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Increased incidence of *Erwinia* soft-rot on calla lilies in the presence of phosphorous

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Abstract

Erwinia soft-rot is an important disease of many ornamental potted crops and is one of the most limiting factors in greenhouse calla lily (*Zantedeschia* spp.) production. Experiments were conducted to test the effect of phosphorous added to soil-less mixes or to nutrient solutions used for irrigation on soft-rot caused by *Erwinia carotovora* subsp. *carotovora* (Ecc). Soft-rot incidence increased to 51% when soil-less mix was amended with superphosphate in comparison to regular soil-less mix (no superphosphate added) (31%). In contrast, addition of phosphorous in the nutrient solution met the phosphorous needs of the plant without enhancing soft-rot. Plant height, fresh mass, and number of flowers per plant were greater in calla lilies irrigated with nutrient solution containing phosphorous than no phosphorous treatments. Similar results were obtained in tests conducted in a commercial greenhouse with larger sample size. No statistical differences were found between tubers sprayed with water (control) or with a 0.5 mM solution of KH_2PO_4 in laboratory experiments to determine the effect of phosphorous on tuber root development. In other experiments, tubers were sprayed with either water, a bacterial cell suspension 1×10^2 cfu ml^{-1}, a solution of 0.5 mM KH_2PO_4, or a suspension of bacteria in KH_2PO_4. The results from these tests showed a significant increase of soft-rot development in tubers treated with the suspension of Ecc prepared in a solution of KH_2PO_4 relative to other treatments. Further laboratory tests indicated that enzymatic activity (polygalacturonase and pectate lyase) of Ecc increased when grown in the presence of phosphorous. These experiments suggest that increased soft-rot in the presence of phosphorous is due to increased virulence of Ecc.

Introduction

Soft-rots caused by *Erwinia carotovora* subsp. *carotovora* (Ecc) are responsible for considerable losses in the floriculture industry. In the Niagara Region (Ontario, Canada) yearly economic losses of up to $1 million (US) from soft-rot of calla lily, cyclamen, and poinsettia, are common. Soft-rot on calla lilies (*Zantedeschia* spp.), which is considered the most susceptible of these crops, accounts for half of these losses. The primary infection of calla lily tubers occurs in field production areas. The tissue of the tuber and roots become macerated after infected bulbs are forced in the greenhouse.

Diseases caused by soft-rot erwinia are the manifestation of the activity of a number of plant cell wall and membrane degrading enzymes produced by these bacteria (Barras et al., 1994). Ecc and other

members of this group excrete pectinases, cellulases, proteases, phospholipases, and xylanases which have the potential to degrade plant cell wall and membrane components (Collmer and Keen, 1986; Kotoujansky, 1987). Pectinases play an important role in pathogenesis as demonstrated by genetic and biochemical studies (Roberts et al., 1986; Payne et al., 1987; Ried and Collmer, 1988; Barras et al., 1994). Mutants deficient in the production or excretion of one or more of these pectic enzymes showed decreased pathogenicity while addition of genes encoding certain pectinases enabled *Escherichia coli* to macerate plant tissue. In addition, purified pectinases from soft-rot erwinia macerated plant tissue (Mount et al., 1970; Basham and Bateman, 1975; Stephens and Wood, 1975). Ecc and other soft-rot erwinia produce a number of pectinases including pectin lyase, pectate lyase (PL), pectin methylesterase, and polygalacturonase (PG). Multiple isozymes of certain of these enzymes are produced by various strains (Barras et al., 1994).

After calla lily tubers are dug in the field and cured, calla lily tubers are usually sprayed with a mixture of compounds to prevent diseases and provide optimal conditions for plant growth and flower production during forcing. Copper-based compounds are currently used to reduce losses due to Ecc and other soft-rot erwinia (Peter Beckman, Golden State Bulb Growers, CA, pers. comm.). The authors observed that phosphorous had an adverse effect on disease in preliminary experiments where copper-based compounds were used for the control of Ecc in closed sub-irrigation systems. In addition, significant differences in disease incidence were observed on the control treatments (no copper added) between plants grown with and without phosphorous. The objectives of the present research are to (1) establish whether phosphorous enhances soft-rot disease incidence, and (2) determine whether phosphorous increases tuber susceptibility or enhances virulence of Ecc.

Materials and methods

Soft-rot incidence in calla lilies planted in soil-less mix with or without phosphorous and irrigated with or without phosphorous

Untreated commercial-grade (4.5–5.5-cm diameter) calla lily tubers (*Z. elliottiana*, cv. 'Hybrid Yellow'), were forced in the greenhouse for 10 weeks in the summer of 1999. Tubers were sprayed with Promalin (1.8% benzyladenine w/w and 1.8% GA_{4+7} (Gibberellic acid 4 and Gibberellic acid 7), Abbott Laboratories, N. Chicago, IL, USA) at 100 mg l^{-1} GA_{4+7} and Silwet L-77 (Helena Chemicals Co. Memphis, TN, USA) at 0.13 ml l^{-1} until run-off 3 days prior to planting in 11.5-cm diameter plastic pots. A soil-less mix (60% peatmoss, 20% vermiculite, and 20% styrofoam; v/v) amended with 900 g limestone and 900 g gypsum per m^3 was used for planting. Soil-less mix was used as described above (control) or was amended with 1.5 kg of superphosphate (0–20–0) per cubic metre. Plants were maintained at 22/20 °C (day/night) and sub-irrigated in a closed recirculating irrigation system. Two nutrient solutions were used for irrigation: without phosphorous (mmole l^{-1}: 14 NO_3^-, 1.6 NH_4^+, 5 K^+, 5.0 Ca^{2+}, 1.8 Mg^{2+}, 1.8 SO_4^{2-}, 0.025 Fe^{2+}, 0.005 Mn^{2+}, 0.0025 Zn^{2+}, 0.020 B, 0.0008 Cu^{2+}, 0.0008 MoO_4^{2-}) or with phosphorous (mmole l^{-1}: 14 NO_3^-, 1.6 NH_4^+, 1.5 $H_2PO_4^-$, 5 K^+, 5.0 Ca^{2+}, 1.8 Mg^{2+}, 1.05 SO_4^{2-}, 0.025 Fe^{2+}, 0.005 Mn^{2+}, 0.0025 Zn^{2+}, 0.020 B, 0.0008 Cu^{2+}, 0.0008 MoO_4^{2-}). Both nutrient solutions had an electrical conductivity (EC) of 2.3 mS cm^{-1}. No growth retardant treatments were applied. Pots were placed on six troughs, with each trough having its own fertilizer tank and pumping system for a recirculating sub-irrigation. Three troughs were irrigated with the nutrient solution containing phosphorous and three troughs without phosphorous. Each trough contained pots with plants grown in soil-less mix either with or without superphosphate. There were three replicates per treatment and 20 plants per replicate. Diseased plants were removed from the troughs when plants showed soft-rot symptoms and their numbers recorded. After 10 weeks of forcing, the following parameters were measured on the remaining plants: plant height (from edge of pot to tip of tallest leaf), number of flowers, and fresh mass (weight of foliage above soil line). The experiment was performed twice with the second planting started 1 week later.

Soft-rot incidence in calla lilies planted in soil-less mix with or without phosphorous in a commercial operation

This experiment was performed in the summer of 2000 in a commercial greenhouse (Niagara Under Glass Inc.) in the Niagara Region, Ontario, Canada. Treatments consisted of tubers planted in soil-less mix with or without 1.5 kg of superphosphate per cubic metre. Each

treatment had 2400 plants divided into six replicates, each replicate (400 plants) was placed in an individual bench (container) with sub-irrigation. The nutrient solution contained phosphorous (see above for formula of nutrient solution). The experiment was carried out for a 10-week period. The number of diseased plants was recorded on a weekly basis and plants were removed from containers according to commercial practices. At the end of the experiment, the following parameters were measured on 10 plants per treatment of the six replicates; plant height, number of flowers per plant, and fresh mass of the plant. The experiment was conducted once.

Bacterial isolate

A culture of Ecc was obtained from diseased calla lilies grown in a local greenhouse. The tubers originated from a field in California suspected of being naturally infested with Ecc. The isolate was identified as Ecc by M. Sabourin at the Pest Diagnostic Clinic, Guelph, ON, using the GN microplate system (Biolog Inc., Hayward, CA) (Jones et al., 1993). Ecc was cultured on nutrient broth (Difco Labs, Detroit, MI, USA) for 24 h with constant shaking at 150 rpm. The bacterial concentration was adjusted using a spectrophotometer (Beckman DU®640, Fullerton, CA) to a final absorbance of 0.14 (wavelength of 650 μm). At this absorbance, the concentration of bacterial cells was determined to be $\sim 6 \times 10^7$ colony-forming units (cfu) ml^{-1} by dilution-plating (Dhingra and Sinclair, 1995). Sufficient bacterial suspension was added to each tank to obtain a final concentration of 1×10^2 cfu ml^{-1}.

Effect of phosphorous on root development

Tubers were spray-treated with 5 ml of either sterile water (control) or a 0.5 mM of KH_2PO_4 solution per tuber. Individual tubers were then placed in a closed plastic container and incubated for 14 days at 22 °C in the dark. The number of roots per tuber was determined daily. There were 25 tubers per treatment and the experiment was performed twice.

Tuber decay from Ecc in the presence or absence of phosphorous

Tubers were spray-treated with 5 ml of either sterile water (control), a suspension of Ecc (1×10^2 cfu ml^{-1}) in sterile water, a 0.5 mM of KH_2PO_4 solution, or a suspension of Ecc (1×10^2 cfu ml^{-1}) in 0.5 mM of KH_2PO_4. Tubers were incubated for 14 days at 22 °C in the dark. Daily observations were made and tubers were rated for the presence or absence of macerated tissue. Each treatment consisted of 25 tubers and the experiment was performed twice.

Enzymatic assays

Culture filtrate was prepared by growing Ecc in minimal salts liquid medium (LSM) containing ($g\,l^{-1}$): $MgSO_4 \cdot 7H_2O$ (0.2); KCl (0.2); NH_4NO_3 (1.0); $FeSO_4 \cdot 7H_2O$ (0.002); $ZnSO_4 \cdot 7H_2O$ (0.002); $MnCl \cdot 4H_2O$ (0.002) (Ridout et al., 1986) plus 0.5% sodium polypectate (Sigma, Chemical Company, St Louis, MO, USA) with or without KH_2PO_4 (0.5 mM) at 26 °C and 150 rpm for 24 h (Lab-Line Instruments, Inc., Melrose Park, IL, USA) and continuous fluorescent light. To determine the effect that treatments had on bacterial population after incubation, 100 μl of the bacterial suspension were plated in a modified agar selective for *Erwinia* spp. (Miller and Schroth, 1972). After incubation, bacteria were removed by filtration (0.2 μm) and enzyme activity was determined on the filtrate. For PG activity, culture filtrate was mixed with 0.1 M sodium acetate buffer, pH 5.3, 10 mM EDTA, 0.1% (w/v) polygalacturonic acid and incubated at 30 °C. Reducing sugars released due to PG activity were detected by the method of Somogyi (1952) with D-galacturonic acid as standard. One unit of PG activity was defined as the release of 1 μg of D-galacturonic acid per hour per milliliter of filtrate. PL activity was determined by the method of Collmer et al. (1988). Culture filtrate was mixed with 0.1 M Tris(hydroxymethyl) aminomethane (Tris)–HCl buffer, pH 8.5, 1.5 mM calcium chloride, and 0.1% (w/v) polygalacturonic acid. One unit of PL activity was defined as the increase of 1 unit of absorbance at 232 nm min^{-1} ml^{-1} of filtrate.

Statistical analysis

Statistical analysis were conducted using the Statistical Analysis System (SAS Institute, Cary, NC) using the PROC GLM procedure to determine the significance of the effect of all factors. Differences among treatments when significantly different based on the PROC GLM procedure were identified using either the Duncan's

multiple range test or a least significant differencess (LSD) test.

Results

Soft-rot incidence in calla lilies planted in soil-less mix with or without phosphorous and irrigated with or without phosphorous

More plants died when tuber were planted in soil-less mix amended with superphosphate than without phosphorous ($P \leq 0.05$) (Table 1). No effect on mortality was observed when phosphorous was added to the nutrient solution and there was no significant interaction in any parameter measured between the two treatments (Table 1). Mortality of plants grown in the soil-less mix amended with superphosphate was 51% while those grown without superphosphate was 31% (Table 2). Plants grown in the soil-less mix amended with superphosphate were taller, produced more flowers, and had larger fresh mass ($P \leq 0.05$) (Table 2). Although there was no effect of the presence of phosphorous in the nutrient solution on plant mortality; plant height, fresh mass, and the number of flowers per plant were greater in plants irrigated with the nutrient solution containing phosphorous ($P \leq 0.05$) (Table 2). Mortality caused by Ecc was confirmed by isolating the pathogen from diseased tissue of dead tubers.

Soft-rot incidence in calla lilies planted in soil-less mix with or without phosphorous in a commercial operation

Mortality trends were similar for experiments in the research greenhouses as in a commercial greenhouse trial with a large sample size (2400 plants per treatment). Fifty-one percent of tubers died when planted in the soil-less mix with superphosphate compared with 43% when superphosphate was absent. However, this difference was not statistically significant ($P > 0.05$). In these tests, there were no differences in the quality of the plants between tubers planted in soil-less mix with or without superphosphate (Table 3). As in the previous experiments, Ecc was confirmed as the causal agent of soft-rot of tubers by isolating the pathogen from diseased tissue.

Effect of phosphorous on the root development of the tubers

Most roots started to grow at the base of the axillary shoot around day 5 or 6 after the initiation of the experiments. After 14 days, the average number of roots per tuber was 22 ± 20.5 for the control (−P) and 29 ± 26.5 in tubers sprayed with a solution of

Table 2. Percent plant mortality, plant height, plant fresh mass, and no. of flowers of calla lily ('Hybrid Yellow') plants grown with and without phosphorous added to soil-less mix or to nutrient solution[a]

Phosphorous treatment		Percent plant mortality	Plant height (cm)	Fresh mass (g)	No. of flowers per plant
Soil	Nutrient solution				
P+	P+	49 a	44.2 a	79.1 a	1.4 a
P+	P−	53 a	41.4 b	69.4 b	1.1 b
P−	P+	36 b	39.9 b	62.7 c	1.2 ab
P−	P−	25 b	37.4 c	55.0 d	0.9 b

[a]Values followed by the same letter within a column are not statistically different at the 0.05 level of significance according to a Duncan multiple range test.

Table 1. Analysis of variance on the effect of phosphorous in soil-less mix or in nutrient solution on plant mortality, plant growth, and flower production[a] of calla lily ('Hybrid Yellow')

Phosphorous treatment	Mean square			
	Percent plant mortality	Plant height (cm)	Fresh mass (g)	No. of flowers per plant
Source				
Soil	7692***	3546***	82 803***	9.1**
Nutrient solution	233	1447***	37 888***	12.7**
Soil × nutrient solution	912	4	1206	0.01

[a]Significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 3. Effect of phosphorous on the incidence of soft-rot in calla lilies ('Hybrid Yellow') (percent mortality), plant growth and flower production in an experiment conducted in a commercial operation[a]

Phosphorous	Percent mortality	Plant height (cm)	Fresh mass (g)	No. of flowers per plant
P+	51 a	40.0 a	74.5 a	1.2 a
P−	43 a	40.3 a	71.3 a	1.4 a
LSD	9.8	3.5	6.1	0.5

[a]Means within a column followed by a different letter differed at the 0.05 level of significance according to a protected LSD test.

phosphorous (+P). This difference was not statistically significant ($P > 0.05$).

Tuber decay from Ecc in the presence or absence of phosphorous

Phosphorous had a significant effect on the level and speed at which tubers were decomposed by Ecc. The percent decayed tubers increased very rapidly during 2–5 days after tubers were sprayed with the combined solution of Ecc and phosphorous than the other three treatments (Figure 1). After day 5, maceration increased similarly in all treatments (slopes of all curves were similar). There were no significant differences among the other three treatments, although all three treatments showed a linear increase in decay over time.

Enzymatic activity of Ecc in the presence and absence of phosphorous

Polygalacturonase and PL activities were detected in culture filtrates from Ecc grown in the presence and absence of phosphorous (Table 4). Significantly greater PG (3.75 times) and PL (2.75 times) activities were detected in the presence of phosphorous on a per milliliter culture filtrate basis than without phosphorous. Populations of Ecc grown in LSM amended with phosphorous were approximately double to those in LSM without phosphorous but this difference was not statistically significant. On a per cfu basis, PG activity was approximately double when cultures were grown on LSM containing phosphorous (1.7×10^{-7} vs. 0.9×10^{-7} units cfu^{-1}). PL activity, on a per cfu basis, was less than double in the presence of phosphorous in the solution (1.8×10^{-9} vs. 1.3×10^{-9} units cfu^{-1}, Table 4).

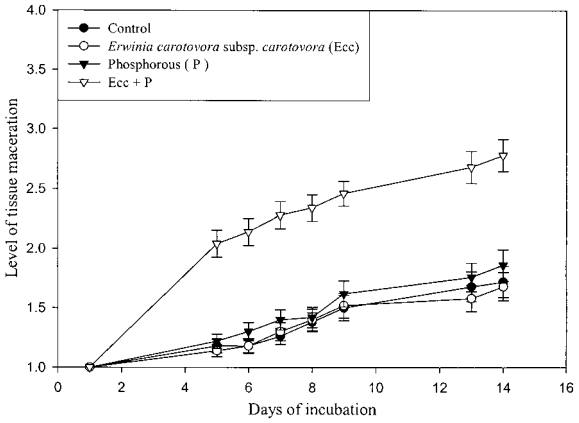

Figure 1. Decay of calla lily ('Hybrid Yellow') tubers sprayed with either sterile water, a suspension of Ecc (1×10^2 cfu ml^{-1}) in sterile water, a 0.5 mM of KH_2PO_4 solution, or a suspension of Ecc (1×10^2 cfu ml^{-1}) in 0.5 mM of KH_2PO_4 and incubated for 14 days in a plastic container at 22 °C in the dark. Tubers were rated daily using the following scale: 1 = healthy, 2 ≤ 25% of tuber tissue macerated or rotting smell, 3 = 50% of tuber tissue macerated, 4 = tuber tissue completely macerated.

Table 4. Growth and enzymatic activity of Ecc in a minimal LSM with or without mono-potassium phosphate (0.5 mM, KH_2PO_4)[a]

Phosphorous treatment	Colony-forming units per milliliter	PG activity[b]	PL activity[c]
$KH_2PO_4^+$	3.6×10^9 a	614.4 a	6.48 a
$KH_2PO_4^-$	1.8×10^9 a	164.4 b	2.33 b
LSD	2.7×10^9	152.8	3.11

[a]Means within a columns followed by a different letter differed at the 0.05 level of significance according to a protected LSD test.
[b]Units. A unit is defined as an increased of 1 μg of D-galacturonic acid per milliliter of filtrate per hour.
[c]Units. A unit is defined as an increase in 1.0 in optical density at 232 nm per milliliter of filtrate per minute.

Discussion

There are conflicting reports in the literature on the effect of phosphorous on disease incidence caused by various plant pathogens. Some researchers have observed a decrease in disease incidence (Reuveni et al., 2000) while others have reported increased levels of disease in the presence of phosphorous (Van den Heuvel and Waterreus, 1985; Zimand et al., 1996). Our experiments established that superphosphate added to a soil-less mix increased the development of *Erwinia* soft-rot of tubers of calla lilies.

The negative impact of phosphorous was not as pronounced when phosphorous was added through the nutrient solution. For growers, this is an important finding, since the phosphorous requirements of the plant can be fulfilled through the nutrient solution without a significant increase in soft-rot disease incidence. Plant growth and flower production were significantly reduced when no phosphorous was provided as either a pre- or post-plant application. When phosphorous was provided via the irrigation system, plants were able to grow adequately and produce as many flowers as the healthy plants in soil-less mix amended with superphosphate.

The rapid decay of tubers when sprayed with an Ecc suspension prepared in a phosphorous solution was very dramatic compared to the other treatments tested. When a suspension of Ecc was applied without phosphorous, the level of soft-rot was similar to the water treatment (control). This is an important indication that phosphorous is enhancing the activity of this pathogen. The increased soft-rot incidence on calla lilies also correlated with increased pectolytic enzyme activity by Ecc. Both PG and PL activities increased in the presence of phosphate. In addition, there was no increased root formation in the presence of phosphorous. Increased root formation would lead to sequential wounding, increased susceptibility of calla lilies, and easier ingress into tuber tissue by Ecc through these wounds. Therefore, we conclude that the increased disease incidence in the presence of phosphorous is due to increased virulence of Ecc, rather than increased susceptibility of the tuber.

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