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Short communication

Survival of the anthurium blight pathogen, *Xanthomonas axonopodis* pv. *dieffenbachiae*, in field crop residues

Brion Duffy
College of Agriculture, Forestry and Natural Resource Management, University of Hawaii at Hilo, Hilo, Hawaii, 96720-4091 USA; Phytopathology Group, Institute of Plant Sciences, Swiss Federal Institute of Technology, Universitätstrasse 2, CH-8092 Zürich, Switzerland (Fax: +411 632 1108; E-mail: brion.duffy@ipw.agrl.ethz.ch)

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Abstract

Bacterial blight, caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (*Xad*), is a major threat to the anthurium cut flower industry worldwide. Two field trials in Hawaii evaluated the long-term persistence of *Xad* in artificially-infested crop residues. *Xad* survived in leaf, petiole, and root residues for as long as 4 months when tissues were left on the surface or buried 15 cm deep. Survival was considerably shorter (approximately 20 days) outside of residues. *Xad* that was recovered from residues over a period of 4 months retained pathogenicity. *Xad* was isolated from living roots of naturally-infected plants which further suggests that roots left in the field after culling may be particularly important, but overlooked, inoculum source. This information is key to determining minimum fallow periods before replanting devastated fields.

Abbreviation: Xad – Xanthomonas axonopodis pv. *dieffenbachiae.*

Anthurium (*Anthurium andraenum* Linden ex André) is the most important cut flower crop (and tenth most important agricultural commodity overall) in Hawaii bringing over 7.4 million US$ annually (Hawaii Agricultural Statistics Service, 1999). The single most serious threat to this industry is systemic bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (*Xad*, formerly *X. campestris* pv. *dieffenbachiae* Vauterin et al., 1995). The disease was first observed in the early 1970s on the island of Kauai and has since spread to epidemic proportions throughout the state (Hayward, 1972). Blight is also a devastating problem in California (Cooksey, 1985), the Carribean (Rott and Prior, 1987), Florida (Hoogasian, 1990), The Netherlands (Sathyanarayana et al., 1997), and the Philippines (Natural, 1990). Management strategies for anthurium blight focus on the use of axenically-propagated plants, sanitation, and prevention (e.g., cull diseased plants, disinfected cutting implements, reduce water-splash, remove alternate hosts) (Nishijima, 1989). Biological control of *Xad* using non-pathogenic bacteria and bacterial mixtures (Fukui et al., 1999) has made considerable progress since it was first investigated by Fernandez et al. (1989), however, no commerical products are currently available. Severely blighted fields are typically cleared and replanted immediately or after a brief fallow (< 2 months) (Higaki et al., 1994). However, the ability of the pathogen to survive in infested crop debris has never been clearly established. Such basic epidemiological information is critical for determining minimum fallow periods and has proven invaluable for controlling *Xanthomonas* diseases on other field and greenhouse crops (Gilbertson et al., 1990; Jones et al., 1986). The objective of this study was to determine the persistence of *Xad* in anthurium debris, both foliar and root, left in a production field.

Survival of *Xad* in anthurium residues was monitored in 1988–89 and 1992–93 at a production field of the College of Agriculture, Forestry and Natural Resource Management, University of Hawaii at Hilo (UHH). In both trials, mature plant tissues were taken from *Xad*-free axenically-propagated anthurium plants (cv. Marian Seefurth), and artificially infested with *Xad* by soaking for 24 h in a bacterial suspension prepared as follows. Bacteria were grown 48 h at 20–24 °C on nutrient agar (NYD; Difco® nutrient broth, 0.8%; yeast extract, 0.5%; dextrose, 1%; Difco® agar, 1.5%; pH 6.5), suspended in sterile distilled water with a few drops of Tween surfactant, and spectrophotometrically adjusted to a cell density of 10^8 colony-forming units (CFU)/ml. Infested residues were placed in 10 × 6 cm litterbags made of fine-mesh nylon (1 mm pores). For each treatment replicate, all tissues to be sampled during the experiment were placed in one litterbag. Litterbags were left on the surface or buried 15 cm deep in a typical anthurium production field of volcanic cinder with high-drainage and daily over-head irrigation (Higaki and Imamura, 1985). To avoid background contamination by indigenous *Xad*, trials were conducted in an isolated area of the field that had not been planted to anthurium or any alternate hosts.

Trial 1 in 1988–89 evaluated survival in leaf discs (0.6 cm diameter), petiole sections (0.5 cm diameter × 1.0 cm long), and root sections (0.5 cm diameter × 1.0 cm long). Artificially infested tissue were sampled periodically for persistence of *Xad* by removing nylon-mesh litterbags from the cinder, extracting tissue pieces with sterile forceps, and immediately replacing bags. Tissue pieces were transported to the laboratory in sterile petri dishes, brushed to remove loosely adhering cinder particles, and placed on CSM selective medium (Norman and Alvarez, 1989). The percentage of tissue pieces with *Xad* growing from the edges was scored after 5 days incubation in darkness at 20–24 °C, and a representative number of putative *Xad* isolates were subjected to confirmatory bacteriological tests (Lelliot and Stead, 1987). Samples consisted of 20–25 tissue pieces taken from each mesh bag at sampling dates 0–58 days, and thereafter 5–10 tissue pieces. Bags containing non-infested leaf tissue were sampled as controls for indigenous *Xad* but data were not considered in the analysis. Treatments (buried vs. surface placement) were replicated three times in a randomised complete block design. Percentage data were arcsine transformed prior to analysis. Relationships between pathogen survival and time were evaluated using SAS linear regression analysis (SAS Institute, Cary, NC, USA).

Pathogenicity of *Xad* isolates recovered from infested tissues was determined for three isolates at each sampling date in Trial 1. Isolates were tested for pathogenicity by syringe-injecting a bacterial suspension, prepared as above, into the leaf margins of axenically-propagated anthurium cv. Marian Seefurth, which is highly susceptible to both foliar and systemic infection (Fukui et al., 1998). Isolates were tested on at least two plants. Sterile water was injected into the same leaves as a negative control, and symptoms never developed. Inoculated plants were grown in individual pots kept in an isolated area of a shade-cloth production house and monitored 2–3 weeks for development of typical blight symptoms (e.g., water-soaked yellow lesions followed by necrosis).

Trial 2 in 1992–93 evaluated survival in leaf tissues using a strain of *Xad* marked with spontaneous rifampicin-resistance in order to facilitate selective recovery (*Xad*^{Rif}). The marked strain, obtained by plating on increasing concentrations of rifampicin in NYD, was not different from the wild-type for growth rate in NYD broth or pathogenicity on anthurium. Rifampicin is a stable and selective marker for bacterial studies in soil and other environments (Glandorf et al., 1992). In this experiment, the population decline of *Xad*^{Rif} was monitored over time based on CFU per leaf disk. Three leaf disks were periodically sampled from litterbags as above, and macerated in sterile water. Appropriate serial dilutions were plated onto NYD amended with 100 μg/ml rifampicin (NYD^{Rif}). In the first sampling at day 17, dilutions were also plated onto CSM without rifampicin as in Trial 1. An additional treatment of *Xad*^{Rif} added directly to cinders was included to evaluate pathogen survival outside of residues. The detection limit was approximately log 2 CFU per leaf disk or per g of cinder. Treatments (buried vs. surface placement vs. cinder) were replicated three times in a randomised complete block design. CFU data were log-transformed prior to analysis. The relationship between survival and time was evaluated using SAS linear regression analysis (SAS Institute, Cary, NC, USA). Plate counts on NYD^{Rif} vs. CSM media at day 17 were not different according to a Student's *t*-test. This indicates that when only CSM was used in Trial 1, recovery of the artificially inoculated *Xad* was sufficiently selective.

The prevalence of living roots harbouring *Xad* was evaluated in three surveys at the UHH production field

in 1988. Root pieces (approximately 2 cm long) were taken from naturally-infected anthurium plants with extensive foliar blight symptoms. Tissues were rinsed in running water, surface disinfested for 60 s with 1.5% NaOHCl, and dissected to remove the internal vascular tissues. Tissue samples were placed on CSM agar. After 5 days, percent tissue pieces positive for *Xad* was determined and representative samples were verified as above.

Xad survived for as long as 129 days in artificially-infested leaf, petiole, and root debris (Figure 1). Survival time was similar in the different tissue types; about 20% of infested plant residues retained *Xad*. Although the isolation medium used was only semi-selective, the fact that plate counts on NYD^{Rif} vs. CSM media in Trial 2 were not statistically different ($P = 0.3122$), and that no *Xad*-like colonies were observed in non-infested control tissues indicates that bacteria recovered from the residues were indeed the introduced pathogen. An almost identical decline in *Xad* was observed in Trial 2 using a stable rifampicin-resistant marked strain (*Xad*^{Rif}) which enabled quantitative determination of bacterial survival in the residues. Bacterial numbers dropped steadily in the first 58 days from approximately log 7.4–2.5 CFU/leaf disk, but then remained at low but detectable levels (approximately log 2 CFU/leaf disk) for 89 days (Figure 2). This second trial clarified the first, that eventhough the pathogen survived for over 4 months in tissues, it persisted only at a very low level.

At present, the threshold level of inoculum capable of inciting an epidemic is unclear. The fact that blight often remains latent for many months after infection (Norman and Alvarez, 1994), however, means that infection of even one plant by a relatively small amount of inoculum surviving in residues could develop into an epidemic via transfer from asymptomatic plants during flower harvest or other movement within the field. The risk of rapid spread is compounded by Hawaii's mild climate and year-round susceptibility of anthurium to infection. This study has shown that bacteria surviving in tissues were not only viable, they remained pathogenic causing typical foliar blight

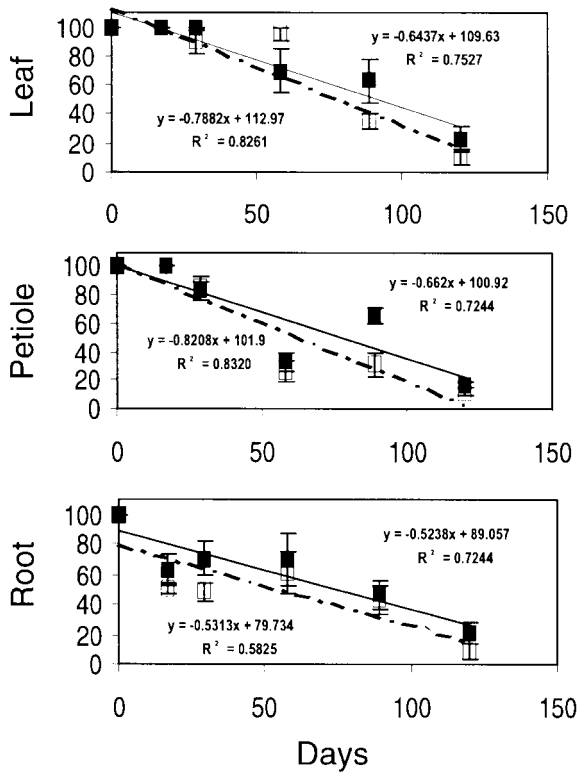

Figure 1. Survival of *Xad* in anthurium residues in the field. Leaf disks, petiole sections, and root sections were artificially infested with the pathogen and placed in nylon-mesh litterbags on the surface (□) or buried 15 cm (■) in volcanic cinder beds typically used in commercial production in Hawaii. Values represent the percentage disks or sections from which the pathogen was detected (*y*-axis) at each sampling time. Each treatment was replicated three times. Standard errors are represented by vertical bars. Day 0 = 7 February 1988.

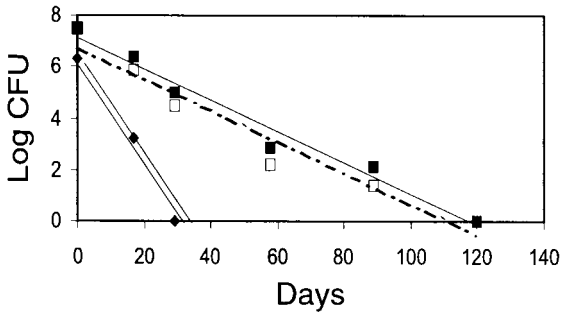

Figure 2. Survival of a rifampicin-resistant *Xad* strain in anthurium leaf disks placed on the surface (□) or buried 15 cm (■), or *Xad* applied directly to cinders (♦). Values represent the bacterial CFU per leaf disk or per g cinder over time. Each treatment was replicated three times. The magnitude of standard errors was smaller than the symbols in all cases. Linear regression lines approximate $y = 7.14 - 0.06x$ for 'surface' (solid line; $r^2 = 0.9610$); $y = 6.76 - 0.06x$ for 'buried' (dashed line; $r^2 = 0.9417$); and $y = 6.5 - 0.22x$ for 'cinder applied' (double line; $r^2 = 0.9869$). Day 0 = 16 January 1992.

symptoms when inoculated on a highly susceptible anthurium cultivar just as rapidly and intensely as fresh *Xad* isolates. The currently recommended fallow period of 2 months or less may be adequate to avoid bacteria that are free in cinders because these survived for only a short time (< 20 days; Figure 2), as previously speculated (Higaki et al., 1994). However, these results clearly show that a more extended fallow of 4 months or longer will be needed when residues are present.

Surprisingly, burying residues only slightly hastened *Xad* decline (Figures 1 and 2). Studies in Florida with *X. axonopodis* pv. *citri* (Graham et al., 1987) and *X. axonopodis* pv. *vesicatoria* (Jones et al., 1986) in citrus and tomato crop residues found that burying tissues in soil contributed to a more dramatic decline in pathogen survival compared to leaving tissues on the soil surface. This minor discrepancy may have something to do with the greater porosity and lower contact area, and thus less opportunity for microbial degradation, found in volcanic cinders used to cultivate anthurium compared to soil used in the other studies.

The observation that roots are commonly infested with *Xad* justifies some concern for long-term survival of the pathogen in residues. In three surveys, $23 \pm 9.2\%$ ($n = 347$) of roots sampled from plants suffering heavy foliar blight (but not sufficient to warrant culling) were positive for the pathogen. Roots appear to be an important but overlooked potential source of inoculum. While it may be possible to remove foliar plant residues which remain primarily on the surface, eradicating buried roots is more impractical. *Xad* has previously been shown to infect via roots, particularly after wounding from transplanting (Fernandez et al., 1989; Fukui et al., 1998), and possibly from nematode damage (Huettel et al., 1986). Roots are a potential infection site that would be in close contact with infested residues. Residue sanitation as proposed by Pfender et al. (1993), may be an alternative niche for biocontrol of anthurium blight, a control strategy that has received renewed attention (Fukui et al., 1999) since it was first investigated by Fernandez et al. (1989).

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