Infection of Juniperus virginiana and J. scopulorum by Phomopsis juniperovora

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ABSTRACT

Nonwounded new foliage of *Juniperus virginiana* and *J. scopulorum* was highly susceptible to infection by *Phomopsis juniperovora*; lesions did not develop on old foliage. Infection occurred with 7 hr incubation at 100% relative humidity, 24 C. Infection occurred over the range 12 to 32 C; intensity was greater at 24 and 28 C. Symptoms, consisting of small, light-colored lesions, developed 3 to 5 days after inoculation. Disease development was enhanced by high postincubation temperatures (32 C). Pycnidia with viable spores developed 3 weeks after inoculation. Germination of spores, germ-tube development, and growth in culture were optimum near 24 C. Spores from tendrils exposed to high (43 C) and low temperatures (-22 C) still germinated. Spores that had been hydrated, then desiccated, subsequently caused infection when conditions became suitable. Light had no effect on spore germination, germ-tube development, growth of the fungus in culture, or infection.

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Phomopsis juniperovora Hahn commonly infects Cupressaceae species in nurseries and landscape plantings through the Midwest, New England, and much of the South (1). This fungus is especially damaging to eastern redcedar (*Juniperus virginiana* L.) and Rocky Mountain juniper (*J. scopulorum* Sarg.) seedlings, and entire beds of 1st- and 2nd-year seedlings are commonly lost. Infected *J. virginiana* stock which survives in the nursery has low survival when outplanted (3, 5).

For many years, control of this disease with fungicides had been unsatisfactory. Recently, good control in *J. virginiana* seedling beds has been obtained with phenyl mercury fungicides such as Puratized Agricultural Spray (6, 7). Control has required frequent (usually weekly) applications of fungicide during the growing season.

Because of the high cost of frequent fungicide applications and the possibility of toxic residue buildup in the soil, control methods involving less frequent application of fungicides are needed. More information is required, however, about the factors related to infection by *Phomopsis juniperovora*. The intensity of infection within and between seasons is highly variable, but the conditions under which epidemics develop are not known. This paper reports on work undertaken preliminary to epidemiological studies of *Phomopsis* blight.

MATERIALS AND METHODS.—*Growth in culture.*—Mass spore isolates from eastern redcedar seedlings were used in growth studies. Stock cultures were maintained on cornmeal agar containing 2% sucrose. Dishes were seeded in the center with 4-mm^2 discs from margins of 5- to 8-day-old cultures on cornmeal sucrose agar. Four Difco culture media, Falcon plastic petri dishes (100 × 15 mm), and incubators with ± 0.5 C control were used. Tests were run in triplicate.

Spore germination.—Spores were obtained from pycnidia on eastern redcedar seedlings. Pycnidia-bearing tissues were placed in dewpoint chambers (24 C) to obtain spore tendrils. Tendrils were placed in distilled water and agitated to disperse spores. Spore concentrations were determined with a hemacytometer. Spores were incubated on agar media in plastic petri dishes.

Spores were placed in the media by dipping a glass rod (8 mm diam) into a spore suspension, which was then touched to the agar surface. Spores were incubated for 18 hr in incubators with ± 0.5 C control, then killed with mercuric chloride. Germ tubes (30) were measured with an ocular micrometer, and germinated spores (no. in 100) were counted at × 430 magnification in each of three replications.

Seedling infection.—Eastern redcedar and Rocky Mountain juniper seedlings grown in greenhouses from seed were used in infection studies. Inoculations were made with spores suspended in water. Spore suspensions (ca. 5×10^5/ml) were applied to seedlings with an atomizer. Each seedling was sprayed ca. 10 sec. Inoculated seedlings were placed in growth chambers (ISCO, E-3, 16 hr/day light, ca. 1,200 ft-c); after incubation, they were placed in greenhouses with night temperatures about 24 C or in growth chambers with temperatures controlled and 40% relative humidity.

RESULTS.—*Growth in culture.*—Growth of *Phomopsis juniperovora* on the 4 different agar media was optimum near 24-26 C (Fig. 1). The rate of growth from 3 to 9 days was linear with time. Growth was greater on potato-dextrose agar (PDA) and cornmeal sucrose agar than on prune and malt extract agars. Colony diameters of 27 isolates after 6 days at 24 C on cornmeal sucrose agar ranged from 5.3 to 8.0 cm. Seventeen isolates had colony diameters between 7 and 8 cm; 8 were between 6 and 7 cm. Growth was as rapid in the dark as it was in the light (ca. 100 ft-c).

All isolates developed a yellow color in advance of the colony periphery. The color intensity of all isolates was the same whether grown in light or dark. Orange-red crystals formed in all media. The compound forming these crystals has recently been identified as altersolanol A (9).

Sporulating pycnidia were more numerous in cultures grown under fluorescent illumination than in

cultures grown in the dark. Most of the pycnidia produced in the dark were infertile.

Spore extrusion and germination.—Spores were extruded from pycnidia in tendrils or in globules. Globules usually formed when free water was present on tissues containing pycnidia. Temperature affected the extrusion of spores from pycnidia on branches kept in the dark at 100% relative humidity. Spores were extruded within: 23 hr at 24 and 28 C; 30 hr at 20 C; 45 hr at 16 C; and 93 hr at 12 C. Spores were not extruded at 32 or 36 C after 165 hr. Spores were extruded at similar rates whether pycnidia-bearing

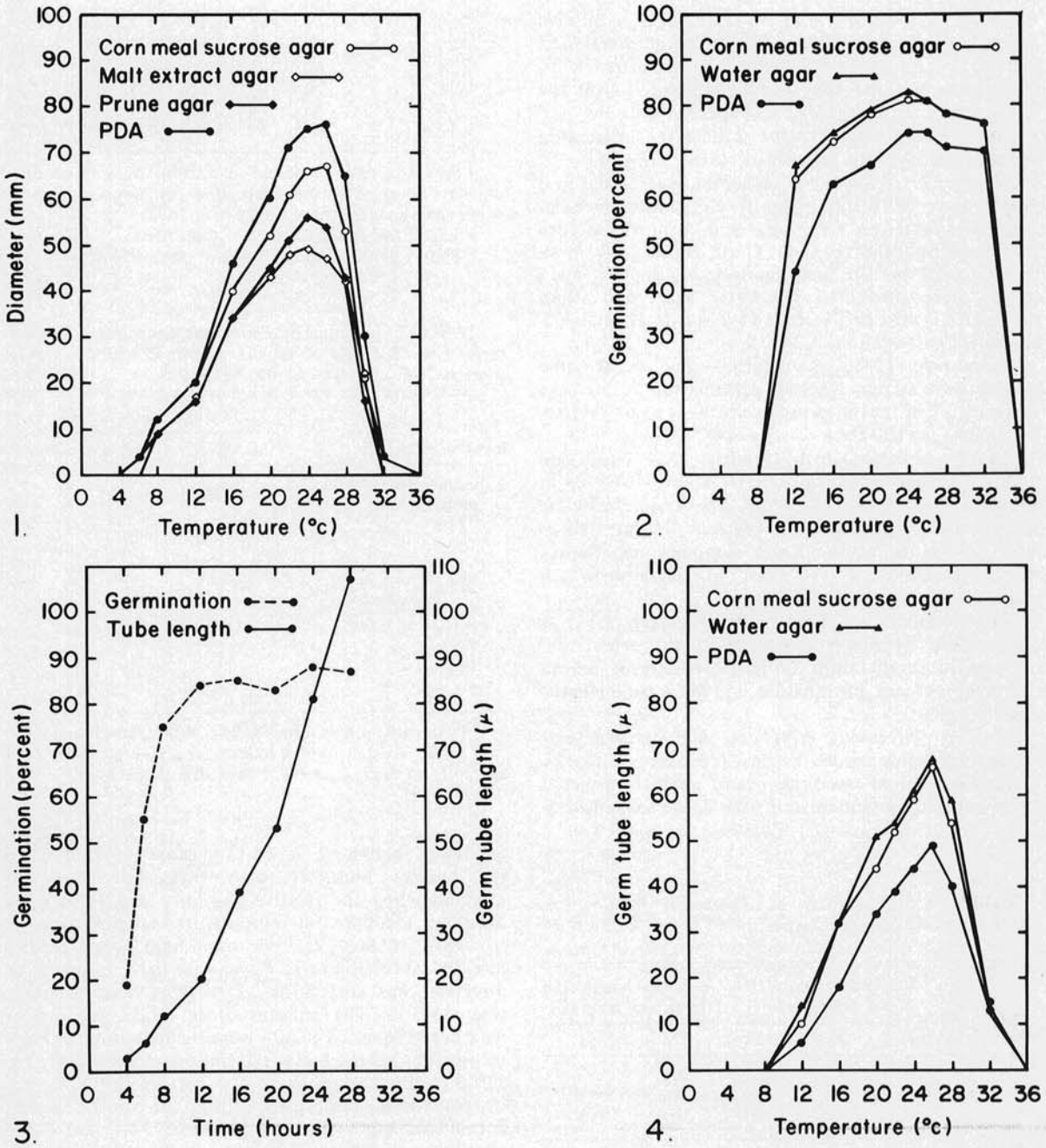

Fig. 1-4. 1) Effect of temperature on colony diameter of *Phomopsis juniperovora* grown for 6 days on different agar media. **2)** Effect of temperature on germination of *Phomopsis juniperovora* spores incubated for 18 hr on different agar media. **3)** Germ tube length and percent germination of *Phomopsis juniperovora* spores incubated for various periods of time on water agar at 24 C. **4)** Effect of temperature on length of germ tubes of *Phomopsis juniperovora* spores incubated on various agar media for 18 hr.

tissues were incubated in light or dark at 24 or 28 C and 100% relative humidity.

Spores in tendrils were tightly bound by a matrix. Considerable agitation was required to disperse spores in tendrils placed in water. Spores were not dispersed after 7 hr when tendrils were placed in water and not agitated.

Alpha spores of *Phomopsis juniperovora* germinated over the temperature range 12 to 32 C when incubated for 18 hr on water agar, cornmeal sucrose agar, and PDA (Fig. 2). Spores germinated at 8 C after incubation for 34 hr but not after 18 hr. More than 60% of the spores germinated over the temperature range 12 to 32 C (Fig. 2).

Germ tubes were evident 4 hr after incubation started. Germ tubes developed slowly from 4 to 12 hr; from 12 to 28 hr, development was rapid and nearly linear with time (Fig. 3). Germ tube development was better on water agar and cornmeal sucrose agar than on PDA (Fig. 4). Germ tubes grew more rapidly, and percent germination was higher when spores were incubated on water agar than when incubated in distilled water (44 μ versus 10 μ; 100% versus 15% after 18 hr at 24 C).

Percentage spore germination and germ tube lengths were similar whether germination occurred in the dark or in incandescent (ca. 100 ft-c) or fluorescent light (ca. 100 ft-c).

Spores obtained from tendrils that had been exposed to temperatures of 1, 10, and 24 C for 24 hr germinated 100%; exposure at 33-43 C resulted in 80% germination and germ tubes about one-half as long (25 μ) as at the lower temperatures. Spores subjected to -22 C in water or in tendrils still germinated.

Spores which had been hydrated, then dried at temperatures ranging from 4 to 54 C, still germinated. Increase in temperature or length of drying period decreased percent germination and germ tube lengths (Tables 1, 2).

Seedling infection, effect of temperature, and humidity.—Nonwounded eastern redcedar and Rocky Mountain juniper seedlings were readily infected when foliage was inoculated with spore suspensions.

TABLE 1. Germination of *Phomopsis juniperovora* spores following drying of spores for 12 hr and 24 hr at 24 and 54 C[a]

Preincubation drying period (hr)	Germination[b] (%) by drying temp (C)		Germ tube length[c] (μ) by drying temp (C)	
	24	54	24	54
0	97		92	
12	57	36	46	23
24	13	26	22	13

[a] Spores initially dispersed in distilled water, then dried on glass slides, then deposited by inverting slides on water agar and incubated for 18 hr at 24 C.
[b] Basis: 100 spores on each of three plates.
[c] Basis: 30 germ tubes on each of three plates.

TABLE 2. Germination of *Phomopsis juniperovora* spores following drying of spores for different periods at 4, 24, and 38 C[a]

Preincubation drying period (hr)	Germination[b] (%) by drying temp (C)			Germ tube length[c] (μ) by drying temp (C)		
	4	24	38	4	24	38
0		100			48	
1	100	84	90	38	41	39
2	68	92	80	42	42	32
3	82	81	90	38	40	40
4	62	72	80	38	38	38
6	70	67	68	41	37	33
8	80	79	70	30	31	30
12	67	47	56	32	24	24

[a] Spores initially dispersed in distilled water, then dried on glass cover slips, then deposited (by inverting slips on water agar) and incubated for 18 hr at 24 C.
[b] Basis: 100 spores on each of three plates.
[c] Basis: 30 germ tubes on each of three plates.

TABLE 3. Infection by *Phomopsis juniperovora* of *Juniperus virginiana* and *J. scopulorum* incubated for various times at 24 C and 100% relative humidity[a]

Experiment and incubation period	Number of lesions[b]	
	J. virginiana	*J. scopulorum*
Experiment No. 1		
6 hr	0	0
7 hr	2	1
8 hr	6	4
9 hr	9	6
24 hr	81	60
Experiment No. 2		
1 day	122	95
2 days	125	84
3 days	122	88
4 days	70	45

[a] Postincubation conditions: 24 C, 40% relative humidity.
[b] Basis: average number of lesions on 10 branchlets, 2 cm in length, on each of 2 trees, 7 days after inoculation.

Seedlings incubated at 24 C became infected when the relative humidity was 100%, but were not infected when the relative humidity was 91 or 82%. Seedlings incubated at temperatures ranging from 16 to 32 C (100% relative humidity) were readily infected. Infection at 12 C was very light. Intensity of infection was greater at 24 to 28 C than at other temperatures. The amount of infection was similar whether inoculated plants were incubated in the dark or in the light (2-day continuous fluorescent, ca. 1,200 ft-c).

Incubation period.—Seedlings of both species became infected when incubated at 24 C and 100% relative humidity for 7 hr but not for 6 hr (Table 3). The numbers of lesions on seedlings incubated for 1, 2, or 3 days were similar; seedlings incubated for 4 days had fewer lesions. Incubation for 9 hr at 28 and 32 C did not result in more lesions than at 24 C.

TABLE 4. Effect of length of incubation at 100% relative humidity and postincubation temperature on number of lesions[a] developed on *Juniperus virginiana* and *J. scopulorum* inoculated with *Phomopsis juniperovora*

Incubation			Number of lesions	
Temp (C)	Days	Postincubation	*J. virginiana*	*J. scopulorum*
20	7	None	34	0
20	2	Greenhouse[b], 5 days	66	37
24	7	None	36	0
24	2	Greenhouse[b], 5 days	93	40
28	7	None	38	43
28	2	Greenhouse[b], 5 days	51	38
32	7	None	44	24
32	2	Greenhouse[b], 5 days	46	28

[a] Average number of lesions on 10 branchlets, 2 cm in length on each of three seedlings, 7 days after inoculation.
[b] Postincubation temperature: *J. virginiana* test: average, 24 C (range 14-35); *J. scopulorum* test: average, 27 C (range 18-37).

TABLE 5. Effect of continuous low-temperature incubation at 100% relative humidity and high temperatures during postincubation on number of lesions on *Juniperus virginiana* and *J. scopulorum* inoculated with *Phomopsis juniperovora*

Incubation			Number of lesions[a]	
Temp (C)	Days	Postincubation	*J. virginiana*	*J. scopulorum*
20	2	Greenhouse, 5 days[b]	62	43
20	7	None	6	4
20[c]	7	Greenhouse, 3 days[d]	30	38
24	2	Greenhouse, 5 days[b]	74	75
24	7	None	20	27
24[c]	7	Greenhouse, 3 days[d]	50	49

[a] Average number of lesions on 10 branchlets, 2 cm in length on each of three seedlings. Lesions counted after 7 days on seedlings receiving the first two treatments at each incubation temperature.
[b] Greenhouse temperature averaged 26 C (range 17-37).
[c] Lesions counted after 10 days on the same seedlings which had been evaluated after 7 days' incubation.
[d] Greenhouse temperature averaged 27 C (range 18-37).

Postincubation effects.—Postincubation conditions had a striking effect on disease development. At lower temperatures (20-24 C), plants incubated for 2 days and then placed in a greenhouse had many more lesions than plants incubated for 7 days (Table 4). At higher temperatures (28-32 C), lesion numbers were similar when plants were incubated for 2 or 7 days. Trees of both species incubated at low temperatures (20-24 C) for 7 days subsequently developed considerable numbers of lesions after 3 days' exposure to higher temperatures in a greenhouse (Table 5). Accordingly, it seems that higher temperatures do not influence infection per se, but rather the rate at which disease development proceeds. Inoculated plants that had low-temperature (24 C) incubation interrupted by 3, 12, and 24 hr of high temperature incubation, progressively had more lesions with increasing length of high-temperature incubation (Table 6).

Influence of drying on infection of inoculated plants.—Inoculated junipers kept in a drying atmosphere (24 C, 40% relative humidity) for up to 24 hr immediately after inoculation became infected following incubation for 3 days at 24 C and 100% relative humidity, but number of lesions were fewer as drying time increased. Furthermore, inoculated seedlings incubated for 2, 4, or 6 hr (24 C, 100% relative humidity), then placed in a drying atmosphere (24 C, 40% relative humidity) for 2, 4, or 8 hr, then incubated for 3 days (24 C and 100% relative humidity) became infected (Table 7).

Disease development.—Small, light-colored lesions were observed on both species as early as 3 days after inoculation. Lesions formed on juvenile, whip, and spur leaves [leaf terminology of Hall (2)], but only on newly developed leaves; older leaves of the three types were resistant to infection. The newly developed leaves were susceptible only during the period when they were light green; as they turned dark green, they became resistant. However, the fungus spread from young leaves to stem tissue and older leaves. Spread was more rapid from juvenile leaves than from whip and spur leaves. Pycnidia with viable spores were observed 3 weeks after inoculation; production was extensive after 4 weeks.

Infection of wounded stems.—Infection resulted

when stems were inoculated by (i) placing mycelium or spores beneath bark flaps; and (ii) puncturing or slicing stems which had spores deposited on them.

Canker development was studied on eastern redcedars of two diameter classes which had been inoculated by placement of mycelium beneath bark flaps. More terminals were killed after 3 and 12 months on the smaller-diameter seedlings than on the larger seedlings (Table 8). Cankers extended only a short distance below the point of inoculation (Table 8).

In another experiment, the fungus was recovered 2.5 cm above the inoculation point 16 days after inoculation, 5.0 cm above after 28 days, and 7.5 cm above after 42 days. The fungus was recovered 2.5 cm below the inoculation point 28 days after inoculation.

TABLE 6. Effect of short periods of high (32 C) postincubation temperature on number of lesions on *Juniperus virginiana* and *J. scopulorum* inoculated with *Phomopsis juniperovora* and incubated at 24 C and 100% relative humidity for 2 days

Postincubation (40% relative humidity)	Number of lesions[a]	
	J. virginiana	*J. scopulorum*
24 C, 4 days	64	51
32 C, 3 hr; 24 C, 3 days, 21 hr	77	67
32 C, 12 hr; 24 C, 3 days, 12 hr	90	70
32 C, 24 hr; 24 C, 3 days	97	80

[a] Average number of lesions on 10 branchlets, 2 cm in length, on each of three seedlings, 6 days after inoculation.

TABLE 7. Infection of *Juniperus virginiana* and *J. scopulorum* which were inoculated with *Phomopsis juniperovora* conidia, then incubated, dried, and reincubated

Incubation period[a]	Drying time[b] after initial incubation (hr)	Number of lesions[c] after 3-day incubation period[d]	
		J. virginiana	*J. scopulorum*
2 hr	2	46	88
	4	51	55
	8	26	34
4 hr	2	27	27
	4	22	12
	8	10	21
6 hr	2	24	20
	4	18	20
	8	12	8
Check	0	45	69

[a] 24 C, 100% relative humidity (RH).
[b] 24 C, 50% RH.
[c] Basis: average number of lesions on 10 branchlets, 2 cm in length, on each of two trees.
[d] 24 C, 100% RH.

In a similar experiment with Rocky Mountain junipers, girdling was less extensive than in eastern redcedar. Stems averaging 5.7 or 6.7 mm in diam were not girdled 4 months after inoculation.

The fungus persisted in inoculated trees. After 15 months, it was isolated from the point of inoculation on 22 of 40 trees, and from the lower edge of cankers on 24 of 40 trees. After 24 months, the fungus was isolated from the point of inoculation on 2 of 40 trees, and from the lower edge of cankers on 13 of 40 trees. Pycnidia were not observed on tissues before or after plating, indicating that the fungus persisted as mycelium.

TABLE 8. Canker development and girdling on 20 *Juniperus virginiana* seedlings of two diameter classes, wound-inoculated on stems with *Phomopsis juniperovora*

Item	Diameter classes	
	6.7 mm	9.5 mm
Terminals dead above inoculation point		
At 3 months	75%	65%
At 12 months	95%	70%
Extent of canker below inoculation point		
At 3 months	2.1 cm	2.5 cm
At 12 months	2.5 cm	3.1 cm
Extent of canker above inoculation point[a]		
At 3 months		6.9 cm
At 12 months		8.0 cm

[a] Average from six trees with terminals not killed.

DISCUSSION.—The results show that nonwounded new foliage of both juniper species is susceptible to *Phomopsis juniperovora*. Thus, junipers in seedling beds will need to be protected at those times during the growing season when they have new, susceptible foliage. Since infection can occur after only 7 hr incubation at 100% relative humidity, it is unlikely that limitation of fungicide applications to rainfall periods will control the disease. Systemic fungicides which translocate to new growth will be required if control is to be obtained with fewer applications.

Symptoms (lesions) developed as early as 3 days after inoculation, and were numerous 5 days after inoculation. This contrasts with a report by Pero & Howard (4) that, in Rhode Island, symptoms developed 4 to 6 weeks after inoculation. Results from experiments reported here showed that symptoms were suppressed by continuous incubation at low or moderate temperatures (20-24 C). The rate of disease development was enhanced by higher temperatures (28-32 C). Even short periods (12 hr) of high temperature were effective. Thus, it seems unlikely that low temperatures could account for the difference in rate of disease development. These facts indicate that the time when infection occurred cannot be accurately estimated from the time when symptoms appear.

Lesions never developed on older foliage of either species. The recent work of Pero & Howard (4) is of

interest in this respect. They found that diffusates from young foliage of *J. virginiana* readily stimulated germination of *P. juniperovora* spores, whereas diffusates from dormant foliage did not.

Both juniper species were highly susceptible to *P. juniperovora*, although *J. virginiana* was somewhat more susceptible than *J. scopulorum*. This is in accord with observations of both species in seedling beds (10). Susceptibility comparisons can be misleading if the stage of growth is not considered. Susceptible new foliage develops at different rates. In the greenhouse, new foliage developed sooner on *J. virginiana* than on *J. scopulorum* of the same age.

There is great potential for heavy infection, even in relatively hot and dry growing seasons. Moisture is essential for extrusion of spores; furthermore, spores extruded in tendrils are tightly bound; thus, moisture is needed for dispersion. However, spores in tendrils which have been exposed to both high and low temperature extremes will germinate. Furthermore, spores which were hydrated at time of dispersal, then subsequently desiccated, can still cause infection when moisture and temperature conditions become suitable. Though 24 C is about optimum for spore germination, germ-tube development, growth of the fungus in culture, and infection, disease development proceeds at a higher rate at higher temperatures (32 C).

Light had no influence on spore germination, germ-tube development, or growth of the fungus in culture. This is consistent with infection results; seedlings incubated in the light had about the same amount of infection as seedlings incubated in the dark.

Only stems of small diameter are girdled. Thus, well-established junipers in plantings are not likely to be killed by this fungus. Older trees with extensive infection will be unsightly, however, due to numerous small dead branches. That only small-diameter stems were girdled in the wound-inoculated trees is in accord with measurements made in heavily infected 7-yr-old eastern redcedars. These trees had extensive infection over their entire crowns, yet girdling was confined to branches that were 3 mm or less in diameter.

There is high potential for this fungus to persist for long periods as mycelium. In this study, the fungus was recovered from seedlings in the greenhouse 24 months after inoculation, and Scheld & Kelman (8) reported that the fungus can overwinter as mycelium in North Carolina.

The fungus spreads more rapidly from new juvenile leaves than from new whip or spur leaves; thus, any treatment which causes formation of juvenile needles, such as pruning, will result in more damage.

LITERATURE CITED

1. HAHN, G. G. 1943. Taxonomy, distribution, and pathology of Phomopsis occulta and P. juniperovora. Mycologia 35:112-129.
2. HALL, M. T. 1952. Variation and hybridization in Juniperus. Ann. Missouri Bot. Garden 39:1-64.
3. HODGES, C. S., & H. J. GREEN. 1961. Survival in the plantation of eastern redcedar seedlings infected with Phomopsis blight in the nursery. Plant Dis. Reptr. 45:134-136.
4. PERO, R. W., & F. L. HOWARD. 1970. Activity of juniper diffusates on spores of Phomopsis juniperovora. Phytopathology 60:491-495.
5. PETERSON, G. W. 1965. Field survival and growth of Phomopsis-blighted and nonblighted eastern redcedar planting stock. Plant Dis. Reptr. 49:121-123.
6. PETERSON, G. W., D. NULAND, & J. L. WEIHING. 1960. Test of four fungicides for control of cedar blight. Plant Dis. Reptr. 44:744-746.
7. PETERSON, G. W., D. R. SUMNER, & C. NORMAN. 1965. Control of Phomopsis blight of eastern redcedar seedlings. Plant Dis. Reptr. 49:529-531.
8. SCHELD, H. W., JR., & A. KELMAN. 1963. Influence of environmental factors on Phomopsis juniperovora. Plant Dis. Reptr. 47:932-935.
9. WHEELER, D. M. S., M. M. WHEELER, & G. W. PETERSON. 1971. Identification of altersolanol A as a metabolite of Phomopsis juniperovora Hahn. Nebraska Acad. Sci. Proc. 1971:34-35 (Abstr.).
10. WRIGHT, E. 1942. Cedar blight. Amer. Nurseryman 75:14, 25.