Effect of Leaf Wetness, Fertilizer Rate, Leaf Age, and Light Intensity Before Inoculation on Bacterial Leaf Spot of Chrysanthemum

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ABSTRACT

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Potted Iceberg chrysanthemum plants were grown at four controlled-release fertilizer rates and two light intensities. As fertilizer rate increased, the number of lesions caused by *Pseudomonas cichorii* increased. Levels of nitrogen and phosphorus were positively correlated with disease severity. Lesion diameter and lesion incidence were greater on detached leaves from plants grown under high light conditions. Excised leaves from the bottom one-third of the plant were less susceptible than leaves from the middle or top of the plant. The number of lesions that developed on plants misted before inoculation increased with the duration of the mist period.

Pseudomonas cichorii (Swingle) Stapp causes leaf spot, bud blight, and stem blight of *Chrysanthemum morifolium* Ramat. (10,14). The bacterium has an extensive host range (1,2,4,15,19,20). High moisture is required for disease to develop (11). Preinoculation factors suspected to influence susceptibility are fertilizer rate, light intensity, leaf age, and preinoculation moisture period. In the ornamental plant industry, fertilizer is commonly applied as a controlled-release formulation (5,6,18). The objective of this study was to determine the effects of mist period, rate of controlled-release fertilizer, and light intensity on the susceptibility of *C. morifolium* to *P. cichorii*.

MATERIALS AND METHODS

Five strains of *P. cichorii* were obtained from chrysanthemum stems and were stored for up to a year at room temperature in sterile tap water. Inoculum was produced on medium B of King et al (13) from cultures grown 48 hr at 28 C. Inoculum was prepared in 0.01 M $MgSO_4 \cdot 7H_2O$ solution and adjusted photometrically at a wavelength of 590 nm to 10^8 colony-forming units (cfu) per milliliter.

Influence of light intensity and fertilizer rate. Rooted Manatee Yellow Iceberg cuttings were planted in 15.25-cm-diameter pots (three cuttings per pot) that contained a mixture of Florida peat, sand, vermiculite, and perlite (5:3:3:1, v/v). Calcium carbonate, dolomitic lime, hydrated lime, super phosphate, and Micromax (Sierra Chemical Co., Milpitas, CA) were applied at 5.54, 5.54, 0.549, 1.39, and 0.83 kg/m^3, respectively. Osmocote (18-6-12) fertilizer (Sierra Chemical) was applied at the rate of 5.25, 10.5, 21, or 42 g/pot. The pots were placed on a greenhouse bench in a split-plot design. Each block consisted of two sets of four pots (one pot for each of the four fertilizer rates) placed at 975 μE m^{-2} s^{-1} (greenhouse light) or at 390 μE m^{-2} s^{-1} (60% shade). Each treatment consisted of five replicate pots. Plants were grown for 28 and 33 days before inoculation in experiments 1 and 2, respectively, then gently misted with a suspension of 10^8 cfu/ml of *P. cichorii* and placed in polyethylene bags for 72 hr at 28 C and 12 hr of light in a growth room. After 4 days, total lesions on each plant were counted, plant height and fresh weight were determined, and tissue samples were collected from recently matured leaves. The micro-Kjeldahl technique was used for nitrogen, flame spectrophotometry for potassium, and colorimetric determination for phosphorus.

Influence of light intensity and leaf age. Chrysanthemum plants were grown as described previously in 15.25-cm-diameter pots with 21 g of 18-6-12 fertilizer per pot. Eight pots each were held at 975 and 390 μE m^{-2} s^{-1}. After growing for 28 days, leaves were harvested separately from the top, middle, and bottom third of each plant and inoculated by the pinprick method (10) on nine sites per leaf. A sponge containing inoculum was placed on the abaxial surface while the pins were pressed through the adaxial surface. By gently pressing together the sponge and needles, inoculum was released from the sponge into the wound sites. Inoculated leaves were placed in a moist chamber and incubated 48 hr at 28 C. Percent lesion incidence (defined as the percentage of wound sites that developed lesions) and lesion development (diameter or longest length of the lesions) were recorded after 2 days. This experiment was repeated three times.

Influence of mist. Chrysanthemum cuttings, planted in 10-cm pots (one plant per pot), were grown for 3 wk in a greenhouse. Before inoculation, the plants were placed in a chamber and exposed for 0, 2, 4, 8, 16, or 24 hr to a fine mist generated by a cool-mist humidifier. Inoculation was achieved by spraying 10^8 cfu/ml on each plant to runoff. The plants were then placed in polyethylene bags for 72 hr at 28 C in a growth room. After the incubation period, lesions were counted. The experimental design was a randomized complete block with four replicates. The experiment was repeated three times.

RESULTS

Influence of light intensity and fertilizer rate. Disease severity increased significantly as fertilizer rate increased (Table 1). Although in experiment 1 more disease developed on plants grown under high light intensity, the difference was not statistically significant. Plants grown under high light intensity compared with low light intensity showed increased plant height and plant weight. Disease, height, and weight measurements of plants grown under high light intensity were significantly related to fertilizer rate by linear and/or quadratic equations, whereas plants grown under low light intensity generally were unaffected by fertilizer rate. The interaction between light intensity and fertilizer rate was therefore highly significant for disease severity, height, and weight. Nitrogen and phosphorus contents of foliage of plants grown under low light were positively and significantly correlated with disease occurrence and plant weight (Table 2). With plants grown under high

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Table 1. Effect of light intensity and Osmocote (18-6-12) fertilizer rate on development of leaf lesions on *Chrysanthemum morifolium* leaves inoculated with *Pseudomonas cichorii* and on weight, height, and foliar nutrient content

Fertilizer rate (g/15.25-cm pot)	Disease (no. lesions/plant)		Plant height (cm)		Plant weight (g)		Tissue analysis (mean % dry weight of leaf tissue)					
							Nitrogen		Phosphorus		Potassium	
	Low light[a]	High light	Low light	High light	Low light	High light	Low	High	Low	High	Low	High
					Test 1							
5.25	2.9	2.4	29.8	35.2	51.4	61.8	3.2	2.4	0.44	0.42	5.1	4.4
10.5	4.5	4.3	31.6	38.2	64.8	85.0	3.6	2.7	0.48	0.46	5.2	4.2
21.0	10.1	17.5	32.1	43.4	72.6	115.4	4.3	3.7	0.55	0.54	5.4	4.1
42.0	6.8	28.1	30.6	38.1	63.0	107.0	4.5	4.8	0.64	0.72	5.7	5.1
Significant effects[b]												
Linear	NS[c]	**[d]	NS	NS	NS	**	**	**	**	**	**	**
Quadratic	NS	*	NS	**	NS	**	**	**	**	**	*	**
					Test 2							
5.25	10.1	6.9	30.8	34.8	20.2	26.5	2.8	2.3	0.37	0.41	5.3	4.2
10.5	26.0	10.4	31.6	40.9	25.6	34.9	3.5	2.6	0.43	0.41	5.7	4.5
21.0	47.5	25.1	32.8	42.6	32.0	48.7	4.2	3.3	0.54	0.52	6.2	4.9
42.0	30.2	46.7	32.3	41.4	29.9	52.0	4.7	4.3	0.68	0.66	6.4	5.2
Significant effects												
Linear	NS	**	NS	*	**	**	**	**	**	**	NS	**
Quadratic	*	**	NS	**	**	**	**	**	**	**	NS	NS

	Main effects											
	Disease		Height		Weight		Nitrogen		Phosphorus		Potassium	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Light	NS	NS	**	**	**	**	**	**	NS	NS	**	**
Fertilizer rate	**	**	**	**	**	**	**	**	**	**	NS	NS
Light × fertilizer rate	**	**	**	**	**	**	**	NS	*	NS	NS	NS

[a] Low light is 390 $\mu E\ m^{-2}\ s^{-1}$, high light is 975 $\mu E\ m^{-2}\ s^{-1}$.
[b] Linear and quadratic regression analyses were run for each vertical row at each light level.
[c] NS = not significant at $P = 0.05$.
[d] * = Significant at $P = 0.05$, ** = significant at $P = 0.01$.

light intensity, there were positive correlations of nitrogen and phosphorus contents with height, weight, and number of lesions. Relationships between these variables and potassium content were inconsistent.

Influence of light intensity and leaf age. In experiments conducted at an intermediate fertilizer level (which is also the recommended rate) and at two light intensities, excised leaves from plants grown at the high light intensity developed more and larger lesions than did leaves from plants grown at the low light intensity (Table 3). Leaf age (or position) also affected lesion diameter and lesion incidence; the middle and top excised leaves were significantly more susceptible than the lower leaves.

Influence of mist. Mist before inoculation was not essential for disease development (Table 4); however, increased exposure to mist before inoculation significantly increased the number of lesions per plant.

Table 2. Correlation of plant weight, plant height, and number of lesions on leaves of *Chrysanthemum morifolium* caused by *Pseudomonas cichorii* with nitrogen, phosphorus, and potassium content of foliage

Light intensity ($\mu E\ m^{-2}\ s^{-1}$)	Parameter	Test 1			Test 2		
		Nitrogen	Phosphorus	Potassium	Nitrogen	Phosphorus	Potassium
Low (390)	Disease	0.431	0.412	0.377	0.612**	0.501*	0.301
	Weight	0.282	0.475*	−0.060	0.763**	0.862**	0.491*
	Height	0.039	0.232	−0.027	0.217	0.425	0.069
High (975)	Disease	0.708**[a]	0.732**	0.516*	0.760**	0.774**	0.427
	Weight	0.812**	0.841**	0.149	0.708**	0.853**	0.489
	Height	0.452*	0.561*	−0.124	0.595**	0.501*	0.263

[a] * = Significant at $P = 0.05$, ** = significant at $P = 0.01$.

DISCUSSION

Disease severity increased with an increase in fertilizer rate. In a previous study using coated fertilizers, postharvest deterioration of stems and leaves of *C. morifolium* increased as the fertilizer rate increased (9,18). Nitrogen, when applied at high levels, has been shown to reduce severity of several bacterial leaf spots (7,16,17). With *Philodendron selloum*, susceptibility to *Erwinia chrysanthemi* increased as nitrogen levels increased (8). Only at very high nitrogen levels was there a reduction in disease. Although rates of slow-release fertilizer rather than individual nutrients were varied, disease severity appeared to increase as nitrogen and phosphorus levels increased, since the percentage of phosphorus and nitrogen in leaves correlated with disease severity. The reason for this increased susceptibility to bacterial spot is unclear. Tissue nitrogen level has previously been proposed to be a factor that may affect susceptibility of chrysanthemum to *Botrytis cinerea* by making tissue more sensitive to the fungus (9). In our study, tissue nitrogen correlated with disease severity in plants grown under high light intensity; however, tissue nitrogen was significantly higher in leaf tissue grown under low light intensity. Since disease ratings were significantly higher on plants grown under high light intensity, factors more important than nitrogen are probably responsible for the disease reaction involving *P. cichorii* on *C. morifolium*.

Light intensity before inoculation appears to be relatively important in the development of bacterial spot of chrysanthemum. Lesion incidence was slightly greater, and lesion size was considerably greater on leaves of plants

Table 3. Effect of light intensity and leaf age on lesion incidence and lesion diameter when *Chrysanthemum morifolium* leaves were wound-inoculated with *Pseudomonas cichorii*

Leaf position	Lesion incidence[a] High light[b]	Lesion incidence[a] Low light	Lesion diameter (mm) High light	Lesion diameter (mm) Low light
		Test 1		
Top	99	85	6.9	3.9
Middle	94	83	6.4	3.4
Bottom	90	77	4.8	2.5
		Test 2		
Top	100	91	5.7	2.9
Middle	98	86	5.7	2.9
Bottom	97	72	4.0	5.0
		Significant effects		
	Test 1	Test 2	Test 1	Test 2
Light	*	*	*	*
Leaf position	*	*	*	*

[a]Lesion incidence is defined as the percentage of wound sites that developed lesions. Each value represents eight replicates of two leaves per replicate and nine inoculation sites per leaf.
[b]High light is 975 $\mu E\ m^{-2}\ s^{-1}$, low light is 390 $\mu E\ m^{-2}\ s^{-1}$. * = Significant at $P = 0.05$.

Table 4. Effect of duration of preinoculation mist on susceptibility of detached leaves of *Chrysanthemum morifolium* to *Pseudomonas cichorii*

Hr of mist	Lesion no. per plant Test 1	Test 2	Test 3
0	9.8	6.4	3.4
2	18.6	7.4	5.4
4	24.6	11.0	3.2
8	30.0	8.4	5.4
16	32.8	11.6	8.2
24	32.8	16.4	15.4
	Significant effects		
Linear	**[a]	**	**
Quadratic	**	**	**

[a]** = Significant at $P = 0.01$.

grown under high light intensity. Relatively little work has been reported on the effect of light intensity on plant susceptibility to bacterial pathogens. Gallegly and Walker (3) observed that susceptibility to bacterial wilt of tomato was greatest in plants grown under low preinoculation light intensity. Although plants grown in low light intensity were less susceptible to *P. cichorii*, the overall poor plant growth precludes the use of low light as a feasible means for reducing disease.

Considerably more leaf spots developed on plants that were misted before inoculation than on those not misted. This type of reaction is common with phytopathogenic bacteria (11). It was previously reported that a postinoculation wet period is essential for disease development (12); thus, reducing or preventing leaf wetness would be important for control of bacterial spot of chrysanthemum.

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