# Leaf Spot and Blight of *Strelitzia reginae* (Bird-of-Paradise) Caused by *Xanthomonas campestris*

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**ABSTRACT**

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A newly described bacterial leaf spot disease of *Strelitzia reginae* (bird-of-paradise) is caused by a strain of *Xanthomonas campestris*. Angular, yellow to reddish brown lesions were found on all ages of leaves and petioles. Lesions were frequently bordered by leaf veins and remained less than 1 mm in diameter or length, although lesions coalesced to give leaves a blighted appearance. A strain of *X. campestris* caused these symptoms on bird-of-paradise and *S. nicolai* (bird-of-paradise tree) but not on *Heliconia* spp., *Musa acuminata* (blood banana), or *Ravenala madagascariensis* (traveler's palm). Moderate symptom development occurred between 21 and 27 C, with optimal development at 24 C. Pathogenicity tests with strains of known pathovars of *X. campestris* showed the bird-of-paradise pathogen to be distinct from *X. c.* pv. *dieffenbachiae* (Araceae), *X. c.* pv. *hederae* (Araliaceae), *X. c.* pv. *malvacearum* (Malvaceae), and *X. c.* pv. *poinsettiicola* (Euphorbiaceae) but not distinct from strains of *X. campestris* isolated from *Ficus* (Moraceae) or *Pellionia* and *Pilea* (Urticaceae).

A leaf spot disease of *Strelitzia reginae* Ait. (bird-of-paradise) has been economically important periodically in Florida during the past 3 yr in 90% of nurseries producing these plants. Symptoms are initially characterized by tiny water-soaked interveinal areas on leaves of all ages. Lesions turn yellow, then red-brown, and eventually coalesce to form large areas of necrotic tissue that can encompass entire leaf blades (Fig. 1). Symptoms and initial isolations suggested involvement of a bacterial pathogen. The *Index of Plant Diseases in Florida* lists a species of *Pseudomonas* isolated from "leaf stripe" symptoms on bird-of-paradise (1). The only documented report of a bacterial disease of this plant was bacterial wilt caused by *Pseudomonas solanacearum* in 1963 by Quinon and Aragaki (14). The following research was performed to determine the causal agent of the leaf spot of bird-of-paradise.

## MATERIALS AND METHODS

Isolations from diseased tissue using standard materials and methods for fungi did not indicate involvement of a fungal pathogen, and all methods reported here are for isolation of bacterial pathogens. Symptomatic plants were obtained from six Florida nurseries. Leaves were detached and washed in sterilized deionized water. Leaf pieces about 1 mm long and 0.5 mm wide were crushed in a scintered glass tissue grinder containing 2–3 ml of sterilized 0.01 M $MgSO_4$. The resulting suspensions were streaked onto nutrient agar (Difco, NA), King's medium B (KMB) (9), and crystal violet pectate medium (CVP) (13). Plates were incubated for 4 days at 27 C and examined for bacterial colonies. Single colonies of suspect pathogens were transferred to new NA plates successively three times before establishing stock cultures in tubes containing 5 ml of sterilized tap water. Six strains of the suspect pathogen were maintained in water at about 27 C when not in use.

Pathogenicity of the suspect pathogen to bird-of-paradise was tested using 15-cm tall seedlings of the plant established in 10-cm-square pots containing the following potting medium: Canadian peat and builder's sand (3:1, v/v). The medium was steam-treated for 1.5 hr at 90 C before amending with 4.4 kg of Osmocote (19:6:12, slow-release fertilizer from Sierra Chemical Co., Milpitas, CA), 4 kg of dolomitic lime, and 0.9 kg of Micromax (micronutrient source from Sierra) per cubic meter. Plants were grown in a shadehouse for 1–4 mo before use, with a daily temperature fluctuation between 18 and 36 C and a maximum natural light level of 500 $\mu$mol $sec^{-1}$ $m^{-2}$. They were irrigated by overhead sprinklers to deliver about 5 cm of water per week.

All inoculation trials were performed in a greenhouse with a similar temperature fluctuation and a maximum natural light level of 180 $\mu$mol $sec^{-1}$ $m^{-2}$. Plants were exposed to intermittent misting (5 sec/30 min from 0800 to 2000 hours daily) starting 24 hr before inoculation and continuing until final ratings (up to 30 days). Plants were irrigated by hand once or twice a week as needed. Inocula were prepared from 2-day-old cultures grown at 27 C on KMB as described for isolations. Five plants each were spray-inoculated with a suspension of a suspect pathogen in sterilized 0.01 M $MgSO_4$ ($1\times10^8$ colony-forming units [cfu] per milliliter based on optical density readings) or treated with 0.01 M $MgSO_4$ alone. Plants were placed in polyethylene bags for 3 days and then arranged in a randomized complete block design on the bench. Disease severity was rated by estimating the percentage of the foliage showing symptoms. Reisolation of the suspect pathogen was attempted using the methods described above from symptomatic portions of test plants. This

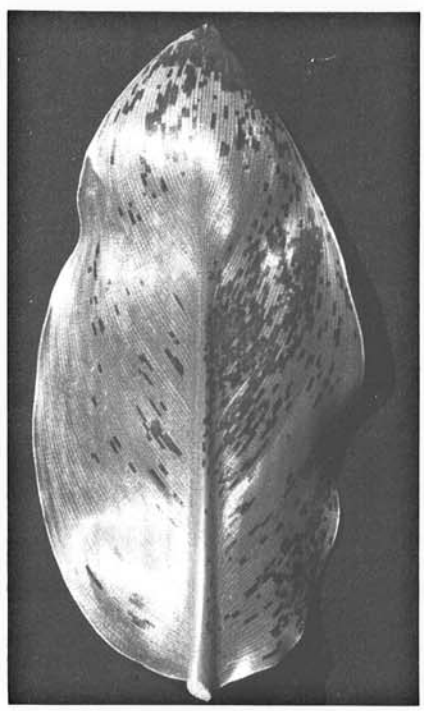

**Fig. 1.** View of abaxial leaf surface of *Strelitzia reginae* (bird-of-paradise) infected with *Xanthomonas campestris*.

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test was performed three times between 1 February and 15 May 1986.

The host range of the suspect pathogen was tested on the following members of the Musaceae family: *Heliconia bicolor* Benth. (*H. angustifolia* Hook), *H. latispatha* Benth. × *H. psittacorum* L.f. 'Golden Torch,' *H. psittacorum* L.f. 'Andromeda' and 'Chocoyana,' *Musa acuminata* Colla (blood banana), *Ravenala madagascariensis* Sonn. (traveler's palm), *S. nicolai* Regel E. Korn (bird-of-paradise tree), and bird-of-paradise. Three to five plants of each type were inoculated with one of three strains of the suspect pathogen or treated with 0.01 M $MgSO_4$ alone, as indicated before. Plants were evaluated for symptoms about 30 days after inoculation, and reisolation of the suspect pathogen was attempted from symptomatic tissue as described. This test was performed three times between 15 May and 15 November 1986.

The effect of temperature on bacterial leaf spot of bird-of-paradise was tested in growth chambers (plant growth chamber E 30B, Percival Manufacturing Co., Boone, IA), using one growth chamber for each continuous temperature tested. The temperatures tested were 18, 21, 24, 27, 30, and 33 C. Relative humidities were 67 ± 10% with light levels of 100 $\mu$mol $sec^{-1}$ $m^{-2}$ from 0800 to 2000 hours daily. Five plants were placed in each growth chamber 1 day before inoculation and irrigated by hand two or three times a week as needed. All plants were inoculated with a suspension of strain X137 as described, except the misting treatment was omitted. Plants were enclosed in polyethylene bags for 3 days. The percentage of leaf area showing symptoms was estimated about 3 wk after inoculation.

Pathogenicity of the suspect pathogen was compared on a variety of ornamental plants with a pathovar or strain of *X. campestris* known to be pathogenic on that plant (Table 1). Five plants of each type were inoculated with strain X137 of the suspect pathogen or a strain of *X. campestris* known to be pathogenic on the plant type, or they were treated with sterile buffer alone. The suspect pathogen and known *X. campestris* pathovars and strains were compared for pathogenicity on bird-of-paradise. Inoculation materials and methods were those described before. Data were recorded about 3 wk after inoculation as an estimate of the percentage of the leaf area showing symptoms.

Biochemical characterization of the suspect pathogen (six strains) was compared with responses of *X. campestris* (six strains) using the following tests: Gram reaction (15), oxygen requirement using Hugh-Leifson's medium (7), asparagine utilization (5), production of xanthomonadin (8), mucoid growth on Wilbrink's medium (11), gelatin or casein hydrolysis (5), and growth on SX medium (15). Hypersensitive reaction was tested on *Capsicum annuum* L. 'Early Calwonder pepper,' *Lycopersicon lycopersicum* (L.) Karst. ex Fariv. 'Bonny Best' and 'Ace,' and *Nicotiana tabacum* L. 'Hick's' tobacco (10). Carbon source utilization was tested for six strains each of the suspect pathogen and *X. c.* pv. *dieffenbachiae* by acid production according to Dye using medium C (6). A strain of *X. c.* pv. *vesicatoria* was compared with bird-of-paradise strains in the carbon source utilization test.

## RESULTS AND DISCUSSION

A yellow, gram-negative, rod-shaped bacterium was consistently recovered from bird-of-paradise showing leaf spot and blight. Typical symptoms developed on inoculated plants about 10–14 days after inoculation. Lesions were initially water-soaked and readily observed on the abaxial leaf surface. Within 1 mo of inoculation, lesions were necrotic and beginning to coalesce into areas of blighted tissue (1–2 cm in diameter). Reisolation of the suspect pathogen was easily accomplished from symptomatic tissue but not from control plants, which remained asymptomatic throughout the tests.

Six strains of the suspect pathogen were also pathogenic on bird-of-paradise tree and caused the same symptoms as on bird-of-paradise. Traveler's palm, blood banana, and the species of *Heliconia* did not develop any symptoms of disease in these trials.

Temperatures between 21 and 28 C were favorable for disease development, with 16–33% of the leaf area developing symptoms. The highest percentage of leaf area with symptoms developed when plants were maintained at 24 C (33%), with only 4% at 18 C and 5% at 33 C.

Host range tests showed that the following strains of *X. campestris* were different from those obtained from bird-of-paradise: *X. c.* pv. *dieffenbachiae* (Araceae), *X. c.* pv. *hederae* (Araliaceae), *X. c.* pv. *malvacearum* (Malvaceae), and *X. c.* pv. *poinsettiicola* (Euphorbiaceae) (Table 1). Typical symptoms did occur, however, on bird-of-paradise inoculated

**Table 1.** Plants and pathogens used for host range tests with pathogen strains from *Strelitzia reginae* (bird-or-paradise)

| Pathogen | Source | Reference | Pathogenic to *S. reginae* |
|---|---|---|---|
| *Xanthomonas campestris* pv. *dieffenbachiae* | *Syngonium podophyllum* 'White Butterfly' | 12,16 | No |
| *X. c.* pv. *hederae* | *Schefflera arboricola* (dwarf schefflera) | 2 | No |
| *X. c.* pv. *malvacearum* | *Hibiscus rosa-sinensis* 'Brilliant Red' | 4 | No |
| *X. c.* pv. *poinsettiicola* | *Codiaeum variegatum* 'Gold Dust Croton' | 3 | No |
| *X. c.* pv. unknown | *Ficus benjamina* 'Weeping Fig' | ...[a] | Yes |
| *X. c.* pv. unknown | *Pilea cadierei* (aluminum plant) | 13 | Yes |

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**Table 2.** Biochemical characteristics of pathogen strains from *Strelitzia reginae* (bird-of-paradise)

| Test characteristic | Reaction (no. positive/six strains tested) | |
|---|---|---|
| | Pathogen from *Strelitzia* | *Xanthomonas campestris* |
| Gram reaction (negative rod) | 6 | 6 |
| Aerobic | 6 | 6 |
| Asparagine utilization | 0 | 0 |
| Mucoid growth | 6 | 6 |
| Hydrolysis of | | |
| Aesculin | 6 | 6 |
| Casein | 6 | 6 |
| Starch | 6 | 6 |
| Gelatin liquefaction | 6 | 6 |
| Growth at | | |
| 27 C (3 days) | 6 | 6 |
| 36 C (1 day) | 6 | 6 |
| 41 C (3 days) | 0 | 0 |
| Urease production | 0 | 0 |
| Xanthomonadin production | 6 | 6 |
| Hypersensitive reaction | | |
| Hick's tobacco | 2 | 4 |
| Bonny Best tomato | 6 | 5 |
| Ace 55 tomato | 6 | 6 |
| Early Calwonder pepper | 4 | 5 |
| Pathogenic on bird-of-paradise | 6 | – |

**Table 3.** Acid production from different carbohydrates for strains of *Xanthomonas campestris* from *Strelitzia reginae* (bird-of-paradise)

| Carbon source | Strain designation | | | | | |
|---|---|---|---|---|---|---|
| | X22 | X137 | X142 | X143 | X144 | Xv12[a] |
| D-Arabinose | +[b] | ++ | ++ | + | ++ | ++w |
| Cellobiose | + | ++ | + | + | + | ++ |
| Fructose | ++ | ++ | ++ | ++ | +w | ++ |
| Galactose | + | + | + | ++ | + | + |
| Glucose | + | + | + | + | +w | + |
| Glycerol | − | + | + | ++ | ++ | + |
| Maltose | + | +w | + | +w | nt[c] | nt |
| Mannitol | − | + | +w | + | nt | nt |
| Mannose | + | ++ | +w | +w | ++ | + |
| Salicin | − | − | − | − | nt | nt |
| D-Sorbitol | − | − | − | − | nt | nt |
| Sucrose | + | + | + | ++ | + | + |
| Trehalose | + | + | + | + | + | ++ |

[a] Xv12 = *X. campestris* pv. *vesicatoria* originally isolated from tomato.
[b] Reactions were negative (−), positive (+ or ++), and weakly positive (+w).
[c] Not tested.

with strains of *X. campestris* isolated from *Ficus* (Moraceae), or *Pellionia* and *Pilea* (Urticaceae). In addition, these plants developed typical symptoms of Xanthomonas leaf spot when inoculated with strains of the suspect pathogen. Control plants did not develop symptoms.

All six strains of the suspect pathogen showed typical characteristics of *X. campestris* (Table 2) (15). Carbon source utilization tests also supported this conclusion (Table 3). Previous host range studies conducted on strains of *X. campestris* from *Pilea* and *Pellionia* spp. indicate that differentiation of *X. campestris* strains from ornamentals is not always clear-cut (13). The tests presented in this paper indicate that a single pathovar may be responsible for bacterial leaf spot of plants from three families. Because the accepted criterion for differentiation of pathovars of *X. campestris* is based on host range, further work is necessary before this pathogen can be named (17).

Control strategies must account for the potential of this *X. campestris* pathovar to spread to other crops. *Ficus, Pilea,* and *Pellionia* plants should be protected, as well as bird-of-paradise and bird-of-paradise tree, if any symptoms of Xanthomonas leaf spot are found on any of these hosts.

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