

able period of 3 or 4 weeks. Those plants that do not move to market make the compost pile shortly thereafter. After considering the implications of these production and marketing conditions, now think of the challenge of outguessing the season to determine when spring will break and sales will soar. If we are conservative and plan for a late spring, the market can leave us in the dust; and we lose that early market surge. If we throw caution to the wind, gamble on that early spring market, and spring breaks 3 weeks later than planned, only our compost pile reaps the benefit. Bedding plant production is a real challenge.

### QUESTIONS FOR DEXTER McDONALD

CHARLIE PARKERSON: Do your 4-inch and pak materials compete with each other in the market? Is the season longer for the 4-inch?

DEXTER McDONALD: Yes, they do compete. The season is not longer for the 4-inch, but the holding time is. They remain salable for a longer period of time.

CHARLIE PARKERSON: Can all types of containers go through the same filling and planting equipment?

DEXTER McDONALD. Yes, with minor adjustments.

CHARLIE PARKERSON: How do you water?

DEXTER McDONALD: We use sprinklers. We have no specific nozzles. Often we hand water as well.

### IRIS SOFT ROT CAUSED BY *ERWINIA CHRYSANTHEMI*, ASSOCIATED WITH OVERHEAD IRRIGATION AND ITS CONTROL BY CHLORINATION

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**Abstract.** Iris soft rot, previously reported to be caused by *Erwinia carotovora* subsp. *carotovora*, was correlated positively with the intensity of sprinkler irrigation rather than iris borer damage in a commercial rhizome production operation in Virginia. *Erwinia chrysanthemi* was consistently isolated from the rotted plants and reproduced the symptoms observed in

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the field in artificially inoculated healthy plants. *Erwinia carotovora* subsp. *carotovora* and garden slug damage were associated with a minor outbreak of soft rot in a greenhouse. Both bacterial pathogens were reduced in viability by exposure to sodium hypochlorite and the incidence of soft rot was reduced on sprouting rhizomes in greenhouse tests, especially when treated with 20mg Cl/liter (20ppm Cl). However, the effectiveness of chlorination was reduced in some water sources or when the number of bacteria suspended in the water was increased.

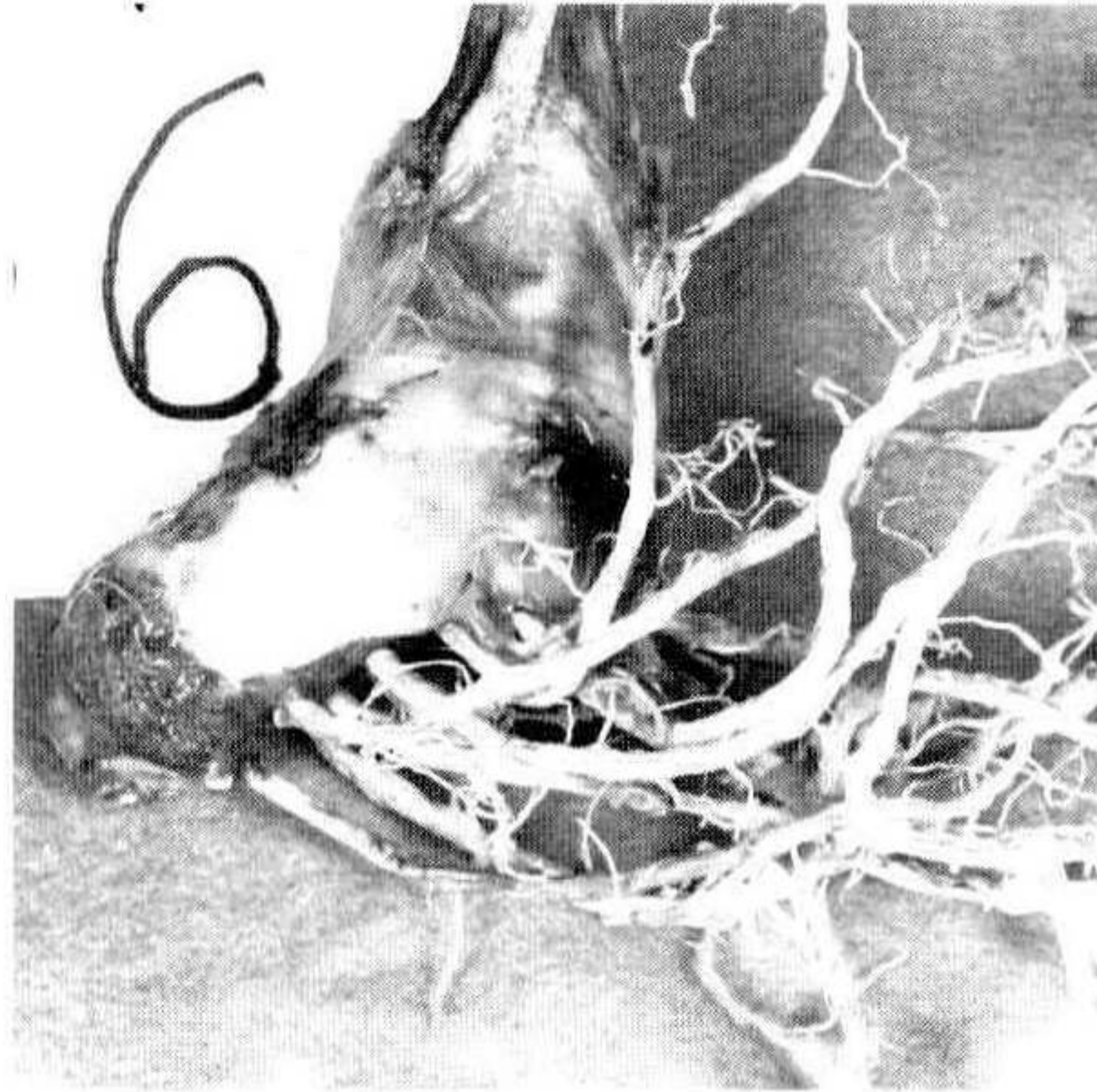
## INTRODUCTION

In the summer of 1980, extensive bacterial soft rot of iris (*Iris germanica* L.) was observed in a commercial rhizome production operation near Fishersville, Virginia. The initial symptoms included water-soaked streaks on the leafblades progressing upward from the base of the leaf fans (Figure 1). Some of these rotted leaves lodged or could be pulled very easily from the fans. Later, leaves that died became dry and brownish-grey in color. The rhizomes, at the base of the fans, were rotted at or near the soil line. The interior of the rhizomes was reduced to a viscous cream-colored mass that was often foul smelling (Figure 2). These symptoms were consistent with those described first by van Hall in 1902 (in ref. 11) and later by Massey in Virginia (11). Thanos (15) confirmed that the pathogen was *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al. and Howard and with Leach (9) established a vector relationship for the pathogen with the iris borer (*Macronoctus onusta* Grote).



**Figure 1.** Leaf symptoms of iris soft rot caused by *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi* in *I. germanica* cv. 'Jesse Viette'.

In this epidemic, however, iris borer damage to the rhizomes was not obvious and an increasing incidence of soft rot was correlated positively with the intensity of overhead irrigation from "Rain-bird" sprinklers. The incidence in non-irrigated areas was <1%, in irrigated areas 5-25%, and 20-40% in areas that received overlapping irrigations. Irrigation water was pumped directly from a small visibly turbid creek that drained a cow pasture and an alfalfa (*Medicago sativa* L.) hay field located adjacent to the iris production area.



**Figure 2.** Rhizome rot symptoms of iris soft rot caused by *Erwinia chrysanthemi* strain L-436 on *I. germanica* cv. 'Jesse Viette.'

*Erwinia chrysanthemi* Burkholder et al. strains capable of rotting wild *Iris* spp. growing on the banks of paddies were isolated from rice (*Oryza sativa* L.) plants with foot rot in Japan (5,6). Therefore, *E. chrysanthemi* may also be a pathogen of domestic iris.

In the past, control of iris soft rot has been accomplished chiefly by control of the iris borer and sanitation (13,17). However, some bacterial diseases such as stalk rot of maize (*Zea mays* L.) (16), soft rot of tomatoes (*Lycopersicon esculentum* Mill.) and potatoes (*Solanum tuberosum* L.) (1), diseases caused by *E. chrysanthemi* and *E. carotovora* subsp. *carotovora*, have been controlled by chlorination of irrigation water, wash water and flume water. Therefore, chlorination should be considered for control of iris soft rot if disease spread is related to irrigation.

This report confirms that *E. chrysanthemi*, as well as *E. carotovora* subsp. *carotovora* (Jones) Bergey, can be a pathogen of cultivated iris, that soft-rotting *Erwinia* spp. may be isolated from irrigation water, and that chlorination reduces the numbers of pathogenic bacteria in water and the incidence of iris soft rot in greenhouse tests.

## MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used in this study are listed in Table 1

**Table 1.** Origins of bacterial strains

Strain designation	Origin	Authority	Host
<i>Erwinia carotovora</i> subsp. <i>carotovora</i> (Jones) Bergey et al			
E32 & E34 <sup>x</sup>	New York	W H Burkholder	<i>Iris</i> sp
SR-53 <sup>y</sup>	Vermont	L R Jones	<i>Daucus carota</i> L
L-441 to L-443 <sup>z</sup>	Virginia	C M Berg	<i>I. germanica</i> L
<i>Erwinia chrysanthemi</i> Burkholder, et al			
E11 <sup>x</sup>	Japan	M Goto	<i>Iris ensata</i> Thunb
SP-26 <sup>y</sup>	Wisconsin	J I Victoria	<i>Zea mays</i> L
L-434 to L-436 <sup>z</sup>	Virginia	G H Lacy	<i>I. germanica</i>

<sup>x</sup> Provided by Dr R S Dickey, Dept Plant Pathol, Cornell Univ, Ithaca, NY 14853

<sup>y</sup> Provided by Dr A Kelman, Dept Plant Pathol, Univ Wisconsin, Madison, WI 53706

<sup>z</sup> Isolated during this study from field-grown iris (L-434 to L-436) and greenhouse-grown iris (L-441 to L-443)

**Media.** Unless otherwise specified, plate count agar (PCA; Difco, Detroit, MI 48232), crystal violet pectate medium (CVP; 14) and King's medium B (KB, 14) were used at 25°C to maintain and culture the bacteria.

**Isolation from plant tissues.** Diseased leaf sections were surface sterilized in 0.5% sodium hypochlorite for 30-60 sec and rinsed with sterile distilled water (SDW). A smaller section was cut aseptically from the leading margin of the lesion and placed in a tube containing 2ml SDW. After 10-30 min, the resulting bacterial suspension was streaked onto CVP medium. Colonies of pectolytic bacteria that caused pitting on CVP were restreaked twice on PCA and then again on CVP.

**Physiological and biochemical characterization of bacteria.** Production of acid from lactose, trehalose, mannitol, inositol, esculin, maltose and raffinose and nitrate reduction was detected using the Minitek® method (BBL, Cockeysville, MD 21030) according to the manufacturer's directions except that incubations were carried out at 25°C rather than 37°C

Phosphatase production was detected using phenolphthalein diphosphate-containing medium (14) and erythromycin sensitivity tests were performed on PCA plates swabbed with bacterial cells and exposed to a paper disk impregnated with 15 µg of erythromycin (Sensidiscs®, BBL). The diameter of the zone of inhibition was measured at 48h. Cytochrome oxidase was detected with Taxo® N disks (BBL). Growth in 5% NaCl-nutrient broth (Difco) was determined by an increase in turbidity seven days after inoculation. Positive and negative controls were used for all tests.

**Greenhouse culture of iris.** During the winter, iris rhizomes, *I. germanica* cv. 'Jesse Viette', were dipped in captan WP-50 (479 g a.i./liter) for 15 min. to control fungal rotting and potted in 25 cm plastic containers with a mixture of one part composted pine bark and one part Weblite® (50 mesh particle size, heat-expanded shale; Weblite Corp, Blue Ridge, VA 24064). The plants were fertilized with about 5g of slow release Osmocote® (Sierra Chem. Co., Milpitas, CA 95035; elemental nitrogen, phosphorus, and potassium in a 14:14:14 ratio by weight). The irises were watered when necessary with tap water and sprayed periodically with diazinon EC-25 (2.5 ml/liter) to control insect pests. The greenhouse temperature range was maintained at 18-28°C during the 16 week growth period.

**Inoculations.** Colonies of bacteria on PCA (48h) were suspended in SDW and diluted to about  $10^7$  colony forming units (cfu)/ml. Plants were inoculated by two techniques. For the first, iris leaves or rhizomes were swabbed with 1% sodium hypochlorite and injected with a total of 1.0 ml of the bacterial suspension through five syringe punctures. Six iris plants were inoculated with each strain of bacteria or SDW as a control.

For the second inoculation method, sprouting rhizomes were wounded by removing a tangential slice with a knife through the leaf fan base and into the rhizome. Plants were sprayed immediately with bacteria suspended in SDW or in sodium hypochlorite solutions of 0.2, 2.0 or 20mg elemental chlorine (Cl)/liter/ using a hand sprayer.

Inoculated plants were placed in clear plastic boxes (open at the bottom) on a wire greenhouse bench and misted with water for 15 sec every 15 min. After 24-26 h, the mist frequency was reduced to 8 h/day. Observations for disease development were made daily.

**Detection of soft-rotting bacteria in irrigation water.** Water collected in sterile 3.8 liter polyethylene containers from a creek, a pond and a well at the iris rhizome production facility was stored at 5°C until assayed. The well water was collected from a flowing tap. Creek and pond water was sampled well away from the sides and bottoms of these water sources.

Seventy ml of water were centrifuged at  $5,000 \times g$  for 15 min. The supernatant was discarded and the pellet was resuspended in Meneley and Stanghellini's enrichment broth (12). After anaerobic incubation (GasPac®, BBL) for 48-72 h, the enrichment broth was centrifuged, the supernatant discarded and the pellet streaked on CVP and incubated aerobically. All Gram-negative, pectolytic, facultative anaerobic bacteria capa-

ble of rotting carrot root (*Daucus carota* L.) and/or potato tissue were considered to be *Erwinia* spp.

**Effect of chlorination on the survival of bacteria in water.** Soft-rotting pathogens or irises were suspended in filter-sterilized (to pass 0.22  $\mu$  filter), pond water, creek water, well water, or SDW and exposed to various concentrations of sodium hypochlorite for 1 min. The titer of viable cfu remaining was determined by trapping cells on 0.45  $\mu$  pore-size nitrocellulose membranes (Millipore Corp., Bedford, MA 01730) and rinsing them with SDW. The membranes were placed on PCA and the colonies that developed were counted after incubation at 30°C for 24-48 h.

## RESULTS

Pectolytic bacteria were isolated on CVP medium from rotting field-grown (FG) iris from near Fishersville and from rotting greenhouse-grown (GHG) garden slug-infested iris cultured at Virginia Polytechnic Institute and State University. The rotting of the GHG rhizomes was centered on the slug wounds but rotting also progressed in streaks upward into the leaves as occurred with rotted FG iris. The bacteria isolated

**Table 2.** Comparison of physiological and biochemical properties of authentic *Erwinia carotovora* subsp. *carotovora* (Ecc) and *E. chrysanthemi* (Ech) strains with those strains isolated from rotting field-grown (FG), greenhouse-grown (GHG) iris, and strains reisolated (RI) from iris inoculated with FG strains of Ech

Test	Authentic Strains		Strains from rotted iris		
	Ecc (3 strains)	Ech (2 strains)	FG (3 strains)	GHG (3 strains)	RI (6 strains)
Production of acid from					
Trehalose	2 <sup>x</sup>	0	0	2	0
Mannitol	3	2	3	3	6
Inositol	3	2	3	3	6
Esculin	3	2	3	3	6
Maltose	0	0	0	2	0
Raffinose	3	2 <sup>y</sup>	3	2	6 <sup>y</sup>
Sensitivity to 15 $\mu$ g					
erythromycin	0	2	3 <sup>z</sup>	0	6 <sup>z</sup>
Phosphatase production	0	2	3	0	6
Nitrate reduction	3	2	3	3	6
Growth in 5% NaCl	3	0	0	3	0
Pectate hydrolysis	3	2	3	3	6
Cytochrome oxidase					
production	0	0	0	0	0
Fluorescence	0	0	0	0	0
Growth at 37°C	3	2	3	3	6

<sup>x</sup> Figures refer to the number of strains with positive reaction to specific tests

<sup>y</sup> One strain was weakly positive

<sup>z</sup> One strain was intermediate in sensitivity

from both FG and GHG iris were Gram-negative and peritrichously flagellated.

The bacteria were characterized biochemically and physiologically as described in Table 2. The strains isolated from rotting iris segregated into two groups similar to the two groups formed by authentic strains of *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*. The three isolates from FG iris are identified here as *E. chrysanthemi* and the three from the slug-infested GHG plants as *E. carotovora* subsp. *carotovora*.

Inoculations of iris plants (cv. 'Jesse Viette') with purified and characterized strains of bacteria indicated that symptom development by *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* were very similar; however, symptoms developed more slowly in *E. carotovora* subsp. *carotovora*-inoculated plants (3). Symptoms developed on plants inoculated with either pathogen within 96 h.

Strains of *E. chrysanthemi* were re-isolated and recharacterized (Table 2) from rotted artificially-inoculated GHG iris. These strains showed the same biochemical and physiological profiles as the parental strains indicating that Koch's postulates for pathogenicity had been fulfilled.

Isolation of pectolytic *Erwinia* spp. from the water of a creek and a spring-fed pond, but not a well, used for irrigation indicated that the numbers of pectolytic erwiniae were extremely low (< 1cfu/ml) at the time of observation several weeks after the epidemic of iris soft rot.

**Table 3.** Percentage of *Erwinia chrysanthemi* strain L-435 and *Erwinia carotovora* subsp. *carotovora* strain L-442 colony forming units (cfu) surviving exposure for one minute to sodium hypochlorite in water

Ppm of elemental chlorine	% of cfu surviving <sup>z</sup>	
	L-435	L-442
0.5	>99.9	>99.9
1.0	1.8	3.6
2.0	1.5	0.9
5.0	1.3	<0.002
10.0	<0.002	<0.002

<sup>z</sup> cfu/ml = L-435,  $1.2 \times 10^9$ . L-442  $1.4 \times 10^9$

In the laboratory, pectolytic bacteria, suspended in SDW, were reduced in viability by chlorination with sodium hypochlorite (Table 3). This reduction varied with the number of cfu suspended in the water. For instance, when  $1.2 \times 10^9$  cfu/ml of *E. chrysanthemi* strain L-435 were suspended for one minute in water containing 0.5 mg Cl (in sodium hypochlorite)/liter (or 0.5 ppm Cl), no reduction of viability was ob-

served. However, at 10 mg Cl/liter (1 ppm), viability was reduced 98%, and it was reduced more than 99% at 10 mg Cl/liter (10 ppm). In contrast, when only  $1.8 \times 10^6$  cfu were suspended in water, 0.2 mg Cl/liter (0.2 ppm) reduced viability 98%.

The source of irrigation water also affected the efficiency of chlorination for reducing the survival of bacteria (Table 4). Well water and SDW were no different in their effect on the survival of bacteria after chlorination (data not shown). However, pond and creek water increased the survival of pathogenic bacteria compared to well water. At collection, creek water was visibly turbid (0.05-0.15 OD at 550 nm), the pond water was clear and light tan in color, and well water was clear and colorless.

**Table 4.** Percentage of *Erwinia chrysanthemi* strain L-436 and *Erwinia carotovora* subsp. *carotovora* strain L-442 colony forming units (cfu) surviving exposure for one minute to sodium hypochlorite in filter sterilized water from various irrigation water sources

Water Source	% cfu/ppm of elemental chlorine			
	1	2	5	10
<i>Erwinia chrysanthemi</i> strain L-436				
Well	0.01	<0.002	<0.002	<0.002
Pond	90.0	38.5	1.2	0.04
Creek	99.6	45.0	5.8	0.58
<i>Erwinia carotovora</i> subsp. <i>carotovora</i> strain L-442				
Well	0.03	<0.002	<0.002	<0.002
Pond	12.8	27.8	24.0	0.19
Creek	96.4	30.0	34.3	0.01

In two greenhouse trials, chlorination with up to 20mg Cl/liter (20ppm Cl) was effective for reducing the incidence of iris rot on sprouting iris rhizomes (Table 5). In this test, bacteria

**Table 5.** Incidence of iris rhizome wounds rotted by *Erwinia chrysanthemi* strain L-436 or *Erwinia carotovora* subsp. *carotovora* strain L-442 treated for one minute with sodium hypochlorite in water

Ppm of elemental chlorine (Cl)	% Wounds rotted <sup>y</sup>			
	L-436 <sup>z</sup>		L-442	
	1	2	1	2
Not inoculated	0	0	0	6.2
Inoculated				
Cl treated	0.2	20.0	68.8	40.0
	2.0	0	25.0	30.0
	20.0	0	0	6.2
Not treated		30.0	81.2	40.0
				43.8

<sup>y</sup> % of 10 wounds (1) and 16 wounds (2)

<sup>z</sup> cfu/ml = L-436,  $3.5 \times 10^7$ , L-442,  $2.4 \times 10^7$

were suspended in SDW to simulate contaminated irrigation water and then exposed for one minute to various concentrations of sodium hypochlorite. The freshly wounded plants, to



simulate pest or cultural wounding, were sprayed immediately with the chlorinated suspensions.

No phytotoxicity was observed on the treated irises. However, more extensive tests with long-term repeated irrigation will be necessary before any firm conclusion about chlorine toxicity for iris can be reached.

## DISCUSSION

Although *E. carotovora* subsp. *carotovora* has been reported as the pathogen causing iris soft-rot, this study indicated that *E. chrysanthemi* also causes an iris soft rot with similar or identical symptoms. *Erwinia chrysanthemi* strains isolated from diseased field-grown irises were characterized biochemically and physiologically, inoculated into healthy plants, found to reproduce the symptoms of iris soft rot, and were re-isolated and re-characterized as being similar or identical to the parental inoculant strains. This confirms, by Koch's postulates, for the first time that *E. chrysanthemi* is also a pathogen of *I. germanica*. Although other workers, including W.H. Burkholder (Table 1) and M. Goto (5,6) have isolated *E. chrysanthemi* from rotting iris tissues, none have reported fulfilling Koch's postulates.

Another interesting feature of this iris soft rot epidemic was that the highest symptom incidence was correlated with overhead irrigation rather than iris borer damage. Since Bald suggested irrigation may help spread iris soft rot (2) and Kameron found that unwounded iris leaf fans could become infected and diseased after exposure to aqueous suspensions of bacteria (10), the irrigation water sources at the site of this epidemic were assayed for the presence of soft-rotting bacteria. Significantly, pectolytic *Erwinia* spp were recovered from the spring-fed pond and creek, but not the well. The populations of bacteria detected were too low to establish these irrigation reservoirs as sources of the pathogenic inoculum. However, both the pond, lined with succulent weeds, and the stream, passing through a cow pasture and an alfalfa field, could be contaminated occasionally with high populations of pectolytic *Erwinia* from decomposing submerged plant tissue. Similar conditions have been reported for *E. chrysanthemi* dissemination by water (8). The iris grower has discontinued use of the creek water and reports that iris soft rot was not a problem during the 1981 growing season.

Garden slugs apparently may act as vectors or provide wounds for bacterial penetration and colonization of iris rhizomes. The only bacterium isolated from the slug-damaged

and rotted plants was *E. carotovora* subsp. *carotovora*. This reconfirms that this bacterium is also a soft-rotting pathogen of iris.

Perhaps, under optimal conditions for disease development, both of these pathogens are able to penetrate unwounded plants. This hypothesis is supported by Kamerman's observation of *E. carotovora* subsp. *carotovora* penetration into unwounded iris (10), and Hartman's evidence for penetration by *E. chrysanthemi* into unwounded maize plants (7). Although iris borers were not associated with disease development during field observations of this epidemic of iris soft rot, damage by other unrecognized organisms (insects, nematodes or slugs) or cultural practices may have provided wounds for the pathogen (3).

Chlorination of water was effective for reducing the populations of pathogenic bacteria surviving in water. However, increased numbers of suspended bacteria and water source may reduce the effectiveness of chlorination (Reviewed in ref. 4). Although both the pond and creek water were filtered to remove indigenous bacteria prior to these chlorination studies, dissolved and suspended inorganic and organic materials may have bound chlorine, thus reducing the actual concentration of chlorine available for chlorination. In greenhouse tests, chlorination was effective for reducing the incidence of iris soft rot caused by both *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*.

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## **INFLUENCE OF SOIL FUNGICIDES ON PRODUCTION EFFICIENCY OF *PEPEROMIA GRISEO ARGENTEA* 'BLACKIE'**

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Soil-borne diseases are important constraints to efficient production of many types of greenhouse crops. Direct plant losses and delayed growth of crops affected with various types of root and stem rotting diseases contribute to higher production costs, unpredictable growth, and reductions in plant quality at the time of sale. Familiarity and use of sanitary growing practices advocated by Baker, et al. (1) are necessary for control of soil-borne diseases. The growing medium and container, the plant used for production and cultural operations are potential avenues of entry for soil-borne pathogens into the crop production cycle. Sanitary production practices can be effectively used to eliminate soil-borne pathogens from greenhouse production programs provided the procedures are uniformly adopted for all facets of the growing operation.

### SANITATION AND THE GROWING MEDIUM

The growing medium is an important and common source of soil-borne pathogens for greenhouse crops. Use of a wide