

A Severe Outbreak of *Xanthomonas* on *Ficus elastica* in South Florida

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A severe outbreak of Xanthomonas blight on *Ficus elastica* was observed in several nurseries in Miami–Dade County during summer of 2010. Bacteria were isolated from diseased plants and maintained on yeast dextrose chalk (YDC) agar for DNA extraction and PCR assays. The 16S rDNA gene was sequenced and results indicated that the isolated strain is 99% identical to that of *Xanthomonas* spp. Pathogenicity was confirmed by spraying a bacterial cell suspension of 1×10^8 colony-forming units (cfu)/mL onto *F. elastica* variety 'Burgandy'. Plants were placed in a modified humidity chamber (polyethylene bags) for 48 hours and then maintained in a shade house where temperature ranged from 23 to 32 °C and 60% to 95% relative humidity. After symptoms developed, the bacteria were re-isolated and identified using methods described above. *Xanthomonas* spp. has been previously reported on other species of *Ficus*; however, this is the first report on *F. elastica*. Further characterization of the pathogen, host range studies, and the effect of temperature and light on disease development are under way.

Introduction

Florida has led the nation in production of foliage plants, accounting for more than 55% of the national wholesale value since the 1960s (Chen et al., 2002). Hot, humid, and rainy conditions (typical summer in South Florida) are highly favorable for plant disease. Xanthomonas is a bacterial plant pathogen that thrives under these environmental conditions and is spread very effectively in water, especially by irrigation sprinklers and windblown rain. Once cells of the bacterium come into contact with the plant, they enter through wounds or natural openings such as stomata or hydathodes. When inside the plant, bacterial cells can move systemically causing a severe blight of the leaves. Xanthomonas campestris pathovar. fici is reported to be the most common bacterial disease affecting species of Ficus. Host studies indicate that several *Ficus* species and their cultivars are susceptible including F. benjamina, F. buxifolia, F. triangularis, F. mexicana, F. maclellandii 'Alli', F. retusa 'California Nitida' and 'Green Gem', and F. grennisland (Chase and Henley, 1993). During the summer of 2010 a severe leaf blight causing small water soaked lesions with irregular borders was observed affecting Ficus elastica. These symptoms closely resembled those caused by X. campestris p.v. fici, but have never been reported on this host.

Materials and Methods

Symptomatic leaf tissue, from *F. elastica* variety 'Burgandy', was collected from local nurseries in Miami–Dade County during the summer of 2010. Affected leaf tissue was macerated in sterile deionized water and 10 mL of the resulting suspension

was streaked on nutrient agar plates. Yellow-pigmented, gramnegative, rod-shaped bacteria were isolated repeatedly from diseased tissue. Bacteria were catalase-positive, cellulolytic, oxidase-negative, amylolytic, proteolytic, and utilized glucose in an oxidative manner.

HYPERSENSITIVE RESPONSE (HR) ON TOMATO AND TOBACCO. After 48 to 72 h, the isolate 10-294 was tested for its ability to induce a hypersensitive response (HR) on tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum* L.) Bacterial suspensions at OD600 = 0.6, which corresponds to a cell density of 1×10^8 cfu/mL, were infiltrated into the leaf mesophyll using a 0.5-mL hypodermic syringe without a needle. Infiltrated zones were observed for development of tissue collapse and necrosis for 24–48 h post-infiltration. The experiments were repeated twice.

KOCH'S POSTULATES. Pathogenicity was confirmed by spraying approximately 100 μ L of a bacterial suspension at 1 × 10⁸ cfu/mL onto leaves of eight ficus plants. Four plants were also inoculated with water controls. Plants were placed in a modified humidity chamber (polyethylene bags) for 48 h and then maintained in a shade house where temperature ranged from 23 to 32 °C and 60% to 95% relative humidity. Disease progress was evaluated after 7 days post inoculation (dpi).

The *Xanthomonas* spp. was re-isolated from diseased ficus plants; the inoculum was grown on YDC agar for 48 h.

In addition, DNA was extracted from the pure culture and PCR was conducted using universal primers for 16rDNA: 1492R (5'-CTACGGCTACCTTGTTACGA-3') and 27F (5'-GAGA-GTTTGATCCTG-3') (Lane et al., 1991).

Results and Discussion

Initial characterization of this bacteria was performed using culture media and biological and molecular assays (Fig. 1).

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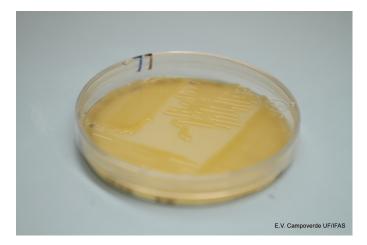


Fig 1. Xanthomonas growth on YDC agar after 48 h isolated from symptomatic *F. elastica* with Xanthomonas blight.

HYPERSENSITIVE RESPONSE (HR) ON TOMATO AND TOBACCO. The area infiltrated with inoculum (Xanthomonas cells) showed plant cell death (tissue necrosis); whereas the negative control (sterile, deionized water) infiltrated leaf tissue remained symptomless.

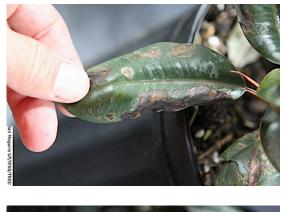
Symptoms developed after 24 h for tomato and 36 h for tobacco plant leaves.

KOCH'S POSTULATES. Symptoms appeared as small, watersoaked, circular lesions with irregular borders near the leaf margin. Symptoms developed after 7 to 14 d of inoculation; lesions enlarged, coalesced, and typically covered large portions of the leaf area. Premature senescence and leaf drop were common for tender plants. Foliar symptoms occurred on all inoculated plants (Fig. 2 A and B).The disease was most severe on younger plants and in some cases plant death occurred (Fig. 3).

The resulting band obtained from PCR with degenerate primers for 16rDNA was sequenced and the sequenced results were blasted in GenBank. The sequence exhibited 99% nucleotide identity to an isolate of *X. axonopodis* (GenBank accession no. AF123091) collected from diseased tomato leaves in Florida.

Preliminary results indicate that this is potentially a new strain of Xanthomonas affecting F. elastica. The outbreak of Xanthomonas that occurred during the summer of 2010 was very challenging for F. elastica growers throughout Miami-Dade County. The disease severely affected plant quality requiring numerous growers to abandon their crop, or drastically cut back the plant canopy, resulting in substantial economic losses. Previous to this disease outbreak, F. elastica was relatively easy to grow, requiring minimal use of pesticides for disease management. As with other bacterial diseases, a preventative disease management approach is the best option. Recommendations include monitoring for favorable weather conditions, scouting for early symptom development, spacing plants to allow for good air movement in the canopy, and allowing for the foliage to dry before evening. Good sanitation, including properly disinfecting tools that come into contact with the plants, covering the ground with cloth and surface disinfesting benches, and maintaining a weed-free nursery are essential for disease control.

To our knowledge, this is the first report of *Xanthomonas* affecting *F. elastica* in Florida. Ongoing experiments include evaluating the impact of light and temperature on disease development and examining host range of the pathogen, specifically other popular foliage plants that are produced under the same conditions in close proximity to *F. elastica*.





B

Fig. 2. Classic symptoms on leaves of *F. elastica* after 7 d post inoculum: (A) adaxial foliar symptoms; (B) abaxial foliar symptoms.



Fig. 3. Xanthomonas blight on juvenile F. elastica after 14 d post inoculum.

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