Septoria Leaf Spot, a Potential Biological Control for Banana Poka Vine in Forests of Hawaii

EDUARDO E. TRUJILLO, Professor, and DAVID J. NORMAN, Assistant Researcher, Department of Plant Pathology, University of Hawaii, 3190 Maile Way, Honolulu 96822; and ELOISE M. KILLGORE, Plant Pathologist, Plant Pathology Quarantine Facility, Biological Control Section, Hawaii Department of Agriculture, Honolulu 96814

ABSTRACT

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Pathogenicity studies in Hawaii have confirmed *Septoria passiflorae* as an aggressive pathogen of banana poka (*Passiflora tripartita* var. *tripartita*). Initial symptoms on inoculated leaves are chlorotic spots that appear 14 days after inoculation and become distinct circular lesions with yellow halos by 20 days. The spots develop necrotic centers 3 mm in diameter 20–24 days after inoculation, and diseased leaves turn bright yellow and dehisce. Host range studies confirmed *P. foetida*, also a weed, as a susceptible host. The economic crops purple and yellow lilikoi and other species tested were immune. Since the Passifloraceae are not native to the Hawaiian islands, *S. passiflorae* is presumed to be an environmentally safe biocontrol agent for these weeds in Hawaii.

Passiflora tripartita (Juss.) Poir var. *tripartita* Holm-Nie. Jörg. & Laws. (= *Passiflora mollissima* Neal), commonly referred to as "banana poka," was first introduced into Hawaii in 1921 as an ornamental (5). This perennial woody vine with attractive pink, pendulous flowers and oblong bright yellow fruit is native to the southern Colombia and northern Ecuador Andean region of South America (12). The ripe, bright yellow fruit resembles a ripe banana, thus its Hawaiian name. In Hawaii it has become established at mid to high elevations, 800–2,200 m, on the islands of Hawaii, Kauai, and Maui. Banana poka is the most aggressive introduced weed of high-elevation forest areas in Hawaii. In 1981 it was estimated to cover more than 500 km^2 of forests on Kauai and Hawaii (14). Banana poka was classified as a noxious weed in 1979 by the Hawaii Department of Agriculture (10).

In 1981 the State of Hawaii Department of Land and Natural Resources, Division of Forestry and Wildlife, funded the exploratory search of South America for potential biological control agents for this weed. Exploration of Colombia and Ecuador Andean regions, conducted during 1983–1984, revealed a number of insect pests and fungal pathogens of *Passiflora* spp. with biological control potential (7,12). The moth *Cyanotricha necyria* Felder, an aggressive phytophagous pest of banana poka (2), was introduced in 1985 and released in 1989 (6), but a breeding population has failed to become established so far. Another moth, *Pyrausta perelegans* Hampton, introduced in 1986 and released in 1989, has become established but has had negligible biological pressure on banana poka. A powdery mildew caused by a *Phyllactinia* sp. and studied by the first author at Providencia, Nariño, Colombia, during 1987–1989 was approved for introduction and release by the Hawaii Board of Agriculture in 1989. However, failure to maintain viability of this pathogen during transportation has precluded its successful establishment in Hawaii.

Hawaii banana poka seedlings planted in Aldana, Nariño, Colombia, in 1991 developed a leaf spot disease caused by a *Septoria* sp. Symptoms of the disease were distinct circular lesions with necrotic centers surrounded by yellow halos. On 19 April 1993, diseased, air-dried leaves were shipped to Hawaii under USDA Permit No. 930095 for isolation, pathogenicity, and host range studies.

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MATERIALS AND METHODS

Isolation and pathogenicity tests. All research was performed under strict quarantine requirements at the Hawaii Department of Agriculture Plant Pathology Quarantine Facility in Honolulu. *Septoria* sp. was isolated from lesions with necrotic centers on diseased banana poka leaves incubated in moist chambers under fluorescent illumination for 2 days at 25 C. Spore cirrhi produced by pycnidia were removed aseptically and streaked with acidified sterile water onto Difco potato-dextrose agar (PDA) plates. After 4 days of incubation at 21 C under continuous fluorescent illumination, bacteria-free cultures were transferred to new plates and grown for 2 wk. Spore suspensions were made by flooding 14-day-old PDA cultures with 10 ml of sterile distilled water and/or a solution of 2% sucrose and 0.5% gelatin (11). Spore suspensions for inoculations were adjusted to approximately 1×10^6 spores per milliliter using hemacytometer counts. All plants were kept in a quarantined, negative pressure, sunlit (40,000–100,000 lux), air-conditioned greenhouse maintained at 24–25 C. Plants were grown in 15-cm-diameter pots filled with equal parts of soil and Fisons's Sunshine Mix 3 (Fison Horticulture Inc., Vancouver, BC, Canada) and fertilized with Osmocote 14-14-14. Two-month-old banana poka seedlings were inoculated by spraying leaves with inoculum to wetness. Control plants were sprayed with water or the sugar-gelatin solution. Sets of four inoculated and four noninoculated plants were incubated for 48 hr at approximately 100% relative humidity and 25 C in terraria 50 cm in diameter and with an adjustable top opening to control humidity (Lawnware Products, Inc., Mortin Grove, IL). Forty-eight

hours after incubation, plants were transferred to the quarantined greenhouse until symptoms developed. Three weeks after inoculation, the number of lesions present on the oldest leaf of each plant was counted. A one-way analysis of variance followed by an LSD test was used to compare mean lesion number between treatments. The pathogenicity test was repeated.

Host range studies. Two-month-old rooted cuttings of banana poka and six of the most common naturalized *Passiflora* spp. found in Hawaii were used in host range determinations. These were *P. edulis* Sims (purple lilikoi), *P. e. flavicarpa* Degener (yellow lilikoi), *P. foetida* L. (love-in-a-mist), *P. laurifolia* L. (yellow granadilla), *P. ligularis* A.L. Juss. (sweet granadilla), and *P. suberosa* L. (huehue haole). Sets of four plants of each *Passiflora* spp. were inoculated as previously described with 1×10^6 conidia per milliliter. Tests were repeated.

RESULTS

Isolation and pathogenicity tests. The *Septoria* sp. isolated from diseased banana poka leaves from Aldana, Nariño, Colombia, was identical in morphology to *Septoria passiflorae* Syd., with conidia 35–52 × 1.5–2 μm (Fig. 1A) and immersed, dark brown to black, globosed pycnidia 72–104 μ in diameter (Fig. 1D). This fungus was originally described in 1937 on *P. mollissima* (Kunth) L.H. Bailey from Pichincha, Ecuador (9,13). The fungus grows readily on PDA and produces abundant pycnidia and conidia after 14 days of incubation at 20 C under continuous fluorescent illumination.

All banana poka plants inoculated with spores in water or sugar-gelatin solution developed symptoms, whereas noninoculated control plants were free of disease. The number of lesions per leaf ranged from one to 39 on plants inoculated with the spore-water suspension and from 56 to 105 on those inoculated with the sugar-gelatin solution, an approximately sixfold increase in the number of spots (Fig. 2). Treatments were significantly different ($P \leq 0.05$).

Symptoms were small, chlorotic leaf spots appearing 14 days after inoculation. Spots enlarged into distinct circular lesions with yellow halos 4–6 days later (Fig. 1B); the centers were necrotic 4 wk after inoculation. When the necrotic centers of lesions were approximately 3 mm in diameter, affected leaves turned bright yellow and dehisced (Fig. 1C). Numerous cirrhi were observed protruding from pycnidia on senescent diseased leaves after 48 hr of incubation in moist chambers at 25 C (Fig. 1D). The pathogen was reisolated in pure culture from all inoculated banana poka plants, confirming pathogenicity.

Host range studies. Besides banana poka, *P. foetida* was the only other member of the Passifloraceae tested that was susceptible to this isolate of *S. passiflorae* from Colombia (Table 1). Purple and yellow lilikoi and yellow and sweet granadilla were not hosts of *S. passiflorae*. All banana poka plants used as controls developed leaf spots 3–4 wk after inoculation. These results were consistently reproduced in repeated tests.

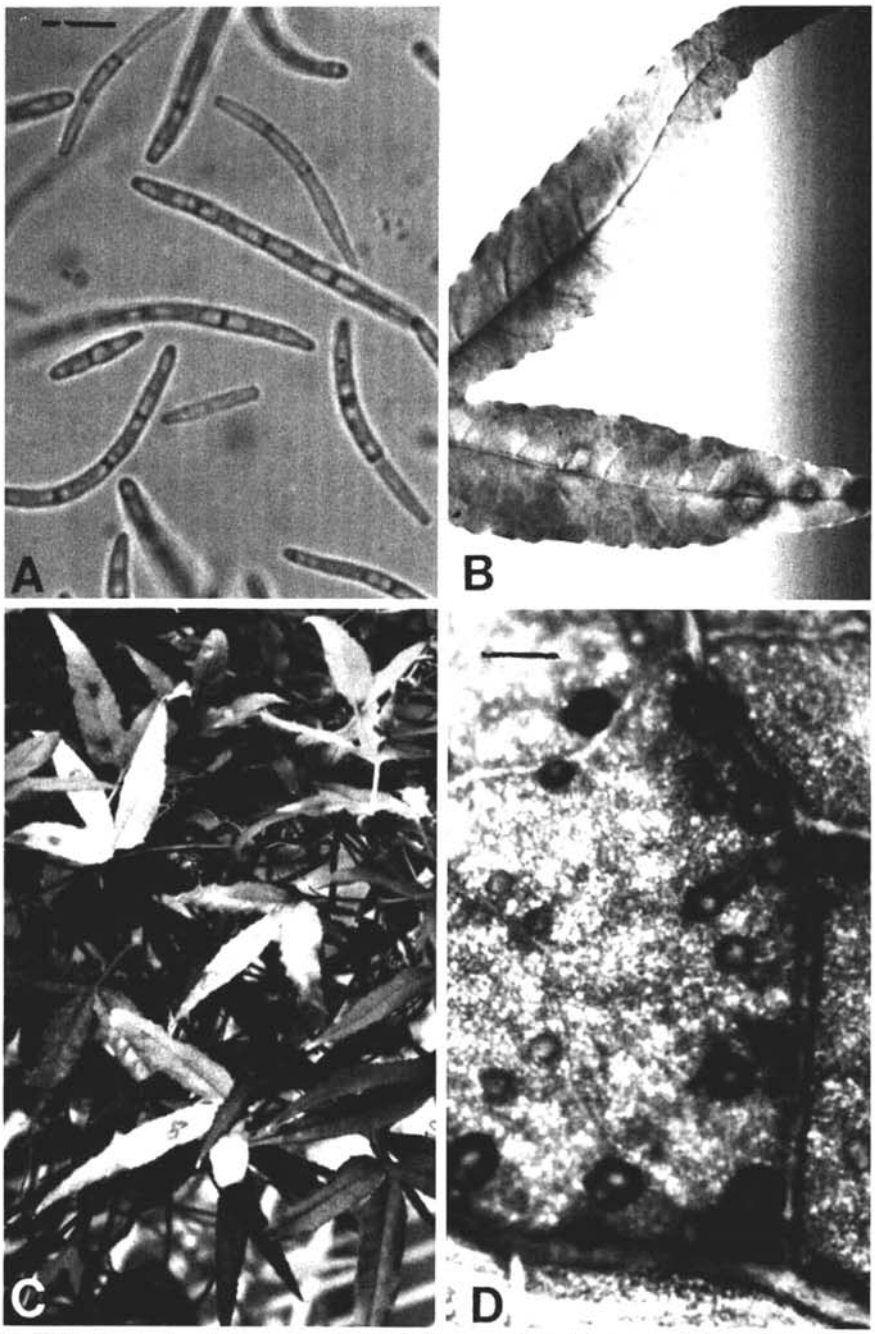

Fig. 1. Septoria leaf spot of banana poka: **(A)** Filiform, multiseptate spores of *Septoria passiflorae*. Scale bar = 8.46 μm. **(B)** Typical spots with halos on banana poka leaf 20 days after inoculation. **(C)** Acute chlorosis before leaf dehiscence on banana poka seedlings 24 days after inoculation. **(D)** Pycnidia formation on the surface of a senescent leaf lesion 2 days after incubation in a moist chamber at 25 C. Scale bar = 150 μm.

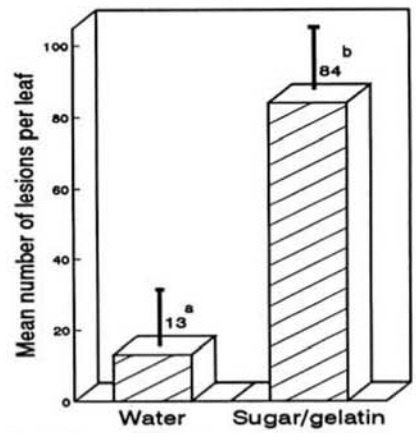

Fig. 2. Comparison of mean number of lesions produced on leaves of *Passiflora tripartita* var. *tripartita* by *Septoria passiflorae* applied in sterile distilled water or 2% sucrose and 0.5% gelatin solution.

Table 1. Susceptibility of seven species of Passifloraceae to *Septoria passiflorae*

Passifloraceae species	No. of plants inoculated	Disease index[a]
Passiflora laurifolia	8	0
P. ligularis	8	0
P. suberosa	8	0
P. edulis	8	0
P. e. flavicarpa	8	0
P. foetida	8	1
P. tripartita var. *tripartita*	8	3

[a] 0 = No symptoms, 1 = few chlorotic lesions per leaf without necrotic centers, 2 = one to five lesions per leaf with necrotic centers, 3 = more than five lesions per leaf with acute chlorosis, blight, and severe defoliation.

DISCUSSION

Septoria fungi have been well documented as host-specific pathogens. In some cases, pathogenicity is limited to a single host species (e.g., *S. tritici* Roberge in Desmaz., which consists of several forma speciales attacking single hosts), or to closely related host species within a genus (e.g., *S. septulata* Beach., *S. helianthi* Ellis & Kellerm, *S. lactucae* Pass., *S. cirsii* Niessl, *S. polygonorum* Desmaz., and *S. apiicola* Speg.), or to a few species in closely related genera within a family of plants (e.g., *S. lactucicola* Ellis & G. Martin, *S. scrophulariae* Peck, *S. malvicola* Ellis & G. Martin, and *S. lycopersici* Speg.) (1,3,4). Our study supports the evidence that *Septoria* spp. are highly specialized. Therefore, they are promising fungi for bioherbicide exploitation (12).

Pathogenicity tests confirmed *S. passiflorae* isolated from diseased banana poka leaves from Colombia as an aggressive pathogen of *P. t. tripartita* in Hawaii. The probability for success of this fungus as a biocontrol agent for banana poka appears excellent, primarily because this pathogen is restricted in its pathogenicity to a few closely related host species within the genus *Passiflora*. Host range studies have demonstrated that *S. passiflorae* isolated from banana poka leaves from Aldana, Nariño, Colombia, is pathogenic to *P. t. tripartita* and *P. foetida*, two introduced weeds of importance in Hawaii. It does not infect the important commercial crops *P. edulis* and *P. e. flavicarpa*. The other species of *Passiflora* tested are not susceptible and have no agricultural significance in Hawaii. *P. manicata* Juss. (red passion fruit), *P. pulchella* Kunth (two-lobed passion fruit), *P. quadrangularis* L. (giant granadilla), *P. subpeltata* Ort. (white passion flower), and *P. vitifolia* Kunth were not tested because they have not become naturalized.

When released on selected banana poka sites in Kauai, Maui, and Hawaiian forests, the *S. passiflorae* isolated from banana poka lesions from Aldana, Nariño, Colombia, is expected to reduce the growth and spread of banana poka significantly. This fungus also infects *P. foetida*, a weedy species found in rocky outcrops and noncultivated land. Since there are no native populations of Passifloraceae in Hawaii (8), and since all members of the family are introduced plants, we recommend the release of this pathogen from Hawaiian quarantine for field testing at selected forest sites.

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