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Host Range of *Puccinia psidii,* a Potential Biological Control Agent of *Melaleuca quinquenervia* in Florida[1]

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The rust fungus *Puccinia psidii* infects the foliage and causes dieback of actively growing tips on several myrtaceous plants in South and Central America. It has recently been discovered in south Florida causing a similar disease on *Melaleuca quinquenervia*. We therefore evaluated *P. psidii* as a potential biological control agent of this invasive tree. Typical disease symptoms on *M. quinquenervia* included distortion and abscission of young foliage and dieback of severely infected tips. Young stems with living bark developed lesions and localized swellings. The stems became brittle and prone to breakage at the point of these swellings. Often, flowers and young seed capsules also developed eruptive pustules. Host range tests were performed on 18 species in 11 genera of Myrtaceae by inoculating expanding leaves with uredospores of two *P. psidii* isolates: MISOL, obtained from *M. quinquenervia*, and PISOL, obtained from *Pimenta dioica*. Results showed *Callistemon viminalis, Eugenia reinwardtiana, M. decora, M. quinquenervia, Myrcianthes fragrans, Myrciaria cauliflora, P. dioica,* and *Psidium guajava* to be susceptible to both isolates. *Eucalyptus grandis, Eugenia paniculatum,* and *Syzygium cumini* manifested chlorotic halos that developed into brown leaf spots but had no sporulation and were therefore considered resistant. The remaining seven species (*Calyptranthes pallens, Eugenia confusa, Eugenia foetida, Eugenia uniflora, Feijoa sellowiana, Psidium cattleianum,* and *S. jambos*) exhibited no symptoms and were considered immune to both isolates. The ability of these isolates to initiate pustules on susceptible hosts differed significantly. Overall, both isolates induced more pustules on *M. quinquenervia, E. reinwardtiana,* and *P. dioica* than on other susceptible species. Based on host range, both Florida isolates of *P. psidii* appear similar to one that infects *Pimenta* spp. in Jamaica. Our studies included a limited number of plant species grown under optimal conditions for disease expression. Field tests will be needed to ascertain their susceptibility under more natural conditions. The *P. psidii* and *M. quinquenervia* pathosystem probably represents a "new association," because of the disparate origins of the two species involved and their adventive status in Florida. © 2001 Academic Press

Key Words: **rust fungus; biological control; exotic weed; invasive weed; augmentation; melaleuca; paperbark tree; coevolution.**

INTRODUCTION

Puccinia psidii G. Wint. (Basidiomycetes, Uredinales) (common name: guava rust) was first described by Winter (1884) on *Psidium guajava* L. in Brazil. This rust has since been reported on 11 genera and 31 species of Myrtaceae in the Caribbean islands and North (Florida), Central, and South America (Laundon and Waterston, 1965; Marlatt and Kimbrough, 1979; Coutinho *et al.*, 1998; Rayachhetry *et al.*, 1997). The known host species include *Callistemon glaucus* (Bonpl.) Sweet, *Eucalyptus camaldulensis* Dehn., *Eucalyptus citriodora* Hook, *Eucalyptus cloeziana* F. Muell., *Eucalyptus grandis* (A.W.) Hill ex Maiden, *Eucalyptus maculata* Hook., *Eucalyptus paniculata* Sm., *Eucalyptus pellita* F. Muell., *Eucalyptus phaeotricha* Blakely et Mckie, *Eucalyptus pirocarpa* Johnson & Blaxell, *Eucalyptus punctata* DC., *Eucalyptus saligna* Sm., *Eucalyptus tereticornis* Sm., *Eucalyptus urophylla* Blake, *Eugenia* sp., *Eugenia brasiliensis* Lam. Skeels, *Eugenia uniflora* L., *Eugenia pyriformis* Camb. var. *uvalha* (Camb.) D. Legrand, *Marlierea edulis* Nied., *Melaleuca leucadendra* (L.) L., *Melaleuca quinquenervia* (Cav.) Blake, *Myrcia* sp., *Myrciaria* sp., *Myrciaria jaboticaba* (Vell.) Berg, *Pimenta recemosa* (Mill.) Moore, *Pimenta dioica* (L.) Merr., *Psidium guineense* SW., *Psidium guajava* L., *Syzygium cumini* (L.), *Syzygium jambos* (L.) Alston, and *Syzygium malaccensis* (L.) Merr. & Perry (Marlatt and Kimbrough, 1979;

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Coutinho *et al.*, 1998; Rayachhetry *et al.*, 1997). In 1996, *P. psidii* was discovered attacking healthy new foliage of *M. quinquenervia* (melaleuca), a noxious weed tree in south Florida (Rayachhetry *et al.*, 1997).

P. psidii has been described as a devastating disease of *Pimenta* spp. (allspice, pimenta, pimento) in some Caribbean and Central American countries (MacLachlan, 1936, 1938). Smith (1935) speculated that the rust attacking allspice was a new strain derived from one known to attack rose apple (Syzygium sp.). Field tests for resistance of 23 provenances in 13 *Eucalyptus* species to *P. psidii* in the Minas Gerais state of Brazil revealed a South African provenance of *E. grandis* that was highly susceptible and 3 provenances, 1 in each of 3 species, that were moderately susceptible (Dianese *et al.*, 1984). A similar study conducted in the Bahia state of Brazil showed 3 provenances (2 in *E. grandis* and 1 in *E. cloeziana*) to be highly susceptible (Dianese *et al.*, 1986). Differential susceptibility among provenances of *Eucalyptus* species were attributed to differences in virulence between the isolates and/or different microenvironmental characteristics of the two geographical areas. MacLachlan (1938) recognized the existence of two strains of *P. psidii,* one from pimento and one from rose apple, each incapable of causing serious disease on the other host.

Figueiredo *et al.* (1984) studied the life cycle of *P. psidii* on *S. jambos.* They produced three spore stages (uredospore, teliospore, and basidiospore) by inoculating leaves of *S. jambos* with pure basidiospores. These produced pustules bearing spiny spores resembling uredospores. It was assumed that some of these uredospore-like structures were aeciospores and that the pycniospores were unrecognizable. Thus, Figueiredo *et al.* (1984) considered the rust to be autoecious on the host *S. jambos* and macrocyclic with all five spore stages.

P. psidii attacks the leaves, petioles, and succulent stems of vigorously growing branches of allspice, eucalyptus, melaleuca, and other hosts (Smith, 1935; MacLachlan, 1936; Marlatt and Kimbrough, 1979; Dianese *et al.*, 1984, 1986; Rayachhetry *et al.*, 1997). As a result, it causes defoliation, twig mortality, and abortion of flowers and fruits (Smith, 1935). Ten to 12 days of incubation result in sporulation (eruption of pustules and exposition of spores) on allspice and melaleuca (MacLachlan, 1936; Marlatt and Kimbrough, 1979; Rayachhetry *et al.*, 1997). Uredospores constitute the dominant spore form which are dispersed mainly by wind and rain. Germ tube formation is initiated within 2 to 4 h after the spores land on a suitable infection court followed by complete differentiation within 6 h (Piza and Ribeiro, 1989) and full growth after about 12 h (Marlatt and Kimbrough, 1979). Pathogenicity and spore germination studies have shown that temperatures ranging from of 13 to 20°C (optimum at 16°C) favor uredospore germination and infection in *Pimenta* species (MacLachlan, 1936). In general, uredospore germination requires high ambient humidity (Smith, 1935). Pustule formation and release of spores occurred within 10 and 12 days, respectively, after inoculation on melaleuca leaves (Rayachhetry *et al.*, 1997).

M. quinquenervia is one of the most serious invasive weeds in the south Florida environment, and biological control of this weed will require a suite of agents. Generally, an average of seven natural enemies (ranging from 1 to >10 species) has been introduced to control each target pest (Hokkanen and Pimentel, 1984). Hence, the natural incidence of *P. psidii*, a potential biocontrol agent, on *M. quinquenervia* in Florida is of interest. Rust fungi are considered suitable biological control agents because of their narrow host ranges, rapid dispersion of inocula (Shishkoff and Bruckart, 1993), ability to attack healthy tissues, and the substantial level of damage they inflict to the host plants. Therefore, the objectives of this research were: (1) to describe *P. psidii* symptoms and impact on infected melaleuca plants and (2) to determine the host range of this rust pathogens within several myrtaceous species that occur in south Florida. Ultimately, we may determine the suitability of *P. psidii* as an augmentative biological control agent to supplement melaleuca control efforts in Florida. Therefore, the studies presented herein constitute an initial step in this process.

MATERIALS AND METHODS

Test Plants

P. psidii is already in south Florida, so no attempts were made to test all the myrtaceous plant species in Florida. The 18 plant species tested in this study include representatives of both exotic and native plants within the family Myrtaceae that occur in Florida (Table 1). Test plants were obtained from local nurseries and grown in 3.8- to 11.5-liter plastic pots containing a commercial potting mix (3002 Potting Mix, Atlas Peat Co., Boynton Beach, FL). These plants were pruned to maintain them at about 1.0 m tall. The pots were placed in full sun, fertilized (60 g/11.8 liter container) with Osmocote Plus (N:P:K, 15:9:12), and watered at 3- to 7-day intervals. These growing conditions induced the new growth (rust-susceptible leaf tissues) needed for inoculation experiments. However, it was difficult to induce synchronized new growth in all species at the same time. Therefore, at the time of inoculation, some species had a few new leaves while others had numerous new leaves. The new leaves on these plants were inoculated in the host range studies described below.

Isolate Collection and Maintenance

Uredospores of the melaleuca isolates (MISOL) of *P. psidii* were obtained from infected melaleuca saplings.

Spore suspensions were prepared by washing the sporulating leaves with sterile deionized water, straining the wash water into a beaker through two layers of cheese cloth, and allowing the spores in the filtrate to settle to the bottom of the beaker. The excess water was then decanted with minimal disturbance to the settled residue (uredospores). A droplet of the surfactant polyoxyethylene sorbitan monolaurate (Tween 20) was added and the suspension was gently stirred to evenly disperse the uredospore aggregates. The uredospores of the pimenta isolate (PISOL) were washed from leaves of infected *P. dioica* saplings obtained from nursery stock and suspensions were prepared as described above.

Uredospore suspensions of either isolate were then misted onto growing tips of their respective original host species until all expanding (young) leaves were completely wet. Plants of either species were then placed in separate growth chambers maintained at 14 to 18°C and covered with a polyethylene bag for 72 h to maintain high humidity. Uredospores produced on the leaves of these saplings were extracted and used for host range tests within 2 h.

Host Plant Inoculation

Eighteen myrtaceous plant species were evaluated in two experiments (Table 1). Test plants of each species were inoculated separately with uredospores of either MISOL or PISOL. Uredospore concentrations in suspensions of both MISOL and PISOL were determined, and the same batch and concentration of these spores of a given isolate were used to treat all of the plants within an experiment.

Experiment I. This experiment was performed from December 1998 through February 1999. The inoculum concentrations of MISOL and PISOL used in this experiment were 3.7×10^5 and 5.0×10^5 uredospores/ml, respectively. Two to three plants of each test species were sprayed separately with the inoculum suspension until the water began to drip from the leaves. The inoculated plants were placed in separate growth chambers (one for each isolate) and maintained under 12 h fluorescent light at 15°C–18°C. The plants were covered with plastic bags to raise the relative humidity to 95%. After 72 h, the bags were removed and the plants transferred to two separate plastic enclosures (1.5 m tall × 2.5 m long × 90 cm wide), one for each isolate, constructed from polyethylene sheeting tacked to a wooden frame on a bench in an air-conditioned room. The plants were watered twice weekly to saturation. During the 4-week experimental period, temperature and relative humidity in the plastic chambers ranged from 19 to 23°C and 65 to 95%, respectively. The plants were maintained under 12-h fluorescent light cycles in a growth chamber. Controls included at least one plant of each species that was not inoculated with *P. psidii*. Control plants were also sprayed with sterile deionized water, covered with plastic for 72 h, and then placed in a separate plastic chamber in the same room as the rust-inoculated plants.

Experiment II. This was performed during March–May 1999. The new growth (2- to 4-week-old with expanding leaves) was misted with water to wet the leaf surfaces. Uredospores were collected in the morning and dusted and/or brushed on the wet surfaces of expanding foliage. The inoculated plants were then placed in two separate growth chambers under 12-h fluorescent light cycles at 14 to 18°C and covered with polyethylene sheets to raise the relative humidity to 95%. The number of MISOL and PISOL uredospores on the wet leaves were estimated to be ca. 10 and 11 spores/mm^2, respectively. After 72 h, the bags were removed and the MISOL- and PISOL-treated plants were transferred from growth chambers to two separate, partially shaded shadehouses. Open, water-filled plastic containers were placed between treated plants, which were surrounded by untreated melaleuca plants, to maintain high ambient humidity. The plants were watered daily to saturation. During the experimental period, temperature and relative humidity in the shadehouse ranged from 14 to 33°C and 65 to 95%, respectively.

Symptoms and Disease Effects on Melaleuca

P. psidii-inoculated plants grown in a shadehouse in Experiment II and naturally infected trees in the field were monitored for symptoms of the disease. These were recorded and photographed as they developed.

Disease Assessment

The criteria employed by Politis *et al.* (1984) to evaluate *P. carduorum* Jacky were modified to determine the susceptibility of test plants to *P. psidii* isolates 14 days after inoculation. These criteria were, 0 = no symptoms (immune), 1 = chlorotic halos and or brown spots but no sporulation (resistant), and 2 = erupted pustules associated with active sporulation (susceptible).

Total length (L) and width at the widest portion (W) of 20 healthy leaves of different ages and dimensions were measured for each test species, and their actual leaf area (L_A) was determined by overlaying a transparent graph sheet. A multiplying factor (M) was developed by using the expression, $M = L_A/(L \times W)$. The multiplying factor developed for each test plant was used to estimate the area of an individual symptomatic leaf of a given test-plant species. Pustule densities (number of pustules/mm^2) and disease severity (percentage of leaf area covered with pustules) were then determined for each leaf selected for evaluation.

Plants treated with uredospores were evaluated for pustule formation, presence of spores, and leaf senes-

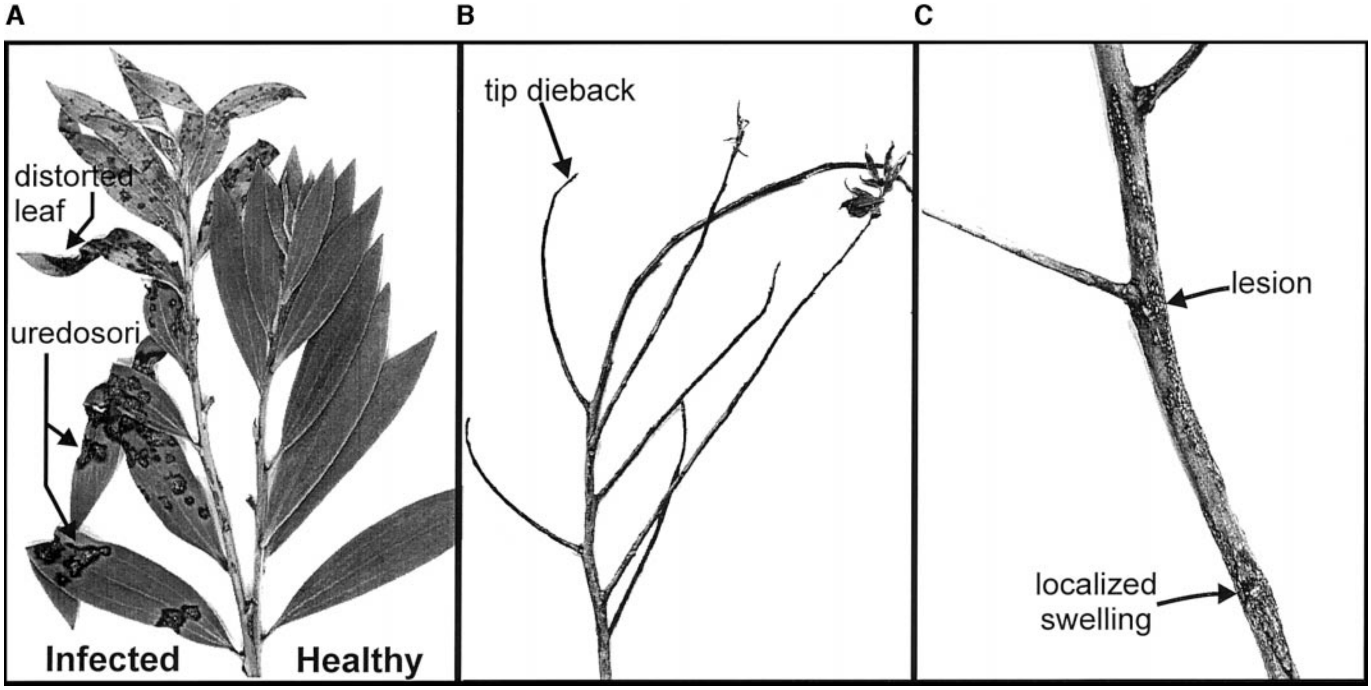

FIG. 1. Effects of *Puccinia psidii* on *Melaleuca quinquenervia* plants. (A) Healthy (right) and infected (left) shoot tips; (B) a severely defoliated twig showing tip dieback; (C) a portion of defoliated twig showing localized swellings and lesions.

cence at 14 days after inoculation. Since the number of inoculated leaves varied with each plant species, evaluations were done as follows. For plants with <10 inoculated new leaves, all inoculated leaves were evaluated. For plants with >10 new leaves at inoculation, only the 10 most severely infected leaves were evaluated to represent the worst case scenario of plant susceptibility under the test conditions. These leaves were tagged and the lengths (L) and widths (W) were measured. On the same day, each leaf was evaluated for the total number of chlorotic halos and/or pustules (densities), the percentage of total leaf area covered by pustules (disease severity), and the senescence of leaf and/or branch tips. These evaluations were repeated for the same leaves at 21 and 28 days after inoculation.

Data Analyses

Analyses of variance (ANOVA) on pustule density and disease-area (disease severity) were performed using SAS software (SAS Institute, 1985). Only plant species with *P. psidii* sporulation on their expanding leaves were included in the ANOVA and mean separations. Mean separations were performed using the Waller-Duncan's *t*-test procedure.

RESULTS

Symptoms and Disease Effects on Melaleuca

Typical symptoms produced by *P. psidii* on melaleuca in field and shadehouse conditions are presented in Fig. 1. *P. psidii* isolates infected expanding foliage as well as succulent twigs of *M. quinquenervia.* After infection, leaves developed minute chlorotic haloes within 7 to 10 days and the initial eruptive pustules appeared within 10 to 14 days. Over time, the pustules rapidly increased in size and coalesced, and the entire leaf became covered with a mass of yellow uredospores. Sporulation occurred on both leaf surfaces. Severe infection (>50% leaf coverage) caused extreme leaf distortion that ultimately resulted in defoliation. Even with light infection (<10% leaf coverage), leaves near growing branch tips quickly lost turgor, desiccated, and then turned gray to black. Severe infection of the succulent stems caused the tips to lose turgor and to die. Severely infected twigs with live bark developed bark-limited lesions that often developed into localized swellings. These twigs were usually completely defoliated and became brittle, breaking easily at the point of swelling. Infected *M. quinquenervia* and *E. reinwardtiana*-reproductive structures (flowers and immature fruits) were often encountered in the field and at nurseries.

Disease Assessment

Despite the differences in the number of new leaves available for inoculation in different plant species, the susceptible tissues of all species received equal amounts of inoculum per unit area. Therefore, differences in symptom expression and diseased leaf area can be attributed to the relative susceptibility of the

TABLE 1

Disease Rating of *Puccinia psidii* on Myrtaceous Plants at 14 Days after Inoculation with Uredospores

		Disease rating[a]			
		Experiment I[b]		Experiment II	
Plant species	Origin	MISOL[c]	PISOL	MISOL	PISOL
Calyptranthes pallens Griseb.	Native	0	0	0	0
Callistemon viminalis (Gaertner) G. Don	Exotic	2	2	2	0
Eucalyptus grandis	Exotic	NT	NT	NT	1
Eugenia reinwardtiana (Blume) DC.	Exotic	NT	NT	2	2
Eugenia confusa DC.	Native	NT	NT	0	0
Eugenia foetida Pers.	Native	NT	NT	0	0
Eugenia paniculatum Gaert.	Exotic	0	0	1	0
Eugenia uniflora	Exotic	0	0	0	0
Feijoa sellowiana (O. Berg) O. Berg	Exotic	0	0	0	0
Myrciaria cauliflora (C. Martius) O. Berg	Exotic	2	2	2	2
Melaleuca decora (Salisb.)	Exotic	NT	NT	2	2
Melaleuca quinquenervia	Exotic	2	2	2	2
Myrcianthes fragrans (Sw.) Mc Vaugh	Native	2	1	2	2
Pimenta dioica	Exotic	2	2	2	2
Psidium guajava	Exotic	2	0	1	0
Psidium cattleianum Sabine	Exotic	0	0	0	0
Syzygium cumini	Exotic	1	1	0	0
Syzygium jambos	Exotic	0	0	0	0

[a] Disease ratings: 0, no disease symptoms; 1, chlorotic halos and/or brown spots but no sporulation; 2, erupted pustules associated with active sporulation (susceptible); and NT, plants not tested in this experiment.
[b] Isolates: MISOL, *Puccinia psidii* isolated from *M. quinquenervia* leaves; PISOL, *P. psidii* isolated from *Pimenta dioica* leaves.
[c] Experiments: I, growing branch misted with uredospores suspension, placed in growth chamber for 72 h, and then transferred to plastic chamber for incubation; Experiment II, dusted and/or rubbed with uredospores, placed in growth chamber for 72 h, and then transferred to shadehouse for further incubation.

plant species. Seven of 18 species inoculated with the two isolates of *P. psidii* during one or both experiments remained symptomless (immune), 3 species only developed chlorotic halos (resistant), and 8 species developed pustules (susceptible) within 14 days after inoculation with at least one *P. psidii* isolate (Table 1). The seven "immune" plant species remained symptomless after 28 days. However, the chlorotic halos on the leaves of 3 "resistant" plant species turned brown within that time period but never sporulated. In general, plants found to be susceptible in Experiment I were also susceptible in Experiment II.

The analysis of variance for pustule densities on the eight susceptible host species indicated that overall pustule densities (pustules/mm^2), as observed at Day 14, did not differ between experiments, but the differences were significant for rust isolates and tree species (Table 2). Therefore, the pustule-density data from the two experiments were pooled and means separations were performed by plant species (Fig. 2). Pustule densities were significantly different among the eight susceptible test species. The pustule densities on MISOL-inoculated leaves were greatest for *E. reinwardtiana* (0.347 pustules/mm^2), and least for *P. guajava* (0.005 pustules/mm^2). Based on pustule densities, both *M. quinquenervia* (0.178 pustules/mm^2) and *P. dioica* (0.166 pustules/mm^2) were relatively less susceptible than *E. reinwardtiana* to the MISOL isolate. The pustule densities on PISOL-inoculated leaves were signif-

TABLE 2

Analyses of Variances for Pustule Densities and Diseased Area on Expanding Leaves of Myrtaceous Plants at 14 Days after Inoculation with Uredospores of the Rust *Puccinia psidii*

Variables	*df*	MS	*F* value	Pr > *F*
Pustules[a]				
Experiment	1	0.0016	0.09	0.7596
Isolates	1	0.0662	3.90	0.0496
Tree species	7	0.1523	8.97	0.0001
Experiment * Isolates	1	0.0364	2.15	0.1445
Experiment * tree species	5	0.0160	0.94	0.4533
Isolate * tree species	7	0.0281	1.65	0.1223
Diseased[b]				
Experiment	1	1092.4190	5.51	0.0197
Isolates	1	1082.3167	5.47	0.0203
Tree species	7	3205.2134	16.20	0.0001
Experiment * Isolates	1	0.3423	0.00	0.9669
Experiment * tree species	5	1253.1784	6.33	0.0001
Isolate * tree species	7	516.3762	2.61	0.0133

[a] Number of pustules/mm^2 of the most severely diseased expanding leaves inoculated with uredospore suspension.
[b] Leaf area (mm^2) covered by the pustules.

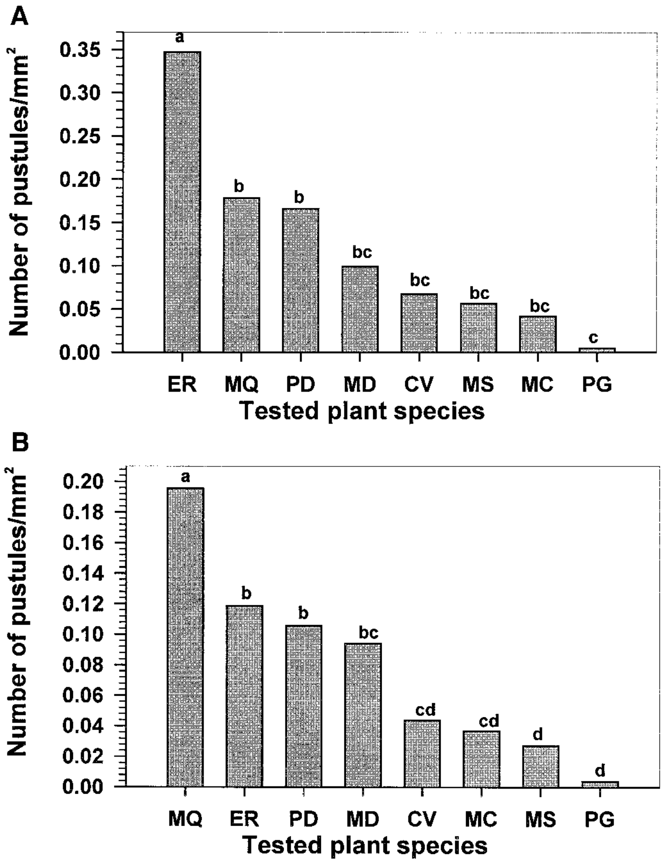

FIG. 2. Pustule density of *Puccinia psidii* isolates as evaluated on Day 14 after inoculation, with Experiments I and II combined. (A) *Melaleuca quinquenervia* isolate (MISOL); (B) *Pimenta dioica* isolate (PISOL). CV, *Callistemon viminalis;* ER, *Eugenia reinwardtiana;* MD, *Melaleuca decora;* MQ, *M. quinquenervia;* MS, *Myrcianthes fragrans;* MC, *Myrciaria cauliflora;* PD, *Pimenta dioica;* PG, *Psidium guajava.*

icantly greater for *M. quinquenervia* (nearly 0.20 pustules/mm^2) than for any other plant species tested. Both isolates caused intermediate levels of disease on *Melaleuca decora, C. viminalis, Myrcianthes fragrance,* and *Myrciaria cauliflora.*

The amount of leaf area covered by eruptive pustules (disease–area) has been used as an indicator of disease severity. This assumes that disease severity is a function of the density and size of the erupted pustules which in turn reflects the host susceptibility. The analyses of variance for diseased leaf area (mm^2) on eight susceptible host species, as observed at Day 14, showed significant effects of experiments, rust isolates, and tree species (Table 2). Therefore, the disease–area means separations were performed by experiment (Table 3). Due to a wide variation in disease–area among leaves of a given test plant, the mean differences between isolates in Experiment I were not significant. In Experiment II, the MISOL isolate produced more disease–area on *Eugenia reinwardtiana* and *M. quinquenervia,* while the PISOL isolate produced more disease–area on *P. dioica* and *M. quinquenervia* plants.

DISCUSSION

Historically, the rust *P. psidii* is neotropical in origin and its host range is limited to the family Myrtaceae (Laundon and Waterson, 1965; MacLachlan, 1936, 1938; Marlatt and Kimbrough, 1979; Coutinho *et al.*, 1998). Therefore, host range tests presented in this study were restricted to common myrtaceous species that occur in the south Florida area. These included four of the eight species that occur naturally in Florida as well as several exotic species used in ornamental plantings. *P. psidii* disease development, pustule morphology, and damage to young foliage and succulent stems of *M. quinquenervia* in our experiments were similar to those described for *Pimenta* species (Laundon and Waterson, 1965; MacLachlan, 1936, 1938; Marlatt and Kimbrough, 1979).

Based on Bruzzese and Hasan's (1986) scoring system, *C. viminalis, E. reinwardtiana, M. decora, M. quinquenervia, M. fragrans, M. cauliflora, P. dioica,* and *P. guajava* were found susceptible to both the MISOL and PISOL isolates. This showed that the Florida isolates of *P. psidii* are capable of infecting these species. However, disease severity was greater on MISOL-inoculated *E. reinwardtiana, M. quinquenervia,* and *P. dioica* plants. Like *M. quinquenervia,* both *E. reinwardtiana* and *P. dioica* are exotic species in Florida, where they are used for ornamental purposes. On the other hand, *E. grandis, E. paniculatum,* and *S. cumini* reacted to *P. psidii* by manifesting chlorotic halos that developed into localized brown spots. Similar phenomenon in the rust *Phragmidium violaceum* (Schutz) Wint. and *Rubus fruticosus* L. pathosys-

TABLE 3

Diseased Leaf Area (mm^2)[a] of the Most Susceptible Plant Species 14 Days after Inoculation with *Puccinia psidii* Uredospores Obtained from Either *M. quinquenervia* (MISOL) or *P. dioica* (PISOL) Plants

	Experiment I		Experiment II	
Test plants	MISOL	PISOL	MISOL	PISOL
Callistemon viminalis	9.02a	6.97a	1.01b	9.42b
Eugenia reinwardtiana	NT	NT	44.53a	10.58b
Melaleuca decora	NT	NT	3.51b	NT
M. quinquenervia	16.43a	12.88a	40.22a	36.35a
Myrcianthes fragrans	9.61a	4.90a	11.90b	7.15b
Myrciaria cauliflora	4.59a	7.34a	13.48b	NT
Pimenta dioica	22.03a	11.48a	NT	37.09a
Psidium guajava	5.64a	NT	NT	NT

[a] Mean values followed by the same letter within a column are not significantly different from each other at $P = 0.05$, as determined by Waller-Duncan k ratio t test.

tem has been considered a resistance reaction (Bruzzese and Hasan, 1986). *E. grandis* provenances are reported to be variable in susceptibility towards isolates of *P. psidii* (Dianese *et al.*, 1984, 1986), and our test plants of this species may have been from a resistant provenance.

P. psidii originally described from *P. guajava* was reported during 1934 as a pathogen on *S. jambos* and *Pimenta* species in Jamaica (Smith, 1935) where it devastated the *Pimenta* industry (Smith, 1935; MacLachlan, 1938). Smith (1935) suggested that the rust strain attacking *Pimenta* species may have evolved from the one reported to cause disease on *S. jambos*. Two strains of *P. psidii*, one that attacks *Pimenta* species and another that attacks *S. jambos* and *Eugenia* species, have been reported by MacLachlan (1936) also. MacLachlan (1938) reported that the *P. psidii* strain from *S. jambos* infected and sporulated on *Syzygium malaccense*, but on *Pimenta* spp. it produced minute lesions that never sporulated. Similarly, *P. psidii* isolates from *Pimenta* sp. sporulated on *Pimenta* species but not on *Syzygium* species (MacLachlan, 1938). Based on previous work in Florida, *S. jambos* reportedly manifests fewer and smaller pustules 23 days after inoculation (Marlatt and Kimbrough, 1979). In our study, *S. jambos* did not develop the chlorotic halos and/or pustules that characterize *P. psidii*. However, *S. cumini* exhibited some chlorotic halos which did not sporulate but developed into shot holes 3 to 4 weeks after inoculation. Based on these pathogenic attributes, *P. psidii* isolates attacking *M. quinquenervia* appeared to be similar to the strain of *P. psidii* that attacked *Pimenta* species in Jamaica (MacLachlan, 1936). In a cross-inoculation study involving single-pustule isolates from *Eucalyptus* sp., *P. guajava*, and *S. jambos*, Coelho (1988) reported three physiologically different groups. Groups I, II, and III infected *Eucalyptus* sp. and *S. jambos*, *P. guajava* and *Eucalyptus* sp., and *P. guajava*, respectively. These findings indicate the existence of physiological races of the rust, *P. psidii*. Gene-for-gene relationships have been demonstrated for various physiological races of important cereal rusts (*Puccinia* spp.) in North America (Kolmer, 1997). Such gene-for-gene systems are considered responsible for coevolutionary interactions leading to the development of host resistance, pathogen pathogenicity, and aggressiveness (Burdon, 1997).

It has been suggested that host specificity tests of microbial pathogens should be conducted under controlled conditions to facilitate optimum infection and disease development on test plants (Watson, 1985). However, these artificial conditions can predispose plants to infection and may artificially increase the perceived host range of the organism being tested (Adams, 1988; Parker *et al.*, 1994; Watson, 1985). Other *Puccinia* spp. evaluated for introduction to North America have exhibited artificially expanded host ranges under controlled environmental conditions (Watson, 1985). In our study, the host range of two *P. psidii* isolates may have artificially expanded. This assumption is supported by the fact that under Florida field conditions, *C. viminalis* and *E. cumini* (determined to be susceptible under controlled environment in our experiments) trees found growing near *P. psidii*-infested melaleuca trees never exhibited symptoms of this rust infection. Similar false positive results have been reported for other rusts and their test-plant systems (Parker *et al.*, 1994; Watson, 1985) under controlled environment.

Analyses of numerous pathosystems have shown that most serious plant diseases are related to pathogens that originate from another host in different geographical areas (Hokkanen, 1985). The examples of such new associations are powdery mildew of grape [(*Uncinula necator* (Schwein.) Burrill] in Europe, swollen shoot disease of cacao in Ghana, and Dutch elm disease caused by *Ophiostoma (=Ceratocystis) ulmi* (Buisman) Nannf. in North America (Klinskowski, 1970). As opposed to an “old association” accounting for coevolution and balanced coexistence (interspecific homeostasis), a “new association” may lack both coevolution and balanced coexistence and therefore result in more successful biological control of pests (Hokkanen and Pimentel, 1984; Hokkanen, 1985). It has also been noted that the deployment of the “new association” principle may increase the array of natural enemies available for utilization in biological control programs (Hokkanen and Pimentel, 1984). However, this “new association” principle has been refuted by Goeden and Kok (1986) as a preferred method of selection of biological control agents. According to Goeden and Kok (1986), the new association principle is mainly based on cactus biocontrol, and the cacti are not representative of other target weeds. Biological control agents involved in new associations are, by definition, not host-specific (Hokkanen and Pimentel, 1984) and, thus, may carry more risk of nontarget effects.

Our host test added one genus (*Myrcianthes*) and three species (*C. viminalis*, *M. decora*, and *M. fragrance*), previously not reported as hosts for *P. psidii* (Coutinho *et al.*, 1998; Marlatt and Kimbrough, 1979; Rayachhetry *et al.*, 1997). *P. psidii* may have been introduced to Florida with susceptible plant materials, and its earliest record on *Pimenta* species in Florida dates back to the late 1970s (Marlatt and Kimbrough, 1979). *P. psidii* was previously recorded in Brazil (Viégas, 1961) on *M. leucadendra* (L.) L., a close relative of *M. quinquenervia*. Despite the presence of *P. psidii* in Florida as a pathogen on *Pimenta* spp., the first outbreak of this rust disease on *M. quinquenervia* was not observed until the Fall of 1996 (Rayachhetry *et al.*, 1997). Considering its history of geographical expansion and increasing numbers of host species in the family Myrtaceae, *P. psidii*–*M. quinquenervia* pathosystem appears to be an example of a "new association," and it may contribute to the *M. quinquenervia* control along with other classical biological control agents, herbicides, and mechanical removal as part of an integrated management program.

Host screening showed that the most susceptible species were exotic. Of the four native species tested, only twinberry stopper (*M. fragrans*) developed minor symptoms of infection, and it appeared to be relatively less susceptible than other plants even in an optimal environment. Therefore, use of *P. psidii* as a augmentative biological control would probably not impact native species, but the breadth of the potential host range requires a cautious approach. However, the realized host range of *P. psidii* in Florida should be determined by exposing these plant species to inoculum sources under natural field conditions.

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