A Wilt and Crown Rot of *Primula* Species Caused by *Erwinia carotovora* subsp. *carotovora*

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ABSTRACT

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A crown rot and wilt of *Primula* × *polyantha* and *P. malacoides* caused significant damage in a commercial greenhouse operation in Georgia in 1985. A rapidly growing bacterium, later identified as *Erwinia carotovora* subsp. *carotovora*, was consistently isolated from the diseased plants, and Koch's postulates were fulfilled. The bacterium attacked *P.* × *polyantha*, *P. malacoides*, *P. obconica*, and *P. vulgaris* (*P. acaulis*), with *P. malacoides* apparently being less susceptible than the other three. Wounds, high temperatures (≥25 C), and high moisture were conducive to infection and disease development. This bacterium is a potential threat to the expanding commercial production of *Primula* spp. Prevention of wounding, maintenance of a low greenhouse temperature (<25 C), and avoidance of excessive moisture may be useful preventive measures.

Primulas are colorful annual or perennial flowering plants that are grown in gardens and pot culture in various areas of the world. They are best adapted to areas having cool summers, and some types will withstand mild winter conditions. Some primulas are sold to be enjoyed for a short time as small houseplants. Primulas are currently very popular in Japan and Europe and are increasingly grown in the United States. Species available commercially include *P. malacoides* Franch., *P. obconica* Hance, *P.* × *polyantha* Hort., and *P. vulgaris* Huds. (*P. acaulis* (L.) J. Hill; *P. hybrida* Schrank). A wide range of hybrids, varieties, and colors occur within these species.

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In 1985, a commercial greenhouse operation in Georgia reported a serious outbreak of a wilt and crown rot on *Primula* spp. and sent plants to the Cooperative Extension Service Plant Disease Clinic for diagnosis. The disease occurred on both *P. malacoides* and *P.* × *polyantha*. Symptoms included marginal leaf scorch and wilting and various degrees of discoloration and rot in the crown area. The roots were free of disease. Sections of diseased crown tissue plated on potato-dextrose agar did not yield a fungus. However, streak-plate isolations from infected tissues on nutrient agar consistently yielded mostly pure cultures of a rapidly growing bacterium. Plants of *P. malacoides* and *P. obconica* wound-inoculated with the bacterium and maintained in a growth chamber at 25 C developed a crown rot and foliage wilt similar to that observed on the original plants. The bacterium was reisolated in essentially pure cultures. Because there was no report of a bacterial wilt and crown rot of *Primula* spp., we conducted a series of tests during 1985–1987 to identify the causal organism, determine susceptibility of various *Primula* spp., and study conditions conducive for disease development. A preliminary report on a portion of this work has appeared (11).

MATERIALS AND METHODS

Isolation and culture maintenance. Plates of nutrient-yeast-dextrose agar (NYDA, 23 g of nutrient agar, 5 g of yeast extract, 10 g of dextrose, 1 L of distilled water, pH 6.8) were used for isolation and inoculum production. Strains used as working cultures were grown at 30 C on slants of NYDA and stored at 6 C. For permanent storage, the strains were maintained in a dehydrated state on glass beads.

Cultural and morphological characterization. Cultural and colony characteristics of the primula strain were described on plates of NYDA, Miller-Scroth (MS) medium (13), yeast extract-dextrose-calcium carbonate (YDC) agar (13), and slants of NYDA. The bacterium was stained by the Hucker modification of the Gram-staining procedure (14), and the morphology and Gram reaction were determined. Flagellation was determined after staining by a silver-plating method (12).

Determination of physiological and biochemical reactions. Initially, the primula strain was characterized by routine bacteriological tests (14). Later, additional tests were run that specifically separate *Erwinia* spp. and subspecies (3–6). Emphasis was placed on tests used to separate members within the "carotovora" *Erwinia* group (9). Three strains of *E. carotovora* subsp. *carotovora* (Jones) Dye from different hosts (dracaena, cyclamen, and chrysanthemum), and one strain each of *E. c.* subsp. *atroseptica* (van Hall) Dye and *E. chrysanthemi* Burkholder et al, from

potato, were used for comparison with the primula strain. The strains of *E. c.* subsp. *carotovora* were obtained from R. S. Dickey, Cornell University, and the strains of *E. c.* subsp. *atroseptica* and *E. chrysanthemi* were from A. Kelman, University of Wisconsin.

Influence of temperature on growth of the primula strain was determined by placing freshly streaked slants at 5–40 C, at 2–5 C intervals, and recording growth after 24 and 48 hr. Standard methods (14) were used to determine relationship to free oxygen, presence of catalase, motility in stabs of semisolid agar, action on litmus milk, citrate utilization in Koser's citrate medium, nitrate reduction, starch hydrolysis (plate test), liquefaction of 15% gelatin medium, and production of acetylmethylcarbinol. Tests to determine arginine dihydrolase activity, oxidase reaction, salt tolerance, lipase production, casein hydrolysis, levan production, β-glucosidase activity, ammonia production, urease activity, aesculin hydrolysis, and oxidative versus fermentative use of glucose, sucrose, and lactose were run as previously described (8). Tests to determine production of acid from dulcitol, xylose, raffinose, α-methyl-D-glucoside, lactose, inositol, melezitose, and palatinose were run at 1% (w/v) in both 1% peptone broth containing 0.7 ml of 1.5% alcoholic solution of bromcresol purple/L (3) and on agar slants of medium C of Dye (4,5). Durham tubes were used in the broth media to detect gas formation. Utilization of the organic acids acetic, benzoic, citric, formic, oxalic, propionic, and succinic, all as sodium salts, was determined using 0.2% (w/v) in the OY medium of Dye (4). Tests for methyl red, indole production, and potassium cyanine (KCN) tolerance were run as described by Dye (4) without 2,3,5-triphenyltetrazolium chloride in the medium. Tests to determine sensitivity to erythromycin, and production of reducing substances from sucrose, phosphatase, and pectate degradation were run as described by Kelman and Dickey for separating members of the "carotovora" *Erwinia* group (9). Pectate degradation was determined in both Beraha's medium (1) and crystal violet-pectate (CVP) medium (2). Potato soft rot was determined by inoculating whole white potatoes. Healthy tubers were washed in running tap water, surface-sterilized for 5 min in a 0.5% sodium hypochlorite solution, allowed to dry, and flamed two times with alcohol. Triangular plugs, approximately 0.8 cm across, were removed with a sterile scalpel from each potato, and cells from a 24-hr NYDA plate were smeared in the cavity. The plugs were replaced, covered with sterile adhesive tape, and incubated in covered 25-cm-diameter polyethylene chambers. The inoculated areas were checked for rot beginning 3 days after inoculation. All the above tests were run at 25–28 C. Tobacco hypersensitivity was determined by infiltrating interveinal areas of mature leaves of tobacco (*Nicotiana tabacum* L. 'Hicks') with a cell suspension (10^8 colony-forming units [cfu]/ml) and observing after 24 hr (10).

Plant inoculations. *General procedures.* Primula plants for the inoculation studies were either obtained as young plants from a commercial grower or were grown from seed. Until inoculation, all plants were grown in a growth chamber at 18–24 C in 10-cm-diameter pots filled with a sterilized commercial mix (Pro-Mix, Premier Brands, Inc., New Rochelle, NY 10801). Plants were 10–12 cm tall and well established at the time of inoculation. All studies were conducted in temperature-controlled growth chambers. In all experiments, treatments were arranged in a randomized complete block design with five replications. All studies were repeated at least once. Disease severity was recorded at appropriate intervals on a 0–5 scale in which 0 = no disease, 1 = discoloration at the point of inoculation in the crown area, 2 = slight decay in the crown area evident but no leaves permanently wilted, 3 = significant decay in the crown area and one or more leaves wilted, 4 = massive decay in the crown area and most leaves wilted, and 5 = complete decay in the crown area and plant dead or near death.

Susceptibility of various *Primula* spp. In one series of studies, plants of *P. vulgaris* 'Dania', *P. malacoides* (mixed variety), *P. obconica* 'Juno mixture', and *P.* × *polyantha* 'Jewel Mixture' were inoculated by puncturing the crown area two times with a sterilized dissecting needle and flooding with 0.5 ml of a turbid suspension (1.2×10^9 cfu/ml) of the primula strain. Plants of *P. vulgaris* 'Dania' and *P. malacoides* (mixed variety) were also inoculated in the same way with four known strains (one each from dracaena, cyclamen, chrysanthemum, and potato) of *E. c.* subsp. *carotovora* to determine whether other strains would cause a disease similar to the primula strain. The dracaena, cyclamen, and chrysanthemum strains were the same as those described and used in the physiological tests. The potato strain was obtained from A. Kelman. Inoculated plants were enclosed in clear polyethylene bags and placed in a 28–30 C growth chamber. The bags were removed after 36 hr and the plants were held for 2 wk to allow disease development, during which time disease ratings were taken.

Effect of wounding, moisture, and temperature on disease development. Plants of *P.* × *polyantha* 'Jewel Mixture' were inoculated with or without wounding (puncturing crown with a dissecting needle as described above) and either were or were not incubated in a high moisture environment (enclosed in polyethylene bag for 36 hr) at 20, 25, and 30 C. Disease was rated at 2- to 3-day intervals for 2 wk.

Effect of wounding on disease development on three *Primula* spp. In one study, injured and noninjured plants of *P. vulgaris* 'Dania', *P. malacoides* (mixed variety), and *P. obconica* 'Juno Mixture' were inoculated with a suspension (10^8 cfu/ml) of the primula strain. Plants were inoculated in the crown area as previously described, enclosed in polyethylene bags for 36 hr, and held in a growth chamber at 28–30 C.

Susceptibility of other plant species to the primula strain. Plants in addition to the *Primula* spp. were inoculated to determine whether the bacterium from primula would cause rot. Tobacco (*Nicotiana tabacum* L. 'Hicks'), tomato (*Lycopersicon esculentum* Mill. 'Marion'), potato (*Solanum tuberosum* L. 'Kennebec'), cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Clay'), and collard (*Brassica oleracea* var. *acephala* DC 'Vates') plants were inoculated by injecting 0.5 ml of a bacterial suspension (10^8 cfu/ml) into stems with a needle and syringe. All plants were young, succulent, and 12–15 cm tall at inoculation. Inoculated plants were placed in a chamber at 28–30 C and were incubated for 7 days.

RESULTS

Cultural and morphological characterization. On NYDA, colonies of the primula strain were rapidly growing, cream-colored, and circular to slightly irregular in outline with entire margins. On MS medium, colonies were initially light orange but turned slightly green in 2–3 days. The bacterium did not produce pink, blue, or other diffusible pigments on YDC. Filiform growth occurred on NYDA slants. Cells were rods, 0.7 × 2.7 μm (mean), with up to seven peritrichous flagella.

Physiological and biochemical tests. The organism was a facultative anaerobe and had minimum, optimum, and maximum temperatures of 10, 26–28, and 40 C, respectively. It was positive in the following tests: motility in semisolid agar, catalase production, casein hydrolysis, citrate utilization, salt tolerance (5% NaCl), nitrate reduction, aesculin hydrolysis, β-glucosidase activity, acetylmethylcarbinol production, tolerance to KCN, and methyl red. In litmus milk it caused slow reduction and a soft curd after 7 days. The organism tested negative in the following tests: oxidase, ammonia production, starch hydrolysis, urease, arginine dihydrolase activity, levan production, lipolytic activity, and indole. It caused rapid acid, but not gas, production from glucose, lactose, and sucrose. It used these sugars fermentatively when tested in Hugh and Leifson's semisolid basal medium (7). It also produced acid from xylose, raffinose, and inositol but not from dulcitol, α-

Table 1. Comparison of the primula strain with known *Erwinia* spp. in physiological and biochemical tests used to separate members of the "carotovora" group[z]

Test	*E. c.* subsp. *carotovora* (from dracaena)	*E. c.* subsp. *carotovora* (from cyclamen)	*E. c.* subsp. *carotovora* (from chrysanthemum)	*E. c.* subsp. *atroseptica* (from potato)	*E. chrysanthemi* (from corn)	Primula strain
Pectate degradation	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+
Potato soft rot	+	+	+	+	+	+
Sensitivity to erythromycin	−	−	−	−	+	−
Reducing substances from sucrose	−	−	−	+	+	−
Phosphatase	−	−	−	−	+	−
Gas from glucose	−	−	−	−	+	−
Acid from lactose	+	+	+	+	+	+
α-Methyl-D-glucoside	−	−	−	+	−	−
Palatinose	−	−	−	+	−	−

[z]The three strains of *E. c.* subsp. *carotovora* were obtained from R. S. Dickey and the strains of *E. c.* subsp. *atroseptica* and *E. chrysanthemi* were from A. Kelman.

Table 2. Effect of temperature and a moisture period after inoculation on the development of crown rot and wilt on wounded plants of *Primula* × *polyantha* 'Jewel Mixture' inoculated with a primula strain of *Erwinia carotovora* subsp. *carotovora*

Temp (C)	Moisture period after inoculation[x]	Disease severity[y] 3 days	7 days	15 days
20	No	0.0	0.0	0.0
	Yes	0.0	0.0	0.8
25	No	0.0	1.2	3.2
	Yes	0.0	2.4	4.5
30	No	2.1	4.0	5.0
	Yes	3.2	4.8	5.0
Analysis of variance				
Temperature		*[z]	*	*
Moisture		*	*	*
Temperature × moisture		*	*	*

[x]Moisture period was provided by enclosing plants in clear polyethylene bags for 36 hr after inoculation. Plants were wounded by puncturing the crown area with a sterile dissecting needle followed by flooding with 0.5 ml of a turbid bacterial suspension.

[y]Based on a 0–5 scale in which 0 = no disease, 1–4 = increasing degrees of crown rot and wilt, and 5 = plant dead or near death with severe decay of the crown and wilt of foliage.

[z]* = Significant at the $P = 0.05$ level, based on a separate analysis of variance for each incubation time.

methyl-D-glucoside, melezitose, or palatinose. It utilized sodium acetate, sodium citrate, sodium formate, and sodium succinate but not sodium benzoate, sodium oxalate, and sodium propionate. In other tests used to separate members of the "carotovora" group of *Erwinia* (Table 1), it was positive for pectate degradation when tested with Beraha's medium (1) and CVP (2), gelatin liquefaction, and potato soft rot. It was negative for sensitivity to erythromycin, production of reducing substances from sucrose, and phosphatase. Results of the tests on the primula strain agreed closely with the results obtained with the three known strains of *E. c.* subsp. *carotovora* (Table 1). The primula strain caused rapid death of infiltrated tobacco leaves that initially appeared similar to a hypersensitive reaction. However, the bacterium later produced a typical disease reaction by causing water-soaking and decay in surrounding tissue.

Susceptibility of *Primula* species. When plants were wounded at the time of inoculation with the primula strain, incubated for 36 hr in high moisture, and held at 28–30 C, crown rot symptoms were observed within 2–3 days on the tested cultivars of *P. vulgaris*, *P. obconica*, and *P.* × *polyantha*. The crown area on many of the plants collapsed as a mushy rot in 5–7 days. The soft rot often progressed from the crown area into the leaf petioles. Most plants of all three species wilted and dried within 7–10 days. Decay and wilt developed less rapidly on *P. malacoides*, usually appearing slower, as a drier rot, and a gradual wilt. The four known strains of *E. c.* subsp. *carotovora* caused rot on *P. vulgaris* similar to the primula strain, but rot developed slowly on the *P. malacoides*. Rot caused by four strains developed more slowly on the two primula species than did that caused by the primula strain.

Effect of wounding, moisture, and temperature on disease development. Wounding at inoculation, a moisture period after inoculation, and temperature each influenced disease development. No disease developed at 20 and 25 C on nonwounded plants even when a 36-hr moisture period was provided. At 30 C, a few nonwounded plants became diseased (mean disease ratings were 1.2 and 2.2 after 7 and 14 days) but only when the moisture period was provided. Disease development on nonwounded plants was erratic; some plants developed disease whereas others remained healthy. On wounded plants, the rate of disease development increased with each increase in temperature (Table 2). Under optimum conditions for disease (wounding and high temperature), the high moisture period increased the rate of disease development, but severe disease eventually developed on wounded plants that did not receive the high moisture period.

Effect of wounding on disease development on three *Primula* spp. Wounding was required for disease development on plants of *P. vulgaris*, *P. obconica*, and *P. malacoides* given a 36-hr moisture period and held at 28–30 C. Nonwounded plants of these species did not show symptoms in 14 days. Disease developed on wounded plants of all three species, but disease severity was lower on *P. malacoides* than on *P. vulgaris* and *P. obconica* (Table 3).

Susceptibility of other plant species to the primula strain. All of the five plant species inoculated showed necrosis. The typical reaction on all hosts was marked discoloration and various degrees of rot at the point of inoculation. Stems of potato had a soft rot, collapsed, and the plant fell over. On tomato, there was marked deterioration of the pith, and the stem collapsed when pressed between the

fingers. On the other hosts, the necrosis often moved from the initial inoculation point through the stem and into the base of the petiole of the nearest leaf, which eventually wilted and collapsed. In some cases, the necrosis appeared to follow veins.

DISCUSSION

The common soft rot bacterium *E. c.* subsp. *carotovora* appears to be a highly virulent pathogen on several species of *Primula*. The primula strain does not appear to be specialized, as it also caused decay on several other host plants when wound inoculations were made. Four other known strains of *E. c.* subsp. *carotovora* from other hosts also caused a crown rot on primula, although the primula strain caused a more rapid decay. Wounding and high temperature (≥25 C) seem to be the most important factors influencing disease development. Nonwounded plants usually did not become diseased regardless of the inoculum level or environmental conditions. In some studies, a few nonwounded plants became diseased at 30 C when a 36-hr high moisture was provided, but these conditions would be unusual in commercial production conditions because primula plants are usually produced at temperatures lower than 30 C. A high moisture period promotes disease development but is not required if a wound is present. In the absence of effective chemical control methods, the most practical control measures appear to be the avoidance of injury during handling and culture and the maintenance of the low temperatures that are suitable for the growth of primulas. The results of our work suggest that species may differ in susceptibility, but more varieties will have to be studied before it can be determined whether the response is at the species or varietal level.

Table 3. Development of crown rot and wilt on wounded plants of three *Primula* species inoculated with the primula strain of *Erwinia carotovora* subsp. *carotovora*[x]

Primula species and variety	Disease severity[y]		
	3 days	7 days	14 days
P. vulgaris 'Dania'	0.8 a[z]	4.0 a	4.3 a
P. obconica 'Juno Mixture'	1.0 a	3.0 a	4.7 a
P. malacoides (mixed variety)	0.0 b	1.0 b	1.8 b

[x] The crown areas of plants were punctured two times with a sterile dissecting needle and flooded with 0.5 ml of a 10^8 suspension. Plants were given a 36-hr moisture period and held at 28–30 C.
[y] Based on a 0–5 scale in which 0 = no disease, 1–4 = increasing degrees of crown rot and wilt, and 5 = plants dead.
[z] Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

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