Influence of Moisture and Inoculum Concentration on Infection of *Philodendron selloum* by *Erwinia chrysanthemi*

R. A. HAYGOOD, Graduate Research Assistant, and D. L. STRIDER, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650

ABSTRACT

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Free moisture maintained on wounded or unwounded *Philodendron selloum* leaves 72 hr before inoculation with *Erwinia chrysanthemi* at 2×10^8 colony-forming units per milliliter had no effect on disease severity 5 days after inoculation. Differences in disease severity were significant between unwounded leaves misted for 0 and 24 hr after similar inoculation and between wounded leaves misted for 0, 6, and 24 hr after inoculation. At the inoculum concentration of 2×10^8 colony-forming units per milliliter, misting for longer than 24 hr after inoculation did not affect disease severity. Differences in disease severity were significant among wounded leaves inoculated with 2×10^8, 2×10^6, and 2×10^4 colony-forming units per milliliter. Differences on unwounded leaves were significant only between leaves inoculated with 2×10^8 colony-forming units per milliliter and those inoculated with the two lower concentrations. Longer misting times after inoculation were required before significant differences were detected on the wounded and unwounded leaves inoculated with the lower concentrations.

Additional key words: bacterial blight, foliar plants

Moisture and inoculum concentration are important factors in disease development when plants are artificially inoculated with pathogenic bacteria. Free moisture on leaves before inoculation often causes water congestion, facilitating the entrance of bacteria via stomata and wounds (3,7). Jones and Strider (6) reported significantly increased disease on zinnias misted 3 hr before inoculation with *Xanthomonas nigromaculans* f. sp. *zinniae*. Lucas and Grogan (7) found that cucumber plants develop more lesions when maintained with free water for 24 hr before inoculation with *Pseudomonas lachrymans*. High water content of tissues before inoculation favors infection of apple twigs by *Erwinia amylovora* (11) and cucumber and peas by *Pseudomonas* spp. (9). Johnson (5) showed that water-congested tobacco leaves are more susceptible than uncongested leaves when inoculated with *Pseudomonas tabaci*.

In studies with *E. chrysanthemi* on various plant species, leaves were misted 24 hr before inoculation and one to several days after inoculation (1,2,4,8,10). However, we are unaware of any studies that compared the influence of mist treatments before and after inoculation with *E. chrysanthemi*.

The influence of inoculum concentrations of *E. chrysanthemi* on *Philodendron selloum* leaves maintained with free moisture for 24 hr after inoculation at different temperatures has been reported (4). Our purpose was to determine the effect of free moisture on wounded and unwounded *P. selloum* leaves 0–72 hr before and after inoculation with *E. chrysanthemi* at 2×10^8 colony-forming units (CFU) per milliliter and to assess the influence of inoculum concentration on disease severity of wounded and unwounded leaves maintained with free moisture for 0–72 hr after inoculation.

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MATERIALS AND METHODS

Seedlings were donated by Speedling, Inc. (Sun City, FL 33586) and were grown in Metro-Mix 220 (W. R. Grace and Co., Cambridge, MA 02138) in 10-cm clay pots for 3 mo before the start of each experiment. N-P-K fertilizer (20-20-20) at 2.6 g/L of water was applied once a week and the plants were watered twice a day by overhead irrigation.

Plants were maintained in a mist chamber for 0, 24, 48, or 72 hr before and after inoculation with *E. chrysanthemi* at 2×10^8 CFU/ml in the first factorial experiment; plants were misted for 6, 12, 18, 24, 30, 36, 42, 48, and 72 hr after inoculation in the second experiment. Inoculum concentrations of 2×10^4, 2×10^6, and 2×10^8 and mist times of 0, 6, 24, and 72 hr after inoculation were used in the third experiment.

Three plants, each with two wounded and two unwounded leaves, were used per treatment. Two fully expanded leaves on each plant were wounded as described (4); the other leaves were not wounded. Young, expanding leaves were not studied because they are less susceptible to *E. chrysanthemi* (4). Treatments were replicated four times in the first experiment and three times in the second and third experiments. The first two experiments were repeated once.

E. chrysanthemi isolate Cu 242, obtained from *P. selloum* leaf tissue in Florida by J. F. Knauss (tissue obtained from R. S. Dickey, Cornell University), was used in all experiments. We prepared inoculum containing 2×10^8 CFU/ml as described previously (4) and applied it evenly to the upper and lower leaf surfaces to runoff by using an atomizer at 10 psi. The atomizer was held about 25 cm from leaves and all inoculations were made between 1000 and 1300 hours. Control plants were sprayed with distilled water. Leaves that were not maintained with free moisture before inoculation were misted a few minutes before inoculation so that inoculum dispersal would be similar for all treatments.

We built a mist chamber of clear plastic on top of a greenhouse bench to reduce the possible influence of light on disease severity. The mist chamber was vented to prevent heat buildup. Free moisture was maintained on the leaves in the mist chamber by intermittent misting with a Herrmidifier (Herrmidifier Co., Inc., Lancaster, PA 17604). The temperature inside the mist chamber and greenhouse was 20–30 C and the ambient relative humidity was 60–100%.

A seven-point rating scale was used as before (4) but was redefined based on the percentage of leaf surface covered with symptoms: 1 = 0%, no disease; 2 = less than 3%, slight disease; 3 = 3–10%, slight to moderate disease; 4 = 11–25%, moderate disease; 5 = 26–50%, severe disease; 6 = 51–75%, very severe disease; and 7 = symptoms covering more than 75% of leaf. Disease severity was rated 5 days after inoculation.

RESULTS

The first experiment yielded no significant interaction between mist treatments before or after inoculation. Disease severity ratings were not significantly different for wounded or

Table 1. Disease severity ratings of *Philodendron selloum* leaves maintained with free moisture after inoculation with *Erwinia chrysanthemi*[x]

Leaves	Misting after inoculation (hr)									
	0	6	12	18	24	30	36	42	48	72
Wounded	4.1 a[y]	5.0 b[z]	5.0 b	4.9 b	5.9 c	5.8 c	6.2 c	6.2 c	6.4 c	6.4 c
Unwounded	1.2 a	2.4 ab	2.4 ab	2.5 ab	3.6 b	3.8 b	3.7 b	3.7 b	3.8 b	3.7 b

[x] At 2×10^8 colony-forming units per milliliter.
[y] Average ratings of 18 leaves were based on a seven-point scale of progressive disease severity.
[z] Means within rows followed by the same letter are not significantly different ($P = 0.05$) as calculated by the method of least significant difference.

Table 2. Disease severity ratings of *Philodendron selloum* leaves maintained with free moisture after inoculation with three concentrations of *Erwinia chrysanthemi*

Inoculum concentration (CFU/ml)	Misting after inoculation (hr)									
	Wounded leaves					Unwounded leaves				
	0	6	24	72	Mean	0	6	24	72	Mean
2×10^4	1.8[y]	1.6	2.2	3.2	2.2 a[z]	1.0	1.0	1.0	1.6	1.2 a
2×10^6	2.8	2.9	3.6	4.4	3.4 b	1.1	1.2	1.5	1.8	1.4 a
2×10^8	3.1	4.2	5.1	5.2	4.4 c	2.3	3.7	3.7	4.4	3.8 b
Mean	2.6 a	2.9 a	3.6 b	4.3 c		1.4 a	2.0 b	2.1 b	2.6 c	

[y] Average ratings of 18 leaves 5 days after inoculation based on a seven-point scale of progressive disease severity.
[z] Means within columns or row followed by the same letter are not significantly different ($P = 0.05$) as calculated by the method of least significant difference.

unwounded leaves maintained with free moisture before inoculation with 2×10^8 CFU/ml. The mean ratings for wounded leaves misted for 0, 24, 48, and 72 hr before inoculation were 5.2, 5.2, 5.2, and 5.4, respectively; for unwounded leaves, the ratings were 3.0, 3.3, 3.2, and 2.6, respectively. Ratings were significantly different for wounded and unwounded leaves misted for 0 and 24 hr after inoculation at 2×10^8 CFU/ml but not leaves misted for 24 and 72 hr after inoculation. Mean ratings for wounded leaves misted for 0, 24, 48, and 72 hr after inoculation were 2.9, 5.8, 6.0, and 6.3, respectively; for unwounded leaves, the ratings were 1.9, 3.1, 3.5, and 3.6, respectively. The same trends were observed when the experiment was repeated.

Disease severity ratings were significantly different for wounded leaves misted for 0, 6, and 24 hr after inoculation at 2×10^8 CFU/ml (Table 1). Ratings were not significantly different for wounded leaves misted 6–18 or 24–72 hr after inoculation. Similarly, ratings did not differ for unwounded leaves misted 0–18 hr or 6–72 hr after inoculation. However, ratings were significantly different among unwounded leaves misted 0 and 24–72 hr after inoculation. The test was repeated with similar results.

No overall significant interaction was detected between misting after inoculation and inoculum concentration (Table 2). However, wounded leaves misted for 24 and 72 hr after inoculation at lower concentrations showed greater differences than those inoculated at 2×10^8 CFU/ml. Because the trend toward interaction was slight, we have interpreted the mean ratings given in Table 2.

On wounded leaves, disease severity differed at all inoculum concentrations. Disease severity was the same on wounded leaves misted for 0 and 6 hr after inoculation, but it was significantly different on leaves misted for 6, 24, and 72 hr after inoculation. As time after inoculation increased, disease severity ratings of wounded leaves inoculated at lower concentrations showed greater differences.

On unwounded leaves, disease severity was significantly different only between those inoculated at 2×10^8 CFU/ml and those inoculated at the two lower concentrations. Severity was significantly different among unwounded leaves misted for 0, 6, and 72 hr after inoculation, but not between leaves misted for 6 and 24 hr after inoculation.

DISCUSSION

Disease severity was not different on plants misted for up to 72 hr before inoculation. This result was not expected, because free moisture before inoculation often causes water congestion that predisposes leaves to bacterial plant pathogens (3,7). The *P. selloum* leaves did not appear water-soaked after misting for 72 hr, perhaps because of a physical barrier such as a thick, waxy cuticle. In the laboratory, detached *P. selloum* leaves are difficult to infiltrate in a vacuum chamber at 25 psi (*unpublished data*).

The free moisture remaining from inoculation with 2×10^8 CFU/ml was enough for infection to occur on wounded leaves. Misting the plants for 6 hr after inoculation significantly increased disease severity, but significant differences did not occur again until the leaves had been misted 24 hr.

Misting times longer than 24 hr had little influence on disease severity of wounded and unwounded leaves inoculated at 2×10^8 CFU/ml. However, severity was significantly different on wounded leaves misted for 24 and 72 hr after inoculation in the third experiment, probably because the inoculum concentration was lower; disease severity followed the same trend as in other experiments on wounded leaves inoculated at 2×10^8 CFU/ml.

Misting from 24 to 72 hr after inoculation influenced disease development more on wounded leaves inoculated with lower concentrations, possibly because of the rapidly increasing bacterial populations in a favorable environment. The influence of moisture on epiphytic populations is under investigation; current studies indicate that *E. chrysanthemi* can reside epiphytically on *P. selloum* leaves for more than 4 mo (*unpublished data*).

This study reinforces the need for growers of *P. selloum* to keep free moisture off leaf surfaces under growing and shipping conditions, since moisture on leaf surfaces for extended periods can greatly increase disease severity. Wounding of leaves should also be held to a minimum, because the free moisture remaining from inoculation was adequate for disease to develop.

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