

Use of Monoclonal Antibodies and Pathogenicity Tests to Characterize Strains of *Xanthomonas campestris* pv. *dieffenbachiae* from Aroids

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ABSTRACT

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Six monoclonal antibodies (MAbs) were used to group 323 strains of *Xanthomonas campestris* pv. *dieffenbachiae*, isolated from 12 genera of aroids and collected from five Hawaiian islands, Florida, California, and Australia over 11 yr. Nine percent of these strains did not react with any of the six MAbs. Of the remaining 91%, 12 major serogroups were identified. Ninety-five percent of the *Anthurium* strains formed seven groups, four of which contained exclusively *Anthurium* strains. Thirty-

six of 43 strains from *Syngonium* reacted with the MAbs, including most of those strains previously considered to be "*X. vitians*" and recently described as a separate pathovar (pv. *syngonii*). However, strains of *X. c. vitians* from lettuce reacted only with *Xanthomonas*-specific MAbs and not with MAbs used to group *X. c. dieffenbachiae* strains. Strains isolated from *Anthurium* had a broader host range and were generally more virulent than strains from other aroids.

Xanthomonas campestris pv. *dieffenbachiae* (McCulloch and Pirone) Dye is the causal agent of Anthurium blight in Hawaii and elsewhere. Initially identified as a pathogen on *Dieffenbachia* (22), *X. c. dieffenbachiae* was first isolated from *Anthurium* by Hayward in Kauai, Hawaii (16). Hayward described the disease as a nonsystemic leaf blight similar to other leaf blight diseases caused by *X. c. dieffenbachiae* (7,20,30,35). On plants such as *Dieffenbachia*, *Philodendron*, and *Xanthosoma*, *X. c. dieffenbachiae* has been described as a leafspot pathogen that enters through the hydathodes or occasionally through the stomata of the host. In 1980, a devastating systemic blight of *Anthurium* was reported on the islands of Oahu and Hawaii (26). In this outbreak, the pathogen not only caused a serious leaf blight, but also invaded the vascular system (32) and eventually killed susceptible plants. The disease has been the major limiting factor to *Anthurium* production in the Hawaiian islands (33). In 1989, estimated losses on the island of Hawaii totaled \$2.74 million (33). Bacterial blight has been reported on *Anthurium* in California (10), Venezuela (15), Guadalupe, and Martinique (18), Jamaica (38), Tahiti (23), and the Philippines (24). In every case, *X. c. dieffenbachiae* was identified as the pathogen that caused foliar and systemic disease.

Control methods now consist primarily of sanitation and exclusion (25). The production of certified pathogen-free planting stock is one long-term solution. To pursue this solution, we have examined the serological and pathogenic properties of a large collection of suspected *X. c. dieffenbachiae* strains isolated over 11 yr from a wide range of aroid hosts and geographical regions

(21). Strains of *Xanthomonas* isolated from *Syngonium*, some that were typical of those described as the proposed pathovar "*syngonii*" (9,11) and others that have the characteristics of *X. c. dieffenbachiae*, were also included. Because many of these strains from *Syngonium* were first identified as *X. c. vitians* (36,37), strains of *X. c. vitians* from lettuce were included. Reactions of these strains to MAbs were used to separate the *X. c. dieffenbachiae* strains into serogroups. Representatives of each group were tested for pathogenicity on six aroid hosts and for virulence on three *Anthurium* cultivars.

MATERIALS AND METHODS

Bacterial strains. The strains tested and their origins are listed in Table 1. The strains were purified on tetrazolium chloride (TZC) medium (19), which was modified by reducing the final concentration of TZC to 0.001%, and tested for growth and starch hydrolysis on cellobiose-starch (CS) (27) or Fieldhouse-Sasser (39) medium. Bacteria were stored in phosphate-buffered saline (PBS) at room temperature or in Luria-Bertani broth with 25% glycerol at -20 C.

Monoclonal antibodies (MAbs). The MAbs were produced as reported previously (1). BALB/c mice were injected with strain A1024-3 from *Dieffenbachia*, and a second injection was made with strain A990-5 from *Anthurium*. Both strains were isolated locally and were pathogenic on their hosts of origin. Selected strains of *X. c. dieffenbachiae*, other xanthomonads, and other bacterial genera (1) were screened against the antibodies. Of these MAbs, three were selected and designated Xcd1 (clone 72E-E3-B9-C5, isotype IgG2a), Xcd3 (clone 72B-F9-B6-E9, isotype IgM), and Xcd7 (clone 72D-E10-C2-D9, isotype IgM).

Three other MABs were used. MAB Xcd2 (clone 97-2-1-1, isotype IgM) was made after immunization with strain 870, which was isolated in the 1984 epidemic of citrus bacterial spot disease in Florida. Xcd2 reacted only with strain 870, Mexican T strains (3), and some strains of *X. c. dieffenbachiae*. The isotypes and reactivities of the other two MABs, T1 and T2, made to Mexican strains of citrus bacterial spot, citrus bacteriosis, and *X. c. dieffenbachiae* strains (3), they were included in the panel of MABs to serotype *X. c. dieffenbachiae*. *Xanthomonas*-specific MABs X1 and X11 (1) also were used.

Serotyping. An ELISA procedure (27) was modified as follows. Plates were coated with cells at approximately 1×10^8 cfu/ml. The plates were washed three times with 0.16 M borate buffer (pH 8.3) and blocked for 30 min with 200 μ l of PBS containing 1% bovine serum albumin (BSA) per well. The reagents were diluted in PBS with 1% BSA: MABs (Xcd3, 1:2,000; T1, 1:16;

Xcd2, 1:32,000; Xcd1, 1:2,000; Xcd7, 1:4,000; T2, 1:8,000; X1, 1:1,000; and X11, 1:4,000), rabbit anti-mouse globulin (1:1,000), and Protein A conjugated to horseradish peroxidase (1:2,000) (BioRad Laboratories, Richmond, CA). Each reagent was dispensed (100 μ l per well) and incubated for 1 h. We determined optimum MAB dilutions from binding curves by using 10–12 *X. c. dieffenbachiae* strains to test each MAB. Each step was preceded by three borate buffer washes. The substrate, 0.04% 5-aminosalicylic acid with 0.003% H₂O₂ in phosphate-EDTA buffer (pH 6.8), was added (100 μ l per well) and incubated 45–60 min. Color reaction was measured at A_{450nm} on a microtiter plate reader (Titertek Multiskan, Flow Laboratories, Inc., McLean, VA) linked to an IBM PC computer. All samples were coated in duplicate, and control plates were run with each assay.

Reactions were compared to known positive and negative controls. Average values for two negative (*Erwinia herbicola* strain Eh1) wells for each antibody were subtracted from average values of two "test wells". From experiments comparing well-to-well variation of samples on a plate, samples were assigned reaction values based on the OD₄₅₀ for each antibody: 0 = -0.1–0.050; 1 = 0.051–0.100; 2 = 0.101–0.200; and 3 = >0.201. For serogrouping purposes, values of 0 and 1 were considered negative, and 2 and 3 were positive. Strains were grouped based on reactions to the six MABs. Strains negative for all MABs were checked by several bacteriological tests (12,34) and rechecked with MABs X1 and X11 (1); thus, nonxanthomonads were eliminated from this study.

Host range and virulence studies. Eighty-five strains from all serogroups and hosts were selected for host range studies on six aroids and one nonaroid. The inoculated plants were *Anthurium andraeanum* 'Mauna Kea', *A. scherzerianum*, *Dieffenbachia maculata* 'Compacta', *Syngonium podophyllum* 'White Butterfly', *Spathiphyllum* 'Tasson', *Epipremnum aureum* 'Marble Queen', and a nonaroid host, *Schefflera arboricola*. The *A. andraeanum* plants were tissue-cultured. The *Spathiphyllum*, *A. scherzerianum*, and *S. arboricola* plants were grown from seed. The others were propagated from disease-free, tissue-cultured stock plants. All plants were inoculated in the following manner. Cells from 24- to 48-h cultures grown on yeast dextrose agar (YDC) were harvested into sterile 0.85% saline, adjusted to A_{600nm} = 0.1, and further diluted to approximately $2-3 \times 10^4$ cfu/ml as confirmed by dilution plating. Within 5 min after harvest, the suspensions were injected into the leaf mesophyll, and the injected area (approximately 2 cm²) was marked. Each strain was inoculated onto one young (first fully expanded) and one mature (third fully expanded) leaf with duplicate inoculations on a second plant. Six to eight strains were tested per leaf. After inoculation, the leaves were covered with plastic bags. After 24 h in the laboratory at 25 C, the plants were removed to the greenhouse and unbagged.

The reaction severity was rated as follows: 0 = no reaction; 1 = discoloration at needle entry point, short-term (3–7 days) water-soaking; 2 = long-term (>7 days) water-soaking or discoloration, no expansion; 3 = lesions and water-soaking, little expansion past injected area; 4 = lesions, water-soaking, expansion up to 0.5 cm past injection zone; 5 = expansion greater than 0.5 cm. A positive test for pathogenicity was an average rating of 2 or higher. The 85 strains were randomized with respect to host and serogroup and inoculated in a series of seven tests over 6 mo. In each of the seven tests, four controls were used. Positive controls selected from preliminary studies were D185, weakly virulent, and B89, highly virulent and streptomycin resistant. *E. herbicola* (strain Eh1) and sterile 0.85% saline were negative controls. Greenhouse temperatures ranged from 21 to 30 C. We attempted to reisolate the pathogen from each host for all of the inoculated strains. A severity index,

$$\frac{\sum(\text{No. leaves in each severity class} \times \text{class no.}) \times 100}{(\text{Total no. leaves}) \times (\text{total classes} - 1)}$$

was used to compare the strains with respect to general virulence on all aroids.

TABLE 1. Strains of *Xanthomonas campestris* pv. *dieffenbachiae* and other xanthomonads tested for reactivity with monoclonal antibodies^a

Number of strains	Original host	Number at each location ^b	Source ^c
174	<i>Anthurium andraeanum</i>	67 H	1,2,3
		51 O	1,2,7
		43 K	1,2
		6 M	2
		4 A	5
		3 FL	4
2	<i>A. scherzerianum</i>	1 O	1
		1 FL	4
2	<i>A. crystalinum</i>	2 K	2
2	<i>A. amnicola</i>	2 O	1
50	<i>Colocasia esculenta</i>	40 K	2,8
		8 O	1
		2 Mo	1
		2 O	1
3	<i>Alocasia</i>	2 Mo	1
		1 O	1
44	<i>Syngonium</i>	22 O	1,2
		16 FL	4
		4 M	2
15	<i>Aglaonema</i>	11 O	1,2
		4 H	1,2
12	<i>Epipremnum</i>	12 O	1,2
		6 FL	4
9	<i>Dieffenbachia</i>	2 O	1
		1 K	1
		6 O	2
2	<i>Xanthosoma lindenii</i>	2 FL	10
6	<i>X. caracu</i>	6 O	1,2
7	<i>Spathiphyllum</i>	6 FL	4
		1 O	1
1	<i>Philodendron</i>	1 FL	4
1	<i>Caladium</i>	1 O	1
1	<i>Rhaphidophora</i>	1 O	1
26	<i>Lactuca sativa</i> ^d	23 M	1
		1 O	1
		2 CA	11
2	<i>Medicago sativa</i> ^d	1 KS	6
		1 FL	6

^aSome strains were eliminated after bacteriological and pathogenicity tests.

^bNumber of strains at each location where strains were originally collected: Islands of Hawaii (H), Oahu (O), Kauai (K), Maui (M), and Molokai (Mo). FL, KS, CA: Florida, Kansas, and California, respectively. A: Australia.

^c1, This laboratory; 2, Plant Disease Clinic, University of Hawaii, Honolulu; 3, W. Nishijima, University of Hawaii, Hilo; 4, A. Chase, USDA-ARS, Apopka, FL; 5, M. Moffett, via A. C. Hayward, University of Queensland, Queensland, Australia; 6, D. W. Gabriel, University of Florida, Gainesville; 7, M. Aragaki, University of Hawaii, Honolulu; 8, J. Ooka, University of Hawaii, Honolulu; 9, C. Kado, University of California, Davis; 10, K. Pohronezny, University of Florida, Homestead; 11, M. P. Starr, Davis, CA. A list of the specific strains used may be obtained on request.

^dNonaroids.

All strains that reacted only with X1 or X11 (group 12 strains) and were not chosen for the host range study were tested for pathogenicity by injecting *D. maculata* 'Compacta' plants. We used the inoculation methods and the 0-5 rating scale just described. The pathogenicity test was repeated for all strains; average reaction severity ratings were <2. Four strains of *X. c. vitians* also were tested on *Dieffenbachia* and *Syngonium*.

Virulence studies on *Anthurium*. To determine if any serogroups corresponded with virulence on *Anthurium* cultivars or if there were any other correlations between serogroups and virulence, 29 strains from *Anthurium* and one from *Dieffenbachia* were chosen. Strains were cultured on YDC. The inoculum was adjusted to 2×10^8 cfu/ml ($A_{600nm} = 0.1$) and misted onto leaves of tissue-cultured cultivars of *A. andraeanum* ('Mauna Kea', 'Anuenue', and 'UH 798') with sterile glass sprayers (Sigma S-3135, Sigma Chemical Co., St. Louis, MO). The plants were covered with plastic bags and kept in the laboratory at 25 C. After 24 h, the bags were removed, and the plants were arranged in the greenhouse in a randomized design.

The experiment was conducted twice with slight variations. In the first test, one strain from *Dieffenbachia* and 21 strains from all serogroups containing strains originally isolated from *Anthurium* were inoculated onto three plants of Anuenue, two plants of Mauna Kea, and one plant of UH 798. In the second test, nine strains, all from serogroup 2 (nonstarch hydrolyzers), were inoculated onto two plants of each cultivar. Positive controls were strain B89 (starch hydrolyzer) in the first test and strain D68 (a nonhydrolyzer) in the second; Eh1 and sterile 0.85% saline were negative controls in both tests. The rating scale for symptoms was: 0 = no symptoms; 1 = slight bronzing or water-soaking; 2 = marginal or central lesions, less than 10% of leaf area; 3 = lesions 10-25% of leaf area; 4 = lesions 26-50% of leaf area; 5 = lesions greater than 50% of leaf area; and 6 = systemic infection in the petiole. Every leaf on every plant was rated; final ratings were made after 5 wk. A two-way analysis of variance of virulence within strains from serogroup 2 was compared with variance of strains from other groups. Cultivar reactions were also compared by using Student's *t* tests.

RESULTS

Monoclonal antibodies. MABs Xcd1 and Xcd3 reacted with 178 and 252, respectively, of 323 *X. c. dieffenbachiae* strains. Xcd1 also reacted with all five strains of an unnamed onion pathovar of *X. campestris* (3), five of seven strains of *X. c. poinsettiae*, three of five strains of *X. c. malvacearum*, four of five strains of *X. c. carotae*, and the one strain of *X. c. phaseoli*. MAB Xcd3 reacted with all five strains of the onion pathogen, three of seven strains of *X. c. poinsettiae*, and two of five strains of *X. c. translucens*. MAB Xcd7 reacted only with *X. c. dieffenbachiae* strains (110 of 323). MABs T1 and T2 reacted with 206

and 10, respectively, of 323 *X. c. dieffenbachiae* strains, and Xcd2 reacted with 111 of 323 *X. c. dieffenbachiae* strains.

Serotyping. Of the 387 strains listed in Table 1, 23 (6%) did not react with any of the eight total MABs. The 23 strains that did not react with the *Xanthomonas*-specific MABs, X1 and X11, were confirmed by bacteriological tests to be nonxanthomonads. The 26 strains of *X. c. vitians* from lettuce reacted only with MABs X1 and X11. The two *X. c. alfalfae* strains tested reacted with X1 and X11, and one also reacted weakly with Xcd1. Thirteen other X1- or X11-positive xanthomonads were eliminated, because they failed twice to cause symptoms on *Dieffenbachia*. The 323 remaining *X. c. dieffenbachiae* strains isolated from all the aroid hosts reacted with X1 and X11.

Based on their reactivity to the six MABs that gave differential patterns (Table 2), the 323 strains of *X. c. dieffenbachiae* were placed into 12 serogroups; 294 strains (91%) reacted with one or more of the six MABs. MAB Xcd3 reacted with the most (252 of 323) strains; 170 (96%) of 177 *Anthurium* strains and 81 of 146 (55%) of the strains from other aroids were Xcd3-positive (Table 2).

Groups 1-6 contained 169 of 177 (95%) *Anthurium* strains and only 27 strains from other hosts. The remaining 119 strains from hosts other than *Anthurium* were in groups 7-12 (Table 3). Strains that reacted only with X1 and X11 were placed in group 12.

Of 177 strains from *Anthurium*, 109 of these were weak or negative for starch hydrolysis, and all except one (B39A, group 12) reacted with MAB Xcd2. Eleven of 146 (7%) strains from other hosts failed to hydrolyze starch; three from *Aglaonema* reacted with Xcd2. None of the 204 strains positive for starch hydrolysis reacted with MAB Xcd2.

No *Anthurium* strains reacted with MAB T2 (group 7 or 8). Those strains that reacted with T2 were weakly pathogenic strains from *Xanthosoma lindenii*, *Spathiphyllum*, *Syngonium podophyllum*, *Epipremnum*, or *Colocasia esculenta*. Most of the slow-growing *syngonii* strains (9,11) were in group 5, 6, or 12; the faster-growing strains from *Syngonium* with characteristics typical of *X. c. dieffenbachiae* strains were in group 8, 11, or 12. Most strains from *Colocasia* were in group 9 (61%) or 11 (29%). Strains from *Philodendron* were primarily in group 10 (57%), and strains from *Dieffenbachia* were primarily in group 12 (63%). Groups 11 and 12 were the most heterogeneous; they contained strains from 10 different hosts (Table 3). No single MAB that reacted only with *Anthurium* strains or with all *X. c. dieffenbachiae* strains was generated; nevertheless, all strains could be grouped with the panel of six MABs.

Host range and virulence studies. The symptoms caused by strains that infected *Dieffenbachia*, *Anthurium*, and *Syngonium* appeared within 5-9 days, beginning with small translucent water-soaked pinpoint spots that later expanded and became necrotic. The most virulent strains spread past the zone of inoculation within 18 days. The symptoms on *Epipremnum* and *Spathiphyllum*

TABLE 2. Grouping of 323 *Xanthomonas campestris* pv. *dieffenbachiae* strains based on differences in reactivity to six monoclonal antibodies

Serogroup	Monoclonal antibodies								Number of strains ^a
	Xcd3	T1	Xcd2	Xcd1	Xcd7	T2	X1	X11	
1	+	+	+	+	+	-	+	+	19
2	+	+	+	+	-	-	+	+	72
3	+	+	+	-	+	-	+	+	5
4	+	+	+	-	-	-	+	+	14
5	+	+	-	-	+	-	+	+	75
6	+	+	-	+	+	-	+	+	10
7	+	+	-	+	-	+	+	+	1
8	+	+	-	-	-	+	+	+	9
9	+	-	-	+	-	-	+	+	32
10	+	-	-	-	-	-	+	+	12
11	-	-	-	+	-	-	+	+	42
12	-	-	-	-	-	-	+	+	30
Total	249	205	110	176	109	10	320	320	320

^aThree strains did not fit into any of the above patterns but were closest in reactivity to groups 4, 7, and 11.

lum developed slowly, forming only pinpoint necrotic lesions. No spread past the zones of inoculation was observed.

Many of the strains from hosts other than *Anthurium* were pathogenic only on *Dieffenbachia* and/or their host of origin. The majority of the *Anthurium* strains were more virulent and infected a broader range of hosts than those from other aroids (Fig. 1). Mean severity indices evaluated by Student's *t* tests for the various serogroups of *Anthurium* strains were not significantly different. However, mean severity indices (Fig. 2) of all *Anthurium* strains were higher ($P = 0.05$) than mean severity indices for strains from all other hosts. Data from the nonaroid host *Schefflera* were not included in the calculation of the severity index. After 4 wk, most strains could be reisolated from leaf disks even if the plants were asymptomatic, indicating that these strains may survive epiphytically or produce latent infections.

Symptoms on *Schefflera* began as small chlorotic pitted lesions and often expanded in size, but not past the zone of inoculation. Bacteria were rarely recovered from lesions.

Twenty-nine strains from group 12, which were not included in the host range study, were tested twice for pathogenicity on

Dieffenbachia. Of these, 16 were pathogenic (rated 2 or higher), and 13 caused no symptoms. Nine of the 13 strains were reisolated after 4 wk in both tests. The others were not reisolated. One *X. c. vitians* strain (XV164A) from lettuce caused moderate symptoms (rating of 2) on one of four *Dieffenbachia* leaves. Two strains (XV164A and D253) from lettuce caused moderate symptoms on two of eight *Syngonium* leaves.

Virulence studies on *Anthurium*. Water-soaking appeared within 7–10 days on the leaf surfaces and margins of misted *Anthurium* plants. These lesions enlarged and became necrotic; they often involved a major portion of the leaf. The lesions were initiated mostly at the hydathodes and occasionally at the stomata. The oldest and youngest leaves usually were the least affected.

No differences were observed in severity ratings among the *Anthurium* strains in different serogroups. However, the three cultivars were differentially susceptible to the 30 strains of *X. c. dieffenbachiae* tested. Mauna Kea was the most susceptible; UH 798 was intermediate; and Anuenue was the least susceptible (Fig. 3). Some control plants (misted with strain Eh1 or sterile saline) showed marginal burning on the older leaves, but water-

TABLE 3. Numbers of strains in each serogroup for each host and total numbers of strains per host

Hosts	Serogroups													Number of strains ^b
	1	2	3	4	5	6	7	8	9	10	11	12	AS ^a	
<i>Anthurium</i>	19	69	5	14	57	4				2	2	4	1	177
<i>Syngonium</i>					15	3		2			14	7	2	43
<i>Aglaonema</i>		3				1					1	6		11
<i>Dieffenbachia</i>					1				2			5		8
<i>Epipremnum</i>					2	1		1	1		2	4		11
<i>Philodendron</i>						1				4		1		7
<i>Colocasia</i>							1		30	3	14	1		49
<i>Xanthosoma</i>									4		2	2		8
<i>Spathiphyllum</i>									2		3			5
<i>Alocasia</i>											2			2
<i>Caladium</i>										1				1
<i>Rhaphidophora</i>											1			1
<i>Medicago sativa</i> ^c											1	1		2
<i>Lactuca sativa</i> ^d												26		26

^aThree strains (two from *Syngonium* and one from *Anthurium*) had atypical serotypes (AS), which were similar in reactivities to groups 7, 11, and 4, respectively.

^bTotal number of strains = 351.

^cTwo strains were tested for reactivity to the antibodies, because they were suspected to be closely related to *Xanthomonas campestris dieffenbachiae* strains (14). One strain reacted with MAb X1, X11, and Xcd1 (serogroup 11); the other only reacted with MAb X1 and X11 (serogroup 12).

^dStrains of *X. c. vitians* were used as controls to compare with strains of "*X. vitians*" isolated from *Syngonium* (9,11,36). All strains of *X. c. vitians* isolated from lettuce reacted only with *Xanthomonas*-specific MAb X1 and X11.

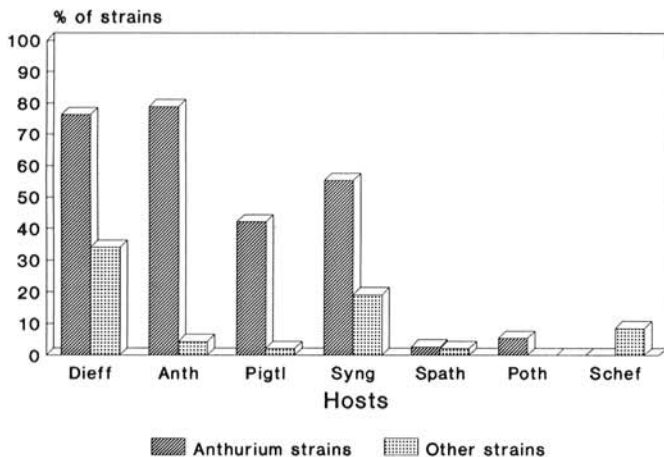


Fig. 1. Percentage of *Xanthomonas campestris* pv. *dieffenbachiae* strains with an average severity rating of 3 or higher on each of seven hosts when comparing 37 strains isolated from *Anthurium* and 48 strains isolated from other hosts. Dieff, *Dieffenbachia maculata* 'Compacta'; Anth, *Anthurium andreaeanum* 'Mauna Kea'; Pigtl (Pigtail anthurium), *Anthurium scherzerianum*; Syng, *Syngonium podophyllum* 'White Butterfly'; Spath, *Spathiphyllum* 'Tasson'; Poth, *Epipremnum aureum* 'Marble Queen'; and Schef, *Schefflera arboricola*.

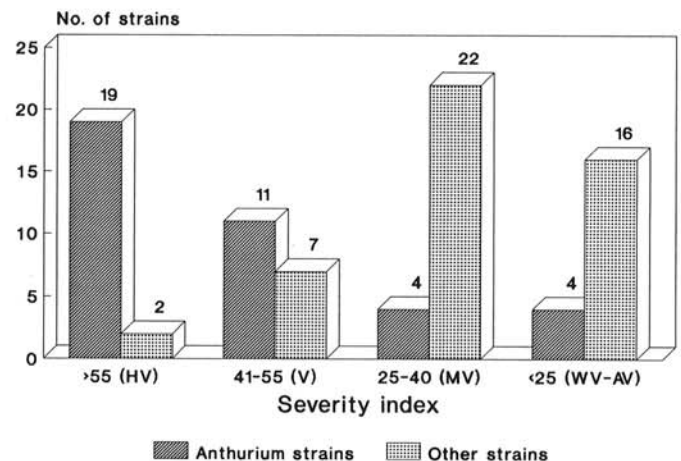


Fig. 2. Numbers of *Xanthomonas campestris* pv. *dieffenbachiae* strains isolated from *Anthurium* and other hosts in each virulence category. The severity index was calculated from the average of five aroid hosts: *Dieffenbachia maculata* 'Compacta', *Anthurium andreaeanum* 'Mauna Kea', *Anthurium scherzerianum*, *Syngonium podophyllum* 'White Butterfly', *Spathiphyllum* 'Tasson', and *Epipremnum aureum* 'Marble Queen'. HV, highly virulent; V, virulent; MV, moderately virulent; and WV-AV, weakly virulent to avirulent.

soaking did not occur, and *X. c. dieffenbachiae* was not reisolated.

DISCUSSION

The pathovar *X. c. dieffenbachiae* consists of a heterogeneous group of bacteria that can cause disease on *Dieffenbachia*. The heterogeneity of *X. c. dieffenbachiae* is apparent in fatty acid profiles (17), virulence, host range (8,29,31), starch hydrolysis (5), carbohydrate utilization (8), and pigmentation (9,11).

In the present study, strains isolated from certain hosts were serologically heterogeneous, but subgroups were identified. Although overlapping occurred, serotypes corresponded loosely to the host of origin. Strains from *Anthurium* were predominantly in serogroups 1-6. They were pathogenic on a wide range of aroid genera and generally were more virulent on these hosts than other *X. c. dieffenbachiae* strains. Rott and Prior (31), working with a small number of strains, had similar results. They produced a polyclonal antibody that differentiated *Anthurium* strains from other *X. c. dieffenbachiae* strains, but not from each other. In addition, their *Anthurium* strains also affected a wide range of hosts.

In this study, many *Anthurium* strains (62%) did not hydrolyze starch as compared to 7% of strains from other hosts. All but one of the nonhydrolyzing *Anthurium* strains and three of 11 from other hosts reacted with MAb Xcd2 (groups 1-4). Thus, reactions with Xcd2 seem to be associated with the inability to hydrolyze starch.

Dickey and Zumoff (11) proposed that strains isolated from *Syngonium* be placed into a separate pathovar (pv. *syngonii*). These strains are highly virulent on *Syngonium* but otherwise have a narrow host range (9,11). In our study, *syngonii* strains were serologically more closely related to strains from *Anthurium* (groups 5 and 6). Like strains from *Anthurium*, the *syngonii* strains were reported to be systemic pathogens (9) and may be the strains described by Wehlburg as "*X. vitians*" (37). In our study, some of these *syngonii* strains did cause symptoms on *Dieffenbachia* but seldom on the other aroids.

A second group of strains from *Syngonium* grew faster than *syngonii* strains and were indistinguishable from other *X. c. dieffenbachiae* strains in bacteriological tests. Serologically, they were similar to *X. c. dieffenbachiae* isolated from *Aglaonema*, *Philodendron*, *Dieffenbachia*, and *Colocasia* (groups 7-12). In our study, both types of *Syngonium* strains reacted with several of the MABs. In contrast, strains of *X. c. vitians* from lettuce did not react with any MABs except genus-specific MABs XI and X11. Hodge et al (17) have found that strains from *Syngonium* cannot be differentiated from other *X. c. dieffenbachiae* strains

on the basis of fatty acid profiles. This evidence suggests that both groups of strains from *Syngonium* should be included in *X. c. dieffenbachiae*.

We reported previously that strains of *X. c. dieffenbachiae* were serologically related to citrus bacterial spot strains from Florida and from bacteriosis of citrus from Mexico (2,3) as well as to *X. c. alfalfae*. Restriction fragment length polymorphism studies (14) also revealed that the heterogeneous citrus bacterial spot strains (previously, the E group of *X. c. citri*) are related to *X. campestris* pathovars *dieffenbachiae* and *alfalfae*. Perhaps these strains come from a common population of weakly virulent strains or strains that survive epiphytically on a broad host range and later adapt to and become more aggressive on separate hosts.

It appears that *X. campestris* pathovars that infect a broad range of host genera do not have a single epitope that all members have in common, whereas pathogens affecting a single genus may share a common epitope that permits identification of all strains of that pathovar with a single MAb (6). Strains of *X. c. dieffenbachiae* are serologically heterogeneous. Some strains have a broad host range, whereas others affect only a few genera. Because of overlapping characteristics and host ranges, these strains are not easily subdivided into separate pathovars. Nevertheless, if subgroups were to be designated by using a variety of methods, as has been done for *X. c. citri* (14), tentative groupings could make up "anthurium" strains, "syngonium" strains, "dieffenbachia" strains, (that infect *Philodendron* and *Dieffenbachia*), and "taro" strains, such as those isolated from *Xanthosoma* and *Colocasia*.

Our study has provided some insight into the serological similarity of *Anthurium* strains of *X. c. dieffenbachiae* from Hawaii, Australia, and Florida. Strains from Jamaica fall within the same serogroups as the Hawaiian strains (F. Young and A. Alvarez, unpublished data). The diversity of *Anthurium* cultivars as well as the diversity of the pathogen and its serotypes is consistent with the long history of *Anthurium* culture in Hawaii. All strains of the pathogen from other geographical regions are represented by the range of serotypes found in Hawaii.

The MABs described in this study have been useful for epidemiological studies on Hawaiian *Anthurium* farms. In addition, biologically distinct (starch hydrolyzing and nonhydrolyzing) strains can be identified and tracked by using the MABs (4,5). These and other MABs are now being used in the development of an indexing program for the production of clean plants for the *Anthurium* industry (13,28).

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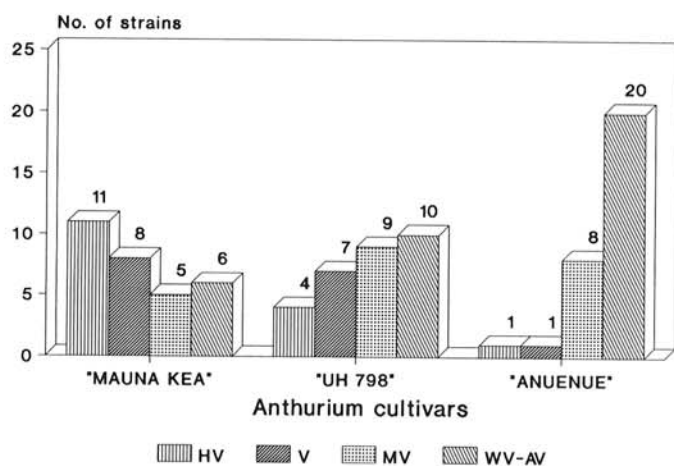


Fig. 3. Virulence of *Xanthomonas campestris* pv. *dieffenbachiae* strains on three cultivars of *Anthurium* (29 strains from *Anthurium* and one strain from *Dieffenbachia*). Virulence was based on a severity rating scale of 0-6: average rating of 4.0 or higher = highly virulent (HV); 3.0-3.9 = virulent (V); 2.0-2.9 = moderately virulent (MV); and 0-1.9 = weakly virulent to avirulent (WV-AV).

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