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FULL RESEARCH PAPER

A quantitative screening method for the detection of foliar resistance to *Xanthomonas axonopodis* pv. *dieffenbachiae* in anthurium

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Abstract Resistance to foliar infection by *Xanthomonas axonopodis* pv. *dieffenbachiae*, the causal agent of the bacterial blight disease (blight) of anthurium (*Anthurium andraeanum*), was investigated in four experiments. Initially, an inoculation method based on vacuum infiltration (15 psi) of leaf discs (2.67 cm diam) was tested at two inoculum densities (10^8 and 10^9 colony-forming units (CFU) ml^{-1}) on 14 anthurium cultivars using three measures of resistance (lesion size, lesion expansion rate and the time taken for lesion to cover the entire leaf disc (TLC)), towards developing an optimised inoculation method capable of differentiating between levels of resistance to blight. The method was repeatable over seasons and was validated by correlation of laboratory results to field resistance scores. An inoculum density of 10^8 CFU ml^{-1} and TLC, were the best inoculum density and resistance measures, respectively, based on (a) their ability to differentiate between various levels of resistance and (b) their correlation to field resistance. Using the method, cultivars can be differentiated into three groups based on TLC: 4–8 days is designated as highly susceptible; 9–13 days as moderately susceptible; and >13 days as resistant. Twenty-two percent of the cultivars screened were resistant to blight. The bacterial multiplication rates were significantly lower in the resistant types compared to susceptible cultivars. Resistance values for foliar (TLC; days) and systemic blight (time to death; weeks) in 45 anthurium cultivars showed poor correlation (r=0.067), showing that they are governed by different mechanisms.

Keywords Differential resistance · Leaf blight · Polygenic resistance · Two-phase-screening

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Introduction

The bacterial blight disease (blight) of *Anthurium andraeanum* (Kamemoto and Kuehnle 1996) caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (XAD) (Vauterin et al. 1995), previously known as *Xanthomonas campestris* pv. *dieffenbachiae* (Bradbury 1986), was attributed to the rapid decline in anthurium production in Hawaii in the early 1980s (Nishijima 1988). The disease has since been reported in most anthurium-producing countries including the mainland USA (Norman and Alvarez 1989; Lipp et al. 1992) and is primarily responsible for the demise of the Caribbean anthurium industry in the late 1980s and early 1990s (Elibox and Umaharan 2007).

The cultivation of anthurium in the Caribbean is based primarily on cultivars imported from the

Netherlands, which for the most part, are highly susceptible to blight. As a result, anthurium cultivation is carried out under capital-intensive structures supported by high levels of management, both of which increase the cost of production (Anaïs et al. 2000). Cultural measures to manage blight at economically feasible levels (Nishijima and Fujiyama 1985; Nishijima 1988, 1989; Norman and Alvarez 1996; Anaïs et al. 2000) have proven to be impractical, while chemical (Nishijima and Chun 1991) and biological control (Fukui et al. 1999a, c) measures have been largely ineffective. The development of blight resistant cultivars through breeding is the only sustainable means of revitalising the anthurium industry (Prior et al. 1985; Anaïs et al. 2000; Elibox and Umaharan 2007).

Blight has two phases, a foliar and a systemic phase (Fukui et al. 1998). In the foliar phase the pathogen invades the foliage of the plant. In susceptible cultivars, the foliar infections can rapidly progress into a systemic phase resulting in plant death. Anthurium cultivars show differential susceptibility to the foliar and systemic phases of blight (Fukui et al. 1998).

The foliar phase of infection can severely decrease farm productivity. Routine removal of diseased leaves as part of the field sanitation practices can result in reduced photosynthetic capacity and stunting of the plant. In cases where most of the leaves have been removed, flowers are not produced, take longer to mature or are unmarketable.

Foliar symptoms of blight usually start at, or close to the leaf margins via hydathodes (Sakai 1990) but occasionally from the centre of leaves (Prior et al. 1985). The first sign of infection is the appearance of irregular water-soaked spots on the underside of leaves and spathes (Prior et al. 1985) and faint chlorosis when viewed from the adaxial surface (Nishijima 1988). As infection proceeds, the spots become brown or black at the centre and bright yellow at the edges (Nishijima 1988) often covering large sections of the leaves. Also, the disease is of greater significance in the wet season compared to the dry (Dilbar and Gosine 2003). Foliar infection may progress into a systemic crown infection, in some cultivars, which results in eventual plant death.

Recently, a rapid screening method for the detection of resistance to systemic invasion by blight was reported (Elibox and Umaharan 2007), but such a method for the detection of resistance at the foliar phase of infection is lacking. Although spray inoculation of leaves has been used successfully to infect anthurium in the Caribbean (Prior et al. 1985) and in Hawaii (Fukui et al. 1996), later studies reported that the spray inoculation method is ineffective in reliably establishing foliar blight infection in anthurium (Anaïs et al. 2000; Dilbar and Gosine 2003). Furthermore, the method is not amenable for the rapid screening of segregating populations or the quantitative assessment of the levels of resistance in breeding programmes.

The aim of this study was to develop and validate a quantitative screening method capable of rapidly differentiating levels of foliar resistance to blight, and to use this method to identify resistance to foliar infection in anthurium.

Materials and methods

Anthurium cultivars and leaf stage

Forty-nine anthurium cultivars (4 year-old) were used in four experiments during the wet (June–December, 2003) and dry seasons (January–April, 2004). Three fully opened but immature (stage-2) leaves (Fukui et al. 1998) with no blemishes were harvested from each cultivar and taken to the laboratory in an ice-chamber and used in the experiments.

Isolation and characterisation of *X. axonopodis* pv. *dieffenbachiae*

Anthurium leaves showing typical symptoms of blight were collected from 14 commercial anthurium fields. The pathogen was isolated, biochemically characterised as *X. axonpodis* pv. *dieffenbachiae* as described by Lipp et al. (1992) and Fukui et al. (1998) and verified using Fatty Acid Methyl Ester (FAME) analysis and the Biolog system. They were then tested for pathogenicity and the positive strains were assessed for their aggressiveness according to Elibox and Umaharan (2007). Distilled water and a non-pathogenic strain of XAD were used as negative controls. The most aggressive strain, X4 (Elibox and Umaharan 2007), was used in all experiments.

Preparation of inoculum and inoculation

Strain (X4) of XAD was placed on 100 ml of Luria agar slants and incubated at 30°C for 48 h. The slants

were washed with sterile distilled water, serially diluted, and inoculum densities determined according to Benson (1990). Fresh inoculum was prepared and diluted to 10^8 and 10^9 colony-forming units (CFU) ml^{-1} prior to each inoculation. A preliminary experiment had shown that infection success was low and took a longer time to establish at concentrations below 10^8 CFU ml^{-1}. Negative controls were used as described before. Inoculations were performed by vacuum-infiltration (15 psi; 5 s) of leaf discs (2.67 cm diam) in a conical flask. The original infiltration area (OIA) of each disc was traced onto clear plastic and transcribed onto brown paper. The area of the cut-outs were determined using a leaf area meter (ΔT Area Meter MK2 model, Delta-T Devices, England), and used as a covariate to correct final lesion size. The discs were incubated at 30°C for 20 days in plastic trays lined with two layers of moist paper towel and enclosed individually in large clear polythene bags.

Experiment 1: developing an optimised screening method

Fourteen anthurium cultivars were studied in two trials (July, 2003 and Oct., 2003) in the wet season. In Trial 1, leaf discs of the 14 cultivars were vacuum infiltrated with inoculum at either 10^8 or 10^9 CFU ml^{-1} and incubated as described before. The 28 (14×2) treatment combinations were arranged in a split-plot design (main plot = inoculation concentration; sub-plot = cultivar) with three replications, and five discs per replicate.

The following data were collected on an individual disc basis: the lesion size at 6, 9, 12 and 16 days after inoculation (DAI); time taken for the expanding lesion to cover the entire disc (TLC) and the lesion expansion rate (LER). Lesion size was measured as described before. The LER over time was determined for each treatment combination and for each replicate separately, from the slope obtained from linear regression analysis (lesion size versus time) using the Number Crunching Statistical System (NCSS 2001).

The significance of the effects of cultivar, inoculum density and interaction between cultivar x inoculum density, were determined by ANOVA (NCSS 2001). For each measure of resistance the following were also calculated: $LSD_{(0.05)}$, range and index of differentiation (ID). ID was calculated as the range divided by $LSD_{(0.05)}$.

Field foliar resistance scores were known for 13 of the 14 cultivars through a previous farmer survey. The association between foliar field resistance and lesion size (6, 9, 12 and 16 days), TLC and LER at 10^8 and 10^9 CFU ml^{-1} inoculum densities were determined by correlation analysis (Spearman's rank correlation; NCSS 2001).

The experiment was repeated (Trial 2) at the optimal inoculum density determined in Trial 1. Data on lesion size at 6, 9 and 12 DAI, TLC and LER were recorded and analysed as before. Repeatability of the results in this trial compared to the previous one was assessed by linear regression analysis of TLC (Trial 1 versus Trial 2) using NCSS (2001) and by comparison of regression lines (TLC Trial 1 versus mean TLC; TLC Trial 2 versus mean TLC), using the COLR Programme (CARDI 1974).

Experiment 2: further screening of anthurium for foliar blight resistance

Thirty-nine anthurium cultivars of which four (Lydia, Mirjam, Pierrot and Venus) were already evaluated in Experiment 1 were evaluated for resistance to XAD (10^8 CFU ml^{-1}) during the wet season (October 2003). The treatments and experimental design were similar to Experiment 1. Data were collected only on TLC (as described before) and subjected to ANOVA (NCSS 2001) to determine the significance of cultivar differences.

All the 49 anthurium cultivars evaluated for resistance to foliar infection by blight in the wet season were categorised based on mean TLC as: susceptible (0–8 days), moderately susceptible (9–13 days), and resistant (>13 days). The correlation between TLC (present study) and systemic resistance reported in a previous study (Elibox and Umaharan 2007) for 45 of the 49 cultivars used in this study was determined.

Experiment 3: repeatability over seasons

Eleven of the 39 anthurium cultivars that were assessed in Experiment 2 were evaluated again in the dry season (February, 2004) using the optimised screening method to determine the repeatability of the screening method over seasons. The experimental design and data collection were similar to Experiment 2. Repeatability of the results over seasons was assessed as in Experiment 1.

Experiment 4: mechanism of foliar blight resistance

Three cultivars, namely Champagne, Local Orange and Pierrot, with TLC (dry season) of 12, 14.2 and 20.2 days, respectively, were inoculated as before (inoculum density:10^8 CFU ml^{-1}). The experiment was arranged in a randomised complete block design with nine replications, with three leaf discs per replicate. On days 5, 7, and 10 of the experiment, three replicates for each cultivar were removed and surface sterilised with 70% ethanol. The CFU per disc was determined by macerating each disc separately in 3 ml of sterile distilled water followed by serial dilution (Willis et al. 1990). The data was transformed to a log scale (Log_{10}CFU per disc) prior to ANOVA (NCSS 2001).

Results

The optimised screening method

The symptoms elicited by XAD on leaf discs were typical of foliar blight in anthurium. TLC showed the best differentiation between cultivars as indicated by its largest ID when compared to the other measures of resistance (Table 1). Further, the ID for TLC at the inoculum density of 10^8 CFU ml^{-1} was approximately twice that at 10^9 CFU ml^{-1}. The second and third most differentiating measures were 'lesion size at day 6' and LER at 10^8 CFU ml^{-1} inoculum density. The ID for lesion size at days 9, 12 and 16 were comparatively much smaller. The correlation coefficient for TLC versus lesion size at day 6 was large and significant (r=−0.75; P<0.01).

The Spearman's rank correlation (NCSS 2001) between field blight resistance scores (CFR) and TLC at the inoculum density of 10^8 CFU ml^{-1} (Table 1), was strong (r=−0.84; P<0.001), and was much larger than that obtained between field resistance and TLC at inoculum density of 10^9 CFU ml^{-1} (r=−0.30) or that obtained between field resistance and 'lesion size at day 6' (r=0.69) at 10^8 CFU ml^{-1}.

When the leaf experiment was repeated in the late wet season using an inoculum density of 10^8 CFU ml^{-1}, again TLC gave the best index of differentiation of 6.2 (Table 2). Further the cultivar means and ranges for the various measures in Trial 2 were quite similar to Trial 1 (Table 2). The Pearson's product moment correlation coefficient (NCSS 2001) between TLC (10^8 CFU ml^{-1}) obtained for the 14 cultivars in Trials 1 and 2 was large and significant (r=0.87; P<0.001). Furthermore, a comparison of regression lines showed that a single regression line could explain the response of the 14 cultivars in Trial 1 and Trial 2. These results in totality indicate that the method was repeatable within season and hence the results for the two trials are presented as mean TLC (Table 3). Further, TLC at 10^8 CFU ml^{-1} inoculum density was consistently the best measure and was used in all subsequent experiments.

Table 1 Mean, index of differentiation (ID), correlation to field resistance (CFR) and other statistics for lesion size at 6, 9, 12 and 16 days; time taken for lesion to cover the entire leaf disc (TLC) and lesion expansion rate (LER) in response to leaf disc inoculation with *Xanthomonas axonopodis* pv. *dieffenbachiae* (10^8 and 10^9 CFU ml^{-1}) in Trial 1

	10^8 CFU ml^{-1}						10^9 CFU ml^{-1}					
	TLC (days)	Lesion size at day (cm^2)				LER (cm^2 day^{-1})	TLC (days)	Lesion size at day (cm^2)				LER (cm^2 day^{-1})
		6	9	12	16			6	9	12	16	
Significance of cultivar (P<)	0.001	0.001	0.001	0.001	NS[a]	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cultivar mean	10.4	3.4	4.1	4.6	5.0	0.28	10.9	4.1	4.2	4.8	5.2	0.24
SE (mean)	0.63	0.30	0.40	0.38	0.21	0.04	0.94	0.24	0.38	0.5	0.49	0.05
Minimum	5.6	2.7	3.5	3.9	5.1	0.18	6.0	0.2	0.7	1.2	0.9	0.09
Maximum	15.7	6.0	5.6	6.1	5.8	0.64	14.0	2.7	3.3	4.0	3.9	0.46
ID[b]	6.7	4.9	2.2	2.3	1.5	4.6	3.6	4.3	2.9	2.4	2.6	2.9
CFR[c]	−0.84	0.69	−0.33	−0.32	−0.15	0.12	−0.3	−0.33	−0.24	0.23	0.11	0.26

[a] Not significant.

[b] Index of differentiation was calculated as range/$LSD_{(0.05)}$.

[c] Spearman's rank correlation between parameters measured and field values from the farmer survey.

Table 2 Mean, index of differentiation (ID) and other statistics for lesion size at 6, 9, and 12 days; time taken for lesion to cover the entire leaf disc (TLC) and lesion expansion rate (LER) in response to inoculation with *Xanthomonas axonopodis* pv. *dieffenbachiae* (10^8 CFU ml^{-1}) in Trial 2

Statistic	10^8 CFU ml^{-1}				
	TLC (days)	Lesion size at day (cm^2)			LER (cm^2 day^{-1})
		6	9	12	
Significance	0.001	0.001	0.001	0.001	0.001
Mean	10.14	3.84	4.37	5.03	0.32
SEM	0.715	0.267	0.307	0.394	0.063
Range	5.1–15.6	2.55–5.18	2.93–5.62	3.77–5.72	0.14–0.71
Id[a]	6.21	4.53	3.71	2.10	3.84

[a] Index of differentiation was calculated as range of cultivars/ $LSD_{(0.05)}$.

Identification of resistance to foliar blight in the wet season (Experiment 2)

There were significant differences ($P<0.05$) in TLC between the 39 cultivars evaluated in the late wet season (Table 3). TLC varied from 4.1 days to 18.8 days with Cross 356 having the longest TLC. By day 8, leaf discs of 35% of the cultivars had deteriorated (4–8 days). These may be considered as highly susceptible. Cultivars with TLC from 9 days to 13 days (43%) were considered moderately susceptible, while those with TLC >13 days (22%) were considered resistant (Alexis, Pierrot, President, Midori, Rosa, Terra, Success, Bianco, Honduras, Acropolis, Cross 356). None of the cultivars tested were immune to the foliar phase of blight.

There was no significant correlation ($r=0.067$; $P>0.05$; Fig. 1) between foliar resistance (TLC values obtained in this study) and systemic resistance assessed as time to death in a previous study for the 45 anthurium cultivars (Elibox and Umaharan 2007). Two cultivars, Honduras and Bianco were highly resistant to blight at both the foliar and the systemic phases. Furthermore, from Fig. 1, it is clear that systemic resistance is qualitative while foliar resistance is quantitative.

Repeatability over seasons

An ANOVA of TLC for the 11 cultivars over the two seasons showed that the cultivar differences were

Table 3 Resistance to foliar blight measured as the time taken for the lesion to cover the leaf disc (TLC) in 49 cultivars of anthurium during the wet season

Time taken for lesion to cover the leaf disc (TLC, days)			
Experiment 1 (mean Trial 1[a] and Trial 2[b])		Experiment 2[c]	
Cultivar	TLC	Cultivar	TLC
Cheers	10.6	Acropolis	18.0
Cuba	10.9	Alexis	13.8
Fantasia	6.3	Andes 115	7.5
Gloria	7.4	Bianco	17.1
Hawaii	9.7	Champagne	10.0
Local Orange	6.0	Cotopaxi	10.5
Lydia	10.3	Cross 356	18.8
Midori	14.5	Cross 2135	5.3
Mirjam	10.0	Cross 2306	11.5
Pierrot	14.4	Cross 3962	7.1
Rosa	14.7	Cross 4835	11.6
Senator	8.8	Cumbia	8.2
Tropical	9.3	Farao	8.4
Venus	11.0	Furore	9.6
		Honduras	17.2
		Ibara	5.1
		Kalapana	4.1
		Laguna	4.8
		Local Mina Pink	10.8
		Local Mina Red	8.3
		Local Mina White	5.5
		Local Pink	11.6
		Local Whitish Pink	10.4
		Lydia	9.8
		Minuet	11.4
		Mirjam	10.1
		Pierrot	14.7
		Pistache	8.5
		President	14.2
		Rapido	5.3
		Safari	5.5
		Sonate	12.6
		Spirit	13.0
		Success	17.0
		Sweety	7.9
		Tequila	8.0
		Terra	14.9
		Venus	11.5
		Victoria	11.5

[a] SEM Experiment 1: Trial 1=0.64.

[b] SEM Experiment 1: Trial 2=0.72.

[c] SEM Experiment 2=0.717.

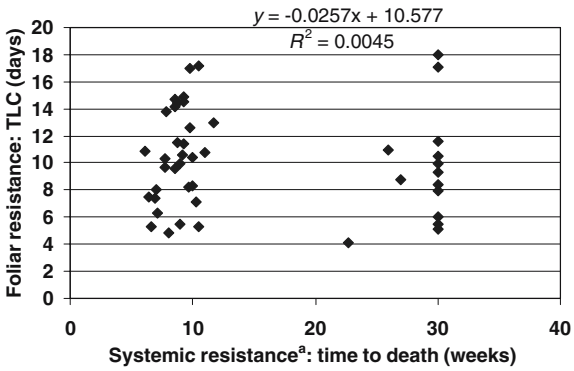

Fig. 1 Relationship between TLC (time taken for lesion to cover the entire leaf disc, days) and systemic blight resistance (time to death, weeks) for 45 anthurium cultivars. [a]At the systemic level, cultivars with no plants that died were given a time to death of 30 weeks

significant at $P \leq 0.001$, the season effects were significant at $P \leq 0.05$ and the cultivar x season interaction effects were not significant ($P > 0.05$). A statistical comparison of linear regression lines showing the responses of the cultivars to inoculation with *X. axonopodis* pv. *dieffenbachiae* in the wet versus the dry season indicated that the they cannot be explained by a single regression line but by two parallel lines. TLC was on average 2 days higher in the dry season compared to the wet in the 11 anthurium cultivars evaluated. Cultivars Acropolis, Cross 356, Honduras, Pierrot and Success consistently had the highest mean TLC over the two seasons. There was a significant positive correlation ($r = 0.75$; $P \leq 0.01$) between TLC for the 11 cultivars evaluated in the wet and dry seasons.

Cultivar differences in bacterial multiplication rates

Cultivar differences in the number of $\log_{10}$CFU/disc were significant ($P \leq 0.05$; Table 4) at 10 days after inoculation (DAI). Tukey–Kramer multiple comparison test showed that $\log_{10}$CFU/disc for cv. Pierrot was significantly different from Champagne. Cultivar Pierrot had significantly lower CFU/disc at 5 DAI, compared to other cultivars with little or no increase in the number of CFU over time. In contrast, cvs Local Orange and Champagne showed 10 and 15-fold increases in the number of CFU/disc at 5 DAI as compared to Pierrot. This value increased a further seven-fold over a 5-day period in Local Orange, and a further 60-fold in Champagne. There was also a strong and significant linear regression ($R^2 = 0.99$; $P < 0.001$) of TLC on tissue bacterial concentration in the three anthurium cultivars.

Discussion

The present research confirmed previous studies in the Caribbean (Prior et al. 1985; Fortune 1990; Young 1990) that the foliar blight of anthurium is capable of causing severe foliar symptoms in susceptible cultivars. The symptoms elicited on leaf discs were typical of blight symptoms on anthurium plants grown under greenhouse conditions.

The study showed that an optimised screening method based on vacuum infiltration of leaf discs (inoculum density=10^8 CFU ml^{-1}; vacuum=15 psi, 5 s) could be used to effectively assess the level of resistance to foliar blight of anthurium cultivars. Vacuum infiltration aided in uniform lesion establishment, reduced the experimental error, and improved the index of differentiation. The time taken for the lesion to cover the leaf disc (TLC), apart from having the highest index of differentiation, was the measure of foliar resistance that best correlated to field resistance and can therefore be regarded as the best and the most valid measure to assess foliar blight resistance. The results showed that TLC could be used in the quantitative assessment of foliar resistance to blight.

Table 4 The effect of cultivar and days after inoculation on the average number of colony-forming units found per leaf disc inoculated with *Xanthomonas axonopodis* pv. *dieffenbachiae* at an inoculum density of 10^8 colony-forming units (CFU) ml^{-1} in anthurium

Cultivar[a]		CFU per disc		
		Day 5	Day 7	Day 10
Champagne	CFU	4.90×10^6	1.41×10^7	2.93×10^8
	Trans.[b]	6.69	7.15	8.47
Local Orange	CFU	2.91×10^6	8.40×10^6	1.92×10^7
	Trans.	6.46	6.92	7.28
Pierrot	CFU	3.10×10^5	7.70×10^5	9.70×10^5
	Trans.	5.49	5.89	5.99

[a] Cultivar differences for $\log_{10}$CFU disc−1 was significant at $P = 0.05$, 10 days after inoculation.

[b] SE of mean for the transformed means=0.338.

The strong and significant correlation of the resistance ranking of cultivars between trials, both within and between seasons, indicated that the method is capable of yielding highly repeatable results. The upward shift of the mean values of TLC (2 days) for the various cultivars in the dry compared to the wet season suggests that screening for resistance should be done within a season for comparability, and preferably in the wet season when anthurium leaves are more susceptible to lesion expansion (Dilbar and Gosine 2003). The results of this study explain the greater problem of this disease in the wet season, when both temperature and humidity are high, than in the dry (Dilbar and Gosine 2003). The faster leaf invasion by the blight pathogen under high humidity and higher temperatures has also been observed in a previous study (Fukui et al. 1999b).

The method is therefore amenable to screening for foliar blight resistance in large breeding populations, since each plant can be screened non-destructively based on replicate leaf discs from stage-two leaves. The assessment is relatively straightforward and involves simply noting the number of days taken for the leaf discs to completely deteriorate. It is also quantitative and highly repeatable.

Although immunity to foliar blight infection was not detected in this study, 22% of the cultivars evaluated were found to possess resistance to foliar blight, with some being highly resistant. The local cultivars fell into the moderately resistant or susceptible categories.

This study, using 45 anthurium cultivars, corroborated an earlier study (Fukui et al. 1998) that indicated differential resistance at the foliar and systemic levels of infection. The correlation between foliar blight resistance of the 45 cultivars in this study and systemic blight resistance reported for the same cultivars in a previous study (Elibox and Umaharan 2007) by the same authors was weak (r=0.07) and not significant (P>0.05). Only two cultivars, Acropolis and Bianco combined blight resistance at both foliar and systemic levels, suggesting that anthurium breeding programmes to date have been inefficient in combining resistance at the foliar and systemic phases of blight infection.

The exact mechanism of resistance to foliar blight infection is not known. An earlier study (Sakai et al. 1991) found that neither structural nor anatomical differences in the hydathodes or adjacent xylem vessels were able to explain differences in resistance to foliar infection and suggested a role for biochemical/physiological mechanisms. Reduced lesion sizes were associated with accumulation of lignin-like phenolic compounds in cell walls during the initial stages of infection in cabbage and rice in response to *X. campestris* pv. *campestris* (Gay and Tuzun 1995) and *X. oryzae* pv. *oryzae* (Reimers and Leach 1991), respectively. In cabbage, greater peroxidase activity in the guttation fluid in resistant compared to susceptible cultivars was suggested as a possible mechanism of resistance (Gay et al. 1996).

There was a 10-fold difference in the bacterial multiplication rate between the susceptible and resistant cultivars of anthurium tested, which suggests that foliar resistance to blight is possibly mediated through the ability of host leaf tissue to suppress the rate of pathogen multiplication. The strong linear regression (R^2=0.99) between TLC and tissue bacterial concentration provides further evidence to this claim. Fukui et al. (1999b) also suggested that the effect of temperature on foliar invasion rate was perhaps mediated through changes in bacterial multiplication rates.

In conclusion, the study outlined a standardised foliar screening method that was able to quantitatively differentiate between levels of foliar resistance to blight on a repeatable basis. The strong rank correlation to field resistance provides confidence of its validity. Furthermore this method is amenable for use in genetic studies and resistance breeding programmes. The study also identified useful sources of resistance to blight.

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