Erwinia carotovora pv. *carotovora*, a Pathogen of *Kalanchoë blossfeldiana*

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ABSTRACT

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A bacterial disease of *Kalanchoë blossfeldiana* that caused a wilt and soft rot of leaves and stems was present in Florida in commercial nurseries in 1983. The disease caused losses as high as 100% in certain plantings and in propagation. Disease was present in 18 cultivars, but severity varied considerably among cultivars. The pathogen was identified as *Erwinia carotovora* pv. *carotovora*. Typical symptoms were produced when the pathogen was inoculated into three cultivars of kalanchoe. The pathogen was pectolytic, gram-negative, a faculative anaerobe, and comparatively tolerant to erythromycin. It liquefied gelatin, was negative for phosphatase production, and did not produce reducing substances from sucrose or gas from glucose. Acid was produced only from lactose but not α-methyl glucoside or palatinose.

The kalanchoe (*Kalanchoë blossfeldiana* Poelln.) is a popular pot plant in the United States and some countries in Europe. This succulent plant with fleshy leaves is a native of Madagasgar and was introduced at Potsdam, Germany, in 1932 by Robert Blossfeld. Both U.S. and European breeders have since been instrumental in developing new types of cultivars with an array of available colors (x4).

Wilting and dying plants were observed in commercial production in Florida in 1983. The disease severity was at a low level during the cooler months of the winter but reached a high level as temperatures rose during April and May. *Erwinia* sp. was isolated from diseased plants. This report details symptomology, etiology, identification procedures, and the importance of this bacterial disease of kalanchoe.

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MATERIALS AND METHODS

Identification of the kalanchoe pathogen. Tissues from the borders of necrotic areas on leaves and stems of kalanchoe plants were ground in phosphate buffer. The resulting suspension was serially diluted and plated onto nutrient-yeast-dextrose agar, King's medium B agar (3), and crystal violet pectate agar (1,2). These media were incubated at 28 C for 48 hr. Five pectolytic colonies on crystal violet pectate were isolated and purified. Suspensions of the pure cultures in buffer were used individually to inoculate media for the biochemical differentiation of the bacteria that had been preliminarily identified as *Erwinia* spp. (2). Previously identified strains of *Erwinia carotovora* pv. *carotovora* (*E. c.* pv. *carotovora*), *E. carotovora* pv. *atroseptica* (*E. c.* pv. *atroseptica*), and *E. chrysanthemi* provided by J. B. Jones (GCREC), A. Kelman (Department of Plant Pathology, University of Wisconsin, Madison), and R. E. Stall (Department of Plant Pathology, University of Florida, Gainesville), respectively, were tested at the same time to provide comparisons. The *Erwinia* isolates were evaluated for 1) aerobic or anaerobic growth, 2) sensitivity to erythromycin, 3) gelatin liquefaction, 4) phosphatase activity, 5) production of reducing substances from sucrose, and 6) production of acid or gas from glucose, lactose, palatinose, and α-methyl glucoside (2).

Inoculation of detached leaves. In experiment 1, mature, healthy leaves of the kalanchoe cultivars Aztec Montezuma, Aztec Sonora, and Mikkel Sensation were injected with 50 μl in serial dilutions at 10^8, 10^6, 10^4, 10^2, and 0 cfu/ml of a 3-day-old culture of *E. c.* pv. *carotovora* isolate K-1 (from the cultivar Forty Niner). Injections at each concentration were made with a 26-gauge needle into the laminae of eight leaves midway between the midvein and edge on each side of the leaf. Leaves in experiment 1 were inoculated on 28 June 1984 and placed under intermittent mist (6 sec/10 min) from 6 A.M. to 8 P.M. on transite plates. Ratings were made after 96 hr by measuring the length of the decay in a direction parallel to the midvein. Another set of two leaves (detached) per inoculum concentration was injected and placed on transite plates directly on the greenhouse bench. Leaves in experiment 2 were

treated and rated in the same manner, except they were inoculated on 3 December 1984.

Inoculation of intact leaves and stems of plants. Plants of the kalanchoe cultivars Aztec Montezuma, Aztec Sonora, and Mikkel Sensation, each growing one per 10-cm pot, were inoculated on 3 December 1984 in a glasshouse by inserting a sterile toothpick that had been dipped in a petri-plate culture of *E. c.* pv. *carotovora* isolate 6162-4 (from the cultivar Forty Niner). Toothpicks were placed into three leaves and three stems on each of four plants. A like number were treated in the same manner but not inoculated with *Erwinia*. The plants were immediately placed under intermittent mist (6 sec/10 min) from 6 A.M. to 8 P.M. Disease ratings (percentage of leaf area decayed or millimeters of stem decayed) were made on 10 December (7 days after inoculation) and again on 15 January 1985 (43 days after inoculation).

RESULTS AND DISCUSSION

Symptoms. Symptoms on infected vegetative, vigorously growing plants consisted of irreversible wilting of leaves and green stems. Subsequently, main veins and adjacent laminar tissue on leaves became chlorotic and/or dark brown to black. Chlorotic areas often developed on laminar tissue of a leaf or as a strip 2–5 mm wide adjacent to necrotic areas. As the disease developed, petioles turned dark brown to black and leaves dehisced. Similarly, dark brown to black sections developed on terminals and stem sections, and eventually, the necrosis spread to the entire plant. Vascular tissue in stems was brown during early stages of wilt development, and bacteria oozed from the vascular ring when a wilting stem was excised. Bacteria also streamed from the vascular tissue when a severely infected stem was suspended in water.

On older, hardened plants, especially flowering plants, soft decay of the base of one or more stems of a multistemmed plant occurred. Affected stems withered and/or wilted and died. The outer cortical tissues decayed and sloughed while the hard, sclerotized and inner tissues remained. When older plants were inoculated, wilting of stems was confined mainly to soft stems; on inoculated leaves, a soft, wet decay occurred and some leaves disintegrated.

A dark necrosis extended up the stem and/or petiole to the main vein on infected cultivars in propagation. Necrosis of the area adjacent to the main vein occurred and expanded to cover the leaf.

The disease was very destructive in commercial nurseries on young, vigorously growing plants. All plants in some plantings wilted and were diseased. The disease possibly was spread by personnel during culture and through vegetative propagation. Cultivars of kalanchoe observed with disease included Aztec Montezuma, Granada, Pueblo, Princess, Mikkel Sensation, Singapore, Aztec Sonora, and Forty Niner. Other cultivars reported by growers to be diseased included Cancun, Yucatan, Pueblo, Goddess, Tabasco, Tijuana, Acapulco, Monterey, Durango, and Eternity.

Identification of the *Erwinia* sp. Compared with the previously identified isolates of *E. c.* pv. *carotovora*, *E. c.* pv. *atroseptica*, and *E. chrysanthemi*, the pectolytic, gram-negative, rod-shaped bacterium from kalanchoe was identified as *E. c.* pv. *carotovora*. All five strains were facultative anaerobes, were comparatively tolerant to erythromycin and liquefied gelatin, did not produce reducing substances from sucrose, and were negative for phosphatase production or gas from glucose (Table 1). Of the other three carbon sources tested, acid was produced only with lactose.

Inoculation of detached leaves (experiments 1 and 2). Leaf injection was a simple method of evaluating differences in decay susceptibility to *E. c.* pv. *carotovora* among the cultivars Aztec Montezuma, Mikkel Sensation, and Aztec Sonora. Aztec Montezuma was consistently and significantly more susceptible to disease at a concentration of 10^8 cfu/ml than were Mikkel Sensation and Aztec Sonora (Table 2). At 10^6 cfu/ml, differences were less pronounced, and concentrations of the pathogen at 10^4 cfu/ml or lower rarely caused decay in any of the three cultivars. Disease did not develop within 96 hr when leaves were placed on the greenhouse bench and remained dry.

Inoculation of intact leaves and stems of plants (experiment 3). Disease developed on the leaves as a soft, wet decay. Severe decay developed on the leaves of Aztec Montezuma and Mikkel Sensation, whereas significantly less decay developed on the leaves of Aztec Sonora. On very susceptible cultivars, some of the leaves disintegrated and fell off after 7 days. Similarly, a moderate amount of decay developed on the stems of both Aztec Montezuma and Mikkel Sensation (no significant difference) but significantly less decay developed in the stems of Aztec Sonora (Table 3). There was relatively little advance in decay and/or disease between the seventh day and the final disease rating on the 43rd day. The lower greenhouse temperatures

Table 1. Identification of a bacterial pathogen from kalanchoe as *Erwinia carotovora* pv. *carotovora*

	E. chrysanthemi	*E. carotovora* pv. *atroseptica*	*E. carotovora* pv. *carotovora*	Kalanchoe isolate[a]
Sensitivity to erythromycin (mm of inhibition)	30	15	15	18
Reducing substances from sucrose	+	+	−	−
Phosphatase	+	−	−	−
Gelatin liquefaction	+	+	+	+
Gas from glucose	+	−	−	−
Acid from				
Lactose	−	+	+	+
α-Methyl glucoside	−	+	−	−
Palatinose	−	+	−	−

[a] Five isolates used.

Table 2. Leaf decay (mm) in detached leaves of *Kalanchoë blossfeldiana* injected with *Erwinia carotovora* pv. *carotovora* and held under intermittent mist

Cultivar	*Erwinia carotovora* pv. *carotovora* (cfu/ml)[y] 10^8	10^6	10^4	10^2	0
	Experiment 1				
Aztec Montezuma	60.0 a[z]	12.2 b	0.0 c	0.0 c	0.0 c
Mikkel Sensation	9.3 b	0.0 c	0.0 c	0.0 c	0.0 c
Aztec Sonora	3.5 c	0.3 c	0.0 c	0.0 c	0.0 c
	Experiment 2				
Aztec Montezuma	56.4 a	15.2 c	0.2 e	0.0 e	0.0 e
Mikkel Sensation	21.0 b	12.3 c	1.1 e	0.2 e	0.0 e
Aztec Sonora	21.3 b	12.3 c	0.1 e	0.0 e	0.0 e

[y] Each number is the mean of 16 measurements taken from eight leaves, each injected once at the midpoint of each side.
[z] Within test columns, numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Decay of leaves and stems of kalanchoe plants inoculated by the toothpick insertion method[y]

Cultivar	Leaf decay (%)	Stem decay (mm)
Aztec Montezuma	33.8 a[z]	6.8 a
Mikkel Sensation	42.5 a	6.0 a
Aztec Sonora	2.4 b	1.3 b

[y] Four replicates with three leaves and three stems per replicate.
[z] Within columns, numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

in late December and early January and the mature condition of plants may have contributed to a finding of less disease.

Conclusions. Soft rot, incited by *E. c.* pv. *carotovora*, is a serious, destructive decay that affected as many as 100% of the plants in some plantings in commercial nurseries. It appears that although considerable variation in susceptibility occurred among cultivars, all were susceptible. In the absence of good chemical controls for *Erwinia*-incited diseases, propagators and growers must use the utmost caution and diligence in using *Erwinia*-free stock and sanitation procedures that will ensure freedom from disease.

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