Cercospora omphakodes Infection and Disease Development in Phlox divaricata

R. W. Judd Jr. and J. L. Peterson

Graduate Assistant and Professor, respectively, Department of Plant Pathology, Rutgers University, New Brunswick, New Jersey 08903. Present address of senior author: 322 North Main Street, Wallingford, Connecticut 06492.

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ABSTRACT

Cercospora omphakodes causes a leaf spot on *Phlox divaricata* in New Jersey. Infectivity was tested under nine light intensities and three temp. Infection was greatest under 1.72×10^4 lux and at 26 and 32 C. Disease development after infection was observed under six light intensities and two temp. The most rapid and severe development occurred under 3.23×10^4 lux and at 32 C. The pathogen enters the host through open stomata, never penetrating directly through the epidermis. The fungus overwintered in infected leaves on the host plant, surviving temp of −20 C. In carbon and nitrogen nutrition studies in liquid shake culture, the organism grew best on fructose and tryptophan, respectively. The optimum temp for growth was between 24 and 28 C.

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Cercospora omphakodes Ell. & Holw. causes a leaf spot on *Phlox divaricata* L. in New Jersey. A 90% loss was sustained from this disease in certain nursery plantings in 1969-71. The pathogen was first reported on *P. divaricata* by Ellis and Holway in 1885 (4). The disease occurs first on the lower, older leaves and progresses up the plant, producing large yellow spots with tan centers. Under ideal conditions, the entire plant can be infected and as many as 10-20 small lesions per leaf can occur. These heavily infected leaves turn yellow, but usually adhere to the plant.

Essentially, little work has been reported on Cercospora leaf spot of phlox. This paper reports effects of certain environmental factors on disease initiation and development, and on the growth of the pathogen in culture.

MATERIALS AND METHODS.—*Effect of light and temperature on infection.*—Preliminary studies and field observations indicated that light intensity, relative humidity (RH), and temp affected disease development. Experiments were conducted in plant growth chambers supplied by twelve VHO cool-white fluorescent lamps and eight 100-W incandescent bulbs. By placing plants in wire cages covered with Saran netting of various weaves, five different light intensities could be maintained simultaneously in each chamber. By the above method light was maintained at 0.39×10^4, 0.54×10^4, 0.73×10^4, 1.08×10^4, 1.29×10^4, 1.72×10^4, 2.15×10^4, 2.8×10^4, and 3.23×10^4 lux.

Young plants in 10.2-cm (4-inch) diam pots were inoculated by atomization with a spore suspension containing approximately 25,000 spores/ml directly upon the upper and lower leaf surfaces until they were covered. After inoculation, the potted plants were individually slipped into an open-topped plastic sleeve the same height as the plant to minimize the drying effects of the circulating air in the growth chamber.

Plants were left in the dark for 8 h after inoculation, followed by a 14 h light and 10 h dark schedule. RH was maintained at 60 ± 2.5% during the light and 97 ± 1.0% during the dark period. Constant temp of 16, 26, and 32 ± 1 C were utilized. Preliminary studies indicated that infection would not occur at 10 C or lower.

Two replications using two plants for each temp and light intensity were made. Disease readings were taken by counting the number of spots on each plant 10 days after

inoculation. A statistical design utilizing the two-way analysis of variance was employed to analyze the data.

The effect of light and temperature on disease development after infection.—Plants were inoculated as described above, and after the 8-h dark period, placed in a humidity chamber at 100% RH and 20 C. The plants were removed after 72 h and placed in the plant growth chambers under 0.39×10^4, 0.73×10^4, 1.08×10^4, 1.72×10^4, 2.15×10^4, and 3.23×10^4 lux at either 16- or 32 C.

Two replications of two plants each were included at each light intensity and temp. Readings were taken 14 days after inoculation by counting and averaging the number of spots on ten fully expanded leaves taken at random from each plant.

Mode of pathogen entrance.—Healthy detached leaves were placed in a Gooch-type, low-form crucible, after spores were placed on the lower epidermis. The crucible was then placed on a wax-coated wire rack over a saturated $CaSO_4$ solution in a 236.6-cc (8-oz), low-form, screw cap jar. The jar was covered and submerged in a water bath at 20 ± 0.05 C. At this temp the saturated $CaSO_4$ solution provided 98% RH in the closed container.

After 48 h the leaves were removed from the jars. The lower epidermis was removed and stained with lactophenol-cotton blue for 45 s and observed under the microscope for fungus penetration.

Overwintering of the fungus.—To determine if the fungus could overwinter in the phlox plant, infected leaves from a previous summer field crop were collected in April 1970. The leaves were incubated in a moisture dish at approx 24 C and observed periodically for fungus growth and sporulation.

The effect of temperature on mycelial growth of the fungus.—Three media were used: potato-dextrose agar (PDA), V-8 agar (9), and a synthetic medium (FGA) containing fructose, 15 g; glutamic acid, 4.5 g; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5g; Fe, 0.2 mg; Zn, 0.2 mg; Mn, 0.1 mg; biotin, 5 μg; thiamine, 100 μg; agar, 15 g and distilled water, 1 liter. Plugs of mycelium (6 mm), taken from the margins of growing cultures, were placed on the media at the dish edge. There were four replications in each treatment. The inoculated dishes were incubated at 12, 16, 20, 24, 28, and 32 C. Growth was measured at 2-day intervals for 21 days.

The effect of nutrition on fungus growth and sporulation.—Fifteen carbon and 11 nitrogen levels were tested at 4.0 g carbon and 0.43 g nitrogen/liter in liquid media. A basal medium (8), was prepared double-strength and 25 ml were dispensed into 250-ml Erlenmeyer flasks after autoclaving. All carbon and nitrogen sources were prepared double-strength, sterilized by passing through a Millipore filter (size, 0.45 μ); and added (25 ml) to the basal medium. Sucrose was used as the carbon source in the nitrogen study. Appropriate controls without carbon or nitrogen were included.

The inoculum was made by homogenizing an actively growing 2-wk-old fungus colony in 100 ml sterile distilled water in a blender for 45 s. Each flask then received 1 ml of the mycelial suspension and was oscillated on a rotary shaker at 200 rev/min.

Duplicate dry wt measurements were taken every 2 days, beginning 6 days after inoculation and continuing

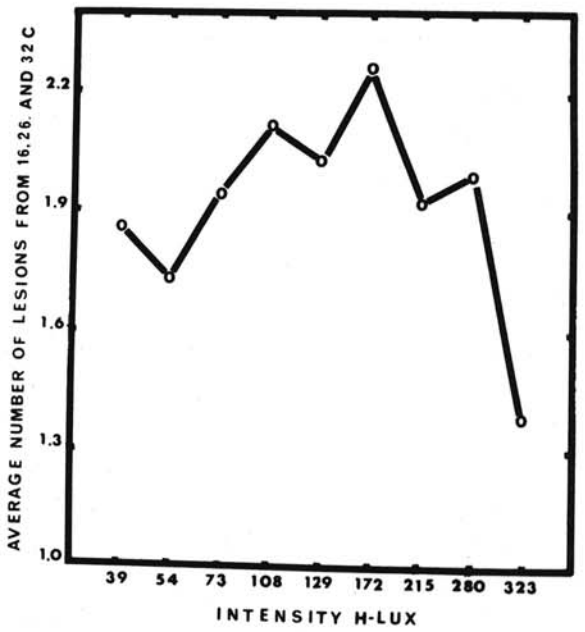

Fig. 1. Effect of light intensity 1.0 lx = (0.093 ft-c) on infection by *Cercospora omphakodes* as determined by averaging the number of infection points per leaf produced at three temp. (Estimate of variance = 0.44).

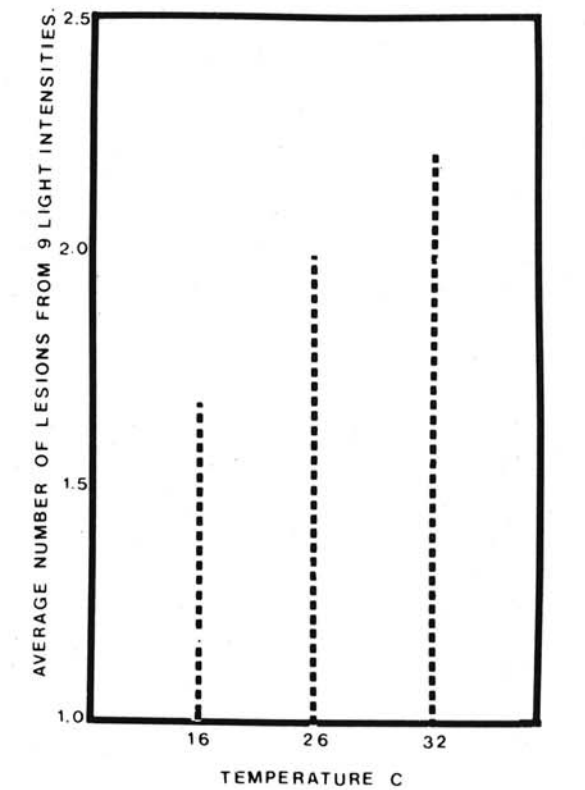

Fig. 2. Effect of temp on infection by *Cercospora omphakodes* as determined by averaging the number of infection points per leaf produced at nine light intensities. (Estimate of variance = 0.31).

TABLE 1. The effect of light intensities and low (16 C) and high (32 C) temp on disease development

Light intensity (10^4 lx)	Lesions[a] 16 C (no.)	32 C (no.)
0.39	1.2	2.0
0.73	0.5	2.7
1.08	1.0	4.2
1.72	1.2	7.3
2.15	0.8	6.7
3.23	1.2	9.4

[a]Average number of lesions on 10 leaves per plant (two replications).

TABLE 2. Growth of *Cercospora omphakodes* in selected carbon sources after 16 days

Carbon Source	Dry wt.[a] (mg)
Fructose	226 A
Sorbitol	221 A
Raffinose	210 AB
Galactose	202 ABC
Xylose	200 BC
Sucrose	198 BC
Glucose	183 C
Mannitol	48
Maltose	29
Ethanol	34
Glycerin	22
Lactose	19
Ribose	21
Sodium acetate	0
Sodium succinate	0
No carbon	0

[a]Means followed by the same letters do not differ, $P=0.05$, by Duncan's multiple range test.

TABLE 3. Growth of *Cercospora omphakodes* in selected nitrogen sources after 24 days

Nitrogen source	Mg dry wt.[a]
Tryptophan	329 A
Glutamic acid	230 B
Glycine	212 B
Lysine	152 B
Leucine	34 C
$(NH_4)_2SO_4$	10
Urea	5
NH_4NO_3	7
$NaNO_3$	5
$NaNO_2$	7
Cysteine	0
No nitrogen	0

[a]Means followed by the same letters do not differ, $P=0.05$, by Duncan's multiple range test.

for 16 days, in the carbon studies. Because of slower growth in the nitrogen studies, dry wt measurements were taken every 3 days for 21 days. All flasks were sampled for spore production when harvested. Data were analyzed with a two-way analysis of variance and Duncan's multiple range test.

RESULTS.—*Effect of light and temperature on infection.*—Light intensity and temp both had a significant effect on infection. Maximum infection occurred at 1.72×10^4 lx when one averaged the number of leaf spots collectively from the 16, 26, and 32 C treatments. At low (0.39×10^4 and 0.54×10^4 lx) and high (3.23×10^4 lx) intensities, infection was significantly less than at 1.72×10^4 lx. Infection occurring between 0.73×10^4 and 2.80×10^4 lx did not differ significantly (Fig. 1). Stomatal opening was greatest at 1.72×10^4 lx.

Maximum infection occurred at 32 C when one averaged the number of leaf spots collectively from the nine tested light intensities. However, this did not differ significantly from infection occurring at 26 C. Significantly more infection occurred at 26 and 32 C than at 16 C (Fig. 2).

Effect of light and temperature on disease development after infection.—The disease developed much faster at 32 than 16 C. After inoculation, lesions appeared in 7 days at 32 C; whereas at 16 C, they were not apparent until 10 days.

The disease was most severe at 32 C and 3.23×10^4 lx (Table 1). Disease development was moderate at 1.72×10^4 and 2.15×10^4 lx and decreased as light intensity decreased. The lesions, under high temp, were small (1-2 mm in diam) and yellow, and did not enlarge. Infection at high temp did, however, cause the leaf to yellow and die rapidly.

Mode of entrance of the pathogen.—The germ tubes entered the leaf through open stomata. However, less than 1% of the spores that germinated penetrated the leaf. Most of the germ tubes grew around and even over the open stomata. No direct host penetration was observed.

Overwintering of the fungus.—The pathogen sporulated heavily on overwintered leaves two days after placement in moist covered dishes. The lowest monthly temp recorded by the U.S. Department of Commerce (10) near the phlox planting during the winter of 1969-70 were −9, −10, −20, −14, −9, and −2 C from November to April, respectively.

Effect of temperature on growth of the fungus.—The optimum temp for pathogen growth on PDA was between 24 and 28 C. On V-8 agar and FGA, the growth optimum was 28 C. Although the fungus grew best on PDA, growth was very slow, attaining only 4 mm in 14 days.

Effect of nutrition on fungus growth and sporulation.—Growth measurements of only seven carbon sources of the fifteen tested were statistically analyzed due to the slow growth rate on the eight other sources. The fungus grew best on fructose, but the growth was not significantly greater than on sorbitol, raffinose, and galactose (Table 2). Lesser growth occurred on xylose, sucrose, and glucose. Little or no growth occurred on mannitol, maltose, ethanol, glycerin, lactose, ribose, sodium acetate, and sodium succinate.

Growth measurements of the fungus on only five

nitrogen sources of the eleven tested were statistically analyzed due to the slow growth rate on the six other sources. The fungus grew significantly better on tryptophan than on the other nitrogen sources (Table 3). Moderate growth occurred on glutamic acid, glycine, and lysine. Leucine was utilized very little, and cysteine not at all. Growth of the fungus was minimal on the inorganic sources and on urea.

No growth occurred in the appropriate control flasks with either a carbon or nitrogen source missing. No sporulation was observed on any of the cultures in the flasks.

DISCUSSION.—Infection and disease development under controlled conditions were correlated with previous disease observations in the field (5). Light infection occurred in the field in late April after a period of rain, cloudy weather, and low temp. These conditions were simulated under controlled conditions of 16 C and 1.72×10^4 lx where light infection also occurred. Heavy infection occurred in the same field in August after a prolonged rainy, humid, and cloudy period with high temp, and was also produced under controlled conditions of 32 C and 1.72×10^4 lx. Disease development in the field was rapid after the infection period as light intensities increased and at high temp. This was correlated with the controlled experiments. These observations are consistent with those on a clover disease caused by *C. zebrina,* which develops best at 28 C (1), and a disease on sugar beet caused by *C. beticola* which develops best at high light intensities (3).

In previous studies (6), *C. omphakodes* spores germinated best at high RH, but not in free water. It was also observed in the present study that stomatal openings were widest at the 1.72×10^4 lx intensity and that open stomata were necessary for fungus penetration. Other *Cercospora* spp. have also been reported to penetrate their hosts only through stomata (1, 2, 7).

Obviously, cloudy periods of high humidity without excessive rain, high temp, and light intensities favor wide stomatal opening, which favors infection. High temp and high light intensities favor disease development after infection. Since the fungus can readily overwinter in old leaves of perennial phlox, a ready source of inoculum is available.

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