New Disease of *Syngonium podophyllum* 'White Butterfly' Caused by a Pathovar of *Xanthomonas campestris*

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ABSTRACT

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A serious new foliar blight of *Syngonium podophyllum* 'White Butterfly' is described. A slow-growing xanthomonad that readily caused symptoms of blight was consistently isolated from affected plants. The bacterium was specific to species and cultivars of *Syngonium*, causing blight within 10 days of misting with a suspension containing 1×10^8 cfu/ml. Standard biochemical tests as well as electron microscopy showed the pathogen to be *Xanthomonas campestris*. Biochemical and biological comparisons of *X. c.* pv. *dieffenbachiae* and *X. c.* pv. *vitians* were performed because both have been reported as pathogens of *Syngonium* spp. In vitro tests, including growth response to temperature, carbohydrate utilization, gelatin hydrolysis, and pectolysis as well as fatty acid analysis, revealed differences among the three groups of organisms. Additionally, symptomatology on *S. podophyllum* and host range varied considerably among the strains.

Plants in the Araceae family are among those most widely grown and include many cultivars and species of *Aglaonema, Dieffenbachia, Philodendron,* and *Syngonium*. Bacterial diseases of foliage plants have caused serious losses over the past 20 yr. Many of the 500 varieties grown in Florida are susceptible to at least one bacterial pathogen from the genera *Erwinia, Pseudomonas,* and *Xanthomonas* (17). *Xanthomonas campestris* pv. *dieffenbachiae* McCull. & Pirone has caused diseases of these plants for many years with the original description made in 1939 by McCulloch and Pirone on *Dieffenbachia maculata* (Lodd.) G. Don (=*D. picta*) (9). Many other important genera in this family are also susceptible to *X. c.* pv. *dieffenbachiae* (3,10,14,18). In addition, an abstract was published in 1969 reporting *X. c.* pv. *vitians*, originally described from lettuce (1), as a pathogen of *Syngonium* spp., but a legitimate description has not been made (19).

In 1982, a serious blight disease was first noted on *Syngonium podophyllum* Schott 'White Butterfly,' a relatively new cultivar. Water-soaking of interveinal tissue was most prevalent during the early morning hours and frequently disappeared as the day progressed. The development and disappearance of water-soaking occurred for up to 10 days in the same portions of leaf tissue before chlorosis and necrosis developed. The development of severe chlorosis and necrosis was very rapid, once initiated; as much as 50% of the foliage area was chlorotic and necrotic within 2 days (Fig. 1). Lesions were nearly always confined within leaf veins and developed a papery appearance when allowed to dry. Lesion expansion, however, did not cease when overhead watering was eliminated, as usually occurs with bacterial diseases of this plant (17).

Disease symptoms suggested a bacterial pathogen as the cause, but attempts to isolate the causal agent within a 3-yr period were unsuccessful. The nature and severity of symptoms, time of year with greatest disease incidence, tolerance to standard bactericides, and difficulty in isolating the causal agent suggested that the disease might not be caused by the bacterial pathogens previously described from *Syngonium*. Therefore, the following research was performed to determine the causal agent of this disease of *S. podophyllum* 'White Butterfly.'

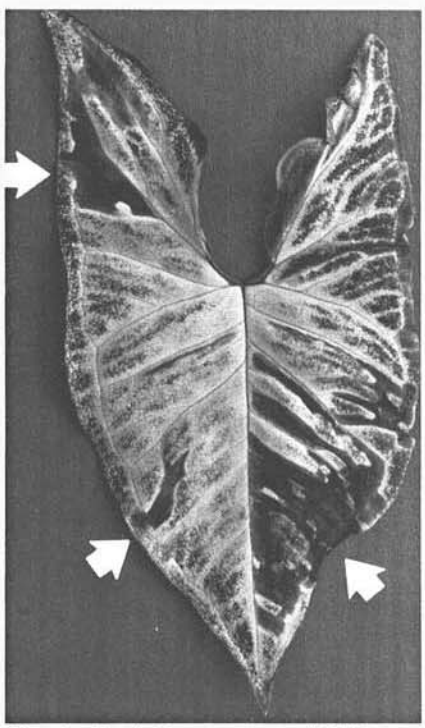

Fig. 1. Typical symptoms of blight on *Syngonium podophyllum* 'White Butterfly' caused by *Xanthomonas campestris*. (Courtesy J. M. F. Yuen)

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MATERIALS AND METHODS

Isolation of suspect pathogen. Symptomatic leaf tissue was collected from 10 nurseries throughout Florida between January 1985 and March 1986. About 0.5 cm^2 of symptomatic leaf tissue was pulverized in a scintered glass tissue grinder containing 2 ml of sterilized 0.01 M $MgSO_4$. The resulting suspension was streaked onto each of the following media: crystal violet pectate medium (CVP) (16), King's medium B (KMB) (6), Difco nutrient agar medium (NA), starch medium (SX) (16), and Wilbrink's agar medium (WA) (8). Other culture media consisting of NA amended with 0.5% sucrose, dextrose, or peptone were used as well. Replicate plates were incubated at 25 and 30 C for up to 10 days. Suspect bacterial colonies were transferred serially three times to KMB, NA, or WA depending on their original isolation medium.

Inoculum production and preparation. Bacterial inocula were produced on either WA or NA amended with 0.5% sucrose (NAS). Streaked plates were incubated at 25 C for 3–5 days. Bacteria were removed from media surfaces by flooding with $MgSO_4$ and gentle rubbing with a sterilized cotton swab. Suspensions were collected and adjusted to 1×10^8 colony-forming units (cfu) per milliliter of $MgSO_4$ using a spectrophotometric method. Inocula were used within 30 min of preparation. Stock cultures of bacteria were maintained on NAS (suspect pathogen) or NA (others) and were transferred twice a week. Prolonged periods between transfers or storage in water resulted in loss of viability for suspect pathogen strains.

Electron microscopy. Naturally infected leaves showing water-soaked margins and bases of lamina were sampled. Water-soaked areas, adjacent normal tissue, and a vein were included with each sample. Leaf pieces were fixed in 2% glutaraldehyde and 1.5% acrolein in 0.05 M PO_4 buffer, dehydrated in a graded series of ethanol, and embedded in Spurr's medium.

Bacteria from a pure culture of the suspect pathogen on NA were washed from plates and diluted in distilled water and stained with 0.25% sodium phosphotungstate, pH 7.0. Other preparations were air-dried and rotary-shadowed with palladium-gold.

Plant production. All *S. podophyllum* 'White Butterfly' were obtained directly from tissue-culture producers to limit potential for exposure to plant pathogens. Explants were established in steam-treated (1.5 hr at 90 C) Canadian peat and pine bark (1:1, v/v) amended with 4.4 kg Osmocote (19:6:12, Sierra Chemical Co., Milpitas, CA), 4.0 kg dolomitic lime, and 0.9 kg Micromax (micronutrient source from Sierra) per cubic meter after steaming. Explants were rooted under intermittent mist (5 sec/30 min, 12 hr/day) for about 3 wk and then removed and irrigated once or twice a week depending on the season. All tests were performed in a glasshouse with temperatures ranging from 18 to 35 C and a maximum natural light level of 200 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. Other plant cultivars or species were obtained as small-rooted cuttings from commercial producers, planted in the potting medium, and grown under the conditions described.

Pathogenicity trials. Two strains of the suspect pathogen were tested on White Butterfly. Inocula and plants were produced as described. Five plants each were inoculated with a bacterial suspension (1×10^8 cfu/ml) or treated with $MgSO_4$ alone using one of the following inoculation methods: 1) misting onto plant foliage without wounding, 2) misting onto plant foliage with one wound per leaf made with a sterilized dissecting needle, 3) injecting an area equivalent to 0.5 cm^2 on each of five leaves per plant, and 4) drenching potting medium at the rate of 10 ml/10-cm pot.

In a separate test, bacterial colonies were transferred directly to wounded leaves, stems, and petioles. Some plants were exposed to intermittent mist (described above) starting 1 day before inoculation and continuing until test completion; others were watered by hand to maintain dry foliage.

All plants were enclosed in polyethylene bags for 3 days after inoculation whether they were exposed to misting or not. Tests were performed using a randomized complete block design. Development of symptoms was monitored for 6 wk after inoculation. Foliar symptoms were rated as the percentage of the foliage area with symptoms of blight. Reisolation of the suspect pathogen from symptomatic tissue was attempted using the methods described for the original isolations and NAS and NA media. These tests, or parts thereof, were performed three times during the fall and winter of 1985–1986.

A second series of tests was performed to compare virulence of nine strains of the suspect pathogen on White Butterfly. Plants were produced and tests were performed as described. Five plants each were inoculated with a strain of the suspect pathogen or treated with $MgSO_4$ by misting onto foliage of unwounded plants. Plants were exposed to intermittent misting 1 day before inoculation and until test completion. Plants were arranged in a randomized complete block design after they were removed from bags. The percentage of foliage area showing symptoms 10–21 days later was estimated for each plant. These methods were used for all subsequent pathogenicity trials on syngonium. This test was performed three times between July and September 1986.

The effect of inoculum concentration on symptom expression was tested using one strain of the suspect pathogen (X157)

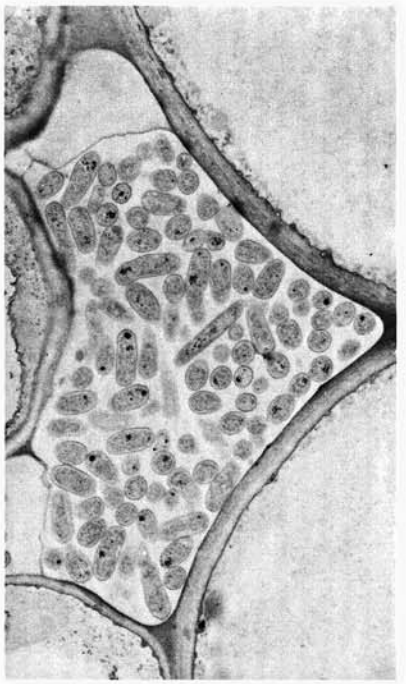

Fig. 2. Ultrathin section of symptomatic leaf tissue of *Syngonium podophyllum* 'White Butterfly' showing intercellular bacteria.

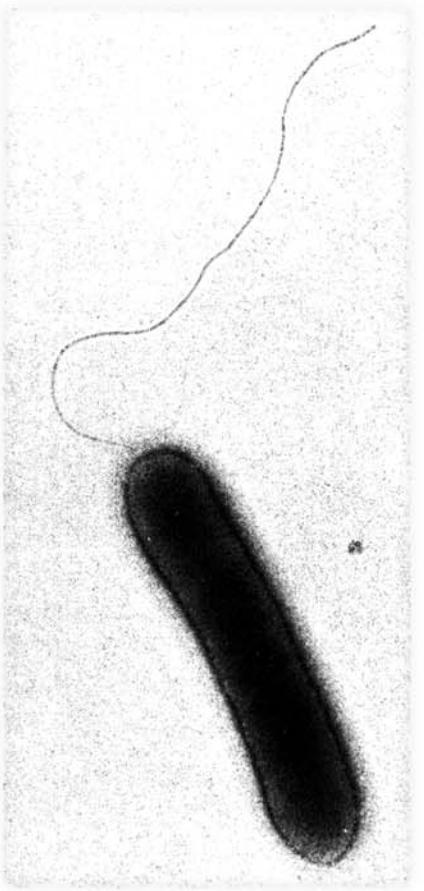

Fig. 3. Negatively stained view of *Xanthomonas campestris* from *Syngonium podophyllum* showing a monotrichous flagellum.

on White Butterfly. Inoculum of X157 was prepared as previously described and diluted to the following concentrations: 1×10^8, 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, and 1×10^2 cfu/ml $MgSO_4$. White Butterfly plants, in 10-cm pots, with about 10 leaves each were inoculated, as described for virulence tests, using 2 ml of the appropriate suspension for each of five plants. The percentage of foliage area showing symptoms of blight was estimated for each plant after 10–21 days, depending on the test. This test was performed twice between April and May 1986 using a randomized complete block design.

Host range of suspect pathogen. The first host range test evaluated pathogenicity of the suspect pathogen on the following members of the Araceae family: *Aglaonema commutatum* Schott 'Silver Queen,' *A. modestum* Schott ex Engl. (Chinese evergreen), *Anthurium andraeanum* Linden (Andrae's flamingo flower), *Dieffenbachia amoena* Bull. 'Tropic Snow,' *D. maculata* (Lodd.) G. Don 'Perfection,' *Epipremnum aureum* (Linden & Andre) Bunt. 'Golden pothos' and 'Marble Queen,' *Monstera deliciosa* Liebm. (Swiss-cheese plant), *Philodendron hastatum* C. Koch & H. Sello, not Hort. 'Red Emerald,' *P. scandens* C. Koch & H. Sello subsp. *oxycardium* (Schott) Bunt. (heartleaf philodendron), *Spathiphyllum* sp. L. 'Vallisii' and 'Bennett,' and White Butterfly. Five plants of each species, obtained from commercial growers, were exposed to intermittent mist and inoculated with X157 or treated with $MgSO_4$ by spraying unwounded foliage to runoff. Plants were placed in polyethylene bags for 5 days after inoculation and then arranged in a randomized complete block design. Plants were incubated for 6 wk before final evaluation. This test was performed twice between February and May 1986.

The second host range test evaluated relative susceptibility of the following cultivars of *S. podophyllum* and the species *S. wenlandii* Schott to X157: Cream, Emerald Gem, Emerald White, Frosty, Maya Red, Noak, Pinkie, Roxanne, Silver Quill, Variegated Emerald Gem, and White Butterfly. Five plants of each cultivar or species were inoculated with the suspect pathogen or treated with $MgSO_4$, placed in polyethylene bags for 3 days, and then arranged in a randomized complete block design. Disease severity was estimated as the percentage of the foliage area with symptoms after 10–21 days. This test was performed twice between March and May 1986.

Identification and characterization of suspect pathogen. Biochemical characterization of the suspect pathogen (nine strains) and comparison with *X. c.* pv. *dieffenbachiae* (four strains) and *X. c.* pv. *vitians* (two strains) were performed using the following tests: Gram reaction (16), utilization of carbohydrates and oxygen requirement using Hugh-Leifson's medium (4), asparagine utilization (2), production of xanthomonadin (5), mucoid growth on WA, gelatin or casein hydrolysis (2), pectolysis on CVP medium (16), and growth on SX medium (16). Induction of a hypersensitive reaction was tested on *Capsicum annuum* L. 'Early Calwonder' pepper, *Lycopersicon lycopersicum* (L.) Karst. ex Fariv. 'Bonny Best,' and *Nicotiana tabacum* L. 'Hick's' tobacco (7). Carbon source utilization was tested for nine strains each of the suspect pathogen and *X. c.* pv. *dieffenbachiae* according to Misaghi and Grogan except that Difco Noble agar was used in place of Ion agar No. 2 (12). Fatty acid analyses were performed by M. Sasser (University of Maryland) on nine strains of the suspect pathogen (11).

Table 1. Relative virulence of strains of *Xanthomonas campestris* isolated from *Syngonium podophyllum* 'White Butterfly' on the plant

	Mean percentage of leaf area with symptoms[y]		
Strain no.	Test 1 (14 July)	Test 2 (13 August)	Test 3 (5 September)
Control	0 a[z]	0 a	0 a
X155	4 ab	33 cd	35 e
X156	46 f	22 bc	22 cde
X157	39 ef	54 e	19 bcd
X158	42 ef	41 de	4 ab
X159	15 bc	23 bc	1 a
X160	23 cd	14 b	24 de
X161	29 de	20 bc	12 abcd
X162	2 ab	29 cd	6 abc
X163	6 ab	33 cd	16 abcd

[y] Values represent a mean for five plants per treatment. Mean percentage of leaf area with symptoms was estimated visually.

[z] Mean separation in columns by Duncan's new multiple range test; values not followed by the same letter differed significantly at $P = 0.05$.

Table 2. Susceptibility of *Syngonium* species and cultivars to *Xanthomonas campestris* isolated from *S. podophyllum* 'White Butterfly'

	Mean percentage of leaf area with symptoms	
Species or cultivar	Test 1 (17 March)	Test 2 (22 April)
S. podophyllum		
Cream	11.6 ab[z]	7.0 a
Emerald Gem	12.3 ab	9.0 a
Emerald White	13.0 ab	8.0 a
Frosty	19.6 ab	28.0 b
Maya Red	45.7 c	7.0 a
Noak	16.6 ab	2.0 a
Pinkie	31.6 bc	12.0 a
Roxanne	16.2 ab	4.0 a
Silver Quill	16.2 ab	15.0 ab
Variegated Emerald Gem	14.3 ab	13.0 a
White Butterfly	17.7 ab	13.0 a
S. wenlandii	9.2 a	14.0 a

[z] Mean separation in columns by Duncan's new multiple range test ($P = 0.05$).

RESULTS

Isolation of the suspect pathogen. A pale yellow to cream-colored bacterium was consistently isolated from symptomatic leaves of White Butterfly. The bacterial colonies grew on WA medium only and were first evident about 5 days after their isolation when incubated at 25 C. No colonies of the suspect pathogen grew on any plates incubated at 30 C. Colonies remained less than 1 mm in diameter (up to 1 mo), were cream-colored, and frequently died unless transferred to fresh plates as soon as they could be seen on the original plates. Subsequent transfers to WA or NAS on a 4- or 5-day interval proved necessary to retain viability. One month after the original isolation, colonies became progressively more yellow and attained faster growth when transferred weekly to fresh NAS or WA plates. Additional isolations directly to WA and NAS from asymptomatic leaves, petioles, stems, and roots of symptomatic plants also yielded the suspect pathogen. Nine strains from these isolations were serially transferred on NAS and used for subsequent tests.

Electron microscopy. Ultrathin sections of symptomatic leaf tissue showed intercellular bacteria (Fig. 2), which was similar to infections produced by strains of *X. campestris*. Some degradation of the cell walls occurred along the cells adjacent to areas of bacterial accumulation. High concentrations of bacteria were present in areas of tissue collapse.

Bacteria were also occasionally observed in xylem vessels. Transport through the vascular system, as well as intercellularly, may account for the systemic movement of the organism from wounded roots to the leaves, with symptoms appearing within about 2 wk under optimum environmental conditions.

Negatively stained bacteria had a single polar flagellum characteristic of *Xanthomonas* (Fig. 3).

Pathogenicity trials. Of the strains tested, only those of the suspect pathogen caused blight symptoms on White Butterfly. Symptom development varied with the inoculation method used. Optimal disease development occurred on plants exposed to misting and spray-inoculated with a suspension of the suspect pathogen. Water-soaking appeared about 10 days after inoculation. Lesions developed interveinally and were water-soaked for as short a time as 2 days or as long as 2 wk before turning chlorotic and necrotic. Water-soaking was most evident in the morning, usually disappearing by noon but reappearing in the same tissue the next morning. Within as little as 2 wk after inoculation, more than 50% of the foliage area could become necrotic. Inoculation by injection followed a similar pattern; injected panels become symptomatic about 10 days after inoculation. Lesions developed in adjacent panels and on uninoculated leaves within 14–21 days of inoculation.

Although intermittent misting affected speed of development, symptoms occurred in the absence of overhead irrigation. Wounding leaves did not affect disease development when plants were spray-inoculated with a bacterial suspension of the suspect pathogen. Stab inoculations of petioles and stems with undiluted bacteria also resulted in development of leaf blight symptoms, although this method was not as rapid or reliable as spray inoculation. Plants inoculated with root drenches of a bacterial suspension also developed foliar symptoms, but occasionally, another phase of disease developed. Individual lower leaves turned chlorotic, wilted, drooped, and dried. When a new leaf unfolded, water-soaking of the entire leaf occurred within 7–10 days. Although distinct lesions did not develop on these leaves, they became chlorotic, wilted, and dried within 20 days of the onset of water-soaking. Reisolation of the suspect pathogen was successful on NAS from asymptomatic and symptomatic tissues on plants showing blight symptoms. Plants treated with $MgSO_4$ did not develop blight symptoms, and the suspect pathogen was not reisolated from them. These observations, as well as electron microscopy and isolation of the causal organism, demonstrate the systemic nature of this pathogen.

Comparisons of nine strains of the pathogen indicated that, although variability in virulence occurred, some strains were consistently more virulent than others (Table 1). Time of year did affect severity of disease expression. Strain source and time maintained in vitro were not correlated to virulence (data not shown).

Inoculum concentration trials demonstrated that as few as about 2×10^4 cfu/plant were necessary for development of blight symptoms within 2 mo of inoculation. Time elapsed between inoculation and onset of symptoms was directly influenced by initial inoculum density. Plants inoculated with 2×10^8 or 2×10^7 cfu showed symptoms 11 days after inoculation, whereas those inoculated with 2×10^6 or 2×10^5 cfu showed symptoms after about 17 days, and plants inoculated with 2×10^4 cfu showed symptoms after 24 days only. Plants inoculated with lower concentrations did not develop symptoms.

Host range of causal bacterium. Spray inoculations of the causal bacterium (1×10^8 cfu/ml) onto either wounded or nonwounded leaves failed to incite disease symptoms on plants other than

Table 3. Biochemical and biological reactions of the causal bacterium from *Syngonium podophyllum* 'White Butterfly,' *Xanthomonas campestris* pv. *dieffenbachiae*, and *X. c.* pv. *vitians*

Test[a]	Causal bacterium (White Butterfly)	*X. c.* pv. *dieffenbachiae* (four strains)	*X. c.* pv. *vitians* (two strains)
Gram reaction (-rod)	9	4	2
Aerobic	9	4	2
Growth in asparagine broth	0	0	0
Xanthomonadin pigment	6	4	2
Casein hydrolysis	5	4	2
Gelatin hydrolysis	0	4	2
Starch hydrolysis	0	0	0
Mucoid growth	8	3	2
Pectolysis on CVP	0	3	0
Growth on SX	0	3	2
Growth on NA	0	4	2
Pathogenic on syngonium	9	3	1
Pathogenic on dieffenbachia	0	3	0
Hypersensitive reaction:			
Bonny Best tomato	9	4	2
Hick's tobacco	8	3	0
Early Calwonder pepper	3	3	1

[a]CVP = crystal violet pectate medium, SX = starch medium, and NA = Difco nutrient agar medium.

Table 4. Carbon source utilization for strains of the causal bacterium from *Syngonium podophyllum* 'White Butterfly' and *Xanthomonas campestris* pv. *diffenbachiae*

Carbon source	*X. campestris* (White Butterfly)	*X. c.* pv. *dieffenbachiae*
Adonitol	–[a]	+
L-Arabinose	+W	–
Citric acid	–	–
D-Fructose	–	++
Galactose	–	++
Glucose	–	++
i-Inositol	+W	+W
2 Keto-D-gluconate	–	+
Lactose	+W	+W
Maltose	+W	++
D-Mannitol	+W	+
Mannose	+W	++
α-Methylglucoside	–	+
L-Rhamnose	–	+W
D-Ribose	–	–
D-Sorbitol	+W	+W
Sucrose	+W	++
Trehalose	+W	+
D-Xylose	+W	+

[a]Reactions were negative (–), positive (+ or ++), and weakly positive (+W).

Table 5. Results of fatty acid analyses[a] for strains of the causal bacterium from *Syngonium podophyllum* 'White Butterfly'

Strain no.	Similarity index for most likely matches			
	Xanthomonas campestris	*X. c.* pv. *manihotis*	*X. c.* pv. *dieffenbachiae*	*X. c.* pv. *vitians*
X155-1[b]	0.586	0.533*[c]	0.529	0.459
X155-2	0.630	0.562	0.485	0.579*
X155-3	0.459	0.427*	0.407	0.338
X156-1	0.564	0.414	...[d]	0.559*
X157-1	0.515	0.379	0.288	0.510*
X157-2	0.509	0.375	...	0.504*
X158-1	0.606	0.558*	0.481	...
X159-1	0.282	0.214	0.258*	0.256
X159-2	0.437	0.372	...	...
X160-1	0.644	0.587	0.597*	...
X161-1	0.597	0.556*	0.523	0.467
X162-1	0.670	0.651*	0.549	...
X163-1	0.668	0.642*	0.538	0.507

[a] Fatty acid analyses were performed by M. Sasser (11).
[b] Three strains were submitted for analysis more than once.
[c] * = Best pathovar fit for each run.
[d] Not one of the three best fitting comparisons.

syngoniums (which developed typical blight symptoms within 14 days).

The causal bacterium incited typical symptoms of blight on all of the species and cultivars of *Syngonium* tested (Table 2). Differences in susceptibility were inconsistent, indicating little if any resistance in the population tested. Blight symptoms were the same on all species and cultivars.

Identification and characterization of causal bacterium. Reactions for the causal bacterium were generally consistent with those described for pathovars of *X. campestris* (Table 3). Significant differences occurred, however, when these strains were compared with *X. c.* pv. *dieffenbachiae* and *X. c.* pv. *vitians* for gelatin hydrolysis, pectolysis, growth on SX and NA, and pathogenicity on dieffenbachia. Strains of the causal bacterium were negative for gelatin hydrolysis, pectolysis, and growth on SX and NA. Carbon source utilization showed a major difference between the causal bacterium and *X. c.* pv. *dieffenbachiae* (Table 4). None of the strains of the causal bacterium grew well under the test conditions, whereas all strains of *X. c.* pv. *dieffenbachiae* were able to utilize many of the carbon sources tested. Fatty acid analysis indicated further differences between the causal bacterium and standard strains of *X. c.* pv. *dieffenbachiae* and *X. c.* pv. *vitians* used for these analyses. Some strains were analyzed multiple times, giving variable results (Table 5). Six of 13 analyses showed the highest similarity index for *X. c.* pv. *manihotis*, whereas only two of 13 were most similar to *X. c.* pv. *dieffenbachiae* and four of 13 were most similar to *X. c.* pv. *vitians*.

DISCUSSION

A strain of *X. campestris* has been found causing a severe new disease of *Syngonium* species and cultivars. Biochemical and biological comparisons of this strain from White Butterfly with strains of *X. c.* pv. *dieffenbachiae* have revealed important differences between the two organisms. Utilization of carbon sources, ability to grow on SX medium and utilize starch, pectolysis, growth on NA, and gelatin hydrolysis each were different for the two organisms. Furthermore, fatty acid analysis has shown a poor degree of similarity between the organisms. Perhaps most important, the two organisms cause distinct symptoms and have different host ranges within the Araceae family.

This blight of syngonium is a new disease that is widespread and devastating. Chemical control of this systemic pathogen has not been successful (A. R. Chase, *unpublished*), although chemical control of certain other diseases caused by a systemic pathovar of *X. campestris* has been at least partially successful (13).

Although these plants are produced in tissue-culture laboratories, they have not been adequately assayed for latent infections because the media employed for growth of most plant-pathogenic bacteria do not adequately support good growth of this pathogen. Even asymptomatic philodendron plants produced in tissue culture were found infected with species of *Erwinia, Pseudomonas,* and *Xanthomonas* (15).

Control of this disease should be based first on exclusion of the pathogen from the tissue-culture stock through adequate indexing, and second, on exclusion of the pathogen from the production environment through diligent and educated scouting and destruction of infected plants.

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