Factors Influencing the Growth of *Phomopsis obscurans* and Disease Development on Strawberry Leaf and Runner Tissue

BRIAN C. ESHENAUR, Graduate Research Assistant, and R. D. MILHOLLAND, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

Eshenaur, B. C., and Milholland, R. D. 1989. Factors influencing the growth of *Phomopsis obscurans* and disease development on strawberry leaf and runner tissue. Plant Disease 73:814-819.

Six representative isolates of *Phomopsis obscurans* from strawberry leaves, petioles, and runners were tested at various temperatures for growth and pathogenicity. The optimum temperature for mycelial growth and germ tube elongation for all isolates was 26–32 C. All isolates tested caused symptoms on both leaf and runner tissue; 30 C was the optimum temperature for disease development. Disease severity increased with increasing inoculum concentration and was greatest at 10^7 conidia per milliliter. Disease development was favored by at least 72 hr of leaf wetness after inoculation. Young leaves and runners were more susceptible to *P. obscurans* than mature strawberry tissue.

Leaf blight of the cultivated strawberry (*Fragaria × ananassa* Duch.) is caused by *Phomopsis obscurans* (Ell. & Ev.) Sutton (syn. *Dendrophoma obscurans* (Ell. & Ev.) Anderson); no teleomorph is known for this species.

P. obscurans is distributed worldwide but is limited to *Fragaria* spp. (cultivated and wild). *P. obscurans* infects strawberry fruit as well as leaves (4). In 1984, infections were recorded for the first time on the petioles, fruit trusses, and stolons (runners) (7) (Fig. 1). Runner blight of strawberry became a serious problem in 1985 in the commercial propagation of strawberry plants in North Carolina. Symptoms on the runners are often confused with strawberry anthracnose caused by *Colletotrichum* spp. (6). Symptoms first appear as elliptical lesions approximately 1–2 mm long. These young lesions are often surrounded by red halos. As the lesions expand, they become dark and slightly sunken and may constrict the plant part.

Leaf symptoms first develop as reddish purple lesions occurring in the interveinal regions and are generally circular and small (1–5 mm in diameter). These lesions resemble those of strawberry leaf spot caused by *Mycosphaerella fragariae* (Tul.) Lindau. Leaf blight lesions on leaf veins cause discoloration along the vein that results in an elliptical shaped lesion.

Lesions often coalesce, forming large distinctive V-shaped lesions (Fig. 2) with purple, red, or yellow outer zones. The central portions of the older lesions become dry and necrotic, and pycnidia develop in the necrotic tissue.

Although strawberry leaf blight has been known for many years, it has not been considered economically important. Under certain conditions when highly susceptible cultivars are planted, however, blighting can be severe and result in almost complete defoliation (3). Significant yield losses can be expected from strawberry plants with even moderate amounts of defoliation from the previous year (5).

Inconsistency in symptom development after artificial inoculation has been a persistent problem in studying this disease (1,2,4). The objective of this research was to identify and quantify factors favoring infection. The effects of temperature, inoculum density, moisture period, isolates, and host tissue maturity on disease development were studied in vivo. Studies in vitro examined the optimum temperature for germination and growth of *P. obscurans*.

MATERIALS AND METHODS

Inoculum. Six isolates of *P. obscurans* were obtained from symptomatic strawberry leaves, petioles, and runners collected from several locations in North Carolina and from California and West Virginia during 1967–1986. PO-1, PO-2, PO-7, and PO-10 were isolated from strawberry leaves and PO-4 and PO-6, from runner and petiole tissues, respectively. Cultures were grown on potato-dextrose agar (PDA) at room temperature under continuous fluorescent lighting (40 μE·s^{-1}·m^{-2}) for 3–4 wk. Conidial suspensions were obtained by flooding the cultures with 20 ml of sterile distilled water, scraping the surface with a razor blade, and filtering the suspension through four layers of cheesecloth. The concentration of conidia was adjusted with the aid of a hemacytometer.

Effects of temperature on growth and conidial germination. The radial growth of five isolates of *P. obscurans* was assessed at the following temperatures: 4, 8, 12, 16, 20, 24, 26, 28, 30, 32, 34, and 36 C. Mycelial plugs, 6 mm in diameter, taken from the margin of 5-day-old cultures of each isolate were placed in the center of 100-mm-diameter
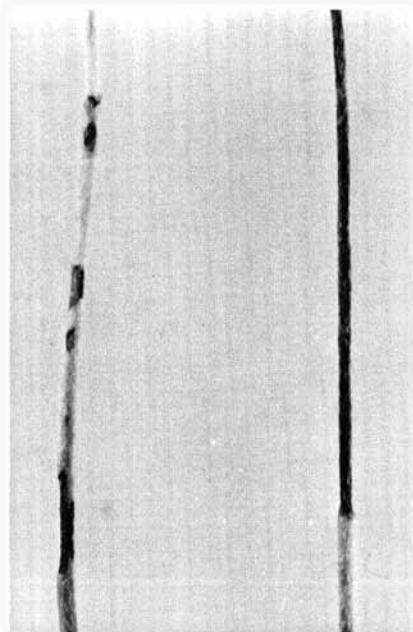

Fig. 1. Necrotic lesions on strawberry cultivar Sparkle caused by *Phomopsis obscurans*.

Fig. 2. Small irregular leaf spots and large V-shaped leaf lesion on strawberry cultivar Sparkle caused by *Phomopsis obscurans*.

petri plates containing approximately 25 ml of PDA. Three replicate cultures of each isolate were incubated in the dark at the above temperatures. Two radial measurements of each replicate were made 84 hr after inoculation. The experiment was repeated three times.

The effects of temperature on spore germination and germ tube elongation were measured at 4, 8, 12, 16, 20, 24, 28, 30, 34, and 38 C. A volume of 0.45 ml of the spore suspension (2.5×10^6 conidia per milliliter) was spread evenly on the surface of a 90-mm-diameter petri plate containing approximately 20 ml of water agar. Two replicate cultures of each isolate were stacked together randomly and incubated in the dark at the above temperatures. The percent germination was measured by counting the number of germinated and nongerminated spores in each of three 0.45-mm-diameter microscope fields per plate after 38 hr. Three representative germ tubes from each plate in each run were measured to assess the mean germ tube length at each temperature. The experiment was repeated three times.

Inoculations. All studies were conducted in controlled-environment chambers and greenhouses in the Southeastern Plant Environment Laboratories at North Carolina State University. Strawberry plants of the cultivar Sparkle were grown in 10-cm-diameter clay pots in a sand-Metromix 220 medium (1:1, v/v) (W. R. Grace & Co., Cambridge, MA). The young plants were placed in a 22/18 C greenhouse 2 mo prior to inoculation and were 3 mo old and well established when inoculated.

Plants were inoculated and incubated with the following procedures unless otherwise noted. A conidial suspension of *P. obscurans* (isolate PO-2) was prepared and adjusted to 2.5×10^6 conidia per milliliter, and two drops of Tween 20 were added to 150 ml of the conidial suspension before inoculation. Three tagged trifoliate leaves on each plant were sprayed with an artist's airbrush to runoff on abaxial and adaxial leaf surfaces. Runners were inoculated by spraying the conidial suspension to runoff on the entire length of tagged runners. Control plants were sprayed with deionized water with two drops of Tween 20 added to 150 ml of water. Inoculated plants were placed in polyethylene bags with two wet paper towels, to maintain free moisture on the inoculated surfaces, and kept at 20 C in diffused light for 5 days. Plants were then placed in growth chambers set at 30 C and a 12-hr photoperiod (350 $\mu E \cdot s^{-1} \cdot m^{-2}$). The treatments were arranged in a randomized complete block design with chambers serving as blocks. All studies were repeated over time in different chambers. Data shown are means of all runs. To test treatment effects, F tests were carried out first to
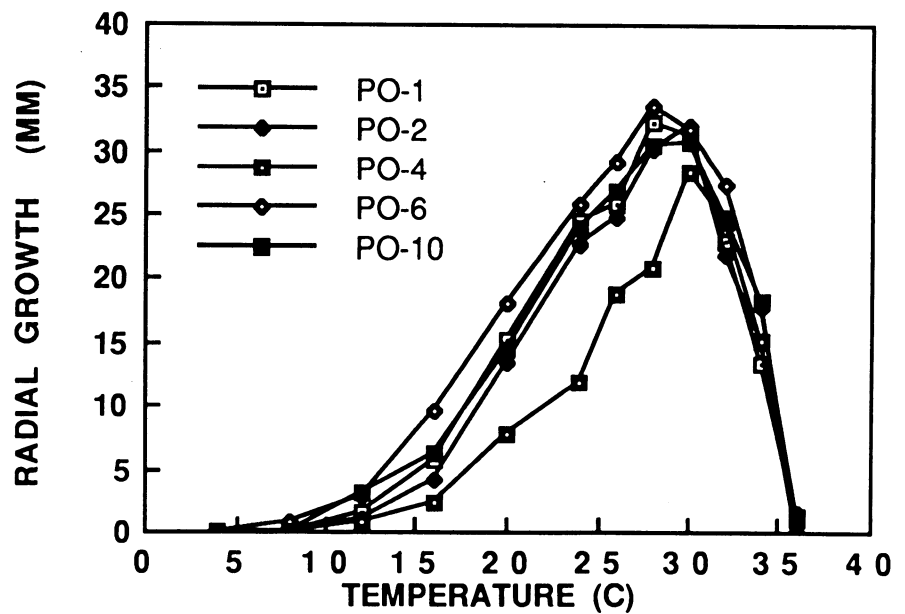

Fig. 3. Effect of temperature on radial growth of five isolates of *Phomopsis obscurans* after 84 hr.
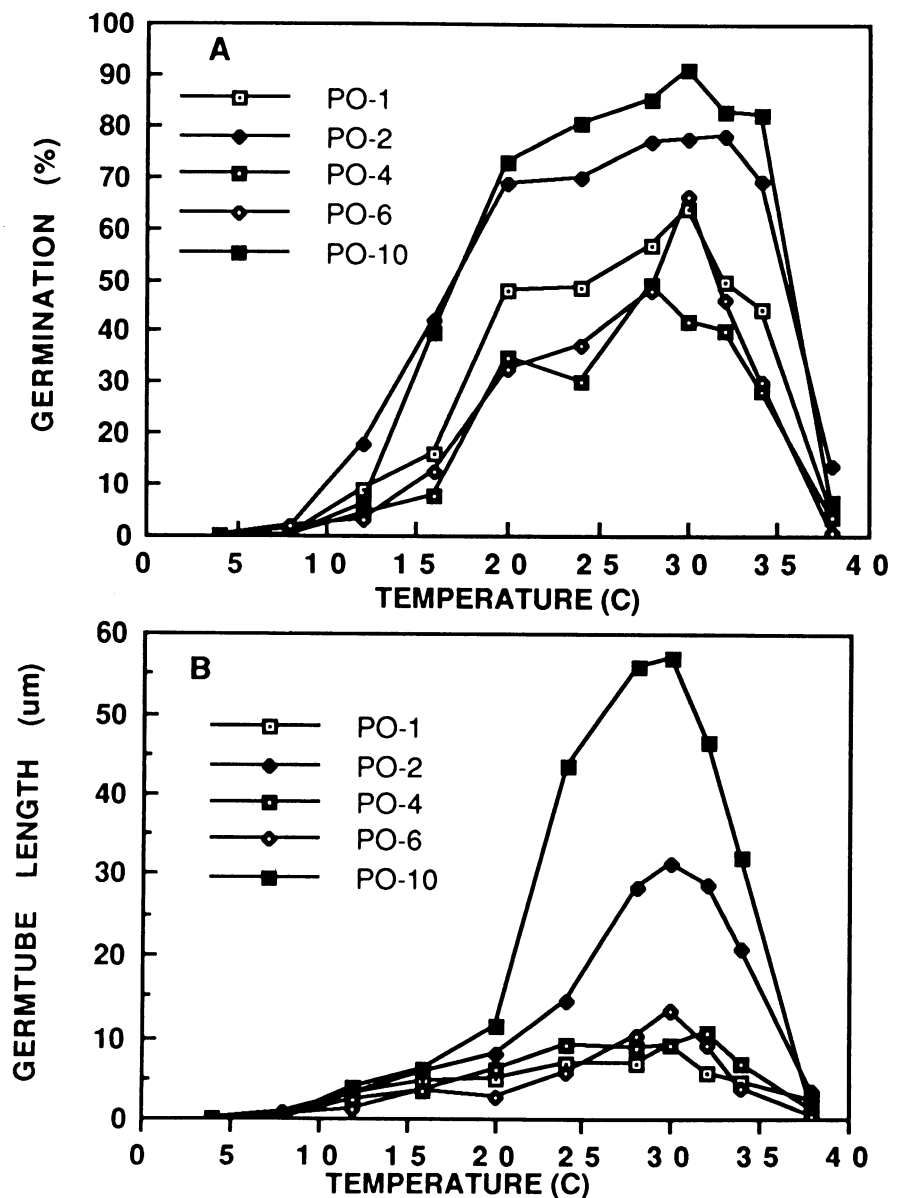

Fig. 4. Effect of temperature on (**A**) germination of conidia and (**B**) germ tube length of *Phomopsis obscurans* after 38 hr.

determine whether to use an error based on replication over chambers or to use an error based on plants within chambers.

Leaf disease severity was recorded for each leaflet at 30 days post inoculation on a 0–5 scale in which 0 = no lesions, 1 = one to five spots, 2 = more than five spots, 3 = elliptical vein lesions, 4 = large V-shaped lesions, and 5 = total necrosis of leaflet. Runner disease was recorded by measuring the total length of the lesions on each runner. Disease severity was expressed as a percentage of lesion development on the inoculated runners' length.

Inoculum density. The effect of inoculum density on disease development was determined on leaf and runner tissue. Conidial suspensions were prepared as described previously and were adjusted to the following concentrations: 5×10^5, 1×10^6, 2.5×10^6, and 1×10^7 conidia per milliliter. Disease severity was recorded for leaves and runners 30 days post inoculation. Five replicate plants with three trifoliate leaves each were inoculated with each inoculum density. Six replicate plants with one runner each were inoculated for each treatment. The experiment was run twice.

Moisture period. The relationship between the duration of leaf wetness after inoculation and disease severity was determined by placing inoculated plants in polyethylene bags with two wet paper towels. The treatment periods in the high humidity were 0, 24, 48, 72, and 120 hr. Four replicate plants with three trifoliate leaves each were inoculated for each treatment. The experiment was done twice.

Leaf and runner maturity. Leaves were removed from plants 45 days before inoculation; new leaves emerging from the crown were tagged and dated daily. Immediately before inoculation, three leaf age categories were identified. Leaves in each category contained an age range of 6 days, and the categories were separated by 7 days. The first category contained only immature leaves that were not fully expanded and ranged in age from −1 to 4 days (leaves less than a day old emerged from the crown and were tagged 1 day after inoculation). The second category contained mature leaves 12–17 days old, and the third category contained mature leaves 25–30 days old. Three trifoliate leaves were inoculated on each of six plants with one leaf in each leaf age category. Disease severity ratings were recorded at 15 and 30 days post inoculation. Analysis was performed by days post inoculation.

Terminals of six runners were marked at regular intervals to establish four different levels of maturity before inoculation. Marks were placed at 2-cm intervals beginning at the growing terminal of the runner and working toward the crowns of the plants. The total length of lesions for each category was measured 15 and 30 days post inoculation. Since the elongation among the segments was not equal (the youngest part of the runner had the greatest rate of elongation), the analysis was performed on the percentage of the total category length that was showing symptoms. The experiment was done twice.

Temperature. The effect of temperature on disease development was determined on strawberry leaves and runners. Following inoculation and the 5-day moisture period, the five replicate plants with three trifoliate leaves each and four replicate plants with one runner each were placed into growth chambers at 15, 20, 25, and 30 C. Plants were monitored for the first appearance of symptoms, and disease severity ratings were recorded at 39 and 49 days post inoculation. The experiment was done twice.

Pathogenicity. The pathogenicity of the six isolates was determined on both leaves and runners. Inoculum was
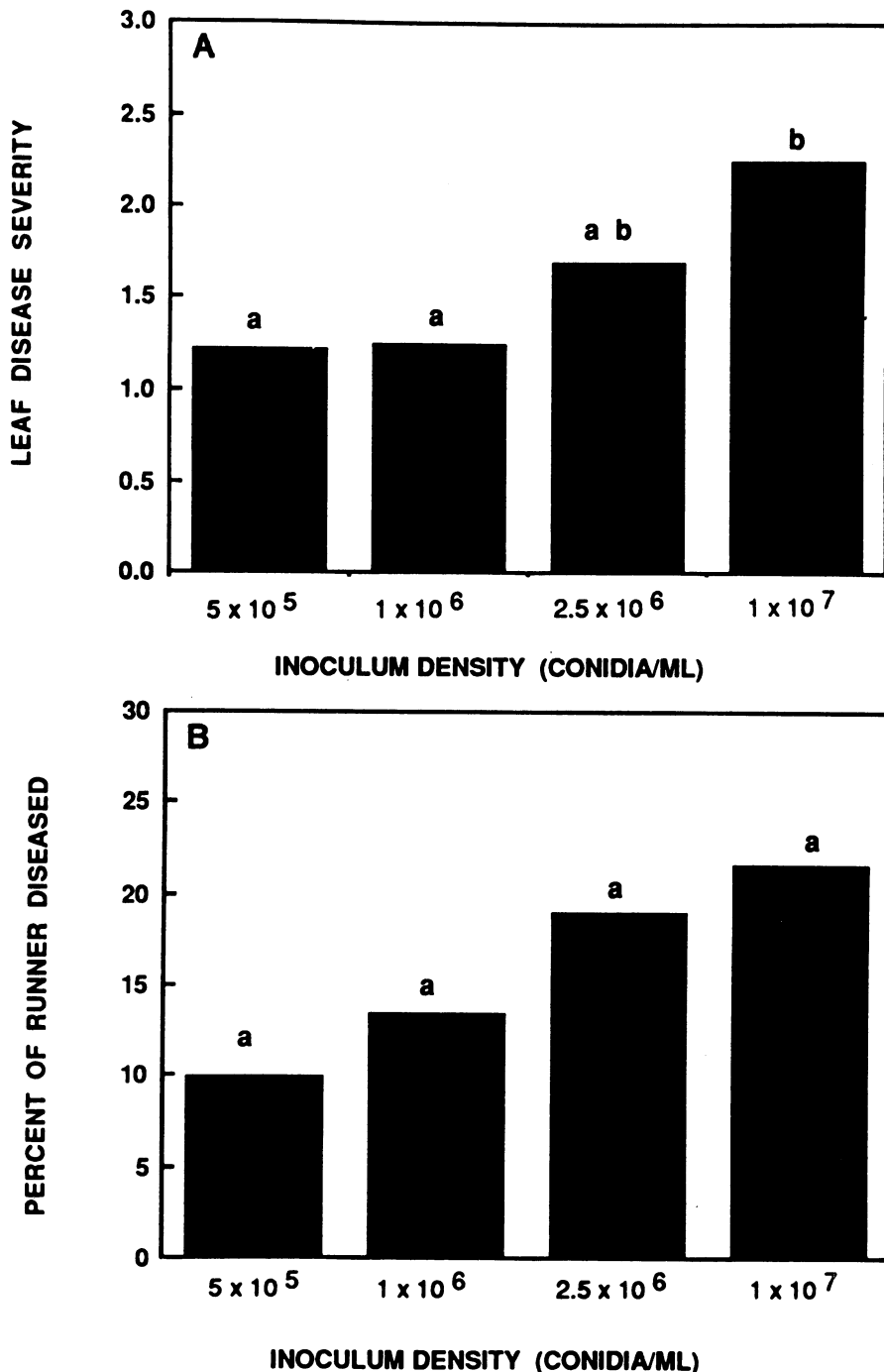

Fig. 5. Effect of inoculum density on (A) leaf disease severity and (B) percentage of runner diseased 30 days post inoculation. Leaf disease severity: 0 = no lesions, 1 = one to five spots, 2 = more than five spots, 3 = elliptical vein lesions, 4 = large V-shaped lesions, and 5 = total necrosis of leaflet. Bars with the same letter(s) are not significantly different according to the least significant difference procedure ($P = 0.05$).

Fig. 6. Effect of postinoculation moisture period on leaf blight severity. Leaf disease severity: 0 = no lesions, 1 = one to five spots, 2 = more than five spots, 3 = elliptical vein lesions, 4 = large V-shaped lesions, and 5 = total necrosis of leaflet.

Fig. 7. Effect of leaf age on disease severity 15 and 30 days post inoculation (DPI). Leaf disease severity: 0 = no lesions, 1 = one to five spots, 2 = more than five spots, 3 = elliptical vein lesions, 4 = large V-shaped lesions, and 5 = total necrosis of leaflet. Bars with the same letter(s) are not significantly different according to the least significant difference procedure ($P = 0.05$).
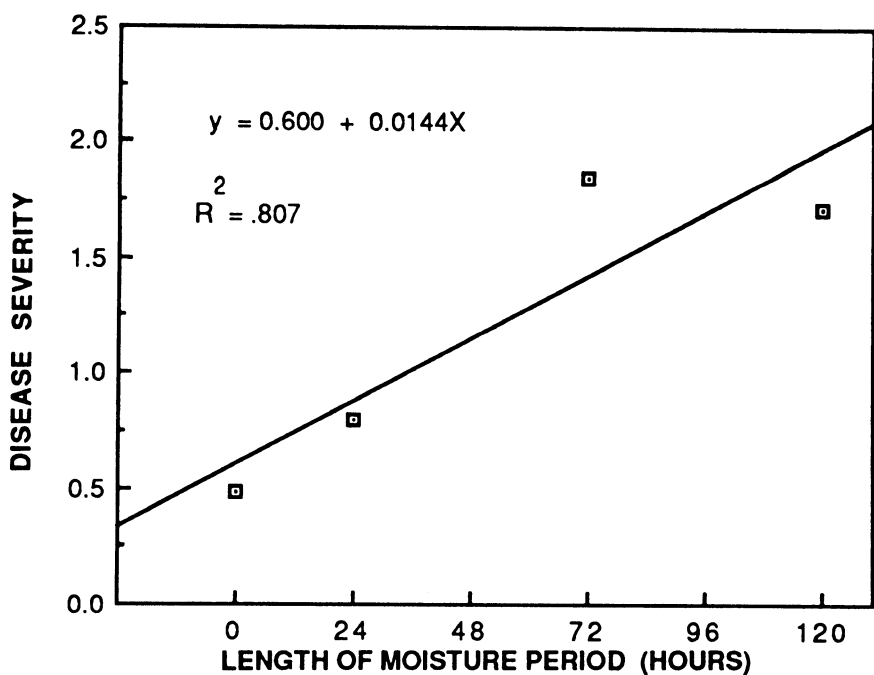
obtained for each isolate and inoculation was carried out as previously described. Six replicate plants with three trifoliate leaves each were inoculated with each isolate. In addition, a single runner from each of eight plants was inoculated with each of the six isolates. Symptoms on the leaves and runners were observed and disease severity ratings were recorded at 15 and 30 days after inoculation. The experiment was done twice.

RESULTS

P. obscurans grew over a wide range of temperatures (Fig. 3). Growth of all isolates occurred from 12 to 32 C. Growth was minimal at 8 and 36 C and maximal between 26 and 30 C.

Conidia germinated at temperatures from 8 to 38 C. Percent germination was highest for isolate PO-10 and lowest for PO-4 (Fig. 4A). Percent germination increased rapidly between 15 and 20 C, remained relatively constant from 20 to 34 C, and declined steeply at 38 C. The optimum temperature for spore germination and germ tube elongation was between 28 and 32 C. Elongation of germ tubes at 30 C was greatest for isolate PO-10 and least for PO-1, PO-4, and PO-6 (Fig. 4B).

Inoculum density. An increase in disease severity on both leaves and runners occurred as conidial concentrations were increased. Low levels of disease resulted when plants were inoculated with 5×10^5 and 1×10^6 conidia per milliliter (Fig. 5). Progressively higher disease severity ratings occurred as the concentration increased to 1×10^7 conidia per milliliter. In the leaf study, because of the large amount of variation in the data, only the highest inoculum density was

significantly different from the others. In the runner study, no significant differences ($P = 0.05$) were detected among the inoculum densities tested.

Moisture period. Disease severity increased with the length of time plants were kept in a saturated environment after inoculation up to 72 hr (Fig. 6). The response of disease severity to the length of moisture period was linear ($R^2 = 0.807$). There was no significant difference between disease severity at 72 hr and at 120 hr.
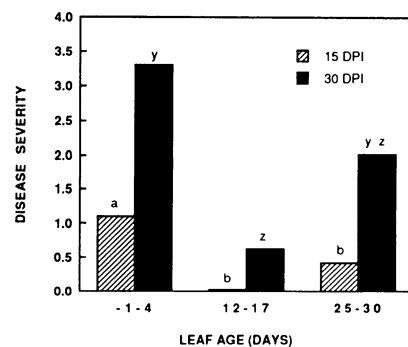
Leaf and runner maturity. The young, immature leaves became symptomatic 7 days after inoculation. Severity of symptoms was also greatest on the young leaves at 15 and 30 days post inoculation (Fig. 7). Leaves in the 25- to 30-day-old category had a higher severity rating than those in the 12- to 17-day-old category, but the difference was not significant.

Total lesion length was greatest on immature runner segments closest to the terminal. The mean lesion lengths at 15 days post inoculation from the most immature segments (0–2 cm) to the most mature (6–8 cm) were 16.3, 5.0, 1.9, and 0.96 mm, respectively (Fig. 8). Total necrosis and death of small inoculated runners sometimes occurred, resulting in only one plant produced per runner
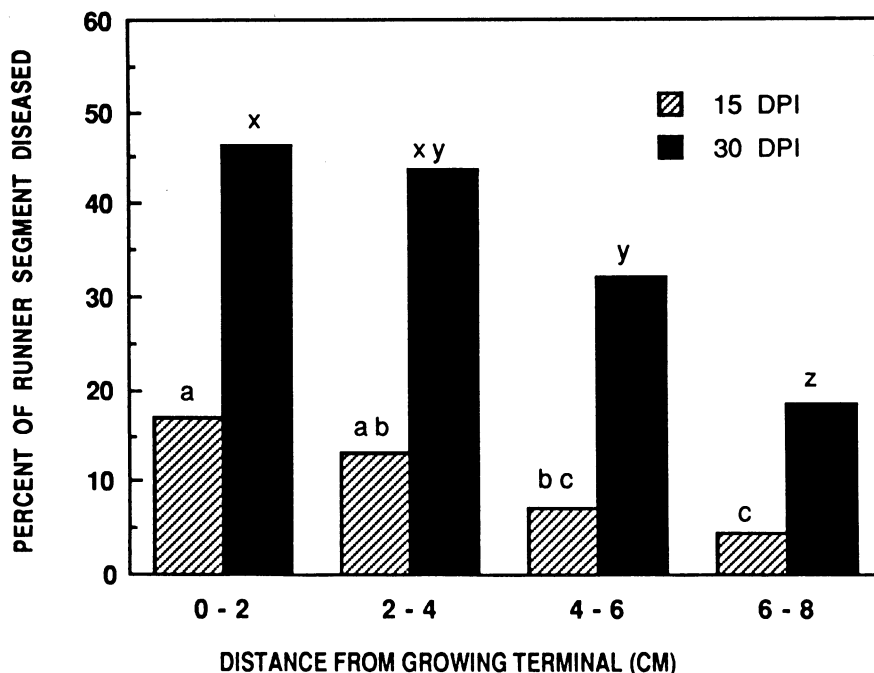
Fig. 8. Effect of runner maturity on disease development 15 and 30 days post inoculation (DPI). Runner segments closest to the terminal (0–2 cm) were the most immature segments and those farthest from the terminal (6–8 cm) were the oldest. Bars with the same letter(s) are not significantly different according to the least significant difference procedure ($P = 0.05$).

Table 1. Severity of symptoms on leaves and runners of the strawberry cultivar Sparkle inoculated with six isolates of *Phomopsis obscurans*

| Isolate | Leaf disease severity[a] | | Percent runner diseased | |
	15 DPI[b]	30 DPI	15 DPI	30 DPI
PO-1	0.18	1.20	6.54	16.82
PO-2	0.17	1.18	8.33	19.97
PO-4	0.34	1.41	5.00	12.34
PO-6	0.10	0.91	2.63	6.53
PO-7	0.22	1.39	4.38	13.72
PO-10	0.22	1.21	10.10	22.67
LSD ($P=0.05$)	NS[c]	NS	NS	9.64

[a] 0 = No lesions, 1 = one to five spots, 2 = more than five spots, 3 = elliptical vein lesions, 4 = large V-shaped lesions, and 5 = total necrosis of leaflet.
[b] DPI = days post inoculation.
[c] NS = not significant.
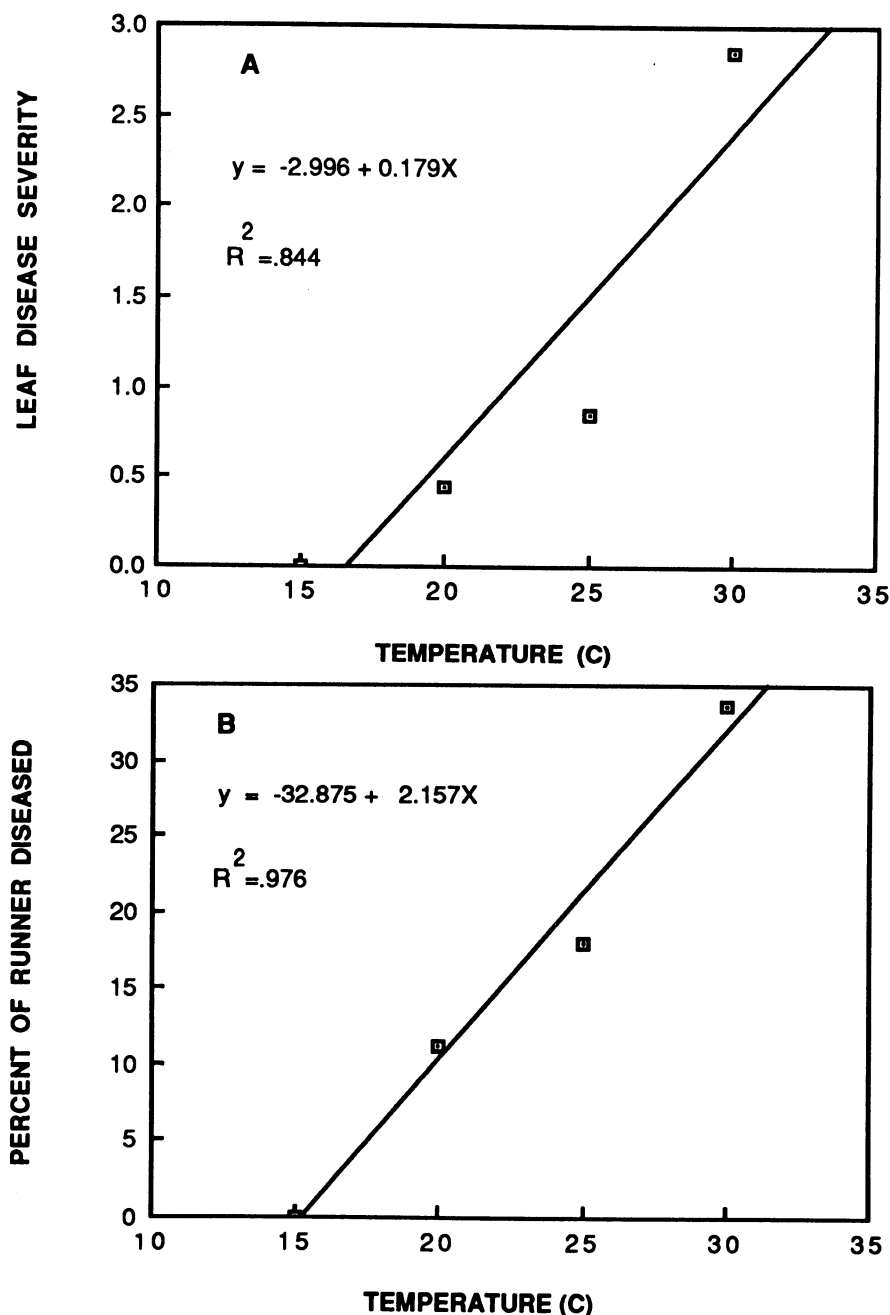
Fig. 9. Effect of temperature on (A) leaf disease severity and (B) percentage of runner diseased 49 days post inoculation. Leaf disease severity: 0 = no lesions, 1 = one to five spots, 2 = more than five spots, 3 = elliptical vein lesions, 4 = large V-shaped lesions, and 5 = total necrosis of leaflet.

(normally, several plants are produced on a single runner).

Temperature. Lesions on leaf and runner tissue developed from 20 to 30 C, with the most rapid development and most severe symptoms at 30 C (Fig. 9). The response of disease severity to temperatures from 15 to 30 was linear on leaves ($R^2 = 0.844$) and runners ($R^2 = 0.976$). Leaf symptoms appeared at 14 days post inoculation. Runner symptoms developed at 12 days post inoculation at 30 C (Fig. 9B). Plants at 25 C expressed both leaf and runner symptoms at 21 days. Runner and leaf symptoms developed at 26 and 34 days post inoculation, respectively, at 20 C. No disease symptoms appeared on plants at 15 C even at 49 days post inoculation. Following the termination of the experiment, however, these plants were maintained at 30 C and symptoms appeared on both leaves and runner within 4 days.

Pathogenicity. All *P. obscurans* isolates tested were capable of causing disease on leaf tissue. There was no difference in the time of appearance of the lesions among the isolates. There was no significant difference in the leaf disease severity rating among isolates at 15 or 30 days post inoculation (Table 1). The same isolates also caused runner lesions. At 30 days post inoculation, there were differences in the percentage of disease on the runners among the isolates. Isolate PO-10 caused the greatest percent infection (22.6) and isolate PO-6 caused the least (6.5).

DISCUSSION

Temperature is an important factor in the infection and development of the leaf and runner blight disease of strawberry caused by *P. obscurans*. Symptoms may not appear for up to 80 days after inoculation in greenhouse tests when temperatures fluctuate greatly (B. C. Eshenaur, *unpublished*). Howard and Albregets (4) also noted that lower incubation temperatures resulted in lower levels of symptom development. All isolates of *P. obscurans* tested grew in vitro over a wide range of temperatures, from 12 to 32 C. The optimum temperature for growth, conidial germination, and germ tube elongation was between 26 and 32 C. Inoculated plants developed symptoms the quickest and became the most severely infected at 30 C. This coincides with the temperatures present when this disease is most severe in midsummer in North Carolina (B. C. Eshenaur, *unpublished*).

In addition to temperature, inoculum density, moisture period, and leaf and runner maturity are all important factors in disease development. Increasing the conidial concentration resulted in an increase in disease severity. The highest disease severity was achieved with a

concentration of 1×10^7 conidia per milliliter. However, a concentration of $2.5-5.0 \times 10^6$ conidia per milliliter would be sufficient for routine inoculations.

An increase in disease severity also was noted when inoculated leaves were exposed to increased durations of leaf wetness after inoculation. After 72 hr, however, the severity of disease appeared to level off and a further increase in disease severity did not occur after 120 hr of free moisture.

Another important factor in disease development is leaf and runner maturity. The youngest leaves, which were not fully expanded, showed symptoms the earliest and had the most severe symptoms throughout the study. The immature runner tissue also was the most susceptible. The immature tissue is probably lacking well-developed cuticles and cell walls, which might allow faster penetration and colonization of host tissue by the pathogen. Although few symptoms developed on the mature leaves 12–17 days old, *P. obscurans* often grew out from leaf sections plated out on PDA. The fungus apparently had infected the leaf tissue but was limited in its colonization. Leaves inoculated at 25–30 days of age had a higher level of symptom development than those inoculated at 12–17 days. These leaves began to senesce naturally 30 days after inoculation, which may have allowed for symptom expression. Tissue maturity is apparently an important factor affecting symptom development. Differences in the age of strawberry leaves inoculated with *P. obscurans* may explain some of the inconsistencies in symptom development in studies by previous investigators (1,2,4). Inoculum density, a leaf wetness period, and an incubation period are other factors to consider when trying to obtain consistent symptom development.

Results of the pathogenicity tests do not appear to indicate the presence of specialization among the isolates. All isolates were capable of causing disease on both leaf and runner tissue. There were no differences in leaf disease severity among the isolates, but there were some differences in disease severity on runners. The isolate that caused the greatest amount of disease on the runners was originally isolated from an infected leaf in 1967, before the reported appearance of the runner disease. Conversely, the isolate that caused the least amount of runner disease was one of the recently collected isolates from an infected petiole. It appears that *P. obscurans* always had the ability to cause lesions on runners and petioles. Symptoms may have been confused with anthracnose (caused by *Colletotrichum* spp.) or possibly leaf scorch (caused by *Diplocarpon earliana* (Ell. & Ev.) Wolf) (6). The runner blight phase may have become more severe in recent years because of a buildup of inoculum caused by a widespread planting of susceptible cultivars. Howard and Albregets (3) reported an increase in incidence of leaf blight in Florida resulting from large plantings of susceptible cultivars.

LITERATURE CITED

1. Anderson, H. W. 1920. *Dendrophoma* leaf blight of strawberry. Ill. Agric. Exp. Stn. Bull. 229. 10 pp.
2. Fall, J. 1951. Studies on fungus parasites of strawberry leaves in Ontario. Can. J. Bot. 29:229-315.
3. Howard, C. M., and Albregets, E. E. 1972. Control of leaf blight in Florida strawberry nurseries. Plant Dis. Rep. 56:23-25.
4. Howard, C. M., and Albregets, E. E. 1973. A strawberry fruit rot caused by *Dendrophoma obscurans*. Phytopathology 63:419-421.
5. Kerkhoff, K. L., Williams, J. M., and Barden, J. A. 1988. Effects of defoliation on growth and yield of 'Redchief' strawberries. Adv. Strawberry Prod. 7:26-28.
6. Maas, J. L., ed. 1984. Compendium of Strawberry Diseases. American Phytopathological Society, St. Paul, MN. 138 pp.
7. Maas, J. L. 1985. New symptoms of strawberry leaf blight disease. Adv. Strawberry Prod. 4:34-35.