became available. During two exploratory searches in 1977 and 1978 for biological control agents for lantana in the American tropics, three fungal pathogens were identified: *Puccinia lantanae* Farl., *Prospodium tuberculatum* (Speg.) Arth., and *Mycovellosiella lantanae* (Chupp) Deighton (E. E. Trujillo, *unpublished progress report 31 October 1983*). All pathogens were tested on Hawaii's weedy lantana, but none was found to be pathogenic. A lantana leaf spot was collected near Ibarra, Ecuador, in 1984 (E. E. Trujillo, *1984 Annual Report to Hawaii Department of Land and Natural Resources*), and was examined at the USDA-ARS, Plant Disease Research Laboratory, Fort Detrick, Maryland. A previously unreported *Septoria* sp. was isolated from infected leaves. Inoculations of the *Septoria* sp. on rooted cuttings of *L. camara* from Hawaii resulted in leaf necrosis and defoliation. Further tests indicated that the *Septoria* sp. also was pathogenic to *L. camara* hybrids, but not to *L. montevidensis,* a widely used ornamental in Hawaii. In 1986, the Board of Agriculture of Hawaii granted permission to introduce two test tube cultures of the lantana *Septoria* from Maryland to Hawaii for host-range determinations. Pathogenicity tests and a limited host-range study done at the University of Hawaii at Manoa are reported here.

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MATERIALS AND METHODS

Isolation, pathogenicity, and characterization. The pathogen was isolated into a pure culture by aseptically removing spore cirri from pycnidia on the surface of leaf lesions and placing them on potato-dextrose agar (Difco, PDA). Spore masses were then streaked on the surface of the PDA plates using a bacterial platinum loop containing a drop of acidified sterile distilled water (SDW). Cultures were stored for 2 to 3 months on PDA slants at 20°C under continuous cool fluorescent light before transferring to fresh medium.

Cultures of the *Septoria* sp. grown for 14 days on PDA and on GCA (100 g of Gerber Mixed Cereal for Baby and 18 g of agar per liter) were used for inoculations. All cultures were grown at 20°C under continuous cool-white fluorescent light. Spore suspensions were made by washing the surface of the cultures with 10 ml of SDW or a solution of 2% sucrose and 0.5% gelatin (7,9). Spore suspensions for inoculations were adjusted to approximately 1×10^6 spores per ml using a hemacytometer. All plants were kept in a quarantined air-conditioned laboratory maintained at 24 to 25°C under 12-h Agro-Lite fluorescent illumination. All plants were grown in 15-cm-diameter pots filled with equal parts of soil and Fison's Sunshine Mix 3 (Fison Horticulture Inc., Vancouver, BC, Canada) and fertilized with Osmocote 14-14-14. Plants 15 cm high were inoculated by spraying leaves to wetness with a conidial suspension. Control plants were sprayed with water or with 2% sucrose–0.5% gelatin solution. Sets of

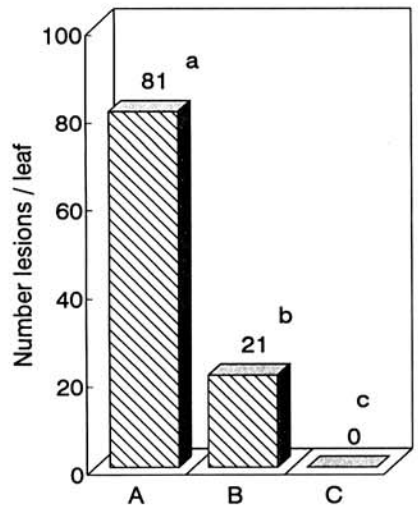

Fig. 1. Number of lesions per leaf caused by a *Septoria* sp. on three different *Lantana* spp. 4 weeks after inoculation. **(A)** *Lantana camara* selection from Maui; **(B)** *L. camara* hybrid Cloth of Gold; **(C)** *L. montevidensis*. Values followed by different letters are significantly different ($P \leq 0.05$) by Tukey's LSD test.

four inoculated and four noninoculated plants were incubated for 48 h at approximately 100% relative humidity and 25°C in 50-cm-diameter terraria (8). Two days after inoculation, plants were removed from the terraria and placed on the bench under Grow-Lite lamps until symptoms developed. Three weeks after inoculation, the number of lesions present on five leaves of each plant was counted. A one-way analysis of variance followed by an LSD test was used to compare mean lesion numbers between treatments. The pathogenicity tests were repeated. After completion of the tests, the plants were disposed of by autoclaving for 30 min.

Host-range determination. Two-month-old rooted cuttings of lantana selections from the islands of Maui, Hawaii, Kauai, Lanai, and Oahu were used as susceptible controls in host-range determinations. For host-range studies, four plants of each species or variety were inoculated with suspensions of 1×10^6 conidia per ml in 2% sucrose–0.5% gelatin. Inoculum was applied to the foliage of test plants to runoff with a 32-oz plastic all-purpose spray bottle. Inoculated plants were incubated in 50-cm-diameter spherical, clear plastic terraria (8). Relative humidity (RH) inside the terraria was kept at 100% for the first 24 h of incubation and at 95% thereafter, following established procedures (8). Tests were repeated. Susceptibility to the *Septoria* sp. was tested on the following plants: *Agavaceae: Cordyline terminalis* (ti plant); *Araceae: Colocasia esculenta* (taro); *Bromeliaceae: Ananas comosus* (pineapple); *Caricaceae: Carica papaya* (papaya); *Chenopodiaceae: Beta vulgaris* (beets); *Convolvulaceae: Ipomea batatas* (sweetpotato); *Cruciferae: Brassica oleracea* var. *capitata* (head cabbage); *Cucurbitaceae: Cucumis sativus* (cucumber); *Poaceae: Saccharum officinarum* var. 7209 and var. Co-290-15 (sugarcane); *Zea mays* (corn); *Leguminosae: Phaseolus vulgaris* (bean); *Solanaceae: Capsicum frutescens* (bell pepper) and *Lycopersicon esculentum* (tomato); and *Verbenaceae: Lantana montevidenses* (trailing lantana), *L. camara* selections from Hawaii, Maui, Lanai, Kauai and Oahu, two hybrids of *L. camara* (Cloth of Gold and Radiation), *Stachytarpheta jamaicensis* (Jamaica vervain), and *S. cayennensis* (Cayenne vervain). Age of inoculated plants varied from 2 weeks to 3 months depending on the growth habit of the species and plant size suitable for terraria incubation.

RESULTS AND DISCUSSION

Isolation, pathogenicity, and characterization. Spore cirri were produced after 3 days of incubation in moist chambers at 21°C from pycnidia of *Septoria* sp. on air-dried diseased lantana leaves collected from Ibarra, Ecuador. The spore horns smeared on PDA surfaces acidified with lactic acid produced pure sporulating cultures of *Septoria* sp. 14 days after transfer. Spore production on the surface of GCA

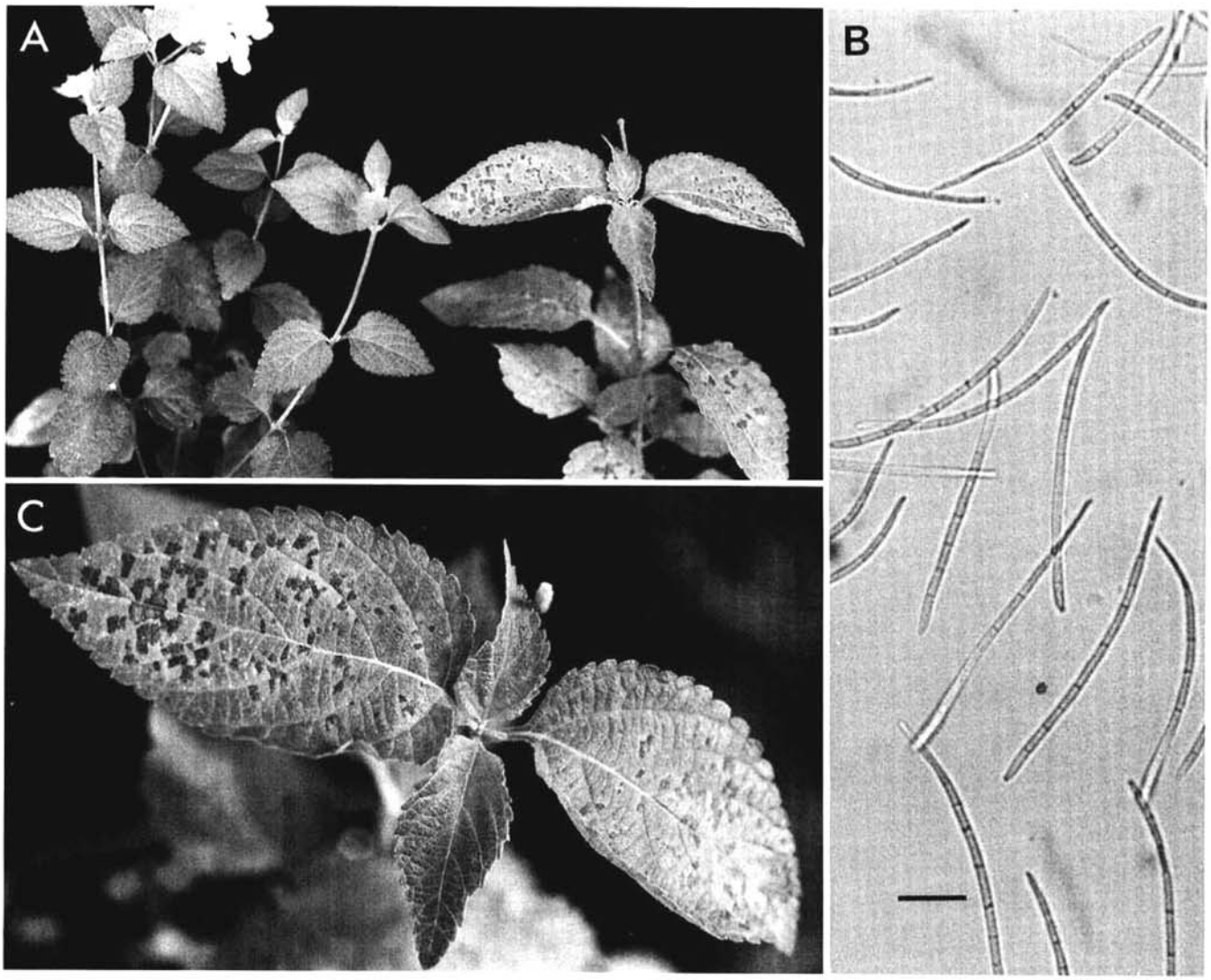

Fig. 2. Septoria leaf spot of lantana. **(A)** Symptoms on two *Lantana* spp. 4 weeks after inoculation: *L. montevidensis*, trailing lantana (left), and *L. camara*, Maui selection (right). Plants were inoculated by spraying to wetness with a suspension of 1×10^6 conidia per ml in 2% sucrose–0.5% gelatin. **(B)** Typical conidia of a *Septoria* sp. from Ibarra, Ecuador, from pycnidia on the surface of an infected lantana leaf (bar = 20 μm). **(C)** Distinctly angular lesions are vein limited, as shown on *L. camara* leaves (close to natural size).

Table 1. Susceptibility of different plant species to a *Septoria* sp. of *Lantana camara* from Ibarra, Ecuador

Family Species	No. plants inoculated	No. plants diseased	Disease index[a]
Agavaceae			
Cordyline terminalis	8	0	0
Araceae			
Colocasia esculenta	8	0	0
Bromeliaceae			
Ananas comosus	8	0	0
Caricaceae			
Carica papaya	8	0	0
Chenopodiceae			
Beta vulgaris	8	0	0
Convolvulaceae			
Ipomea batatas	8	0	0
Cruciferacea			
Brassica oleracea var. *capitata*	8	0	0
Cucurbitaceae			
Cucumis sativus	8	0	0
Poaceae			
Saccharum officinarum			
var. 7209	8	0	0
var. Co-290-15	8	0	0
Zea mays	8	0	0
Leguminosae			
Phaseolus vulgaris	8	0	0
Solanaceae			
Capsicum frutescens	8	0	0
Lycopersicon esculentum	8	0	0
Verbenaceae			
Lantana camara			
Hawaii selection	24	24	3
Maui selection	24	24	3
Lanai selection	16	16	3
Kauai selection	24	24	3
Oahu selection	32	32	3
Hybrids			
Cloth of Gold	16	16	1.3
Radiation	16	16	1.3
Lantana montevidensis	16	0	0
Stachytarpheta jamaicensis	8	0	0
S. cayennensis	8	0	0

[a] 0 = No symptoms, 1 = few necrotic lesions (1–20/leaf), 2 = many necrotic lesions (11–36/leaf), 3 = numerous necrotic lesions coalescing into large lesions (37–130/leaf).

was optimal when the medium was inoculated with 0.5 ml of 1×10^6 conidia suspension in SDW.

All *L. camara* and hybrids of this species inoculated with conidia of the *Septoria* sp. in water or sugar–gelatin suspension developed symptoms, whereas noninoculated control plants were free of disease. The number of lesions per leaf on hybrids inoculated with sugar–gelatin spore suspensions ranged from 1 to 36, and on *L. camara* from 42 to 130, or approximately a fourfold increase in *Septoria* spots (Fig. 1). Treatments were significantly different ($P \leq 0.05$).

Symptoms consisted of small, chlorotic leaf spots appearing 14 days after inoculation. Necrotic spots developed within 3 weeks, and these enlarged into distinct angular necrotic lesions 4 to 5 weeks after inoculation (Fig. 2C). Affected leaves turned light yellow and abscised in 6 to 7 weeks. Numerous cirri were observed protruding from pycnidia on senescent, diseased leaves after 48 h of incubation in moist chambers at 25°C. The pathogen was reisolated into pure culture from inoculated host plants with disease symptoms confirming pathogenicity.

The *Septoria* sp. isolated from diseased *L. camara* leaves from Ecuador differed morphologically from *Septoria lantanae* Gaerman from Puerto Rico (5) on *L. camara*. The conidia of the *Septoria* sp. from Ecuador were 43–106 × 2.5–2.8 µm, or twice the length of *S. lantanae* conidia (24–50 × 2.4 µm) (5). The *Septoria* sp. had filiform conidia, with 6 to 13 septa, broadly curved, and acutely tapered at the apex (Fig. 2B). Pycnidia of the *Septoria* sp. ranged from 70 to 100 µm in diameter and were slightly larger than the pycnidia of *S. lantanae*. The pycnidia were produced sparingly on the surface of the lesions. Further comparative studies with the Puerto Rican fungus must be done to elucidate their taxonomy.

Host-range studies. Besides bush lantana, all hybrids of *L. camara* were susceptible to the *Septoria* sp. from Ibarra, Ecuador. Other members of the *Verbenaceae* tested were immune. None of the species from Hawaii's representative plant families was susceptible. Lantana plants used as susceptible controls developed leaf spots 3 to 4 weeks after inoculation (Fig. 2A). These results were consistently reproduced in repeated tests (Table 1).

Septoria spp. are noted for their narrow host range (1,3,4,9). The *Septoria* sp. from Ibarra appears to be a highly specialized pathogen of bush lantana. Therefore, this is a promising biological control for this weed in Hawaiian forests.

ACKNOWLEDGMENT

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