Characterization of Strains of *Xanthomonas axonopodis* pv. *dieffenbachiae* from Bacterial Blight of Caladium and Identification of Sources of Resistance for Breeding Improved Cultivars

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Abstract. Bacterial blight, caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (*Xad*), is the most common foliar disease of caladium, an ornamental aroid grown for its colorful foliage. The disease can reduce the marketability of caladiums produced as potted plants and lower the yield of caladiums grown for tuber production. Three bacterial strains were isolated from symptomatic caladiums and identified as *Xad* using fatty acid analysis, carbon source use, and the sequence of the 16S-23S spacer, and tested for virulence against three susceptible cultivars. Two strains were virulent to all of the cultivars; however, one strain was differentially pathogenic, virulent against two cultivars, but not to the usually highly susceptible ‘Candidum’. In greenhouse inoculation tests of 17 cultivars and one breeding line, four cultivars were ranked as highly susceptible, nine as moderately susceptible, and five as resistant. Ten of these cultivars were also evaluated with natural infection in the field with good agreement between the results of the greenhouse and field evaluations. Cultivars White Queen, Florida Red Ruffles, Florida Sweetheart, Candidum Jr., and Mrs. Arno Nehrling have been identified as resistant to bacterial blight in greenhouse or field evaluations and can potentially be used in future breeding efforts to produce improved cultivars.

Caladium (*Caladium ×hortulanum* Birdsey) is an ornamental aroid grown as landscape and potted plants. They are valued for their colorful foliage that comes in various leaf shapes and coloration patterns containing shades of red, pink, white, and/or green. The most common foliar disease of caladium is bacterial blight (BB), also known as *Xanthomonas* leaf spot, caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (*Xad*) (formerly *X. campestris* pv. *dieffenbachiae*) (Chase, 1997).

Symptoms of BB begin as small, angular, water-soaked lesions visible on the lower leaf surface. The lesion can either expand in between major veins causing a large V-shaped necrotic area or veins can be infected and cause necrosis of the vein and adjacent leaf tissue. Severe tissue necrosis can lead to

premature defoliation. Under humid conditions, a yellow exudate composed of *Xad* can be observed on the abaxial surface of lesions. The bacteria in this exudate can be splash-dispersed and infect adjacent leaves and plants.

In Florida, where more than 95% of the world’s commercial caladium tubers are produced (Deng et al., 2008), BB is an annual problem because hot, humid, and rainy weather conditions in the summer are ideal for disease development and the spread of *Xad*. Leaf necrosis and defoliation during severe epidemics of BB, especially those occurring earlier in the production season, may decrease tuber sizes and yields in commercial tuber production. Even relatively mild BB symptoms decrease the marketability of potted plants produced in nurseries and greenhouses.

BB is also a major disease in other ornamental aroids such as anthurium (*Anthurium* Schott), dieffenbachia (*Dieffenbachia* Schott), philodendron (*Philodendron* Schott), aglaonema (*Aglaonema* Schott),

colocasia (*Colocasia* Schott), and syngonium (*Syngonium* Schott). There has been considerable research on xanthomonads that cause the BB in these aroids (Chase et al., 1992; Khoodoo and Jaufeerally-Fakim, 2004; Norman et al., 1997, 1999; Robène-Soustrade et al., 2006), but very little has been conducted on xanthomonad diseases of caladium. The main objective of this study was to identify commercial cultivars resistant to BB for use both by growers as a tool for management of BB and in a breeding program as sources of resistance in the development of new cultivars.

Materials and Methods

Isolation and storage of the pathogen. Three strains of *X. axonopodis*, designated by year of isolation and sample number, 05-113, 05-185, and 05-220, were isolated from symptomatic leaf tissue collected from different fields in central Florida during the summer of 2005. To perform isolations, water-soaked tissue from the margin of typical lesions was rinsed under running chlorinated tap water, macerated in sterile water, streaked on plates of nutrient agar (NA) (Difco Laboratory, Sparks, MD), and incubated at 24 °C for 3 to 5 d. Colonies with the typical yellow morphology of *Xanthomonas* sp. dominated the isolations. Several colonies were chosen for further purification through a series of isolation streaks from individual colonies. Each strain was suspended in 20% glycerol and frozen at –80 °C for long-term storage.

Confirmation of pathogenicity. Inoculum of each of the three strains, 05-113, 05-185, and 05-220, was produced on NA, 48 h at 24 °C, and bacteria were suspended in 0.01 M MgSO₄ [O.D._{590nm} (optical density) = 0.1]. Each strain, and 0.01 M MgSO₄ as a non-inoculated control, was sprayed on the leaves of three plants of two caladium cultivars and one breeding line: ‘Candidum’, ‘Gingerland’, and 18-45, all susceptible to BB according to field observations. The experiment was arranged in a randomized complete block design (RCBD) with three replications each containing one plant of each cultivar sprayed with one of the three *Xad* strains or with a 0.01 M MgSO₄ solution as a negative control. Plants were incubated inside plastic bags in a growth chamber and kept a constant 28 °C for 3 weeks. Incidence of symptoms was recorded weekly starting at 3 d postinoculation, and the identity of the pathogen was confirmed by reisolation from arbitrarily selected plants as described previously.

Pathogen identification. To confirm identification, all three strains were subjected to fatty acid analysis (Sasser, 1990), analysis of carbon substrate use patterns using the Biolog GN Microplate (Biolog, Inc., Hayward, CA), and sequencing of the 16S rRNA gene plus the 16S-23S rRNA intergenic spacer. The 16S gene and spacer regions were amplified by polymerase chain reaction (PCR), as described subsequently, sequenced in both directions, and compared

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with sequences in the National Center for Biotechnology Information (NCBI) database. Sequences from all three strains were submitted to GenBank. Primer pair FGPS6/FGPS1509 (Table 1) was originally designed from *Frankia* sp. but has been used to amplify a 1480-base sequence, the majority of the 16S rRNA gene, from other genera (Dickstein et al., 2001; Normand et al., 1992; Ponsonnet and Nesme, 1994). Primers 16S-535 and 16S-1550 (Table 1) were designed in this study from the sequences obtained from the FGPS6/FGPS1509 PCR product and from NCBI sequences for *Xad*, respectively, to amplify a 950-base sequence of the 16S rRNA gene. Primers R16-1 and R23-2R (Table 1) were used to amplify 850 bases, including the 16S-23S spacer region (Nakagawa et al., 1994). The three primer sets produced sequences with overlapping regions (Fig. 1). The sequences were combined for submission to the GenBank but were analyzed as two regions, the 16S rRNA gene and the 16S-23S intergenic spacer. PCR reactions contained 10 mM Tris, 50 mM KCl, 2 mM or 3 mM MgCl₂, 200 µM of each dNTP, 0.5 µM of each primer, and 1 U Taq polymerase. Thermal cycling parameters consisted of 5 min at 95 °C for initial denaturation followed by 30 cycles at 95 °C for 30 s, annealing for 30 s and 72 °C for 90 s, followed by a final elongation of 4 min at 72 °C. Annealing temperature and MgCl₂ concentration varied with primer pairs (Table 1). The thermal cycler model used was the PTC-200 from MJ Research (Waltham, MA).

Greenhouse evaluation of resistance to bacterial blight in caladium cultivars. Seventeen major commercial caladium cultivars and one breeding line (all subsequently referred to as cultivars) were screened for resistance to BB (Table 2). Plants were forced from stored tubers and grown in plastic containers in the greenhouse until they produced several fully expanded leaves. Potted plants were arranged in a RCBD with 10 replicate blocks, each containing one plant of each of the 18 caladium cultivars screened. The experiment was conducted three times. Plants were pruned to remove older and partially expanded leaves, leaving every plant with two (Expts. 1 and 2) or three (Expt. 3) young but fully expanded leaves. The leaves of all plants, both abaxial and

adaxial surfaces, were sprayed with inoculum prepared as described previously, except that the three strains (05-113, 05-185, and 05-220) were mixed in equal proportions after their concentrations were adjusted to O.D._{590nm} = 0.1 to ensure that results will apply to the wide range of *Xad* strains encountered in the field. Inoculated plants were maintained in the greenhouse for 6 weeks. For the first two experiments, a frame was constructed around all the plants and enclosed with a sheet of plastic overnight immediately after inoculation to increase humidity and promote infection. During the third experiment, plants were maintained under misters, 20 s mist every 5 min, for the duration of the experiment to promote initial infection and secondary spread. Disease severity ratings (DSRs) were taken at 3 and 6 weeks postinoculation (WPI) using the 0 to 11 Horsfall-Barrett scale for area of leaf infection, in which 0 = 0%, 1 = 0% to 3%, 2 = 3% to 6%, 3 = 6% to 12%, 4 = 12% to 25%, 5 = 25% to 50%, 6 = 50% to 75%, 7 = 75% to 88%, 8 = 88% to 94%, 9 = 94% to 97%, 10 = 97% to 100%, and 11 = mortality (Horsfall and Cowling, 1978). The identity of the pathogen was confirmed by isolating from symptomatic tissue of arbitrarily selected plants as described previously. The variance in DSRs was analyzed using the PROC MIXED procedure with a Tukey-Kramer adjustment (SAS 9.1; SAS Institute, Cary, NC) and used to categorize the cultivar's susceptibility to *Xad*.

Evaluation of commercial cultivars in the field. The 19 cultivars (Table 3) were grown on fumigated, raised beds under plastic mulch for 5.5 months. The beds (71 cm wide, 21 cm high) were fumigated on 3 Apr. 2007 using a mixture of 67% methyl bromide and 33% chloropicrin (by volume) at 196 kg·ha⁻¹ and covered with white-on-black plastic mulch (white side facing upward). Caladium seed pieces were planted on 16 Apr. 2007 with 25.4 cm between-row spacing and 15.2 cm in-row spacing. A drip irrigation system was used to provide water (≈6 mm per day) and 6N-0.8P-3.9K soluble fertilizer with nitrogen (N) at a rate of ≈1.9 kg·ha⁻¹·d⁻¹ (total N at 290 kg·ha⁻¹ per growing season). Cultivars were arranged in the field in a RCBD, and each cultivar was replicated three times with 30 plants in each experi-

mental unit (plot). BB developed from natural inoculum and was monitored over the growing season. Previous observations indicated that in the field, BB generally reaches its highest severity in late September, at the end of the warm, rainy, and humid summer season and before the cooler, drier fall season. DSRs were taken on 25 Sept. 2007 using a 0 to 5 scale (0 = no BB lesion, 1 = one to five BB lesions per 30-plant plot, 2 = lesions present on less than 50% of leaves, 3 = lesions on 50% to 90% of leaves, 4 = lesions on greater than 90% of leaves, and 5 = lesions on greater than 90% of leaves plus significant defoliation observed). The variance of the DSRs was analyzed using PROC GLM with Tukey's studentized range and the correlation between DSRs in the field and greenhouse was determined by PROC CORR (Pearson's correlation coefficient) using data from the 10 cultivars tested in both trials (SAS 9.1; SAS Institute).

Results

Confirmation of pathogenicity. All three strains tested were pathogenic to caladiums. Symptoms were first visible ≈1 week after inoculation. Three weeks after inoculation, at least two-thirds of plants were symptomatic for each *X. axonopodis* strain–caladium cultivar combination with the exception of strain 05-185 on 'Candidum', which remained healthy. Non-inoculated control plants also remained healthy. The pathogen was recovered from 10 arbitrarily selected, symptomatic plants.

Identification of pathogens. Two strains, 05-185 and 05-220, were identified as *X. axonopodis* by fatty acid analysis (similarity index 0.902 and 0.851, respectively) and a Biolog carbon source use profile (Table 4). Both tests identified *Xad* either as the first or second most similar pathovar (Table 4). Strain 05-113 was not as clearly identified with these assays. Biolog identified this strain as *X. campestris* (similarity 0.73), whereas the fatty acid analysis was inconclusive. The closest matches were *X. axonopodis* (similarity 0.376) and *X. campestris* (similarity 0.257).

Primer pair FGPS6 and FGPS1509 amplified the expected ≈1480-base sequence, the majority of the 16S rRNA gene. However,

Table 1. Polymerase chain reaction (PCR) primers used for the amplification and sequencing of the 16S rRNA gene and 16S-23S spacer.

Primer Pair	Primer Designation	5'-3' Sequence	Location	PCR product	Annealing temperature	MgCl ₂	Reference
1	FGPS6	GGA GAG TTA GAT CTT GGC TCA G	16SrRNA gene	1480 b	54 °C	2 mM	Ponsonnet and Nesme, 1994
	FGPS1509 ²	AAG GAG GGG ATC CAG CCG CA	16SrRNA gene				
2	16S-535	TAA AGC GTG CGT AGG TGG TG	16SrRNA gene	950 b	57 °C	3 mM	This study
	16S-1550	ATT TGT GAG GAC GCC CG	16S-23S spacer				
3	R16-1	CTT GTA CAC ACC GCC CGT CA	16SrRNA gene	850 b	55 °C	2 mM	Nakagawa et al., 1994
	R23-2R	TCC GGG TAC TTA GAT GTT TC	23SrRNA gene				

²Primer FGPS1509 was only used for PCR amplification, because unlike the other primers, it could not initiate a successful sequencing reaction.

only primer FGPS6 could be used to initiate a successful sequencing reaction. These reactions sequenced from 560 to 1000 bases. When FGPS1509 was compared with 16S rRNA gene sequences from *Xad* in the NCBI database, there was a one-base discrepancy between the primer and the gene sequences. Because only FGPS6 amplified products for sequencing, each strain was sequenced twice using products from this primer. Two new primers, 16S-535 and 16S-1550, were designed and used to amplify the remaining portion of the gene. These primers amplified a sequence of ≈ 950 bases, and this overlapped even the shortest 560-base sequence obtained using the primer FGPS6. Together these two primer pairs amplified a sequence of ≈ 1500 bases, the majority of the 16S rRNA gene. The third primer pair, R16-1 and R23-2R, amplified a sequence of ≈ 850 bases. This sequence includes the entire 16S-23S intergenic spacer region along with short sequences from the 16S rRNA and 23S rRNA genes. It overlapped with the sequence of the 16S rRNA gene obtained with the first two primer pairs.

Sequences submitted to NCBI received the following accession numbers: 05-113, #EU203151; 05-185, #EU203152; and 05-220, #EU203153. The 16S gene sequences for strains 05-185 and 05-220 were identical to

each other and to 13 NCBI sequences from *X. axonopodis* (two from pv. *dieffenbachiae*). Strain 05-113 has a two-base difference in its 16S rRNA gene from strains 05-185 and 05-220. Its closest match in the NCBI database, with one base different, is a strain of *Xad*. Although the closest match for all three strains is *X. axonopodis*, the 16S sequence is too conserved to be used for identification to the species level. Numerous other *Xanthomonas* spp. (i.e., *X. campestris*, *X. vasicola*, *X. gardneri*, and *X. oryzae*) are greater than 99% identical, some sequences with as few as three bases different. There are at least six 16S rRNA gene sequences from *Xad* that have three to six bases different from our strains.

The 16S-23S intergenic spacer region is less conserved than the 16S rRNA gene and shows more divergence between *X. axonopodis* and other species of *Xanthomonas*. *X. axonopodis* strains have 2% or less sequence divergence, whereas other species have greater than 3% variance from *X. axonopodis*. The sequences of this spacer region from strains 05-185 and 05-220 were identical to each other and to one NCBI sequence from *Xad*. Strain 05-113 has a spacer sequence identical to eight other *X. axonopodis* strains in the NCBI database, including three *Xad*. Strain 05-113 has four bases different from strains 05-185 and 05-220.

Greenhouse evaluation of resistance to bacterial blight in caladium cultivars. Analysis indicated that experiments were not significantly different; therefore, data from all greenhouse experiments were pooled (Table 2). Significant differences ($P < 0.0001$) in disease severity were observed among cultivars at 6 WPIs. DSRs collected at 3 WPIs gave a similar ranking for cultivars as the data at 6 WPIs; however, these data did not distinguish resistant (R) from moderately susceptible (MS) cultivars because neither group of cultivars had much disease at 3 weeks. Therefore, only the data from 6 weeks are presented.

Although the three experiments were not significantly different, they did have different levels of disease. In Expts. 1 and 2, disease severity varied with the distance that a replicate was from the greenhouse cooling pads (data not shown), likely resulting from increased humidity near the pads. Replicates further from the pads exhibited less disease. Expt. 2 was located further away from the cooling pad than Expt. 1 and, consequently, had less disease. To eliminate the effect of the cooling pads, Expt. 3 was conducted under misters. The highly conducive conditions for disease produced by the mister led to more severe BB in the R and MS cultivars; however, the highly susceptible (HS) cultivars had severities similar to Expt. 1.

A significant interaction ($P = 0.02$) between cultivar and experiment was detected, likely as a result of a few cultivars that had dissimilar results for one of the three experiments. For example, 'Aaron' was clearly susceptible in Expts. 1 and 3 (average ratings of 2.1 and 3.8, respectively), but plants remained healthy in Expt. 2. The average DSR from all three experiments was used to classify cultivars based on susceptibility to BB. Cultivars with an average rating of less than 1 were classified as R, 1 to less than 3 as MS, and 3 or higher as HS (Table 2). All cultivars classified as R had significantly less disease than those classified as HS.

One of the R cultivars, White Queen, was observed to exude *Xad* from the lower surface of an apparently healthy leaf. The bacterium was isolated and pathogenicity confirmed as described previously.

Evaluation of commercial cultivars in the field. Disease severity was significantly different ($P < 0.0001$) among cultivars (Table 3). Similar to the greenhouse experiments, cultivars with an average DSR of less than 1 were classified as R, 1 to less than 3 as MS, and 3 or greater as HS. Two R cultivars, Pink Symphony and White Queen, had no visible symptoms in the field. All R cultivars had significantly less disease than the HS.

Ten of the 19 cultivars included in this trial were also in the greenhouse trials described previously. Analysis of these 10 cultivars confirmed that DSRs from the greenhouse and field were correlated ($r = 0.7785$, $P < 0.01$). The cultivars were classified into resistance categories similar to those for the greenhouse experiments. 'Florida Cardinal', 'Red Flash', and 'Candidum' were categorized as HS in the

Fig. 1. Polymerase chain reaction amplification of the 16S rRNA gene and 16S-23S spacer in overlapping fragments for sequencing using three primer pairs: FGPS6/FGPS1509, 16S-535/16S-1550, and R16-1/R23-2R.

Table 2. Bacterial blight severity from greenhouse inoculations.^a

Cultivar	Leaf type ^y	Expt. 1 Rating ^y	Expt. 2 Rating ^y	Expt. 3 Rating ^x	Avg disease rating	Resistance category
White Queen	Fancy Novelty	0.1	0	1.9	0.7 a ^w	R
Florida Red Ruffles	Lance Red	0.4	0.1	1.6	0.7 a	R
Florida Sweetheart	Lance Pink	0.3	0	2.5	0.9 ab	R
Candidum Jr.	Fancy White	1.8	0.1	0.9	0.9 ab	R
Mrs. Arno Nehrling	Fancy Novelty	0.5	0.3	2	0.9 ab	R
Florida Fantasy	Fancy Novelty	0.2	0.2	3	1.1 abc	MS
Florida Irish Lace	Lance Green	1.2	0.8	2	1.3 abc	MS
Carolyn Whorton	Fancy Pink	0.6	1.1	2.6	1.4 abc	MS
Red Frill	Lance Red	1.2	1.2	2.5	1.6 abcd	MS
Aaron	Fancy White	2.1	0.0	3.8	2.0 abcd	MS
Kathleen (tall)		2.7	1.1	3.4	2.4 abcde	MS
Red Flash	Fancy Red	1.9	1.8	3.3	2.3 abcde	MS
Florida Cardinal	Fancy Red	3.6	1.8	3.0	2.8 bcde	MS
18-45	Fancy Novelty	2.3	1.5	4.3	2.7 bcde	MS
Florida Blizzard	Fancy White	3.6	1.9	3.4	3.0 cde	HS
Rosebud	Fancy Pink	3.7	1.2	4.1	3.0 cde	HS
White Christmas	Fancy White	3.2	3.2	4.1	3.5 de	HS
Candidum	Fancy White	5	3.3	4.1	4.1 e	HS

^aCaladium cultivars were classified as resistant (R), moderately susceptible (MS), or highly susceptible (HS).

^yCaladium cultivars were grouped based on leaf shape (fancy or lance) and primary leaf color [green, white, red, pink or novelty (multicolor)] (Bell et al., 1998).

^xHorsfall-Barrett (HB) scale for area of leaf infection: 0 = 0%, 1 = 0% to 3%, 2 = 3% to 6%, 3 = 6% to 12%, 4 = 12% to 25%, 5 = 25% to 50%, 6 = 50% to 75%, 7 = 75% to 88%, 8 = 88% to 94%, 9 = 94% to 97%, 10 = 97% to 100%, 11 = mortality (Horsfall and Cowling, 1978); average of 10 replications.

^wCultivar ratings followed by the same letter are not significantly different ($P < 0.0001$).

field, whereas they were either HS or MS (with a higher DSR) in the greenhouse. 'Florida Fantasy', 'Florida Sweetheart', 'Candidum Jr.', and 'Red Frill' had the lowest DSR of the MS in this trial and ranked MS, R, R, or MS, respectively, in the greenhouse. 'White Queen', 'Florida Red Ruffles', and 'Carolyn Whorton' were R compared with R, R, or MS (with a lower DSR), respectively, in the greenhouse experiments.

Discussion

The three pathogenic bacterial strains were identified as *X. axonopodis* by the sequence of the 16S-23S rRNA intergenic spacer region. Carbon source use profiles and fatty acid analysis only supported identification for two of the three isolates tested. Previous work with these techniques found that although useful for the identification of many bacterial species, including some species of *Xanthomonas*, *X. axonopodis* strains do not always produce a consistent and unique profile (Vauterin et al., 1995, 1996). The pathovar *Xad* is defined by its host range, pathogens of the family Araceae (aroids). This pathovar has been shown to be a hetero-

geneous group by serology, carbon source use, fatty acid analysis, rep-PCR, and random amplified polymorphic DNA analysis (Chase et al., 1992; Khondoo and Jaufeerally-Fakim, 2004; Rademaker et al., 2005). Strains of *Xad* from different species of aroids are differentiated primarily by host range with strains being generally more pathogenic on the host of origin. However, there is considerable cross-pathogenicity of strains to different species of aroids. Strains from *Anthurium* and *Synгонium* could be differentiated using restriction analysis of PCR amplification products, whereas strains from other hosts were generally difficult to distinguish from each other by physiological, serological, or molecular techniques (Chase et al., 1992; Khondoo and Jaufeerally-Fakim, 2004; Lipp et al., 1992; Robène-Soustrade et al., 2006). Little is known about the host range of strains from caladium, but two strains from caladium were pathogenic to *Anthurium*, *Dieffenbachia*, and/or *Synгонium* (Robène-Soustrade et al., 2006).

One of the three *Xad* strains used in these experiments was differentially virulent among caladium cultivars. Strain 05-185 caused symptoms on 'Gingerland' and '18-45' but not on the HS 'Candidum'. Therefore, it is

important to perform resistance screening with a cocktail of strains to ensure that results will apply to the wide range of *Xad* strains that may be encountered in the field. The variability of the natural inoculum in the field experiment is unknown and the inoculum may have consisted primarily of one *Xad* strain. Thus, additional evaluations should be conducted in other locations to confirm the resistance of certain cultivars. It is also important to evaluate cultivars in different seasons under natural environmental conditions to determine the level of resistance necessary to achieve control in the field. Some cultivars rated as R in greenhouse tests may provide sufficient levels of control for most situations.

There is a wide range of susceptibility to *Xad* among caladium cultivars. Five to eight cultivars were identified as resistant to BB. 'White Queen', 'Florida Red Ruffles', 'Florida Sweetheart', 'Candidum Jr.', and 'Mrs. Arno Nehrling' were identified as R in the replicated greenhouse trials. 'Carolyn Whorton' was classified as R during the natural field epidemic and exhibited one of the lowest DSRs of the MS cultivars in the greenhouse trials. 'Pink Symphony' showed no symptoms during the field observation and was therefore classified as R but might respond differently in other locations with different predominant strains and environmental conditions. None of the cultivars studied in the replicated greenhouse trials was completely R; even the most R cultivars exhibited symptoms, especially in Expt. 3 during which plants were maintained under conditions very favorable to infection and symptom development. At least one leaf rating of 4 or 5 (12% to 50% of leaf tissue symptomatic) was recorded for each of these cultivars (data from individual leaves not presented).

Identification of R cultivars will assist growers in selecting cultivars for use in situations in which the caladiums will be exposed to rain and warm, humid conditions. In our work as well as previous studies (Norman and Alvarez, 1994), it is apparent that cultivars with some level of resistance can have quiescent infections and carry the pathogen without showing symptoms. Thus, care should be taken to avoid planting infected R plants adjacent to HS cultivars. Available R cultivars are not suitable for all uses; therefore, new improved cultivars will have to be developed to provide R cultivars for the various disease problems of caladium. The possibility exists that bacterial strains with virulence to R cultivars will be selected

Table 3. Bacterial blight disease severity from naturally occurring field epidemic.^z

Cultivar	Leaf type ^y	Avg disease severity rating ^x	Resistance category	Resistance category Greenhouse experiments ^w
Pink Symphony	Lance Pink	0.0 a ^v	R	—
White Queen	Fancy Novelty	0.0 a	R	R
Florida Red Ruffles	Lance Red	0.7 ab	R	R
Carolyn Whorton	Fancy Pink	1.0 abc	R	MS
Florida Fantasy	Fancy Novelty	1.3 abc	MS	MS
Red Frill	Lance Red	1.7 bcd	MS	MS
Candidum Jr	Fancy White	1.7 bcd	MS	R
Florida Sweetheart	Lance Pink	1.7 bcd	MS	R
Fannie Munson	Fancy Pink	1.8 bcd	MS	—
Florida White Ruffles	Lance White	2.0 bcde	MS	—
White Wing	Lance White	2.0 bcde	MS	—
Miss Muffet	Fancy Novelty	2.0 bcde	MS	—
Pink Gem	Lance Pink	2.3 cde	MS	—
Gingerland	Fancy Novelty	2.8 def	MS	—
Candidum	Fancy White	3.3 efg	HS	HS
Frieda Hemple	Fancy Red	4.0 fgh	HS	—
Red Flash	Fancy Red	4.0 fgh	HS	MS
Postman Joyner	Fancy Red	4.7 gh	HS	—
Florida Cardinal	Fancy Red	5.0 h	HS	MS

^zCaladium cultivars were classified as resistant (R), moderately susceptible (MS), or highly susceptible (HS) based on disease severity ratings taken 25 Sept. 2007.

^yCaladium cultivars were grouped based on leaf shape (fancy or lance) and primary leaf color [green, white, red, pink or novelty (multicolor)] (Bell et al., 1998).

^xDisease severity rated as: 0 = no BB, 1 = one to five BB lesions per 30 plant/plot, 2 = lesions present on less than 50% of leaves, 3 = lesions on 50% to 90% of leaves, 4 = lesions on greater than 90% of leaves, 5 = lesions on greater than 90% of leaves + significant defoliation observed.

^wOriginal data presented in Table 2.

^vCultivar ratings followed by the same letter are not significantly different ($P < 0.0001$).

Table 4. Identification of pathogenic *Xanthomonas* strains as species/pathovars.^z

Strain no.	Fatty acid analysis		Biolog (carbon source use profile)			16S-23S rRNA spacer sequence	
	Identification	Similarity index	Identification	Probability	Similarity index	Identification	Maximum identity (%)
05-113	<i>X. a.</i> pv. <i>manihotis</i>	0.376	<i>X. campestris</i>	99	0.73	<i>X. axonopodis</i>	100
	<i>X. campestris</i>	0.257					
05-185	<i>X. a.</i> pv. <i>citrumelo</i>	0.902	<i>X. a.</i> pv. <i>dieffenbachiae</i>	100	0.85	<i>X. axonopodis</i>	100
	<i>X. a.</i> pv. <i>dieffenbachiae</i>	0.784					
05-220	<i>X. a.</i> pv. <i>citrumelo</i>	0.851	<i>X. a.</i> pv. <i>begonia</i>	96	0.74	<i>X. axonopodis</i>	100
	<i>X. a.</i> pv. <i>dieffenbachiae</i>	0.696	<i>X. a.</i> pv. <i>dieffenbachiae</i>	4	0.03		

^zFor each identification technique, the two closest matches from the database are listed.

^y*X. a.* = *X. axonopodis*

over time; thus, a continued breeding effort will be required.

Because of their resistance to fusarium tuber rot (FTR), caused by *Fusarium solani* (Mart.) Saa., and to Pythium root rot (PRR), caused by *Pythium myriotylum* Drechs. (Deng et al., 2005a, 2005b; Goktepe et al., 2007), diseases that can significantly affect the production of caladiums, 'Candidum' and 'White Christmas' have been widely used in caladium breeding for improving FTR and PRR resistance. The findings in this study that both cultivars were highly susceptible to BB (Tables 2 and 3) show a strong need to select appropriate parental cultivars and cross combinations for breeding efforts to develop new cultivars with resistance not only to major root and tuber diseases, but also to the major foliar disease, BB. 'Candidum Jr.' and 'Florida Sweetheart' may be good parents to select for disease resistance. Both are R to two of the three major diseases, BB/PRR and BB/FTR, respectively, and only MS to the third. These and other R cultivars identified in this study will serve as valuable sources of resistance in future breeding efforts.

Literature Cited

- Bell, M.L., G.J. Wilfret, and D.A. Devoll. 1998. Survey of caladium tuber producers for acreage of cultivars grown. Proc. Fla. State Hort. Soc. 111:32-34.
- Chase, A.R. 1997. Caladium, p. 27-29. In: Chase, A.R. (ed.). Foliage plant diseases diagnosis and control. APS Press, St. Paul, MN.
- Chase, A.R., R.E. Stall, N.C. Hodge, and J.B. Jones. 1992. Characterization of *Xanthomonas campestris* strains from aroids using physiological, pathological, and fatty acid analyses. Phytopathology 82:754-759.
- Deng, Z., B.K. Harbaugh, R.O. Kelly, T. Seijo, and R.J. McGovern. 2005a. Pythium root rot resistance in commercial caladium cultivars. HortScience 40:549-552.
- Deng, Z., B.K. Harbaugh, R.O. Kelly, T. Seijo, and R.J. McGovern. 2005b. Screening for resistance to pythium root rot among twenty-three caladium cultivars. HortTechnology 15:631-634.
- Deng, Z., B.K. Harbaugh, R.C. Schoelhorn, and R.C. Andrews. 2008. 2003 survey of the Florida caladium tuber production industry (ENH1007). University of Florida, Institute of Food and Agricultural Sciences, Gainesville, FL. 31 Jan. 2008. <http://edis.ifas.ufl.edu/EP258>.
- Dickstein, E.R., J.B. Jones, and D.E. Stall. 2001. Appendix C. Automated techniques, p. 343-352. In: Schaad, N.W., J.B. Jones, and W. Chun (eds.). Laboratory guide for identification of plant pathogenic bacteria. APS Press, St. Paul, MN.
- Goktepe, F., T. Seijo, Z. Deng, B.K. Harbaugh, N.A. Peres, and R.J. McGovern. 2007. Toward breeding for resistance to fusarium tuber rot in caladium: Inoculation technique and sources of resistance. HortScience 42:1135-1139.
- Horsfall, J.G. and E.B. Cowling. 1978. Pathometry: The measurement of plant disease, p. 120-136. In: Horsfall, J.G. and E.B. Cowling (eds.). Plant disease. Academic Press, New York, NY.
- Khodoo, M.H.R. and Y. Juafeerally-Fakim. 2004. RAPD-PCR fingerprinting and Southern analysis of *Xanthomonas axonopodis* pv. *dieffenbachiae* strains isolated from different aroid hosts and locations. Plant Dis. 88:980-988.
- Lipp, R.L., A.M. Alvarez, A.A. Benedict, and J. Berestecky. 1992. Use of monoclonal antibodies and pathogenicity tests to characterize strains of *Xanthomonas axonopodis* pv. *dieffenbachiae*. Phytopathology 82:677-682.
- Nakagawa, T., M. Shimada, H. Mukai, K. Asada, I. Kato, K. Fujino, and T. Sato. 1994. Detection of alcohol-tolerant hiochi bacteria by PCR. Appl. Environ. Microbiol. 60:637-640.
- Norman, D.J. and A.M. Alvarez. 1994. Latent infections of in vitro *Anthurium* caused by *Xanthomonas axonopodis* pv. *dieffenbachiae*. Plant Cell Tissue Org. Cult. 39:55-61.
- Norman, D.J., R.J. Henny, and J.M.F. Yuen. 1997. Disease resistance in twenty *Dieffenbachia* cultivars. HortScience 32:709-710.
- Norman, D.J., R.J. Henny, and J.M.F. Yuen. 1999. Resistance levels of pot anthurium cultivars to *Xanthomonas campestris* pv. *dieffenbachiae*. HortScience 34:721-722.
- Normand, P., B. Cournoyer, P. Simonet, and S. Nazaret. 1992. Analysis of a ribosomal RNA operon in the actinomycete *Frankia*. Gene 111:119-124.
- Ponsonnet, C. and X. Nesme. 1994. Identification of *Agrobacterium* strains by PCR-RFLP analysis of pTi and chromosomal regions. Arch. Microbiol. 161:300-309.
- Rademaker, J.L., F.J. Louws, M.H. Schultz, U. Rossbach, L. Vauterin, J. Swings, and F.J. de Bruijn. 2005. A comprehensive species to strain taxonomic framework for *Xanthomonas*. Phytopathology 95:1098-1111.
- Robène-Soustrade, I., P. Laurent, L. Gagnevin, E. Jouen, and O. Pruvost. 2006. Specific detection of *Xanthomonas axonopodis* pv. *dieffenbachiae* in anthurium (*Anthurium andreanum*) tissues by nested PCR. Appl. Environ. Microbiol. 72:1072-1078.
- Sasser, M. 1990. Identification of bacteria through fatty acid analysis, p. 199-204. In: Klement, Z., K. Rudolph, and D.C. Sands (eds.). Methods in phytobacteriology. Akademiai Kiado, Budapest, Hungary.
- Vauterin, L., B. Hoste, K. Kersters, and J. Swings. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472-489.
- Vauterin, L., P. Yang, and J. Swings. 1996. Utilization of fatty acid methyl esters for the differentiation of new *Xanthomonas* species. Int. J. Syst. Bacteriol. 46:298-304.