Environmental Conditions Required for Infection of Photinia Leaves by *Entomosporium mespili*

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ABSTRACT

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The optimum temperature for infection of photinia leaves by *Entomosporium mespili* was about 20 C, and infection was only slightly retarded at 15 and 25 C. Very little infection occurred at 30 C. Leaf wetness for 9–12 hr was required for substantial infection, although leaf wetness as brief as 5–6 hr sometimes produced a few leaf spots at the optimum temperature. After deposition onto leaves, conidia survived dry periods of 12 hr at 25 C in darkness or outdoors in the shade with little loss of infectivity, regardless of whether the dry period occurred immediately after inoculation or after 5 or 12 hr of leaf wetness. Only bright sun and high temperature resulted in a significant loss of infectivity.

Additional key words: *Diplocarpon maculatum, Entomosporium maculatum, Fabraea maculata, Photinia × fraseri*

Entomosporium leaf spot, caused by *Entomosporium mespili* (DC. ex Duby) Sacc. (=*E. maculatum* Lév., teleomorph: *Diplocarpon maculatum* (Atk.) Jorstad = *Fabraea maculata* Atk.), is an important disease of photinia in nurseries as well as in the landscape. The disease also occurs on other genera in the subfamily Pomoideae of the Rosaceae (3,5). In wet weather, plants may become heavily infected, resulting in defoliation and weakening that render them susceptible to winter kill. Young leaves of *Photinia* × *fraseri* Dress., a commonly grown hybrid of *P. glabra* and *P. serrulata*, are very susceptible to infection, whereas mature leaves sustain few new infections (1). Thus, frequent fungicide sprays are needed to protect young growth when environmental conditions favor infection (2). Knowledge of the environmental conditions required for infection might enable one to improve scheduling of fungicide sprays. Long wet periods of 2–5 days have been used to obtain infection (4,8,10), but the only information on minimum duration of leaf wetness is found in a report of a preliminary study on Entomosporium leaf spot of pear (9). The goal of this paper is to characterize the duration of leaf wetness required for infection at various temperatures and to examine the effects of interruptions in wetness periods.

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MATERIALS AND METHODS

Plant material and inoculation procedures have been described (1) and are briefly summarized here. One- to 2-yr-old *P.* × *fraseri* with young shoots were used. Because leaf age is an important determinant of susceptibility, it was characterized by measuring the leaves just before inoculation and again at symptom evaluation. Only leaves that were inoculated before they reached 90% of their final size (as measured 14 days later) were used in data analysis. Detached leaves were used in several experiments because they required less incubator space and could be inoculated more uniformly. The average lesion density resulting from spray inoculation of detached leaves (396 lesions per 100 cm^2 of leaf surface in four tests) was similar to that for attached leaves (478 lesions per 100 cm^2 in 15 tests). Detached, young leaves expanded very little, so degree of expansion could not be used as a measure of age; only young leaves that could be recognized by color and tenderness were used. Inoculum was prepared from diseased leaves and adjusted to 10^4 conidia per milliliter. For the experiments on duration of leaf wetness, detached leaves on moist filter paper in petri dishes were inoculated by placing drops (av. 30 μl) of conidial suspension on 10 sites per leaf. They were incubated in darkness at 10, 15, 20, 25, and 30 C. After the desired wetness period, the leaves were blotted dry and placed in holes in a Styrofoam plate floating on water so that the petioles were immersed. Lesions were counted after 9 and 14 days. Entire plants were inoculated by spraying the spore suspension over them until droplets began to coalesce. Plants were incubated in darkness in dew chambers or covered with plastic bags in incubators. After the wet period, the degree of leaf wetness was examined and plants were allowed to dry in a stream of air. Symptoms were evaluated 14 days after inoculation. Because the treatment standard deviations were approximately proportional to the means, the data were analyzed and means calculated using the log transformation (6).

RESULTS AND DISCUSSION

Nine days after inoculation of detached leaves, individual infections could be counted as tiny black flecks within the drop areas. When infections were numerous, they usually had coalesced after 14 days, and leaves sometimes turned necrotic. In areas with few lesions, the number of leaf spots after 9 days averaged 91% of that after 14 days. Because very few additional lesions appeared after 14 days (1), counts on the ninth day appeared to give a good indication of the final number of infections (Table 1).

Some infection of detached leaves was

Table 1. Effect of temperature and leaf wetness duration on infection of immature, detached photinia leaves by *Entomosporium mespili*

Temperature (C)	Number of infections per leaf[a] (hours of leaf wetness)				
	6	9	12	18	24
10	···	···	2 (1–7)	4 (4–11)	13 (4–18)
15	3 (0–2)	2 (2–5)	5 (5–11)	31 (15–35)	45 (27–84)
20	1 (1–3)	4 (4–8)	10 (9–18)	41 (30–62)	62 (59–168)
25	1 (1–4)	4 (3–8)	6 (7–14)	32 (17–37)	30 (25–73)
30	···	···	1 (0–5)	···	1 (0–3)

[a] Lesion density 9 days after inoculation. Each leaf was inoculated with 10 drops containing about 300 conidia each. Data are the means of two experiments, each with four replicates, and were analyzed after transformation to log $(x + 1)$. In parentheses are the 95% confidence limits for the mean, calculated using the General Linear Models procedure of SAS (Statistical Analysis System, SAS Institute, Inc., Cary, NC). Significant components of the regression were H (hours of wetness), T^2 (T = temperature, H^2, $T \times H$, T^3, and $T^2 \times H$.

obtained with as little as 6 hr of wetness at 15, 20, and 25 C (Table 1). The optimum temperature for infection was 20 C, but lesion density at 15 and 25 C was only slightly lower. At 10 C, the rate of infection was much lower, and at 30 C, very little infection occurred.

To confirm these results, plants with young shoots were sprayed with inoculum and subjected to dew periods at the same temperatures as before. The results (Table 2) could be compared with those for detached leaves by calculating the infection efficiency, i.e., the number of lesions as a percentage of the number of spores deposited per unit of surface area. Each detached leaf was inoculated with about 3,000 spores, whereas attached leaves, which were inoculated with a conidial spray, retained about 0.54 ml of inoculum (5,400 spores) per 100 cm^2 of leaf surface. Infection efficiency (average for 15, 20, and 25 C) on detached and attached leaves, respectively, was 1.53 and 1.20% with 24 hr of wetness, 0.23 and 0.43% with 12 hr of wetness, and 0.11 and 0.07% with 9 hr of wetness. Although the number of conidia per square centimeter was much higher with drop inoculation than with a spray, the infection efficiency was very similar. The optimum temperature and infection rate paralleled those obtained with detached leaves. In one separate test, the lesion density resulting from 48 hr of wetness at 23–25 C was 18% higher than that resulting from 24 hr of wetness.

Lathhouse tests with natural inoculum confirmed that leaf wetness overnight was sufficient to allow moderate or heavy infection. Plants with young shoots were placed under a photinia heavily infected by *E. mespili*. The plants were left outdoors through a single wetting period, moved into the greenhouse, and observed for symptoms. A wetting period at 18–20 C that began with rain at 1930 hours and ended when the foliage dried the next morning at 0800 hours resulted in five to 30 spots per susceptible leaf. Two wetness periods that began with rain and lasted 16 or 19 hr at similar temperatures resulted in 50–100 spots per leaf.

These results agree with Rosenberger's preliminary paper (9) on Entomosporium leaf spot of pear that reports an optimum temperature between 20 and 25 C and the shortest wetness period for infection as 8 hr. In several of my experiments, a few leaf spots were observed with only 5–6 hr of leaf wetness. The results are also consistent with the observation that penetration could be detected by microscopy within 12 hr of inoculation (1).

The optimum temperature for infection coincided with that reported for conidial germination and colony growth in vitro. Horie and Kobayashi (3) reported the optimum temperature for germination and germ tube elongation as 22–25 C. Optimum temperatures for colony growth reported in the literature are 18–21 C (7), 20–25 C (10), and 22 C (3). Some authors reported substantial growth at 30 C (3,7); others found none (10), suggesting that isolates vary in tolerance to high temperature.

Leaf wetness periods in the field may be interrupted by conditions unfavorable for infection. Horie and Kobayashi (3) reported that conidia that had been allowed to dry out on a glass slide would not subsequently germinate. Thus, it was of interest to determine whether splash-dispersed conidia could survive a subsequent dry period and resume germination and infection after leaves become wet again. After inoculation, plants were either allowed to dry off immediately and then exposed to conditions unfavorable to infection or they were incubated for several hours in a dew chamber and then subjected to an unfavorable period followed by another period in the dew chamber. Interruption of wetness periods by a 5- or 12-hr dry period at 25 C in the dark did not significantly reduce infection compared with uninterrupted wetness of the same total duration. This was true regardless of whether the dry period occurred immediately after inoculation or after 5 or 12 hr of wetness. There was a significant ($P = 0.01$) treatment × experiment interaction, with some experiments showing an increase and others a reduction in leaf spotting in the interrupted leaf wetness treatment.

To test the effect of leaf wetness interruptions under field conditions, plants were inoculated and immediately placed outdoors in full sunlight or under a tree in deep shade (photosynthetically active radiation about 10% of that in full sunlight) between 0900 and 1700 hours and held indoors at 20–25 C during the evening and night to prevent wetting by dew or rainfall. The results of exposure to full sunlight in seven experiments are shown in Table 3. Weather data are from a weather station 1.3 km from the site of the experiments. Significant reductions

Table 2. Effect of temperature and leaf wetness duration on infection of immature, attached photinia leaves by *Entomosporium mespili*

Temperature (C)	Number of infections per 100 cm^2 of leaf surface[a] (hours of leaf wetness)				
	6	9	12	18	24
10	···	···	3 (1–11)	···	78 (22–117)
15	···	0 (0–2)	11 (3–12)	···	22 (21–85)
20	0 (0–1)	11 (3–7)	24 (14–36)	78 (59–193)	137 (45–136)
25	···	1 (2–10)	36 (12–38)	···	36 (22–87)
30	···	···	0 (0–1)	···	0 (0–2)

[a] Lesion density 14 days after inoculation. Data are the means of three inoculated plants (replicates) per treatment and were analyzed after transformation to log $(x + 1)$. In parentheses are the 95% confidence limits for the mean, calculated using the General Linear Models procedure of SAS (Statistical Analysis System, SAS Institute, Inc., Cary, NC). Significant components of the regression were H (hours of wetness), T (temperature), T^2, H^2, $T \times H$, and T^3.

Table 3. Effect of interruption of leaf wetness by a dry, sunny (outdoors) period on infection of attached photinia leaves by *Entomosporium mespili*

Test	Conditions during dry interruption[a]				Wetness preceding (hr)	Leaf spotting[d] (% of control)
	Duration (hr)	Sky	PAR[b]	Temp.[c] (C)		
1	6	Partly sunny	···	20–25	0	100
	24	Partly sunny	···	20–25	0	99
2	24	Sunny	···	26–32	0	2*
3	12	Partly sunny	600–1,970	20–31	0	107
	24	Partly sunny	0–1,970	20–31	0	17*
	36[e]	Sunny	···	17–29	0	3*
4	12	Sunny	600–2,450	24–30	0	56
	24	Sunny	0–2,450	24–30	0	17*
5	5	Partly sunny	400–1,980	16–26	9	34*
6	5	Partly sunny	800–1,060	14–16	0	77
	5	Partly sunny	800–1,060	14–16	5	62
7	5	Partly sunny	400–1,340	22–24	0	250
	5	Partly sunny	400–1,340	22–24	5	203

[a] Plants were inoculated, then placed outdoors from 0900 (first four tests) or 1200 (last three tests) to 1700 hours and held indoors (at 20–25 C) during the evening and night.
[b] Photosynthetically active radiation ($\mu E\ m^{-2}\ s^{-1}$) given as the range of hourly averages during the period of exposure.
[c] Temperature (C) as the range of hourly means during the period of exposure.
[d] Control plants were held in a dew chamber at 25 C for 24 hr immediately after inoculation. Asterisks indicate that the number is significantly different ($P = 0.05$) from the control. There were two to five replicates per experiment.
[e] Conditions on this line are for the second day (no PAR record because of equipment malfunction).

in leaf spotting (compared with plants that received an uninterrupted leaf wetness period) were obtained mainly with dry periods of 24 hr or more on warm days. There was little difference in the lesion density resulting from dry periods applied immediately after inoculation compared with dry periods applied after 5 hr of wetness. None of the shade treatments caused a significant reduction in leaf spotting compared with an uninterrupted wetness period, indicating that the air temperatures encountered in these experiments (Table 3), even those above the maximum for infection, did not reduce survival of conidia. In three additional experiments, leaf wetness interruptions by a 12-hr dark, dry period at 35 C (after 30 min, 12 hr, or 24 hr of leaf wetness at 25 C) reduced conidial survival slightly (to an average of 60%, range 22–106%, of that with uninterrupted wetness periods or wetness periods interrupted by a dry period at 25 C).

Conidia of *E. mespili* appeared moderately resistant to drying and sunlight, although it is possible that many of the conidia surviving one day under sunny conditions were those on shaded leaves or on the lower leaf side. However, long dry periods caused a greater reduction in leaf spotting than shorter periods (Table 3), and it appeared that few conidia would survive several days under summer conditions.

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