Effects of Temperature and Leaf Wetness on Development of Bacterial Spot of Geraniums and Chrysanthemums Incited by *Pseudomonas cichorii*

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ABSTRACT

Jones, J. B., Raju, B. C., and Engelhard, A. W. 1984. Effects of temperature and leaf wetness on development of bacterial spot of geraniums and chrysanthemums incited by *Pseudomonas cichorii*. Plant Disease 68:248-251.

Florida Marble and Polaris chrysanthemum stems and leaves and Sprinter Scarlet geranium leaves were inoculated with *Pseudomonas cichorii*. The number and size of lesions of bacterial spot of chrysanthemum leaves and stems and geranium leaves increased when temperatures were increased from 16 to 28 C but were greatly inhibited at temperatures higher than 28 C. Leaf lesions continued to expand under high-moisture conditions in polyethylene bags but ceased to expand in a low-moisture environment (unbagged). Stem lesions reacted similarly to leaf lesions. However, stem lesions developed on inoculated plants placed in the low-moisture environment, whereas leaf lesions were completely inhibited on inoculated plants placed immediately in the low-moisture environment.

Pseudomonas cichorii (Swing.) Stapp is an extremely destructive pathogen with a wide host range that includes many ornamental (1,5,10,11) and vegetable crops (2,6,12–14). The bacterium causes a leaf spot on geranium (*Pelargonium* × *hortorum* L. H. Bailey) (5), whereas on chrysanthemum (*Chrysanthemum morifolium* Ramat.), it incites leaf lesions, bud blight (10), and stem necrosis (8).

The role of relative humidity in development of several bacteria-induced diseases is fairly well understood; greatest disease occurs in plants exposed to long periods of high relative humidity (4). Bolick (1) and McFadden (10) indicated that *P. cichorii* induced disease only during periods of high humidity, with disease incidence coinciding with heavy and frequent rains. Bolick (1) observed that disease spread ceased when plants were removed from a moist chamber. The disease also was associated with sprinkler-irrigated fields in California (6).

The importance of temperature in bacterial spot development on chrysanthemum and geranium needs clarification. Bolick (1) indicated that disease development was dependent on high temperatures (20–27 C); however, McFadden (10) did not overly emphasize temperature compared with moisture, assuming bacterial spot to be a wide-temperature disease because it occurred in southern Florida throughout the year.

The objectives of this study were to determine the effect of leaf wetness on disease development and to determine the role of temperature on infection and disease development.

MATERIALS AND METHODS

Inoculum production. *P. cichorii*

Florida Agricultural Experiment Stations Journal Series No. 4493.

Accepted for publication 9 September 1983.

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Table 1. Effect of temperature on lesion diameter and incidence of *Pseudomonas cichorii* on Sprinter Scarlet geranium leaves for three incubation periods

	Incubation period					
	24 Hr		48 Hr		72 Hr	
Temperature (C)	Lesion diameter[a] (mm)	Incidence[b] (%)	Lesion diameter (mm)	Incidence (%)	Lesion diameter (mm)	Incidence (%)
16	0.0	0.0	0.4	18.3	0.6	16.7
20	0.3	28.3	1.9	65.0	2.3	65.0
24	0.9	56.6	2.1	61.7	2.4	61.7
28	1.8	100.0	4.0	100.0	5.1	100.0
32	0.0	0.0	0.2	15.0	0.3	16.7
36	0.0	0.0	0.0	0.0	0.0	0.0
Significant effects						
Linear	*[c]	*	*	*	*	NS
Quadratic	*	*	*	*	*	*
R^2	0.52	0.46	0.47	0.64	0.46	0.48

[a] The average diameter of the leaf spot at three inoculation points on a leaf (for 15 leaves per temperature).

[b] Incidence is the percentage of inoculation points positive for disease development.

[c] * = Statistical significance at $P = 0.01$ and NS = not significant.

strain 5359 isolated from chrysanthemum was used in all tests. The strain was grown at 25 C on medium B of King et al (9) for 48 hr. For leaf inoculations, the bacterial growth was suspended in deionized water and adjusted to 10^8 colony-forming units per milliliter. For stem inoculations, the bacterial growth was removed directly from the petri dish so that a mass of bacterial growth was placed on the end of an insect pin. Techniques for leaf and stem inoculation were as described previously (8). For leaf inoculation, a small sterile sponge soaked with the inoculum was placed on the abaxial surface. This served as inoculum as pins were pushed through the leaf from the adaxial surface. After inoculation, plants were placed in clear polyethylene bags to maintain high-moisture conditions.

Plant material. Six-week-old geranium seedlings were transplanted in 4-in. pots and grown under greenhouse conditions 3–4 wk before inoculation. Rooted cuttings of Florida Marble and Polaris chrysanthemum were transplanted in 4-in. pots and grown 3–4 wk in a greenhouse at ambient temperatures ranging from 20 to 30 C.

Temperature studies. Polaris and Florida Marble chrysanthemum stems and leaves and Sprinter Scarlet geranium leaves were inoculated with *P. cichorii* strain 5359. Three of the most recently matured leaves were inoculated with three pinpricks per leaf. Stems were inoculated by pinpricking recently matured stem tissue and inserting the mass of bacteria. Inoculated plants were placed in clear polyethylene bags and set in growth chambers adjusted to 16, 20, 24, 28, 32, or 36 C and a 12-hr daily photoperiod. Four plants per temperature were used. Plants remained in polyethylene bags throughout the experiment. At various time intervals after inoculation, the plants were temporarily unbagged and lesion numbers and size were recorded.

Relative humidity studies. Polaris leaves and Florida Marble leaves and stems were inoculated with *P. cichorii* strain 5359 exactly as in the temperature studies section. After inoculation, the plants were placed at 28 C and subjected to high moisture (accomplished by placing in polyethylene bags), low moisture (unbagged), or high-moisture periods alternating with low-moisture periods (Figs. 1 and 2). Lesion length was determined at various intervals after inoculation. Each treatment was replicated four times.

RESULTS AND DISCUSSION

The optimum temperature for lesion expansion on chrysanthemum leaves and stems and geranium leaves was 28 C (Fig. 3, Table 1). No leaf infection occurred at 36 C with either host (Tables 1 and 2, Fig. 3). Lesion expansion generally increased as temperatures increased from 16 to 28

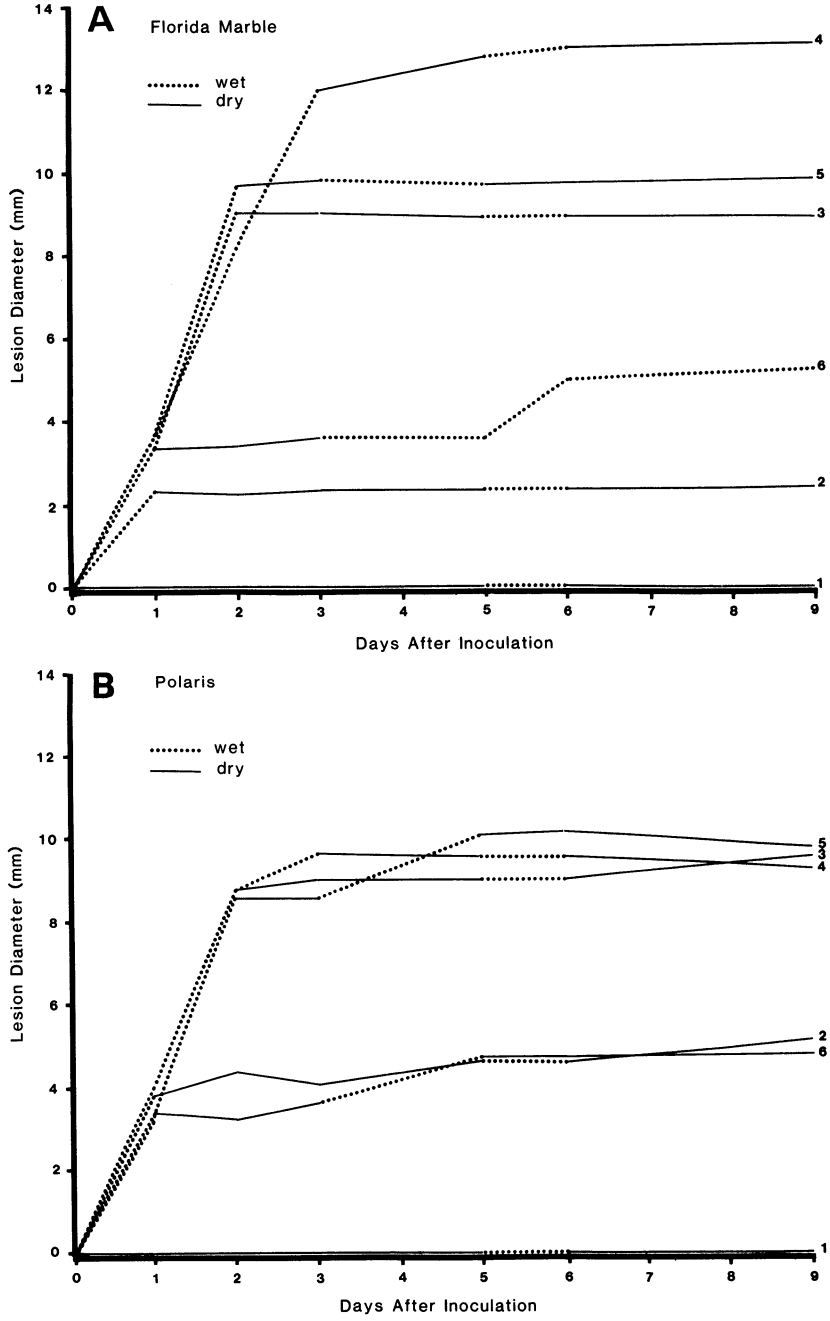

Fig. 1. Effect of intermittent dry and wet periods on average lesion diameter of **(A)** Florida Marble and **(B)** Polaris chrysanthemum leaves inoculated with *Pseudomonas cichorii*.

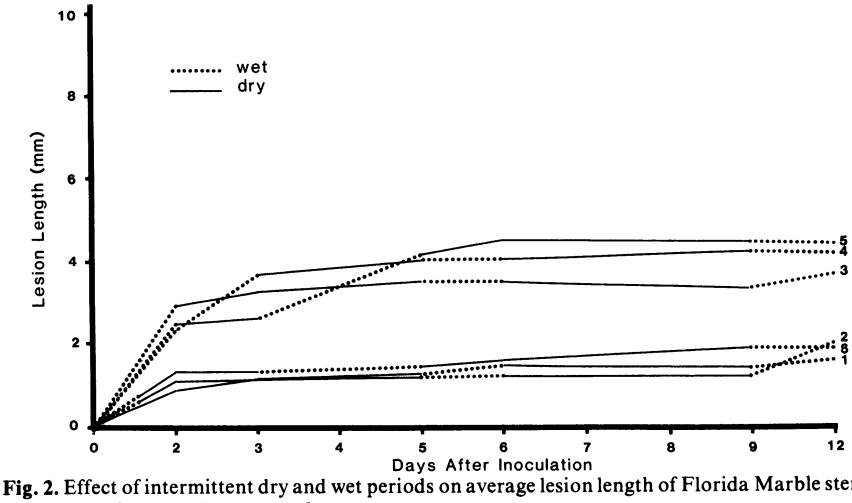

Fig. 2. Effect of intermittent dry and wet periods on average lesion length of Florida Marble stems inoculated with *Pseudomonas cichorii*.

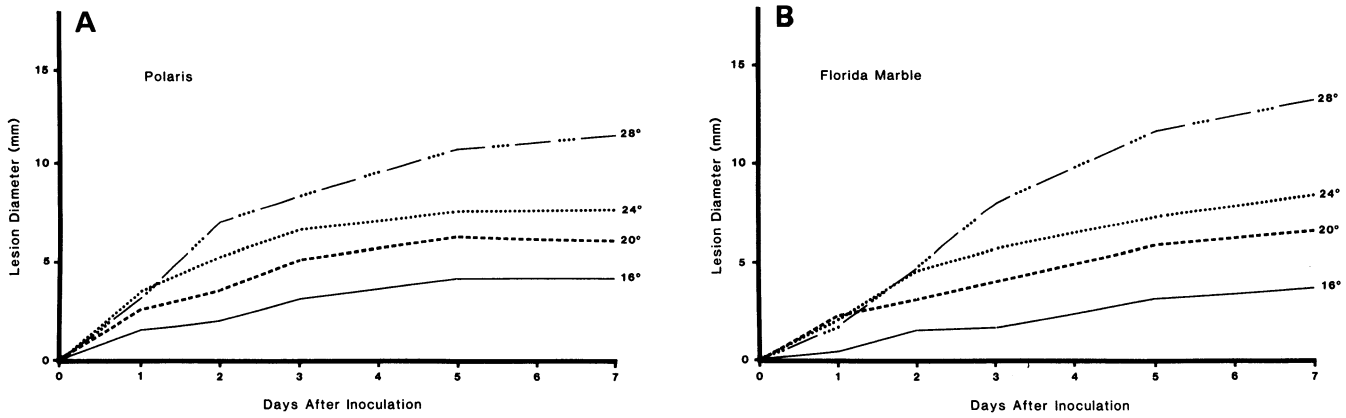

Fig. 3. Effect of ambient temperature on average lesion diameter of **(A)** Polaris and **(B)** Florida Marble chrysanthemum leaves inoculated with *Pseudomonas cichorii*

Table 2. Effect of temperature on incidence of *Pseudomonas cichorii* on Polaris and Florida Marble chrysanthemum leaves for three incubation periods

	Incubation period					
	48 Hr		72 Hr		168 Hr	
Temperature (C)	Polaris	Florida Marble	Polaris	Florida Marble	Polaris	Florida Marble
16	5.6[a]	0.0	11.1	8.3	19.4	13.8
20	25.0	30.6	38.9	38.9	47.2	63.9
24	22.2	44.4	41.7	44.4	44.4	100.0
28	100.0	91.7	100.0	91.7	100.0	100.0
32	0.0	0.0	0.0	0.0	0.0	2.8
36	0.0	0.0	0.0	0.0	0.0	0.0
Significant effects						
Linear	NS	*[b]	NS	*	NS	**
Quadratic	NS	*	*	*	*	*
R^2	0.10	0.64	0.40	0.51	0.38	0.75

[a] Percentage of inoculation points positive for disease development.
[b] * = Statistical significance at $P = 0.01$, ** = statistical significance at $P = 0.05$, and NS = not significant.

Table 3. Effect of temperature on development of bacterial spot incited by *Pseudomonas cichorii* on Polaris and Florida Marble chrysanthemum stems for three incubation periods

	Polaris (days)			Florida Marble (days)		
Temperature (C)	2	3	7	2	3	7
16	4.0[a]	4.5	4.8	3.8	5.5	9.3
20	5.8	5.6	6.5	15.0	18.5	20.3
24	7.0	7.3	7.3	12.5	17.5	17.3
28	9.3	9.6	10.0	16.8	35.8	48.8
32	4.3	4.3	4.3	2.8	3.0	5.0
36	0.0	0.0	0.8	1.3	1.3	2.3
Significant effects						
Linear	*[b]	*	*	*	**	**
Quadratic	*	*	*	*	*	**
R^2	0.59	0.62	0.56	0.41	0.30	0.27

[a] Values are the mean length of four stem lesions as measured in millimeters.
[b] * = Statistical significance at $P = 0.01$ and ** = statistical significance at $P = 0.05$.

C. At 32 C, lesion expansion was sparse on geranium and Florida Marble chrysanthemum leaves and no development occurred on Polaris chrysanthemum leaves. Stem lesions developed at all temperatures (Table 3). Optimum development occurred at 28 C, whereas very sparse development occurred at 16 C or higher than 28 C. Incidence (percentage of inoculated sites with lesions) for chrysanthemum and geranium leaves increased as temperatures were increased from 16 to 28 C but was greatly retarded at temperatures higher than 28 C (Tables 1 and 2). Incidence also increased with the increased moisture period.

Bacterial spot of geranium and chrysanthemum was shown in this study to develop over a wide temperature range; the optimum temperature was 28 C. Lesion numbers were greatest at 28 C. At temperatures higher than 28 C, lesion incidence sharply declined, with very few lesions at 32 C and none at 36 C. There was an interaction between incubation temperature and the high-moisture period; both were important for maximum lesion numbers and size. Lesion development on stems was less affected by temperature, with the least development at 32 and 36 C. The optimum temperature range for disease development was 20–28C, which is in agreement with the range of 20–27 C predicted by Bolick (1). McFadden (10) indicated that temperature within certain limits was less critical than moisture, with which we agree; however, we found temperature had a significant effect on both lesion incidence and expansion.

High-moisture periods of 48–72 hr directly after inoculation resulted in much greater lesion development than a 24-hr period or a continuous low-moisture period (Figs. 1 and 2). Lesions on leaves expanded under high-moisture conditions but ceased to expand under low-moisture conditions. Moisture conditions had a similar effect on the development of stem and leaf lesions, except initial stem lesion development was not dependent on high moisture.

High humidity is an important factor in many bacterial diseases (3,4,7,13), and bacterial spot of chrysanthemum and geranium is no exception. When chrysanthemum plants were removed from the high-moisture environment, there was complete or almost complete cessation of lesion expansion. Thus, *P. cichorii* is dependent on high moisture (and 20–32 C temperature) for infection and lesion expansion. It is also evident from the temperature studies that the longer the period of moisture, the greater the incidence of disease.

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