Survival of *Erwinia chrysanthemi* in Association With *Philodendron selloum*, Other Greenhouse Ornamentals, and in Potting Media

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ABSTRACT

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A rifampin-resistant strain (R_4) was used to study the survival of *Erwinia chrysanthemi* (*Ec*). R_4 was similar to the rifampin-sensitive strain in numerous bacteriological tests and was as virulent as the wild type on *Philodendron selloum* (*Ps*) and 22 other greenhouse ornamentals. An enrichment technique was used that enabled isolation of the bacterium from detached artificially infected leaves of *Ps* 11–13 mo after they had been placed either upon a greenhouse bench, upon and buried in potting media, or in a laboratory. The pathogen was detected from potting media with and without the presence of living *Ps* roots 12 mo after infestation. R_4 survived for 5–6 mo in association with leaves of 23 host and nonhost greenhouse ornamentals and was recovered from symptomless leaves of *Ps* 12 mo after inoculation. R_4 was recovered after 13 mo from artificially infested seed stored at 5 C. *Ec* was detected from artificially and naturally infested seed of *Ps* by germinating seed under conditions of high relative humidity and high temperature.

Additional key words: bacterial leaf blight, floral crops, foliage plants.

Erwinia chrysanthemi McFadden, Burkholder, and Dimock (*Ec*) is an important plant pathogen in North Carolina and is the most destructive bacterium affecting foliage plants in Florida (15). Bacterial leaf blight (BLB), which is caused by *Ec*, is a limiting factor in the production of *Philodendron selloum* Koch (*Ps*).

Knauss and Miller (16) suggested that *Ec* was systemic after isolating it from disinfested, symptomless basal stems and petioles of *Ps*. They isolated *Ec* from fleshy seed coverings of rotted fruits and found that streptomycin dips did not control Erwinia root rot. Antibiotic sprays have provided good control of BLB under experimental conditions (24), but poor control is often experienced by growers (16).

Haygood and Strider (9,10,12) investigated the influence of inoculum concentration, wounding, temperature, moisture, and nutrition on BLB of *Ps*. Further research on the epidemiology of BLB of *Ps* (in particular, studies of survival of *Ec*) was needed for the formulation of a sound control program.

Survival of Erwinia soft rotters (ESR) is not completely understood, probably due to the methods used to detect small populations of bacteria. Few reports on the survival of *Ec* exist. *Ec* was isolated from soil and soil debris in a sweet potato field (30). Lim (21) reported that *Ec* could survive in mineral soil for 18 days, but was undetectable in unsterile peat 1 day after infestation. He detected *Ec* 14 days after infestation of pineapple leaves (22). Garibaldi (7) found that *Ec* could survive in artificially infested soil and in infected carnation debris in numbers sufficiently high to induce symptoms in carnation plants after 25 and 70 days, respectively. Using a selective medium, he detected *Ec* in infested soil for 4–5 mo. Hoitink (13) was unable to detect *Ec* from infected plant parts that had been buried in a compost pile for 10–12 wk.

The survival of other ESR has been more thoroughly investigated. ESR can overwinter in contaminated plant residues remaining in the soil after harvest and can persist as long as the plant material is not completely decomposed (28). *Erwinia carotovora* (*Eca*) persists in some soils for only a few months while in other soils it is considered to be endemic (28).

Plant pathogenic bacteria can survive in or on healthy host or nonhost leaves (19). Epiphytic populations of *Eca* increased during the growing season on leaves and stems of tobacco plants (32). *Eca* was detected by using an enrichment procedure up to 7 wk after infestation of potato leaves, but without enrichment it was detected only up to 3 days (27).

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ESR are usually absent in true or botanical seeds (28); however, *Eca* and *Ec* were isolated from tobacco seed (23,31). Seed collected from diseased plants can be contaminated externally, but the bacteria tend to die out rapidly as the seed dries.

ESR can survive almost indefinitely in the rhizosphere of some plants (4,28). The growth of low populations of bacterial pathogens might be stimulated when the roots of host plants grow into the microenvironment of the pathogen (8).

The importance of survival of *Ec* at low populations should not be underestimated because only a few cells can function as primary inoculum. Selective media (eg, crystal violet pectate [CVP] medium [5]) are available for the detection of ESR; however, it was our opinion that the use of an antibiotic-resistant strain of *Ec* would provide more accurate information on the population dynamics of *Ec* in potting media and in association with greenhouse plants and plant debris. Antibiotic resistance has been used in ecological studies of several bacterial plant pathogens (33). Rifampin resistance has been used in studies of *Agrobacterium tumefaciens* (1,25), *Xanthomonas phaseoli, X. phaseoli* var. *fuscans* (33,34), and *Ec* (35).

The objective of this study was to measure the survival of *Ec* in five environments: (i) in two greenhouse potting media; (ii) in infected *Ps* leaf tissue maintained in the greenhouse and in the laboratory; (iii) in association with roots of *Ps*; (iv) in association with *Ps* seed; and (v) in association with leaves of symptomless *Ps* and other host and nonhost greenhouse ornamentals.

MATERIALS AND METHODS

Ps seed used in this study was supplied by Gloeckner, Inc. (15 East 26 Street, New York, NY 10010), Bodger Seed (P. O. Box 5090, El Monte, CA 91734), or by a commercial *Ps* grower in Apopka, FL. *Ps* seedlings were donated by Speedling, Inc. (P. O. Box 7098, Sun City, FL 33586). Other greenhouse ornamentals used in this study (Table 1) either were supplied by Yoder Brothers, Inc. (P. O. Box 230, Barberton, OH 44203) or were purchased locally. Plants were transplanted into Metro Mix 220 (M-mix: W. R. Grace and Co., Cambridge, MA 02138) in 10-cm pots, fertilized weekly with Peters' 20-20-20 (2.6 g/L water), and overhead-irrigated twice a day for 8–12 wk before they were inoculated. When needed, plants were repotted in M-mix in 15.2-cm pots. Unless otherwise stated, plants were grown and survival studies were conducted in a greenhouse with temperature and relative humidity (RH) ranges of 20–30 C and 30–100%, respectively.

M-mix and a pine bark:peat moss:sand (3:1:1, v/v) mix amended with 1.14 kg of 20% superphosphate, 5.45 kg of dolomitic limestone, and 0.91 kg of hydrated lime per cubic meter were chosen for this study because they were representative of the greenhouse potting media used in North Carolina.

Rifampin-resistant strains of *Ec*. Colonies of many *Ec* strains are distinctive on lima bean agar (LBA) and lima nutrient agar (LNA; 11.5 g dehydrated LBA [Difco] and 11.5 g of dehydrated nutrient agar [Difco] per liter) by a dark-pigmented center and the presence of a halo around the colonies when grown at 30 C. LNA was used throughout this study since preliminary work showed that *Ec* colonies grew more uniformly and rapidly on this medium than on LBA at 30 C.

Rifampin agar medium (RAM) was prepared by dissolving 5 mg of rifampin and 5 mg of cycloheximide in 5 ml of methanol. The solution was added to 1 L of LNA prior to pouring of petri plates. Naturally occuring rifampin-resistant strains were selected from the wild-type strain Cu 242 (isolated from tissue of *Ps* in Florida by J. F. Knauss and obtained from R. S. Dickey, Cornell University) by spreading $\sim 10^{10}$ colony-forming units (cfu) on RAM and incubating at 30 C for 96 hr. Five rifampin-resistant strains were subcultured twice on LNA and RAM and stored at room temperature in distilled water, on LNA slants, on LNA slants covered with sterile mineral oil, or lyophilized and stored at 5 C.

Pathogenicity of 48-hr cultures of the five strains was compared with the wild-type strain by inoculating wounded and unwounded

TABLE 1. Response of 23 greenhouse ornamentals to a rifampin-resistant *Erwinia chrysanthemi* strain R_4 and the recovery of R_4 from leaves 150 and 180 days after inoculation

Plant species	Recovery of R_4 at: (days)[a]		Disease rating of wounded leaves exposed to three inoculum concentrations (cfu/ml)		
	150	180	2×10^6	2×10^8	NI[c]
Aglaonema commutatum Schott.	+	+	–[b]	+	+
Aglaonema commutatum Schott. 'Treubii'	+	+	–	+	++
Aglaonema modestum Schott.	+	+	–	–	–
Asplenium nidus L.	+	+	+	++	++
Brassaia actinophylla Endl.	+	+	+	++	++
Caladium × *hortulanum* 'Birdseye'	+	–	–	–	–
Chrysanthemum × *morifolium* Ramat. 'Bright Golden Anne'	+	+	–	–	–
Coleus blumei Benth.	+	–	–	–	–
Crassula argentea Thurg.	+	+	+	++	++
Dianthus caryophyllus L.	+	+	–	–	+
Dieffenbachia sp. 'Exotica'	+	–	–	–	+
Dracena marginata Lam.	+	+	–	+	++
Dracena sanderiana Sander.	+	+	–	–	+
Epipremnum aureum (Linden and Andre) Bunt.	+	–	–	+	++
Hoya carnosa R. Br.	+	–	–	–	++
Maranta leuconeura E. Morr.	+	–	–	–	–
Pepperomia argyreia E. Morr.	+	+	–	–	++
Pepperomia caperata Yurck.	+	+	–	–	–
Philodendron scandens C. Koch and Sello subsp. *oxycardium* Schott. Bunt.	+	+	–	–	–
Philodendron selloum Koch.	+	+	+	++	++
Pilea cadieri Gagnep and Guill.	+	+	–	–	–
Saintpaulia ionontha H. Wendl. 'Helga'	+	+	–	+	++
Syngonium podophyllum Schott.	+	+	–	–	–

[a] Isolations were made 150 and 180 days after inoculation of unwounded leaves with 1×10^7 cfu/ml.

[b] Wounded leaves were rated as positive (+) or negative (–), where – = water-soaked lesions absent or restricted to the wounded area; + = water-soaked lesions developed 0.1–1.0 cm outside the wounded area, and ++ = water-soaked lesions developed >1 cm outside the wounded area.

[c] Nondiluted inoculum (NI) consisted of bacterial cell masses.

attached leaves of *Ps* with three inoculum concentrations as previously described (11). The isolates were tested for phosphatase production (2), gas from glucose (29), utilization of sodium malonate (6), and pit formation on CVP (5). Strain R_4 was selected for use in this study owing to its close similarity to the wild-type strain in pathological and physiological traits.

Inoculum concentrations of 2×10^8 and 2×10^7 cfu of R_4 per milliliter were determined turbidimetrically and serial dilutions were made to obtain the lower inoculum concentrations throughout the study.

General techniques for isolating R_4. A standard procedure was developed for the recovery of R_4. Infested seed and media and infested or infected leaf tissue discussed in the following sections were placed in test tubes containing rifampin broth (RB), which consisted of 10 ml of yeast extract-dextrose broth (YEDB; 10 g of each per liter of distilled water) amended with 50 μg of rifampin, cycloheximide, benomyl, and etridiazole per milliliter. Plating efficiency was not affected by the addition of the inhibitors. The broth tubes were held at room temperature and shaken twice daily for 10 days with a vortex mixer. Loopfuls of turbid broth were streaked on RAM. One to 2 ml of the turbid broth was also poured onto a wounded *Ps* leaf (9) that had been soaked in 0.5% sodium hypochlorite for 5 min, rinsed in distilled water, and placed on moistened filter paper in a petri plate. Uninoculated RB was poured on *Ps* leaves to serve as controls. After 2 days of incubation at room temperature, water-soaked lesions of leaves in petri plates were placed in sterile distilled water (SDW), shaken, and streaked on RAM and LNA. The RAM was incubated at 30 C for 48 hr when distinctive R_4 colonies were observed.

On each isolation date, a loopful of R_4 cells stored in the lab in SDW was streaked on RAM to compare colony formation with the recovered R_4 cells. Randomly selected R_4 colonies were compared with the control strain for pathogenicity to *Ps* leaves and utilization of malonate.

Survival of R_4 in association with *Ps* leaves and roots. *Ps* seedlings were removed from germination flats after 17 days and inoculated with a bacterial suspension (2×10^8 cfu/ml) in three different ways: roots of 56 seedlings were dipped in the bacterial suspension and planted in either M-mix or 3:1:1 mix in 7-cm-diameter clay pots; 50 ml of bacterial suspension was added to 100 g dry weight 3:1:1 mix and 100 ml of the suspension was added to 225 ml of distilled water and then mixed with 100 g dry weight M-mix. One gram dry weight of each mix contained $\sim 1 \times 10^8$ cfu as determined by serial dilutions on RAM. Fifty-six seedlings were planted in the infested mixes; and 28 seedlings were planted in M-mix and 3:1:1 mix and sprayed to runoff with the bacterial suspension. Control plants were sprayed with SDW and planted in uninfested mixes.

Isolations were made monthly after inoculation from inoculated leaves and roots of two randomly selected plants of each treatment. Several roots and leaf disks (0.8 cm diameter), were removed from the sampled plants and placed in RB. Leaves from which leaf disks were taken were detached and discarded.

Survival of R_4 in *Ps* leaf tissue. Wounded leaves of 10 6-mo-old *Ps* plants were inoculated with R_4 (2×10^8 cfu/ml) as previously described. Plants were placed in a mist chamber for 24 hr and then on a greenhouse bench for 5 days. Rotted leaves were detached and placed on a bench to dry for 48 hr. Leaves were then placed in four locations: 10 leaves were placed in two plastic boxes and stored in a laboratory; 20 leaves were placed on three different areas of a greenhouse bench; five leaves were placed on the surface of M-mix in three metal flats ($20 \times 20 \times 5$ cm); and 10 leaves were shredded and mixed with M-mix in three metal flats. In each of the two latter methods, the metal flats were placed in three different locations on a greenhouse bench. Stock *Ps* plants were also grown on this bench. Thus, the treatments in the greenhouse were maintained in typical normal greenhouse production conditions.

Isolations were made monthly the first 4 mo after inoculation and bimonthly thereafter for 1 yr. For each isolation, 10 samples of leaf tissue were taken from the laboratory and nine samples were taken from each treatment in the greenhouse and incubated in RB.

Detection of *Ec* from commercial seed lots. To determine if *Ec* was transmitted naturally on seed, three seed lots were assayed by four methods: five samples containing 0.5 g of seed from each lot were placed in 10 ml of YEDB amended with 50 μg of cycloheximide per milliliter, shaken daily, observed for 2 wk, and loopfuls of 10^{-1} and 10^{-2} dilutions of the broth were streaked on LNA and CVP and incubated at 30 C; five samples containing 0.5 g of seed from each lot were ground in 10 ml of SDW with mortar and pestle. The slurry was allowed to settle for 10 min and loopfuls were streaked on LNA and CVP. One to 2 ml of the slurries and broth from the first method above were poured on five wounded *Ps* leaves. The leaves were placed on moistened filter paper in petri plates and incubated at room temperature; 400 seeds from each lot were plated directly on CVP (20 seeds per petri plate); and 200 seeds were planted in sterile M-mix in 20 separate sterile metal flats and watered. One half of the flats were placed in plastic bags. Three hundred seeds were planted in sterile M-mix in 10 different sterile $8 \times 38 \times 55$-cm wood flats, half of which were covered with plastic.

Seedlings were fertilized biweekly with 4 g of Peters' 20-20-20 fertilizer per liter and otherwise were watered as needed. Eight to 17 wk after germination, portions of leaves with BLB symptoms were removed, placed in SDW, and streaked on LNA. Attempts were made to isolate *Ec* from symptomless seedlings grown in flats not covered with plastic by placing 10 leaves per flat in YEDB. Colonies suspected of being *Ec* and that had been isolated by the four methods were tested for pathogenicity on *Ps* leaves, for malonate utilization, for phosphatase production, for pitting on CVP, and for rotting of potato slices.

Survival of R_4 in association with artificially infested seed. An R_4 suspension (greater than 10^{10} cfu/ml) was prepared from 48-hr LNA cultures. Two thousand seeds were soaked in the suspension for 10 min. One-half of the seeds were placed in a vacuum chamber at -20 psi for 10 min. The bacterial suspension was drained through cheesecloth, and the seeds were placed in a microvoid overnight to dry. Seeds were germinated in sterile M-mix contained in sterile wood flats.

The inoculation procedure was repeated using 2,000 seeds planted 200 seeds per flat, in sterile M-mix in metal flats, which were placed in plastic bags. Seedlings were watered and fertilized in the same manner as before.

Isolations from roots and leaves of five seedlings per flat were made 6 wk after germination as previously described. Eight weeks after germination a frogging device (14) was used to wound leaves of 20 seedlings in four flats that had not been covered with plastic. The flats were placed in a mist chamber for 96 hr and observed for BLB symptoms. Isolations were made from water-soaked lesions as before.

Two thousand seeds were infested with R_4 as previously described. Five hundred seeds were placed in each of four seed packets and stored at 5 C. The same procedure was repeated at three later times. Assays of seed were made bimonthly by placing 50 seeds from each packet into 10 ml of RB.

Survival of R_4 in association with host and nonhost plants. Twenty-three greenhouse ornamentals were selected for this study (Table 1). The common names and susceptibility of the plants to strain Cu 242 are presented elsewhere (11). Inoculum concentrations of 2×10^6 and 2×10^8 and undiluted inoculum (NI) of R_4 were applied to wounded and unwounded attached leaves of plants as previously described (11).

To determine survival capabilities of R_4 on leaf surfaces of host and nonhost plants, a suspension of R_4 (2×10^7 cfu/ml) was applied to both leaf surfaces of two plants of each species with an atomizer at 3 psi. One month later, SDW was sprayed on leaves of 15 additional *Ps* plants. Five were grown on the same part of the bench as the plants inoculated with R_4. Ten of the *Ps* plants inoculated with SDW were separated from the R_4 inoculated plants by a plastic screen to prevent splashing of water during irrigations. Plants were placed randomly on a greenhouse bench for 6 mo and watered twice daily by overhead irrigation. The temperature range was 20–30 C and the RH varied from 40–100%. At monthly intervals, five leaf disks (0.8 cm in diameter) were taken from two leaves of each plant and placed in RB. Plants were randomly placed

back on the bench after each isolation.

Population trends of R_4 in potting media. Survival of R_4 was studied in M-mix and 3:1:1 mix under four different conditions: in sterile mixes maintained at 0.1 bar soil moisture tension (SMT); in unsterile mixes maintained at 0.1 bar SMT; in the mixes amended with 3% air-dried *Ps* leaf tissue and maintained at 0.1 bar SMT; and in unsterile mixes maintained at 0.005 bar SMT.

Sterile treatments were prepared by autoclaving the mixes at 121 C and 15 psi for 2 hr on two consecutive days. A water retention curve based on SMT versus percent water content by weight was prepared for both media by a pressure membrane apparatus (29). Measured amounts of a bacterial suspension containing R_4 at 2×10^8 cfu/ml and SDW were added to the media so that each treatment contained $\sim 1 \times 10^8$ cfu/g dry weight mix (GDWM) at the designated SMT. Three hundred grams of each medium were placed in three plastic ziplock bags, weighed, and placed under a greenhouse bench. The bags were maintained at the appropriate weights by adding SDW. Serial dilutions were made immediately and at 2, 5, 9, 14, 21, 31, 45, and 75 days after infestation of the media. Five GDWM from each bag were placed in 50 ml SDW to serve as the 10^{-1} dilution. One milliliter from the 10^{-1} dilutions and 0.1-ml aliquots from each of the other dilutions were spread on RAM with a bent glass rod. On previous isolation dates and at 30-day intervals thereafter, 1.0 GDWM from each bag was placed in 10 ml of RB. When R_4 was recovered only by the enrichment technique, less than 10 cfu/GDWM were assumed to be present.

To determine whether R_4 could survive in M-mix alone under a normal production environment, the medium was placed in nine 10-cm-diameter clay pots, and 20 ml of R_4 (2×10^8 cfu/ml) were poured into each pot. The pots were placed on a greenhouse bench and watered twice daily. Isolations were made monthly for 7 mo by placing 1.0 GDWM sample from each pot in RB.

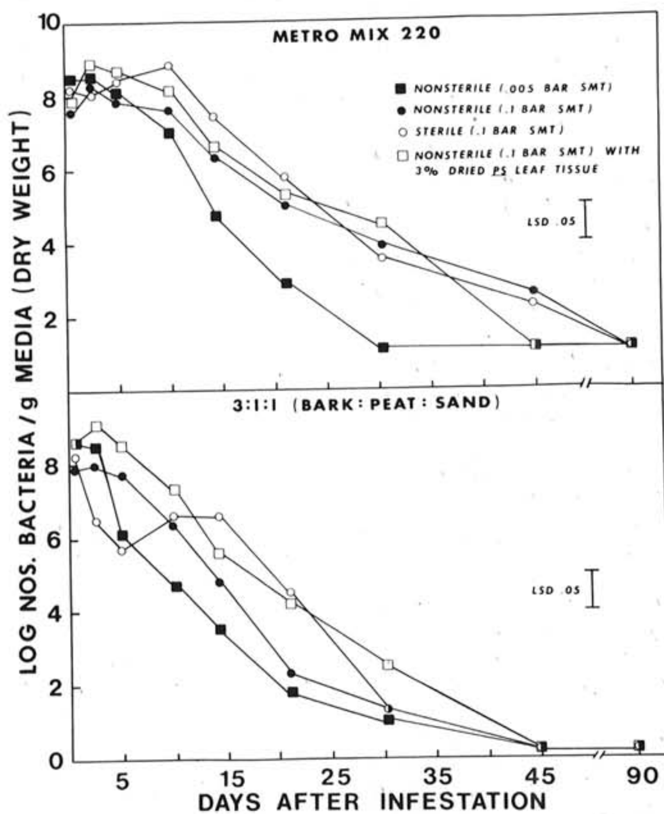

Fig. 1. Population trends of a rifampin-resistant *Erwinia chrysanthemi* strain in Metro Mix 220 and a bark:peat:sand (3:1:1, v/v) potting medium under four different conditions: in unsterile media maintained at 0.005 bar soil moisture tension (SMT); in unsterile media maintained at 0.1 bar SMT; in sterile media maintained at 0.1 bar SMT; and in media containing 3% air-dried *Philodendron selloum* (*Ps*) leaf tissue maintained at 0.1 bar SMT. Each data point represents the mean of 15 isolations.

RESULTS

On *Ps* leaves, two rifampin-resistant *Ec* strains were pathologically indistinguishable from the wild-type strain Cu 242. These two strains were also very similar to the wild type in numerous physiological tests, but in particular for phosphatase production, gas from glucose, and utilization of sodium malonate. Strain R_4 was selected for use in this study due to its close similarity to the wild-type strain in physiological and pathological characteristics.

The isolation technique developed for recovery of R_4 was simple and could detect R_4 at very low populations. All randomly selected R_4 isolates recovered from the various survival treatments were pathogenic on *Ps* leaves and utilized malonate.

Survival of R_4 in association with *Ps* leaves and roots. During this study, BLB symptoms developed on a few leaves, which were removed when symptoms were observed. R_4 was recovered for up to 12 mo from symptomless leaves.

M-mix or 3:1:1 mix had no apparent effect on the ability of R_4 to survive in association with *Ps* roots. The bacterium was recovered after 12 mo from 25% of the roots that had been dipped in the R_4 suspension and planted in the media as well as from roots of seedlings that had been planted in media infested with R_4.

Survival of R_4 in leaf tissue. R_4 was recovered from 20% of the isolations from dried infested leaf tissue maintained in the laboratory and in the greenhouse 2 mo after inoculation. After 11 mo, the pathogen was detected in two of 10 isolations from tissue in the lab and two to three of nine isolations from tissue maintained on a bench, on M-mix, or mixed with M-mix in the greenhouse. R_4 was detectable 15 mo after inoculation in tissue maintained in the laboratory and on the greenhouse bench. Tissue maintained in or on M-mix was badly decomposed after 11 mo and isolation attempts were discontinued.

Detection of *Ec* from commercial seed. *Ec* was not recovered directly from seeds regardless of the method employed. However, BLB symptoms appeared within 8–11 wk on leaves of seedlings grown in sterile M-mix in three of 10 metal flats placed in plastic bags and two of five wood flats covered with plastic. Isolates obtained from these flats were pathogenic on *Ps* leaves, rotted potato slices, caused pitting on CVP, utilized malonate, produced phosphatase, and had characteristic colony morphologies of *Ec* on LNA. *Ec* was not isolated from symptomless leaves of seedlings grown in flats not covered with plastic.

Survival of R_4 in association with artificially infested seed. Similar results were obtained in the recovery of the pathogen from seeds that were placed in a vacuum at −20 psi during infestation and from seeds that were not placed in a vacuum. R_4 was isolated from leaves and roots of at least one seedling per flat 6 wk after germination. Water-soaked lesions on seedlings from which R_4 was recovered appeared after 8 wk in four of 10 flats that were incubated in plastic bags.

Seedlings in one of four flats that had not been incubated under plastic developed BLB symptoms after they were wounded and placed in a mist chamber. R_4 was recovered from the rotted leaves.

The pathogen was recovered from 2/4 and 1/4 of the seed packages stored at 5 C after 2 and 6 mo, respectively. R_4 was detected from seed in two of 10 seed packets assayed 13 mo after infestation.

Survival of R_4 in association with host and nonhost plants. Results for the recovery of R_4 from 23 greenhouse ornamentals 5 and 6 mo after inoculation are presented in Table 1. The pathogen was recovered from all species 5 mo after inoculation and from at least one leaf of each species 6 mo after inoculation except from *Crassula argentea, Maranta leuconeura, Caladium* × *hortulanum, Hoya carnosa, Dieffenbachia* sp. 'Exotica,' *Coleus blumei*, and *Epipremnum aureum*. R_4 was also recovered from two leaves of five *Ps* plants inoculated only with SDW and grown among the plants inoculated with R_4. R_4 was not isolated from leaves of *Ps* plants inoculated with SDW and grown on a part of the bench separated from the R_4 inoculated plants by a plastic screen divider.

Responses of the 23 plants to inoculation of R_4 at 2×10^6 and 2×10^8 cfu/ml and NI are also presented in Table 1. More species

were susceptible to R_4 when wounded leaves were inoculated with NI than with the lower inoculum concentrations. Only *Asplenium nidus, Crassula argentea,* and *Ps* developed BLB symptoms when wounded leaves were inoculated with 2×10^6 cfu/ml.

Unwounded leaves of most plants were not susceptible to R_4 regardless of inoculum concentrations applied. However, water-soaked lesions appeared on unwounded leaves of *A. nidus, C. argentea,* and *Ps* after inoculation with the higher inoculum concentrations.

Population trends of R_4 in potting media. Population trends of R_4 in M-mix and 3:1:1 mix under four different conditions 0–90 days after infestation of mixes are presented in Fig. 1. Results for the recovery of R_4 from the media by enrichment 90–360 days after infestation are presented in Table 2.

Similar population trends were observed for the respective treatments in each medium. Initial populations of 2×10^8 cfu/GDWM decreased to populations of 1×10^1 cfu/GDWM or lower 90 days after infestation. Somewhat higher populations were detected 30–45 days after infestation in M-mix treatments than in respective 3:1:1 mix treatments. The addition of 3% dried leaf tissue of *Ps* or the use of sterile media did not enable R_4 to survive at higher populations or for longer periods of time than R_4 populations in unsterile media maintained at 0.1 bar SMT. Lower R_4 populations were generally recovered from unsterile media maintained at 0.005 bar SMT than unsterile media maintained at 0.1 bar SMT 15 days after infestation in 3:1:1 mix and after 45 days after infestation in M-mix.

R_4 was detected in all 3:1:1 mix and M-mix treatments by the enrichment technique 90 and 180 days after infestation of the media. However, the pathogen was recovered by the enrichment technique only from the M-mix treatments 270 days after infestation and only from the unsterile M-mix treatments maintained at 0.1 and 0.005 bar SMT 360 days after infestation. All randomly selected colonies recovered from the media throughout the study were pathogenic on *Ps* leaves.

DISCUSSION

Rifampin was selected for use in this antibiotic-resistance selection system for several reasons: rifampin has a wide spectrum of antibacterial activity and is highly toxic (33); rifampin has only limited usefulness in human chemotherapy (33); previous studies have shown that rifampin-resistant isolates of *Ec* and *Xanthomonas* sp. are stable and very similar to wild-type strains in morphological and pathological characteristics (32–34); and only a few bacteria isolated from potting media and phyllospheres of greenhouse ornamentals were resistant to rifampin in preliminary studies, and R_4 could be easily distinguished from these bacteria by their characteristic colony morphology on RAM.

Strain R_4 used in this study was indistinguishable from wild-type strain Cu 242 in physiological characteristics as well as in pathogenicity on 23 greenhouse ornamentals. We would not expect all *Ec* strains to have the same survival capabilities of R_4. But the *Ec* strains present under natural conditions probably have been selected by survival pressures and can survive for periods at least as long as the R_4 strain.

Most survival studies of *Ec* in the past have not relied upon enrichment techniques to recover the pathogen from soil and plant debris (7,21). We believe that enrichment techniques are essential in survival studies of *Ec* because only a few bacterial cells may be required to initiate soft rot lesions in susceptible plants under the most conducive environmental conditions. Results on the survival of *Ec* in peat, mineral, and sandy loam soils were based on the recovery of *Ec* by selective media and the ability of *Ec* in the soil to cause symptoms on cut pineapple leaves and carnation roots dipped in soil suspensions (7,21). With the selective medium, Garibaldi (7) could detect *Ec* only if the populations were above 1×10^5 cells per gram of soil. Although 4×10^5 cells per gram of unsterile soil were recovered 151 days after inoculation of the sandy loam soil, no symptoms developed on carnation when dipped in a suspension of that soil. It is probable that this concentration of cells could easily cause soft rot lesions on *Ps* leaves should they come in contact with the soil when conditions were conducive to disease development.

Leben (19) has referred to plant pathogenic bacterial cells in a state of reduced metabolism as hypobiotic cells. He suggested that hypobiotic cells survive in dry leaf, stem, and root lesions of annual plants and that they could live longer without added nutrients and would be more likely to survive the physical and chemical stresses that cause the death of actively metabolizing cells. This phenomenon may explain why 4–7 days often are required for RB to become turbid when R_4 is isolated in low populations from potting media, leaf tissue, etc.

Results of previous studies show that *Ec* survives longer in sterile than in unsterile peat and soils (7,21) presumably due to the action of antagonistic microorganisms in unsterile media. However, in soilless potting mixes similar trends in populations of R_4 were detected in sterile and unsterile treatments of both mixes. Perhaps microorganisms naturally present in soilless mixes are not as antagonistic to *Ec* as microorganisms found in soils.

The addition of 3% dried *Ps* leaf tissue had little effect on the survival of R_4 in either mix. It was expected that the presence of *Ps* tissue would allow R_4 to survive at higher populations for longer periods of time. Although R_4 populations were lower in mixes maintained at 0.005 bar SMT than those in media maintained at 0.1 bar SMT, the differences in SMT had little effect on survival of R_4 in the media. Garibaldi (7) found that *Ec* generally survived longer at soil moisture levels 40–50% of field capacity. Differences may be attributed to use of different *Ec* strains and media.

In general, R_4 survived better in M-mix than in 3:1:1 mix. The pathogen was recovered from all treatments 180 days after infestation but only from M-mix treatments 270 days after infestation. R_4 was detected only in nonsterile M-mix treatments maintained at 0.1 and 0.005 bar SMT 360 days after infestation. Possible explanations include a more favorable texture of the M-mix, the availability of more nutrients in M-mix, or the release of toxic substances from the components of 3:1:1 mix.

R_4 has been recovered from infested M-mix maintained in 10-cm-diameter clay pots under a normal production environment for at least 7 mo. Thus, *Ec* is able to survive in low populations for extended periods of time in a soilless potting medium.

It was not surprising that R_4 was recovered from artificially infected *Ps* leaf tissue maintained in the lab and in the greenhouse for 11 mo or longer, since ESR can generally persist in plant

TABLE 2. Recovery of a rifampin-resistant *Erwinia chrysanthemi* strain from four Metro Mix 220 and 3:1:1 mix treatments 90 to 360 days after infestation

	Metro Mix 220[a]				3:1:1[b]			
Days after infestation	Unsterile (0.005 bar)	Unsterile (0.1 bar)	Sterile (0.1 bar)	Unsterile w/3% organic matter (0.1 bar)	Unsterile (0.005 bar)	Unsterile (0.1 bar)	Sterile (0.1 bar)	Unsterile w/3% organic matter (0.1 bar)
90	+[c]	+	+	+	+	+	+	+
180	+	+	+	+	+	+	+	+
270	+	+	+	+	–	–	–	–
360	+	+	–	–	–	–	–	–

[a] Metro Mix 220 is a commercially prepared potting mixture.
[b] Pine bark, peat moss, and sand (3:1:1, v/v), respectively.
[c] Isolations were mady by using an enrichment technique.

residues if the plant material is not completely decomposed (28). It was also expected that R_4 could survive in association with *Ps* roots, because *Ec* is pathogenic on *Ps* roots and many ESR can survive long periods in the rhizosphere of plants (4,28).

Knauss and Miller (16) isolated *Ec* from fleshy seed coverings of rotted *Ps* fruits, indicating that infestation of seed by *Ec* is likely. We were unable to recover *Ec* directly from commercial seed lots by similar methods. Bacteria identified as *Ec* were isolated from rotted leaves of seedlings grown in sterile media in flats covered with plastic. A similar technique was used to detect *Pseudomonas glycines* in soybean seed lots (26). No attempt was made to quantify the number of infected *Ps* seedlings per flat because the infection of only one seedling in a flat would rapidly provide inoculum for other seedlings in such a conducive environment for disease development.

R_4 was readily recovered by enrichment in RB at 2 and 6 wk after germination from both leaves and roots of artificially inoculated seed. Soft rot symptoms developed on seedlings 8 wk after artificially and naturally infested seeds were germinated in a water-saturated atmosphere. *Ec* was probably undetectable directly from naturally infested seed due to a low number of infested seed, an extremely low population of *Ec* on the infested seed, and the rapid growth of other microorganisms from the seed when placed in YEDB.

Ec can survive and remain pathogenic on *Ps* seed for extended periods as indicated by the recovery of R_4 from artificially infested seed after 13 mo of storage at 5 C. Isolation of R_4 from roots and shoots of seedlings grown from infested seed indicates that infested seeds are sources of primary inoculum and that the bacterium can multiply and spread on the seedling, especially under conditions of high humidity and temperature. *Xanthomonas vesicatoria* was also found to multiply under these conditions on tomato seedlings (17). Previous studies have shown that surface contamination (infestation) of seed is common (18).

R_4 was detected for 1 yr on *Ps* leaves inoculated as seedlings and maintained on a greenhouse bench. It would be difficult to determine if R_4 was surviving in or on leaves. Studies with soybean leaves indicated that bacteria are found in intercellular spaces as well as on the leaf surface (3). Bacteria probably survive in aggregates in protected positions such as deep depressions between epidermal cells (19) or in substomatal chambers. It is likely that more bacteria survive on the undersides of the leaves where they would receive less exposure to direct sunlight. Leben et al (20) have shown that pathogenic as well as nonpathogenic bacteria adhere to cell walls and are not easily removed by repeated washings. When plants are placed under a "conducive" environment for bacterial growth, surviving bacteria can initiate an epiphytic or resident phase on the healthy plant, or if they are plant pathogens, they can infect the plant (19).

This study supports other findings that plant pathogenic bacteria can survive on nonhost leaves (19). R_4 was capable of surviving on leaves of nonhost as well as host plants for 5–6 mo. Responses of the 23 greenhouse ornamentals to R_4 and wild-type strain Cu 242 at three inoculum concentrations were very similar.

R_4 was recovered from leaves of *Ps* plants sprayed with SDW and grown among the R_4 inoculated plants. It is probable that splashing water from one plant to another accounted for the transmission of R_4 to these leaves. In commercial operations, it is possible that primary inoculation of *Ec* can originate from *Ec* cells surviving on nonhost or symptomless host plants and be spread by splashing water.

Several practical implications are apparent from this study. *Ec* can survive in association with infested plant debris, infested seed, symptomless leaves of host and nonhost plants, in potting media, and in the rhizosphere of *Ps* for extended periods of time. The likelihood that *Ec* survives in protected sites of leaves as hypobiotic cells may explain why better control of BLB was obtained when antibiotics were applied to *Ps* leaves prior to rather than after inoculation of leaves with *Ec*.

Ec cells surviving in potting media, leaf debris, or either in or on seed might be stimulated by seed and root exudates. Bacteria surviving in the rhizosphere could be carried by emerging cotyledons to the aboveground portion of the plant. *Ec* would be able to survive on leaves until conducive environmental conditions prevailed and populations increased. The bacteria could be easily distributed by splashing water and thus, infestation of only one seed or seedling could result in an epidemic.

Since artificially and naturally infested seedlings did not develop symptoms of BLB until 8–10 wk after germination in a conducive environment, it is probable that *Ec* is present on many commercially produced *Ps* seedlings.

Until more effective chemicals are introduced and techniques are developed to provide *Ps* seed free of *Ec*, it is essential that growers use strict sanitation measures to reduce losses caused by BLB.

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