Bacterial Blight of Carrots: Interaction of Temperature, Light, and Inoculation Procedures on Disease Development of Various Carrot Cultivars

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The authors thank J. Barnard for statistical analysis, J. Armitage, W. Sherring, and T. Kenny for technical assistance and the Joseph Harris Seed Co., Rochester, N. Y. for some of the seeds.

Approved by the Director as Journal Paper No. 2056, N. Y. State Agricultural Experiment Station, Geneva.

Accepted for publication 26 December 1973.

ABSTRACT

High temp and high light intensity favored foliar symptom expression in carrot (*Daucus carota* L. var. *sativa* DC.) cultivars inoculated with *Xanthomonas carotae* (Kendr.) Dows. Photoperiod had little or no effect. As a group, 'Danvers', 'Waltham HiColor', and P.I. 306588 expressed greater tolerance to leaf blight than did 'Spartan Sweet', 'Spartan Bonus', and a susceptible breeding line (2106). When leaf tips were inoculated, the pathogen and lesions were confined to the area of inoculation. Although foliar symptoms were less severe at 20 C than at 25 C or 30 C, there was no corresponding reduction in the number of bacterial cells recovered from the affected tissue. When roots were wounded and immersed in suspensions of *X. carotae*, the bacteria caused extensive damage to the vascular system, much more at 30 C than at 20 C. In all tests, inoculation with the pathogen caused injury to tops and roots, expressed as a reduction in dry weight.

Phytopathology 64:746-749.

Additional key words: Daucus carota, Xanthomonas carotae.

Bacterial leaf blight of carrots, caused by *Xanthomonas carotae* (Kendr.) Dows., is virtually indistinguishable from the blights caused by *Alternaria dauci* (Kuehn) Groves & Skolko and *Cercospora carotae* (Pass.) Solh., based on foliar symptoms alone. Positive identification requires microscopic examination or isolation.

X. carotae was first reported by Kendrick (1) in California, where the disease was particularly damaging in fields used for carrot-seed production. The disease has been found in Australia and Canada (4), Idaho (5), and most recently in Wisconsin (3). The bacterium was also isolated in upstate New York in the summer of 1972.

We conducted a factorial experiment designed to study varietal response to infection by *X. carotae*, together with the effects of different inoculation procedures, light intensities, and temp on symptom expression and development of the disease.

MATERIALS AND METHODS.—Carrot cultivars used in this study were selected on the basis of a preliminary survey conducted in the field at Geneva, N. Y. in 1972. The survey indicated varietal differences in susceptibility to *X. carotae* among cultivars and breeding lines. Seeds of 'Waltham HiColor' (tolerant), 'Danvers' (tolerant), P.I. 306588 (tolerant), 'Spartan Bonus' (moderately susceptible), 'Spartan Sweet' (susceptible), and a susceptible breeding line (2106) were planted in 27 × 54 × 7-cm flats containing a pasteurized soil:peat:sand mixture (2:1:1, v/v). A total of 72 flats were used, each flat containing a single row of each of the six cultivars. The order of the rows was randomized among flats. Three weeks after planting, the plants were thinned to eight plants per row, so that each flat contained 48 plants.

A culture of *X. carotae* was obtained from E. K. Wade, University of Wisconsin. Subsequently, bacterial cultures used for inoculation were grown on nutrient agar (Difco Laboratories, Detroit) for 48 h, washed with sterile distilled water, vigorously shaken, centrifuged 15 min at 755 *g*, and resuspended in sterile distilled water.

After 4 wk of growth in the greenhouse, plants in 36 flats were sprayed to runoff with a suspension of ca. 10^7 cells/ml of *X. carotae* (2), while plants in the remaining 36 flats were sprayed with sterilized distilled water. Plants in all flats were then maintained at 100% relative humidity (RH) for 72 h (3) at 24 C under a light intensity of 4,300 lux and a 12-h photoperiod.

Then 24 flats (12 inoculated and 12 noninoculated) were moved into each of three growth chambers maintained at 20 C, 25 C, and 30 C. Each chamber was divided into four cells, two at 30,140 lux and two at 21,520 lux (cool-white fluorescent mixed with incandescent light, with 5.3-W fluorescent light for every watt of incandescent light). Within each light-intensity level, two photoperiods (9 h and 15 h) were maintained. Three flats each of inoculated and noninoculated were randomized within each cell. A constant RH of 60-65% was maintained in each chamber. The flats were placed in shallow trays and watered from the bottom, thereby avoiding dispersal of the pathogen by overhead watering. Foliar disease severity was estimated visually for all experimental plants by using a scale of 0-4.

Dry wt of roots and foliage from inoculated and noninoculated plants was recorded from all cultivars. The top portion of the plants included the stem plate and 2-5 mm of the root. The plant parts were dried at 66 C for 7 days.

Since Spartan Bonus plants showed varying degrees of symptom expression under the different temp and light regimes used (Table 1), various plant parts of this cultivar were separated and examined in detail. Individual plants were divided into the following parts: originally inoculated leaves, leaves that had developed after inoculation, stems (5 mm of root and the lower 2 mm of the petioles), roots, and in some cases root epidermal layers were pared away from the remainder of the root. Five and 10 wk after inoculation, viable-cell counts of *X. carotae* were determined in different plant parts by dilution plating in nutrient agar. The tissue was macerated with a mortar and pestle in 4 ml of sterile distilled water plus some sand to aid in maceration.

TABLE 1. Disease ratings of various carrot cultivars inoculated with *Xanthomonas carotae* and grown at different light and temp regimes

Treatment	Cultivar						
	Danvers	Waltham HiColor	P. I. 306588	Spartan Sweet	Spartan Bonus	Susceptible breeding line-2106	Row means LSR 5% = 0.34
30 C at 30,140 lux	0.75[a]	0.93	1.23	1.70	2.40	2.10	1.51
30 C at 21,520 lux	0.55	0.62	0.45	1.20	1.20	1.20	0.87
25 C at 30,140 lux	0.43	1.40	0.56	2.00	1.51	2.12	1.34
25 C at 21,520 lux	0.45	0.41	0.22	1.50	1.10	1.35	0.84
20 C at 30,140 lux	0.10	0.38	0.16	0.55	0.26	0.56	0.34
20 C at 21,520 lux	0.20	0.17	0.38	0.80	0.13	0.58	0.38
Column Means LSR 5% = 0.34	0.48	0.65	0.50	1.29	1.10	1.31	

[a]Disease ratings are the average of a minimum of 24 plants of each cultivar and treatment at 5 wk, and are expressed as 0, no leaf necrosis; 1, 1-25% leaf surface necrotic; 2, 26-50% leaf surface necrotic; 3, 51-75% leaf surface necrotic; and 4, 76-100% of leaf surface necrotic.

To determine the effects of leaf injury on disease development, noninoculated and inoculated plants of Spartan Bonus (8 wk old) were kept at 30 C with a 9-h photoperiod under 30,140 lux of light. In a separate experiment, disease development was determined in injured inoculated roots of intact plants kept at either 30 C or 20 C with a 9-h photoperiod under 30,140 lux of light. Leaf tips were injured by piercing the tissue with a needle, whereas roots were injured by a series of punctures. Injured and uninjured leaf (3 cm) and root tips (5 cm) were placed in a bacterial suspension of ca. 10^7 cells/ml for 48 h. Roots of plants kept in the suspension were constantly aerated by a small aquarium pump. Roots of the intact plants were then removed from bacterial suspensions and placed in 15-cm diam pots containing a pasteurized soil:peat:sand mixture (2:1:, v/v). In control plants, noninoculated injured leaf and root tips were immersed in sterile water and placed in pots containing a similar soil mixture. Viable-cell counts of *X. carotae* were determined as described earlier for injured and uninjured leaf tips, and in the entire petiole immediately below injured leaf tissue, 2 wk after exposure to the bacteria. Bacterial-cell counts in injured inoculated roots were determined 2, 3, and 4 wk after inoculation.

All results presented represent averages from at least seven different plants, unless otherwise noted.

RESULTS.—With the aid of analysis of variance, significant variations were distinguished between means of disease ratings among cultivars grown under different regimes. As a group, Danvers, Waltham HiColor, and P.I. 306588 were more tolerant to leaf blight than were Spartan Sweet, Spartan Bonus, and the susceptible breeding line (2106). Disease ratings were higher at 30,140 lux than at 21,520 lux, but only at growth temp of 25 C and 30 C (Table 1). Differences under different photoperiods were not significant, so data for these two treatments were combined. The nature of the design did not permit formal F tests for temp effects. However, the magnitude of the mean square was so large that the effect of temp on disease was undoubtedly significant. Regardless of light intensity, disease ratings were much lower at 20 C than at 25 C or 30 C (Table 1). Disease ratings taken at 10 wk after inoculation were not different from ratings taken at 5 wk, and in some instances ratings decreased.

Leaves of Spartan Bonus that were present at the time of inoculation showed symptoms, and contained numerous bacteria based on viable-cell counts. However, leaves produced after inoculation showed no symptoms and were free of the pathogen (Table 2). Cell counts in the stem were somewhat lower than those from inoculated leaves, but were generally higher than those from roots. However, when epidermal root tissue was removed and assayed, it contained 4×10^5 *X. carotae* cells/g fresh wt, whereas the remaining portion of the root contained a mean of 2 cells/g fresh wt. In all plant parts, cell counts at 10 wk after inoculation usually were similar to those at 5 wk (Table 2). Results were similar under all light regimes. *X. carotae* was never isolated from noninoculated plants.

In all cultivars, inoculation with the pathogen caused a reduction in dry wt in comparison with noninoculated controls. However, in absence of inoculation, dry wt of Danvers was significantly higher than that of Spartan Bonus, and the magnitude of the loss of dry wt caused by inoculation was no greater in Spartan Bonus than in Danvers. Reduction of dry wt of roots and foliage of both cultivars resulting from inoculation was not significantly different within each temp (Table 3).

Previously noninoculated leaf tips of Spartan Bonus plants immersed 48 h in bacterial suspensions developed typical lesions after removal from the suspension. The lesions were restricted to those portions of the leaves immersed in the suspension. Two wk after inoculation, these portions contained a mean of 4.1×10^6 cells/g fresh wt. However, a mean of only 11 cells/g fresh wt were isolated from the petioles and leaves located immediately below (ca. 20 cm) the injured and uninjured leaves. Thus, when leaves were inoculated, only local infection resulted. Leaves immersed in sterile distilled water developed no symptoms of bacterial infection.

TABLE 2. Viable-cell counts of *Xanthomonas carotae* obtained from different parts of 'Spartan Bonus' carrots grown at different temp[a] 5 and 10 wk after inoculation with 10^7 cells/ml

	Viable cells/g fresh weight (log base 10)[b]							
	Inoculated leaves		Leaves produced after inoculation		Stem[c]		Roots[d]	
Temp (C)	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk
30	7.1	8.0	0	0	6.4	6.5	4.9	5.0
25	7.2	7.2	0	0	6.7	6.6	5.8	5.8
20	3.3	7.2	0	0	6.2	6.9	8.1	5.3

[a]In all tests, the plants received 30,140 lux of light 9 h/day.
[b]Results represent averages obtained from three plants.
[c]Stem tissue included the stem, the upper 5 mm of the root, and lower 2 mm of petioles.
[d]Bacteria shown to be confined to epidermal root tissue.

TABLE 3. Dry wt of roots and foliage of carrot cultivars 'Danvers' (tolerant) and 'Spartan Bonus' (susceptible) carrots noninoculated or inoculated with *Xanthomonas carotae* and grown at different temp

	Danvers				Spartan Bonus					
	Roots		Foliage		Roots		Foliage		Mean dry wt per plant LSR 5% = 0.38	
Temp (C)	Inoc.	Noninoc.	Inoc.	Noninoc.	Inoc.	Noninoc.	Inoc.	Noninoc.	Inoc.	Noninoc.
30[a]	0.46[b]	0.66	0.83	1.20	0.32	0.60	0.49	0.80	0.52	0.82
25	0.83	1.30	0.79	0.95	0.68	1.10	0.47	0.72	0.69	1.02
20	0.92	1.10	1.10	1.30	0.89	0.92	0.95	1.10	0.90	1.11
Mean dry wt per plant	0.74	1.02	0.91	1.15	0.68	0.87	0.64	0.87		
LSR 5% = 0.18										

[a]In all tests, the plants received 30,140 lux of light 9 h/day.
[b]Plants were harvested 12 wk after the data of inoculation and their dry wt (grams/plant) represent the average of at least 22 plants of each cultivar and temp.

Vascular deterioration in wound-inoculated roots occurred at 30 C, but not at 20 C, 2 wk after inoculation (Fig. 1). In severely damaged roots, the vascular cylinder was necrotic or decayed through much of the length of the root. Occasionally, the pathogen was isolated from leaves that had been initiated after the time of inoculation, but only when roots were severely damaged. Frequently more than 10^8 cells of *X. carotae*/g fresh wt were isolated from roots at 30 C, whereas 10^4 to 10^5 cells/g fresh wt of root were recovered at 20 C. No symptoms were observed, and the pathogen was not isolated from roots immersed in sterile distilled water.

DISCUSSION.—Plants grown at 20 C had fewer foliar symptoms and lower disease ratings than those grown at 25 C or 30 C. Disease ratings taken 10 wk after inoculation were similar to or sometimes lower than those taken at 5 wk. This was probably caused by the loss of old diseased leaves.

Foliage was darker green, tops were shorter, and roots were larger in all plants grown at 20 C than at the two higher temp. Despite this, there was no significant difference in dry wt (compared to plants grown at 25 C or 30 C), and bacterial counts were as high in inoculated plants kept at 20 C as in those kept at 25 C and 30 C. Dry

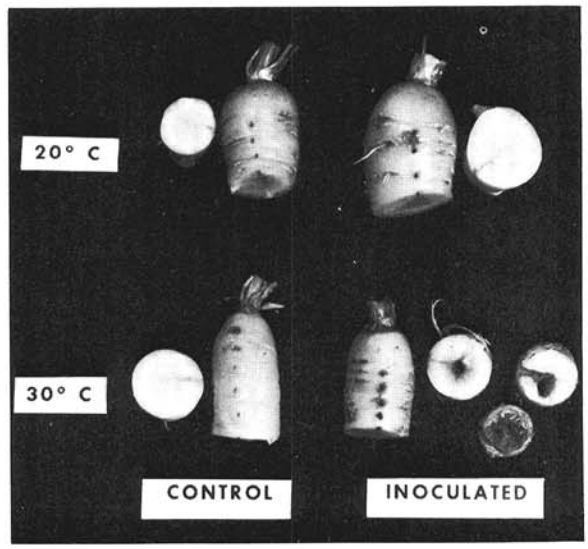

Fig. 1. Symptoms produced by *Xanthomonas carotae* 2 wk after roots were wounded by being pierced with a needle, then immersed in a bacterial suspension of 10^7 cells/ml.

wt of all cultivars were similarly reduced after inoculation. However, Danvers, a tolerant cultivar as judged by foliar symptoms, would be expected to show less reduction in dry wt than Spartan Bonus, a susceptible cultivar. That such a difference was not detected in these experiments was possibly because of lack of dispersal and secondary infection by the pathogen after the initial inoculation, so the disease did not increase. However, under field conditions favoring dispersal of the pathogen, the relative amount of injury to the tolerant vs. the susceptible cultivars might be greater.

Temperature exerted a profound effect on disease development in injured, inoculated roots of Spartan Bonus. In every instance, inoculated roots of intact plants (Spartan Bonus) grown at 30 C developed extensive vascular deterioration, often involving the entire length of the root. Yet the surrounding cortical tissue remained intact and no evidence of deterioration was noted; thus the vascular tissue may be more susceptible to *X. carotae*. Occasionally, the bacteria were isolated from leaves that had initiated after inoculation, but only in those plants with severely damaged roots. However, the evidence is insufficient to conclude that disease development is systemic.

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