Characterization of *Xanthomonas campestris* Strains from Aroids Using Physiological, Pathological, and Fatty Acid Analyses

A. R. Chase, R. E. Stall, N. C. Hodge, and J. B. Jones

Professor of plant pathology, University of Florida, Central Florida Research and Education Center, Apopka, 32703; professor and senior biological scientist, Department of Plant Pathology, University of Florida, Gainesville, 32611; and professor of plant pathology, University of Florida, Gulf Coast Research and Education Center, Bradenton, 34203, respectively.
Florida Agricultural Experiment Station Journal Series R-01509.
Special thanks to Geoff Harris for aid in computer analyses.
Accepted for publication 23 March 1992.

ABSTRACT

Chase, A. R., Stall, R. E., Hodge, N. C., and Jones, J. B. 1992. Characterization of *Xanthomonas campestris* strains from aroids using physiological, pathological, and fatty acid analyses. Phytopathology 82:754-759.

One hundred and forty-nine strains of *Xanthomonas campestris* pv. *syngonii* and *X. c. dieffenbachiae*, obtained from ornamental and agronomic aroid plants, were characterized by pathogenic and physiologic reactions. Strains were originally isolated from plants in the following genera: *Aglaonema, Anthurium, Colocasia, Dieffenbachia, Epipremnum, Philodendron, Syngonium*, and *Xanthosoma*. In pathogenicity tests with *Aglaonema, Anthurium, Dieffenbachia, Philodendron*, and *Syngonium*, strain groups were more virulent on their host of origin than on other plants, but they were not host-specific. Similarly, multiplication of bacterial populations in leaf tissue for each strain was greatest, although strains grew to significant levels in other hosts as well. Although symptom expression varied from strain to strain, no correlation could be drawn between a strain group and symptom expression. Characteristics of strains from some host genera were different based on minimum pH for growth, pectolytic activity, and starch utilization on four media, although no combination of characteristics could be used to separate one host-strain group from the rest. Carbon source oxidation via the Biolog system showed that the majority of strains could be determined to their host of origin with the exception of strains from *Anthurium* and *Dieffenbachia*. Based on fatty acid profiles, strains from *Anthurium, Colocasia, Epipremnum, Philodendron*, and *Syngonium* had similar ratios (2:1) of the predominant fatty acids, 15:0 iso and 15:0 anteiso, and those from *Aglaonema* and *Dieffenbachia* had proportions of 1:1 and 3:1, respectively. Subgroups were based on quantitative differences among other unsaturated and hydroxy acids. None of the subgroups was consistently associated with strains from a particular host genus. The profiles of the strain group from *Xanthosoma* differed from all other strain groups by the unique 1:2 ratio of the 15:0 iso to 15:0 anteiso fatty acids. The degree of differences in pathogenic and physiologic reactions and the fatty acid profiles indicate the heterogeneous nature of *X. c. dieffenbachiae*, but do not support separation into different pathovars at this time.

Plants in the Araceae family (aroids) are among the most widely grown for use as indoor foliage. They include species and cultivars of *Aglaonema, Anthurium, Dieffenbachia, Epipremnum, Philodendron, Spathiphyllum*, and *Syngonium*. Other aroids are utilized for food in the subtropics including *Colocasia* (taro) and *Xanthosoma* (cocoyam or malanga). The most serious diseases these plants have in common are caused by strains of *X. campestris*. *Xanthomonas* diseases of aroids are characterized by marginal chlorosis and necrosis, interveinal necrosis and, in some cases, a systemic infection resulting in plant death. Serious outbreaks of diseases caused by xanthomonads have been reported on the majority of these plant genera with the exceptions of *Epipremnum* and *Spathiphyllum*. Disease incidence as high as 100% in some greenhouses have been reported for *Syngonium* (6) and losses exceeding $5 million for 1987 alone were reported by the *Anthurium* cut flower industry in Hawaii (1).

The pathogen(s) responsible for diseases caused by xanthomonads of aroids have been identified as one or more pathovars of *X. campestris*. *X. c. dieffenbachiae* McCull. & Pirone was described as the causal organism of a disease of *Dieffenbachia maculata* (Lodd.) G. Don (syn. *D. picta*) in 1939 (19). Between 1963 and 1972, *Aglaonema* (20,21), *Philodendron* (14,22,32), and *Syngonium* (15) were also described as hosts of *X. c. dieffenbachiae*. In addition, an abstract published in 1969 reported *X. c. vitians* as a pathogen of *Syngonium* spp., but a complete description of the disease and causal organism was not made (33). In 1988, a foliar disease of *S. podophyllum* Schott 'White Butterfly' was described by Dickey and Zumoff, who designated the causal organism by a new pathovar name, *X. c. syngonii* Dickey & Zumoff (8). Host range, symptomatology, and some physiological differences from *X. c. dieffenbachiae* strains were employed to establish these strains as a new pathovar. A similar study, also reported in 1988, described both foliar and systemic phases of this disease on *Syngonium* in Florida (6).

One of the most devastating *Xanthomonas* diseases of an aroid is found on *Anthurium*. The disease was first reported from Hawaii in 1972 by Hayward (11) as a foliar disease but has since been reported to have both foliar and systemic phases. Additional reports of Anthurium blight have been made from California (7), Florida (5), the French West Indies (13,25,26), and Venezuela (10). At approximately the same time, a disease of *Xanthosoma sagittifolium* (L.) Schott (malanga) was described by Berniac, who recommended that the causal organism (as well as other *X. c. dieffenbachiae* strains) be called *X. c. aracearum* (3). However, recent research by Pohronezny et al cited the pathogen of *Xanthosoma caracu* Koch & Bouche in Florida as *X. c. dieffenbachiae*, based on physiological and pathogenicity tests (23,24). This disease has been found to have both foliar and systemic phases as well.

Although most of the preceding reports included some characteristics of the causal organism, few were complete with respect to host range, symptomatology, and physiological comparisons with strains from other aroids. Most researchers, before 1980, relied on the methods used by Dye (9) to characterize xanthomonads. However, as Dye noted, these physiological tests could not distinguish between pathovars of *X. campestris*. The convention therefore has been to name the pathovar according to previous research on diseases of closely related plants (18). Extensive host range studies are prohibitive if multiple plant families are to be represented, so many studies employed only a few genera or species in the same family as the new host with the result that the information on that group of strains is also limited. Since the 1970s, new methods have been employed to distinguish between strains of *X. campestris*, including monoclonal antibodies (2), restriction fragment-length polymorphism (16,17), and fatty acid analysis (28,29). Additionally, DNA

homology studies have also addressed the pathovar system for xanthomonads (12). The following research was performed to characterize strains of *X. campestris* isolated from eight aroid hosts to better describe *X. c. dieffenbachiae* as well as to show the validity of naming strains from *Syngonium* as a separate pathovar (*syngonii*). Host range, symptomatology, physiological characteristics, and fatty acid profiles were performed.

MATERIALS AND METHODS

Strain collection and maintenance. Strains of *X. campestris* were collected from 25 cultivars of aroids grown in Florida and Hawaii between 1984 and 1990. They were tentatively assigned to strain groups according to the host genus from which they were originally isolated. Strain groups of fewer than four were deemed too limited to include in the following study. Strains were stored in glycerol-nutrient broth at −70 C. Since strains from *Syngonium* grew poorly if at all on nutrient agar (NA) (6), all strains were grown on nutrient agar amended with 0.5% sucrose (NAS). In preliminary tests, two types of strains occurring on syngonium were found; those with poor growth on NA (6,8) were designated as "typical" to conform to *X. c. syngonii* as described by Dickey and Zumoff, and the others were designated as atypical. Atypical strains from *Syngonium* grew well on NA and produced abundant xanthomonadin, unlike typical *Syngonium* strains. Thus, the strains from aroids were divided into nine groups with at least four representatives per group (total of 149 strains).

Inoculum preparation. Inocula for physiological and pathogenicity tests were prepared from 48-h-old cultures on NAS grown at about 30 C. Inocula for pathogenicity tests were prepared by suspending bacteria in 0.01 M $MgSO_4$ and adjusting to 1×10^8 cfu/ml using a spectrophotometer (50% transmission at $A_{600} = 0.3$). Inocula were used within 30 min of preparation.

Plant production and inoculation. All plants used in host range and symptomatology tests were obtained from commercial growers as rooted cuttings or tissue-cultured plantlets and established in 10-cm plastic pots containing a peat-pine bark medium (1:1, Canadian peat and pine bark). The medium was steam-treated for 1.5 h at 90 C and amended with 4.4 kg Osmocote 19:6:12, 4.0 kg of dolomitic lime, and 0.9 kg of Micromax (micronutrient source) per cubic meter. Plants were grown on a greenhouse bench receiving approximately 500 $\mu mol \cdot s^{-1} \cdot m^{-2}$ light and a temperature range from 15 to 35 C depending upon the time of year. Following a 24-h exposure to intermittent mist (15 s/30 min, 12 h/day) plants were inoculated with a bacterial suspension using a Crown Spra Tool (fine mist generated by CO_2 propellant) and enclosed loosely in a plastic bag for 24 h. Three plants per host for each strain were inoculated. Controls heated with 0.01 M $MgSO_4$ were included in each test. One day later, bags were removed; plants remained under intermittent mist for 4–6 wk, and symptoms were monitored twice weekly.

Host range and symptomatology. Five genera of aroids were used for host range and symptomatology tests: *Aglaonema commutatum* Schott 'Maria,' *Anthurium* L. 'Southern Blush,' *Dieffenbachia maculata* (Lodd.) G. Don 'Camille,' *Philodendron scandens* C. Koch & H. Sello ssp. *oxycardium* C. (Schott) Bunt. (heartleaf philodendron), and *Syngonium podophyllum* Schott 'White Butterfly.' Each strain was included on one plant each of the five listed above in at least three experiments between January 1988 and January 1990. In the first test for each strain, reisolation from symptomatic tissues was performed on NAS (6). Symptoms were evaluated according to type (marginal vs. interveinal and chlorotic vs. necrotic) and severity, with data from all tests on each of the five hosts averaged for each strain. Analysis of variance and Duncan's new multiple range tests were performed on these means to determine whether or not strain source affected host range or symptom expression.

Hypersensitive reaction (HR) of 40 strains was compared to disease response in the first test on the five hosts listed above. Plants used for HR induction were: 1) *Capsicum annuum* L. 'Early Calwonder' pepper, 2) *Lycopersicon lycopersicum* (L.) Karst. ex Fariv. 'Bonny Best' tomatoes, 3) 'Ace' tomatoes, and 4) *Nicotiana tabacum* L. 'Hick's' tobacco. Two leaves per plant were injected with the same bacterial suspension used to inoculate the host range plants. HR induction was ranked from 0 to 8 positive responses at 48 h (8 were the total possible if all plants and sites were positive).

Comparative growth rates in plant tissues. One strain each from four host genera was chosen (X162 from *Syngonium podophyllum*, X186 from *Dieffenbachia maculata*, X328 from *Anthurium* sp., and X376 from *Aglaonema commutatum*) for comparative growth studies. The four host plants were *S. podophyllum* 'White Butterfly,' *D. maculata* 'Camille,' *A. andraeanum*, and *A. commutatum* 'Maria.' Inoculum was produced as described above. One plant of each type was inoculated with each of the four strains by injection-infiltration of a 1×10^4 cfu/ml suspension (a 1-cm^2 area on each of 10 mature leaves per plant). Plants were placed on a greenhouse bench without overhead misting. The populations that developed were determined on weekly intervals four times starting 1 wk after inoculation. Symptoms were described before each sampling date and samples were chosen from areas exhibiting the average degree of symptoms for that plant-strain combination. At each sampling two disks (0.5 cm^2 each) were ground in 2.0 ml of 0.01 M $MgSO_4$ and serially diluted to the appropriate level. Suspensions were transferred to nutrient agar or NAS (X162 only) with the number of colonies per plate counted after 3–5 days. Five replicate plates were used for each dilution at each sampling date. This experiment was performed twice.

Physiological tests. The following physiological tests, with the exception of NaCl tolerance, were performed on all strains: mucoid growth, gelatin hydrolysis, aesculin hydrolysis, casein hydrolysis, urease production, growth in asparagine medium, production of xanthomonadin pigment, starch utilization, pectolytic activity, tolerance to NaCl, and minimum pH for growth in nutrient broth. The first five tests were included to verify that strains were *X. campestris* (30). The remaining tests were chosen to characterize potential differences between strain groups. Sensitivity to pH was tested in nutrient broth (0.3 g beef extract, 1.0 g peptone, and 1 L water) adjusted to a pH from 4.5 to 6.0 with 1 N HCl (total of seven treatments). Five tubes (containing 5 ml) each for each pH were inoculated with 10^6 bacteria and placed in the dark at 30 C on a shaker for 72 h. Transmittance was measured periodically using a spectrophotometer at (A_{600}). Salt tolerance was tested similarly with the following concentrations: 0, 1, 2, 3, 4, and 5% NaCl in nutrient broth. Thirty strains originally isolated from five host genera were evaluated for salt tolerance. Pectolytic activity was tested in crystal violet pectate medium (30). Growth and hydrolysis was tested in four starch based media: cellobiose starch (23), basal starch cycloheximide agar (called BSCAA in ref. 30), selective xanthomonad medium (30), and nutrient starch cycloheximide agar (called NSCAA in ref. 30). The presence of clear zones surrounding the bacterial colony was ranked as follows: 1 (negative), 2 (slight), 3 (moderate), and 4 (strong). Each test was performed twice for each strain and results were averaged. Analysis of variance and mean separation by Duncan's new multiple range test were performed on these means to determine whether or not host-strain group affected physiological test responses.

Carbon source oxidation. Strains of *X. campestris* from aroids were tested for ability to oxidize carbon sources using Biolog GN microtiter plates and proprietary software (Microlog 2 from Biolog Inc., Hayward, CA). Strains were grown for 24 h on tryptic soy agar (Difco, Detroit, MI) at about 27 C. Bacterial suspensions were made in sterile saline (0.85% NaCl) to a final OD (A_{590}) between 0.175 and 0.20. Microliter plates were inoculated with 150 μl per well and incubated at 30 C for 24 h. They were then read with a plate reader. A library was developed from the patterns of carbon source oxidation based on the host of origin, and the ability to determine the host of origin by this library was evaluated.

Fatty acid analysis. Colonies of each strain grown on NAS were inoculated onto fatty-acid-free Difco Trypticase soy broth amended with 1.5% Difco Bacto agar. After 48 h growth at 30 C,

approximately 40-mg cells (wet weight) were transferred to a 13 × 100 glass tube fitted with a Teflon-lined screw cap. Methods for extracting the fatty acids have been described (29).

Profiles of fatty acids were stored in the computerized Microbial Identification System (Microbial ID, Inc., Newark, DE). Of the 200 profiles obtained, 149 strains were chosen to establish a library. Pattern recognition programs within the MIDI Library Generation Software statistically developed 17 distinct groups of fatty acid profiles for these *X. campestris* strains (27).

RESULTS

Strain collection. In Florida and Hawaii, outbreaks of *Xanthomonas* diseases of many of the aroids appear to be relatively uncommon, with only 10 strains recovered from *Aglaonema* spp., 10 from *Dieffenbachia* spp., six from *Epipremnum aureum*, and 16 from *Philodendron* spp. since 1984 (Table 1). The two most common aroid hosts of *Xanthomonas* were *Anthurium* spp. (58 strains) and *Syngonium* spp. (27 strains). The relatively low numbers of strains from *Colocasia esculenta* (eight strains) and *Xanthosoma sagittifolium* (11 strains) were attributable to the localized production of these crops, because occurrence of *Xanthomonas* diseases on these crops is high for *C. esculenta* in Hawaii and *X. sagittifolium* in Florida. We did not think using more strains from such a limited geographic base would have dramatically improved our tests. Host range and symptomatology tests were conducted with at least 25 strains each from *Anthurium* and *Syngonium* and all strains from the other hosts.

Host range and symptomatology. The symptoms that developed on each host plant were the same regardless of the origin of the strain used to inoculate the plants. *Aglaonema* developed some marginal necrosis and chlorosis as well as necrotic lesions scattered across the leaf surface. Symptoms on each of the other hosts were similar except that two types of symptoms developed on *Syngonium*. The first type was similar to those described for the other aroids and generally occurred when plants were inoculated with strains from hosts other than the *Syngonium* cultivar White Butterfly. The second symptom type has been called a blight and is characterized by severe water-soaking and necrosis that frequently remains interveinal. Typical blight symptoms also developed on *Syngonium* inoculated with strains from White Butterfly but also with a few strains from *Aglaonema, Anthurium, Dieffenbachia*, and *Philodendron*. Severity of blight symptoms (for those strains causing them) was similar regardless of the strain origin.

Symptom severity was affected by both the strain origin and the host plant. In general, *Syngonium* developed the most severe symptoms; *Anthurium* and *Dieffenbachia* also developed moderate symptoms (Table 2). Both *Aglaonema* and *Philodendron* were apparently resistant to these *X. c. dieffenbachiae* strains, because they developed slight symptoms. There was apparently a limited degree of host specificity in that strains from one host developed slightly more severe symptoms on that host than strains from another host (Table 2). This difference was not statistically significant for any strain group on any host.

Hypersensitivity to tobacco, tomato, and pepper was tested at the same time as the first host range test. Positive reactions were recorded and added to give an HR rating from 0 (no HR) to 7 (7/8 positive HR—none of the strains gave 8/8 HR). No relationship existed between the HR rating and the disease reaction on the host of origin with as many highly virulent strains giving a low HR rating as a high HR rating.

Comparative growth rates in plant tissues. Results from the two tests were similar, and only those from the first test are presented. Most of the test strains multiplied to at least 1×10^5 cfu/cm^2 tissue regardless of the host of origin (Fig. 1). The most obvious exception was strain X162, originally from *Syngonium*, which multiplied in *Syngonium* and *Aglaonema* only. The highest populations were found when a strain was inoculated to its host of origin many times achieving populations a log unit higher than the same strain in the other three hosts.

Physiological tests. Table 3 lists the number of strains from each host that were included in physiological tests. Although some differences between groups were noted, few were distinct enough to be statistically significant. The minimum pH for growth in vitro varied somewhat from a low of about 4.7 for atypical *Syngonium* strains to about 5.5 for typical *Syngonium* strains (Table 3). Because of transitivity, only the typical *Syngonium* strains could be separated from the rest of the *X. c. dieffenbachiae* strains. Hydrolysis of both pectin and starch have potential as characteristics for differentiation of *X. campestris* pathovars. Some of the *X. c. dieffenbachiae* strains were highly pectolytic, such as strains from *Colocasia, Dieffenbachia*, and *Philodendron*. Strains from *Xanthosoma* and the typical *Syngonium* strains were, however, nonpectolytic for the most part. For most strains, the degree of starch hydrolysis was similar on the four starch media employed although CS medium supported the best growth and SX the least growth for most strains. Again, significant overlapping in strain group reactions for these characteristics occurred, and differentiating any group of strains using one or more of

TABLE 1. Strains of *Xanthomonas campestris* from aroids

Host identification	Source[a] (number of strains)
Aglaonema sp.	Alvarez-HI (4)
Aglaonema commutatum 'Maria'	Chase-FL (2)
	PDD-FL (2)
Aglaonema commutatum 'Silver Queen'	PDD-FL (1)
Aglaonema 'Romana'	PDD-FL (1)
Anthurium sp.	Alvarez-HI (6)
	Chase-FL (2)
	DPI-FL (3)
	PDD-FL (4)
Anthurium 'Bird's nest'	Chase-FL (1)
Anthurium 'Lady Jane'	PDD-FL (1)
Anthurium 'Southern Blush'	PDD-FL (3)
Anthurium amnicola	Alvarez-HI (1)
Anthurium andraeanum	Alvarez-HI (27)
	Chase-FL (1)
	PDD-FL (1)
Anthurium cristalinum	Alvarez-HI (1)
	Chase-FL (1)
Anthurium scherzeranum	Alvarez-HI (1)
	Chase-FL (2)
	PDD-FL (3)
Colocasia esculenta	Alvarez-HI (8)
Diffenbachia sp.	DPI-FL (1)
D. maculata	UF-FL (2)
	Yoders-FL (6)
D. maculata 'Bali Hai'	PDD-FL (1)
Epipremnum aureum	Alvarez-HI (3)
	Chase-FL (2)
	DPI-FL (1)
Philodendron sp.	DPI-FL (1)
	UF-FL (2)
Philodendron 'Lynette'	PDD-FL (2)
Philodendron 'Velvet leaf'	Chase-FL (3)
P. scandens ssp. *oxycardium*	Chase-FL (6)
	DPI-FL (2)
Syngonium sp.	Alvarez-HI (7)
	Daughtrey-NY (1)
S. podophyllum	Chase-FL (1)
	Yoders-FL (5)
S. podophyllum 'White Butterfly'	Chase-FL (14)
	Dickey-NY (1)
Xanthosoma sigittifolium	Pohronezny-FL (12)

[a]Strains were supplied by the following laboratories: A. Alvarez, Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822; M. Daughtrey, Long Island Horticultural Research Laboratory, Riverhead, NY 11901; R. Dickey, Department of Plant Pathology, Cornell University, Ithaca, NY 14853; (DPI) J. Miller, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL 32602; (PDD) D. Brunk, Plant Disease Diagnostics, Inc., Apopka, FL 32703; K. Pohronezny, University of Florida, Tropical Research and Education Center, Homestead, FL 33031; (UF) G. Simone, Department of Plant Pathology, University of Florida, Gainesville, FL 32611; and Yoder Bros. Inc., Alva, FL 33920.

these tests was not possible.

Carbon source oxidation. The majority of strains could be identified to their host of origin based on pattern of carbon utilization (Table 4). Only the strains from *Epipremnum* could be completely separated using this library, which may have been attributable to the low number of strains tested. In addition, strains from both *Anthurium* and *Dieffenbachia* were apparently more diverse in their carbon source utilization patterns, since the majority of strains in either host group were not accurately identified.

Fatty acid analysis. Profiles of xanthomonads are complex, consisting of 20–27 acids. Several hydroxy acids and the 15:0 iso and 15:0 anteiso compounds have been identified as the criteria for discriminating pathovars of *X. campestris* (28,29). Relative ratios of 16:1 cis 9 and 16:0 are also used to distinguish strains. The two branched chain isomers 15:0 iso and 15:0 anteiso constitute 32–55% of the total profile. Strains from *Anthurium, Colocasia, Epipremnum, Philodendron*, and *Syngonium* all have similar ratios (2:1) of these acids, and those isolated from *Aglaonema* and *Dieffenbachia* have proportions of 1:1 and 3:1, respectively. The unique 1:2 ratio of 15:0 iso/15:0 anteiso differentiated the 12 strains recovered from *Xanthosoma*.

Principal component (PC) analyses statistically assess the relationships within a set of variables (27). Representing 95% of the variance of samples in a test group, a histogram of PC 1 depicts outlying strains. The elements of PCs are: GC operating conditions, calibration setpoints, and the Microbial Identification System catalog of over 200 FA compounds and their chroma-

TABLE 2. Virulence of *Xanthomonas campestris* strains on five aroid hosts according to host of origin

Original host genus	Virulence index[a]					
	Aglaonema	*Anthurium*	*Dieffenbachia*	*Philodendron*	*Syngonium*	Mean
Aglaonema	1.9 a[b]	1.4 c	1.8 ab	1.2 a	3.2 a	1.9
Anthurium	1.6 ab	2.6 ab	2.4 a	1.3 a	2.6 ab	2.1
Colocasia	1.0 b	1.4 c	...	...	...	1.2
Dieffenbachia	1.4 ab	2.1 abc	2.4 a	1.3 a	2.1 ab	1.9
Epipremnum	1.1 b	1.7 c	1.4 b	1.6 b	1.6 b	1.5
Philodendron	1.7 ab	1.5 c	1.4 b	1.2 a	2.3 ab	1.6
Syngonium	1.0 b	1.9 bc	2.1 ab	1.4 a	3.1 a	1.9
Syngonium (atypical)[c]	1.7 ab	2.7 a	1.7 ab	1.3 a	2.3 ab	1.9
Xanthosoma	1.6 ab	1.7 c	1.2 b	...	2.7 ab	1.8
Mean[d]	1.4	1.9	1.8	1.3	2.5	

[a] Virulence was rated 1 (avirulent), 2 (weak), 3 (moderate), or 4 (strong). The plants tested were as follows: *Aglaonema commutatum* 'Maria,' *Anthurium* 'Southern Blush,' *Dieffenbachia maculata* 'Camille,' *Philodendron scandens* ssp. *oxycardium*, and *Syngonium podophyllum* 'White Butterfly.'

[b] Numbers in the same column followed by different letters were significantly different (DNMRT, $P = 0.05$).

[c] Atypical *Syngonium* strains grow well on nutrient agar and produced the normal yellow pigmentation, which typical *Syngonium* strains did not.

[d] The mean pathogenicity index was determined for each host inoculated and for each group of strains according to original host by averaging all of the values for that host or strain source. Means are given for three plants in each of three experiments (9 total).

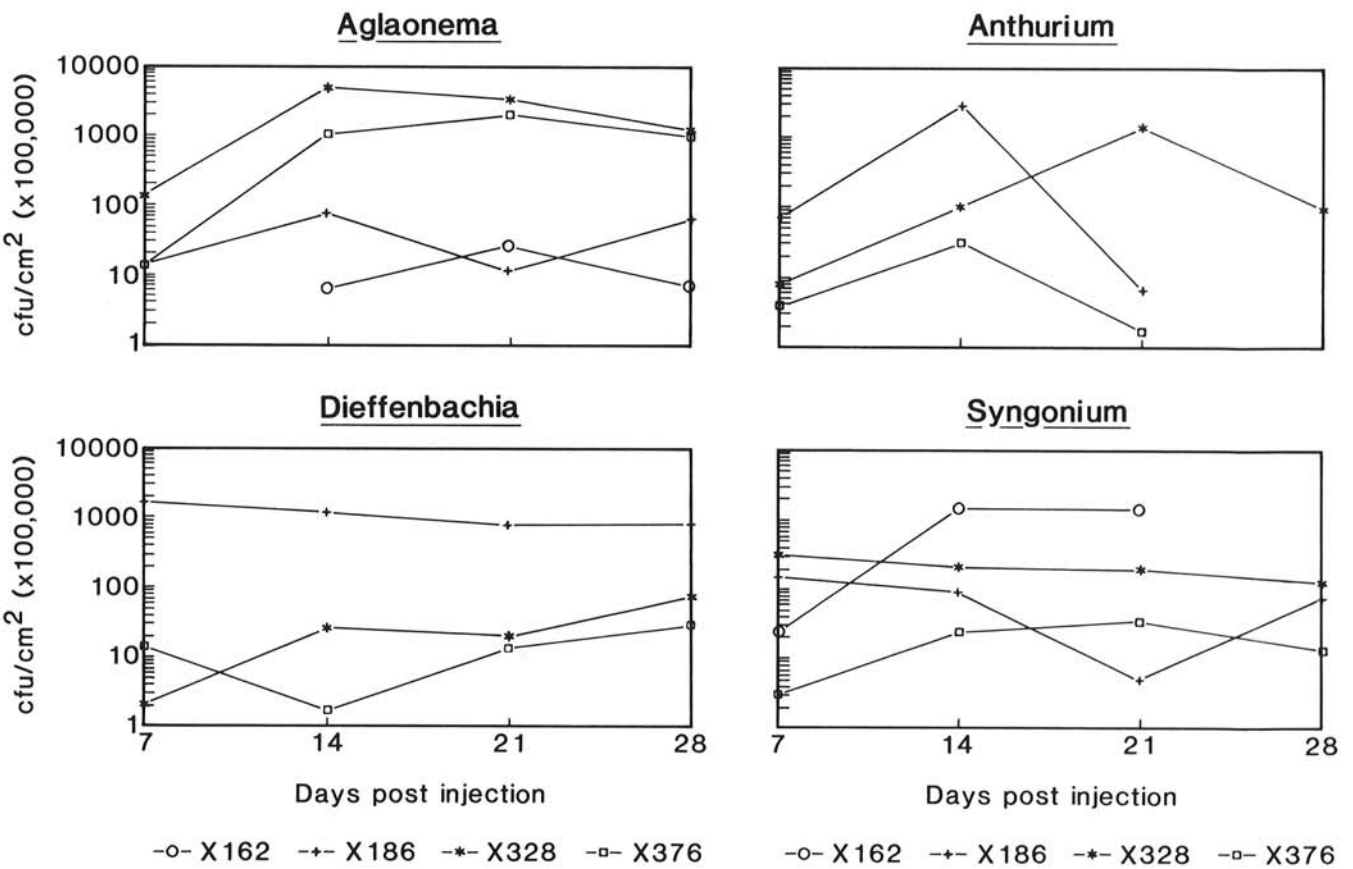

Fig. 1. Population dynamics of four strains of *Xanthomonas campestris* from aroids in *Aglaonema commutatum* 'Maria' (X376 isolated from *Aglaonema* sp. 'Romana'), *Anthurium andraeanum* (X328 isolated from *Anthurium* sp.), *Dieffenbachia maculata* 'Camille' (X186 isolated from *D. maculata*), and *Syngonium podophyllum* 'White Butterfly' (X162 isolated from *S. podophyllum* 'White Butterfly').

tographic features. Using PCs 1 and 2 (accounting for 99% of the variance of a population) the MIS computer generated two-dimensional plots. On this type of plot, similar strains cluster and segregate from other atypical strains. Strains from *Xanthosoma* are in a realm situated beyond one standard deviation from the mean of PC 1. The MIS 10% rule is used to form GC subgroups when the standard deviation divided by the mean of major acids has a value greater than 0.10. Xanthomonads from *Xanthosoma* separate into three subgroups and those from *Anthurium* and *Syngonium* into six and four subgroups, respectively (Table 5). A three-dimensional representation of the relation of xanthomonads from aroids is created using the values of the three major fatty acids as the axes (Fig. 2). GC subgroups within *Xanthosoma* clearly segregate from all other xanthomonads from aroids although other GC subgroups, such as those for *Anthurium,* and *Syngonium* do not segregate from the others or each other.

DISCUSSION

Several of the differentiation methods used in this study were successful in separating one group of *X. campestris* strains from the others. The most common method for identifying a pathovar of *X. campestris* has been host range (18). Our tests have demonstrated that although some degree of host specificity occurs with these strains, some strains from each host can infect, multiply, and cause typical symptoms of disease in plants belonging to other genera. Most of the typical strains from *Syngonium* that have been called *X. c. syngonii* cause a more severe symptom in *Syngonium* (blight) than other strains from aroids. However, two strains from *Aglaonema* and one strain each from *Anthurium, Dieffenbachia*, and *Philodendron* caused blight symptoms in *Syngonium*. Similarly, most strains of *X. c. syngonii* did not cause severe symptoms in other aroids, although they were able to multiply to high levels in these plants. Clearly, host range data do not show major differences between *X. c. syngonii* and other strains from aroids.

Physiological differences were also investigated. Minimum pH for growth in nutrient broth can separate all typical strains from *Syngonium* from all other aroid strains. Other physiological tests failed to clearly differentiate the strains to their host of origin, although some trends were noted. Although starch utilization can differentiate some pathovars of *X. campestris* from others, nearly all of the aroid strains could utilize starch on CS medium, which, overall, supported their best growth. Similarly, most strains produced some pectolytic action on CVP with the exception of those from *Xanthosoma*. Care must be taken when testing such factors to do so on a medium that will allow growth of the strain. This was especially important for strains such as those from *Syngonium*, which are more fastidious than others from aroids.

Ability to oxidize different carbon sources using the Biolog system gave the highest degree of differentiation of *X. campestris* strains from aroids. Approximately 66% of the strains tested could be accurately identified to their host of origin using this system. Strain groups showed differing degrees of heterogeneity. Strains from *Anthurium* and *Dieffenbachia* were least likely to be identified to their host of origin. Strains from *Anthurium* that were not identified correctly were identified to be five of the eight remaining subgroups. Those from *Dieffenbachia* were identified to be of the same five subgroups. In contrast, the strains from *Epipremnum* were all identified correctly and only one of the eight typical strains from *Syngonium* was not identified to *Syngonium*. Since strains for this study were collected from both Florida and Hawaii, this factor was considered as a potential source of variation. Strains from *Colocasia* all originated in Hawaii and all strains from *Xanthosoma* originated in south Florida. Strains in the other host groups were obtained from both Hawaii and Florida. No correlations between either the geographic source or host species of each strain and correct identification by the carbon source occurred.

Fatty acid analysis reveals a very basic difference in the ratio of 15.0 iso/15.0 anteiso in *Xanthosoma* strains and other strains from aroids, as well as other pathovars of *X. campestris* (R. E. Stall, *unpublished*). Unlike the strains from *Xanthosoma*, typical strains from syngonium were not distinguished from the heterogeneous group of xanthomonads from aroids by fatty acid analysis.

X. c. dieffenbachiae appears to encompass a heterogeneous group of strains. The separation of the *Syngonium* strains into

TABLE 3. Response of *Xanthomonas campestris* strains in physiological tests according to host of origin

Original host genus (number of isolates)	Biochemical test[a]					
	pH	CVP	CS	BS	SX	NS
Aglaonema (10)	4.98 bc[b]	1.8 cde	1.9 b	1.5 cd	1.4 e	1.6 e
Anthurium (32)	4.92 bcd	2.1 cd	2.2 b	1.7 cd	1.7 de	1.7 de
Colocasia (8)	4.78 de	3.4 a	3.2 a	2.9 ab	3.1 ab	2.9 bc
Dieffenbachia (10)	4.98 bc	3.2 ab	3.0 a	3.5 a	3.5 a	4.1 a
Epipremnum (6)	4.83 cde	2.5 bc	2.7 ab	1.8 cd	2.2 cd	2.0 cde
Philodendron (16)	4.92 bcd	3.3 a	3.0 a	2.9 ab	3.1 ab	3.2 ab
Syngonium (17)	5.47 a	1.5 de	1.9 b	1.1 d	1.3 e	1.1 e
Syngonium (7) (atypical)[c]	4.71 e	2.0 cd	1.9 b	2.1 bc	2.4 bc	2.4 bcd
Xanthosoma (12)	5.04 b	1.1 e	2.2 b	1.4 cd	1.2 e	1.7 de

[a] Sensitivity to pH was tested in nutrient broth adjusted to pH levels from 4.5 to 6.0. CVP tested pectolytic activity. CS, BS, SX, and NS media tested starch hydrolysis. Zones of clearing surrounding bacterial colonies were ranked as follows: 1 (negative), 2 (slight), 3 (moderate), and 4 (strong). Each test was performed twice and results were averaged.
[b] Numbers in the same column followed by different letters were significantly different (Duncan's new multiple range test, $P = 0.05$).
[c] Atypical *Syngonium* strains grow well on nutrient agar and readily produced the normal yellow pigmentation, which typical *Syngonium* strains do not.

TABLE 4. Identification of *Xanthomonas campestris* strains from aroids using the Biolog system for carbon source utilization

Original host genus	Number of strains tested	Percent identified to host	Mean fit for correct choice (number of strains)	Mean fit for other choices (number of strains)
Aglaonema	10	80	0.680 (8)	0.617 (2)
Anthurium	23	48	0.597 (11)	0.527 (12)
Colocasia	7	86	0.799 (6)	0.459 (1)
Dieffenbachia	6	33	0.844 (2)	0.622 (4)
Epipremnum	4	100	0.728 (4)	...
Philodendron	11	64	0.566 (7)	0.534 (4)
Syngonium	8	88	0.866 (7)	0.674 (1)
Syngonium (atypical)[a]	6	67	0.656 (4)	0.770 (2)
Xanthosoma	11	73	0.819 (8)	0.454 (3)

[a] Atypical *Syngonium* strains grew well on nutrient agar and readily produced the normal yellow pigmentation, which the typical *Syngonium* strains do not.

TABLE 5. Distribution of *Xanthomonas campestris* strains from aroids into 15 GC subgroupings derived from fatty acid analysis

Plant genus	Fatty acid GC subgrouping (number of strains)[a]														
	1	2	3	4	5	6	9	10	11	12	13	14	15	16	17
Aglaonema (7)										1	2		2	2	
Anthurium (27)	16		1	3		1			2		2	1			1
Colocasia (4)											3				1
Dieffenbachia (7)	2	1	2						1	1					
Epipremnum (4)						1			1		1				1
Philodendron (8)	4	1		1	1				1						
Syngonium (17)		1			1		2				3	4	6		
Syngonium (5) (atypical)[b]			1		1				3						
Xanthosoma (11)		3						7		1					

[a] Based on principal components.
[b] Atypical *Syngonium* strains grew well on nutrient agar and readily produced the normal yellow pigmentation, which the typical *Syngonium* strains do not.

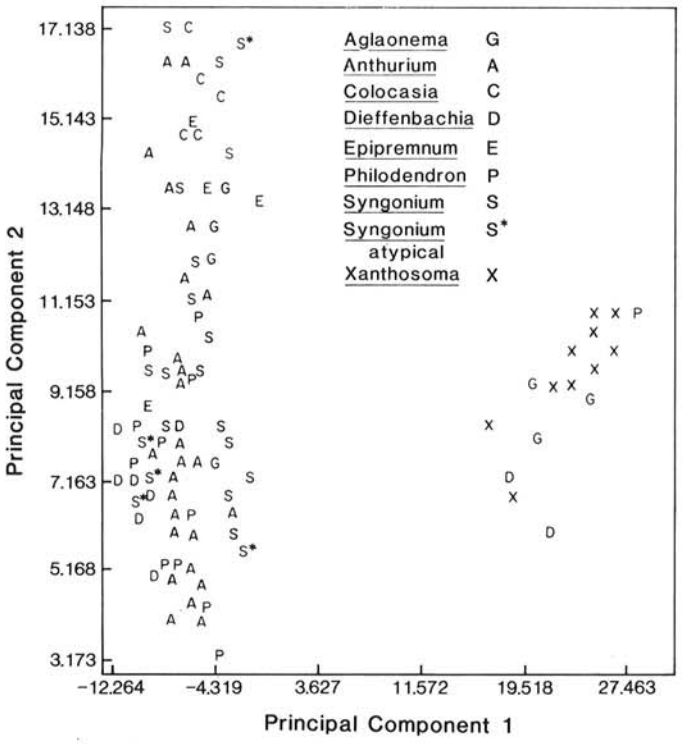

Fig. 2. Two-dimensional plot principal components 1 and 2 from fatty acid analyses of strains of *Xanthomonas campestris* from aroids.

the pathovar *X. c. syngonii* was made on the basis of host range, symptomatology, and physiological differences (6,8) and later by temperature optima (4). Dickey and Zumoff reported differences in NaCl tolerance, gelatin hydrolysis, and growth on SX as well as utilization of a series of carbon sources (8). The current study has demonstrated the extreme variation in *X. c. dieffenbachiae*. The strains included by Dickey and Zumoff were too few to adequately demonstrate the value of the first three physiological tests they relied on for differentiation of *X. c. syngonii* from the remaining *X. c. dieffenbachiae* strains. The differences they found in carbon source utilization were supported by our work using the Biolog system. Although temperature optimum differed slightly for both growth and symptom development of *X. c. syngonii* vs. *X. c. dieffenbachiae*, it was not significantly different.

It remains to be demonstrated whether or not the strains from *Syngonium* comprise a distinct taxonomic group compared to strains from other aroids. None of the tests reported here could be used to demonstrate that *X. c. syngonii* strains should be designated as a separate pathovar from *X. c. dieffenbachiae*. One of the best tools for determining the relationships between groups of xanthomonads remains DNA-DNA homology (12). A combination of DNA-DNA homology, genomic fingerprinting, and restriction fragment length polymorphism (16,17) analyses will be needed to fully describe the relationships between strains from one aroid host and the others. With this information the current examination of the pathovar system will improve categorization and naming of xanthomonads in the future (31).

LITERATURE CITED

1. Alvarez, A. 1988. Proceedings of the first Anthurium Blight conference. HITAHR, College of Tropical Agriculture and Human Resources, Honolulu, HI.
2. Alvarez, A. M., Benedict, A. A., and Mizumoto, C. Y. 1985. Identification of *Xanthomonads* and grouping of strains of *Xanthomonas campestris* pv. *campestris* with monoclonal antibodies. Phytopathology 75:722-728.
3. Berniac, M. 1974. Une maladie bacterienne de *Xanthosoma sagittifolium* (L.) Schott. Ann. Phytopathol. 6(2):197-202.
4. Chase, A. R. 1988. Effects of temperature and preinoculation light level on severity of Syngonium blight caused by *Xanthomonas campestris*. J. Environ. Hortic. 6(2):61-63.
5. Chase, A. R., and Poole, R. T. 1986. Effects of host nutrition on growth and susceptibility of *Anthurium scherzeranum* to Xanthomonas leaf spot. Nurserymen's Digest 20(6):58-59.
6. Chase, A. R., Randhawa, P. S., and Lawson, R. H. 1988. New disease of *Syngonium podophyllum* 'White Butterfly' caused by a pathovar of *Xanthomonas campestris*. Plant Dis. 72:74-78.
7. Cooksey, D. A. 1985. Xanthomonas blight of *Anthurium andraeanum* in California. (Abstr.) Plant Dis. 69:727.
8. Dickey, R. S., and Zumoff, C. H. 1987. Bacterial leaf blight of Syngonium caused by a pathovar of *Xanthomonas campestris*. Phytopathology 77:1257-1262.
9. Dye, D. W. 1962. The inadequacy of the usual determinative tests for identification of *Xanthomonas* spp. N.Z. J. Sci. 4:393-416.
10. Guevara, Y. M., and Debrot, E. 1987. Bacterial blight of Anthurium in Venezuela. Page 764 in: Plant Pathogenic Bacteria. E. L. Civerolo, A. Collmer, R. E. Davis, and A. G. Gillespie, eds. Martinus Nijhoff Publishers, Dordrecht, Netherlands.
11. Hayward, A. C. 1972. A bacterial disease of *Anthurium* in Hawaii. Plant Dis. Rep. 56:904-908.
12. Hildebrand, D. C., Palleroni, N. J., and Schroth, M. N. 1990. Deoxyribonucleic acid relatedness of 24 Xanthomonad strains representing 23 *Xanthomonas campestris* pathovars and *Xanthomonas fragariae*. J. Appl. Bacteriol. 68:263-269.
13. Hostachy, B., Prior, P., Rott, P., and Ferreol, F. 1986. Le deperissement de L' Anthurium—Symptomes et moyens de lutte actuels. B. T. I. 409/411:505-512.
14. Knauss, J. F. 1972. Resistance of *Xanthomonas dieffenbachiae* isolates to streptomycin. Plant Dis. Rep. 56:394-397.
15. Lai, C. M. 1977. Leaf rot disease of *Syngonium*. Plant Pathol. Rep. 1:7.
16. Lazo, G. R., and Gabriel, D. W. 1987. Conservation of plasmid DNA sequences and pathovar identification of strains of *Xanthomonas campestris*. Phytopathology 77:448-453.
17. Lazo, G. R., Roffey, R., and Gabriel, D. W. 1987. Pathovars of *Xanthomonas campestris* are distinguishable by restriction fragment-length polymorphism. Int. J. Syst. Bacteriol. 37:214-221.
18. Leyns, F., de Cleene, M., Swings, J.-G., and de Ley, J. 1984. The host range of the genus *Xanthomonas*. Bot. Rev. 50:308-356.
19. McCulloch, L., and Pirone, P. P. 1939. Bacterial leaf spot of *Dieffenbachia*. Phytopathology 29:956-962.
20. McFadden, L. A. 1962. Two bacterial pathogens affecting leaves of *Aglaonema roebelinii*. (Abstr.) Phytopathology 52:20.
21. McFadden, L. A. 1963. Leaf spot of *Aglaonema roebelinii* (Schmismatoglottis). Univ. Fla. Agric. Ext. Serv. Orna. Hortic. Rep. 2:1.
22. McFadden, L. A. 1967. A *Xanthomonas* infection of *Philodendron oxycardium* leaves. (Abstr.) Phytopathology 57:343.
23. Norman, D., and Alvarez, A. 1989. A rapid method for presumptive identification of *Xanthomonas campestris* pv. *dieffenbachiae* and other Xanthomonads. Plant Dis. 73:654-658.
24. Pohronezny, K., Volin, R. B., and Dankers, W. 1985. Bacterial leaf spot of cocoyam (*Xanthosoma caracu*), incited by *Xanthomonas campestris* pv. *dieffenbachiae*, in Florida. Plant Dis. 69:170-173.
25. Prior, P., Hostachy, B., Sunder, P., and Rott, P. 1986. Bacterial Blight (*X. campestris* pv. *dieffenbachiae*) and Bacterial Leaf Spot (*Pseudomonas* sp.) of *Anthurium* in the French West Indies. Institute National de la Recherche Agronomique, Station de Pathologie Végétale, Paris, France.
26. Rott, P., and Prior, P. 1987. Un deperissement bacterien de l'anthurium provoque par *Xanthomonas campestris* pv. *dieffenbachiae* aux Antilles francaises. Agron. Trop. 42:61-68.
27. Romesburg, H. C. 1990. Cluster Analysis for Researchers. Robert E. Krieger Publishing Co., Malbar, FL.
28. Roy, M. A. 1988. Use of fatty acids for the identification of phytopathogenic bacteria. Plant Dis. 72:460.
29. Sasser, M. 1990. Identification of bacteria through fatty acid analysis. Pages 199-204 in: Methods in Phytobacteriology. Z. Klement, K. Rudolph, and D. Sands, eds. Akademiai Kiado, Budapest.
30. Schaad, N. W., ed. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd Ed. American Phytopathological Society, St. Paul, MN.
31. Vauterin, L., Swings, J., Kersters, K., Gillis, M., Mew, T. W., Schroth, M. N., Palleroni, N. J., Hildebrand, D. C., Stead, D. E., Civerolo, E. L., Hayward, A. C., Maraite, H., Stall, R. E., Vidaver, A. K., and Bradbury, J. F. 1990. Towards an improved taxonomy of *Xanthomonas*. Int. J. Syst. Bacteriol. 40:312-316.
32. Wehlburg, C. 1968. Bacterial leaf spot and tip burn of *Philodendron oxycardium* caused by *Xanthomonas dieffenbachiae*. Proc. Fla. State Hortic. Soc. 81:394-397.
33. Wehlburg, C. 1969. Bacterial leaf blight of *Syngonium podophyllum*. (Abstr.) Phytopathology 56:1056.