Effect of Benomyl on *Phomopsis juniperovora* Infection of *Juniperus virginiana*

J. D. Otta, D. J. Fiedler, and V. H. Lengkeek

Associate professor, former assistant-in, and former graduate research assistant, respectively, Department of Plant Science, South Dakota State University, Brookings, SD 57007.
Journal Series Paper 1633 of the South Dakota State Agricultural Experiment Station.
Accepted for publication 23 July 1979.

ABSTRACT

OTTA, J. D., D. J. FIEDLER, and V. H. LENGKEEK. 1980. Effect of benomyl on *Phomopsis juniperovora* infection of *Juniperus virginiana*. Phytopathology 70:46-50.

Growth of *Phomopsis juniperovora* was reduced in vitro by benomyl concentrations as low as 0.25 mg/L. Benomyl applied as a soil drench to *Juniperus virginiana* in the greenhouse was translocated to the foliage. Foliage was protected from Phomopsis blight whenever fungitoxicant in the new growth exceeded 3 μg/g of fresh weight. Benomyl applied as a spray to the foliage was not translocated to portions of the foliage protected from the spray. Benomyl applied as a spray to *J. virginiana* during their third growing season in a nursery significantly reduced: the incidence of Phomopsis blight, the disease severity on infected trees, the length of diseased tissue (mm) on infected trees with pycnidia of *P. juniperovora*, and the percent of pycnidia containing spores. None of the benomyl spray or drench treatments prevented spore germination or initial infection. Infection studies in the field indicated that *J. virginiana* was susceptible to *P. juniperovora* throughout the growing season.

Additional key words: Phomopsis blight.

Phomopsis blight is often a serious disease problem in nurseries producing junipers and related conifers (15,16,19). The causal fungus, *Phomopsis juniperovora* Hahn, is widely distributed throughout the Midwest, New England, and much of the South (6,7). Although it is especially damaging in nursery production of eastern redcedar (*Juniperus virginiana* L.) and Rocky Mountain juniper (*J. scopulorum* Sarg.), the fungus can attack other junipers and species in the genera *Chamaecyparis, Cupressus,* and *Thuja* (4–7).

Symptoms of Phomopsis blight on eastern redcedar seedlings in nursery beds include blighting of shoot tips of the current season's growth, stem dieback, and death of seedlings (8). Only the new foliage is susceptible to infection (10,13). The fungus invades young stem tissue, causing blight symptoms characterized by girdling and death of branches 3 mm or less in diameter (13). Diseased terminals and branches turn light green, then red brown, and finally, ashen gray with tiny black pycnidia.

A list of fungicides tested for control of Phomopsis blight of junipers was reported (12). More recent investigations indicate control of Phomopsis blight may be obtained with the systemic fungicide benomyl (3,9,16,19).

Our preliminary studies consisted of one or two benomyl sprays applied to potted *J. virginiana* followed by inoculation with *P. juniperovora*. No disease control was achieved with the treatments, suggesting that the field control reported (9) was not achieved by a toxic surface layer. Therefore, the objectives of this study were to determine what amount of benomyl in *J. virginiana* tissue prevents Phomopsis blight development, determine the level of benomyl on or in *J. virginiana* tissue after repeated applications of benomyl, compare benomyl to several other fungicides for efficacy in controlling Phomopsis blight in the field, and determine at what period(s) during the growing season *J. virginiana* is susceptible to Phomopsis blight. By achieving these objectives the number of benomyl sprays currently recommended (9) in production beds might be reduced.

MATERIALS AND METHODS

In vitro toxicity of benomyl to *P. juniperovora*. Benomyl-amended agar media were used to determine the concentration of benomyl necessary to completely suppress growth of *P. juniperovora* in vitro. Commercially prepared benomyl (Benlate 50WP, E. I. duPont de Nemours & Co., Wilmington, DE 19898) was suspended in water and appropriate quantities of the suspension were added to potato dextrose agar (PDA) to give concentrations of 0, 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 mg/L calculated on a w/v active ingredient basis. The PDA was autoclaved after addition of benomyl (17). Twenty-five milliliters of each concentration of benomyl-supplemented medium were poured into each of eight petri dishes. A culture of *P. juniperovora* with no previous exposure to benomyl was used to inoculate plates. Mycelial-agar disks 5 mm in diameter were taken from the periphery of 7-day-old colonies growing on PDA. These disks were inverted and transferred to the center of four plates of each concentration of benomyl-amended PDA and to four control plates. In addition, pycnidial spore extrusions from 6-wk-old colonies growing on PDA were suspended in sterile water and diluted to 2×10^6 spores/ml. A single loopful of the spore suspension was applied to the center of remaining plates. All plates were incubated at 24 C with 12 hr of fluorescent lighting (600 lux) daily.

Benomyl spray of potted eastern redcedar. The efficacy of benomyl when applied as a foliar spray for control of Phomopsis blight was evaluated on potted eastern redcedar in a greenhouse. Two-year-old trees 25–35 cm in height were transplanted in early November into 13-cm diameter clay pots containing a sandy loam soil. Sixteen trees were selected for uniformity of new growth 1 mo after initiation of top growth. The top 7 cm of eight trees were inserted into a 16 × 100-mm test tube during treatments to prevent direct contact with the benomyl sprays. The eight trees were sprayed weekly for 7 wk with a suspension containing 600 mg benomyl and 0.3 ml of spreader-sticker per liter. The surface of each pot was covered with a plastic bag during the sprays to prevent benomyl from reaching the soil. The remaining eight trees served as controls.

After the third and fourth spray treatments all 16 trees were inoculated twice with a *P. juniperovora* spore suspension. The spores were obtained from 6-wk-old pure cultures growing on PDA at 24 C with 12 hr of fluorescent lighting daily. An aqueous spore suspension containing 1×10^6 spores per milliliter was sprayed onto the trees with an aerosol propellant. The high humidity necessary for infection (13) was obtained in the greenhouse by placing a 29 × 32-cm plastic bag over two inverted V-shaped wire frames inserted into the soil in each pot. The bags were sealed around the top of each pot with rubber bands. The trees were incubated under the plastic bags on greenhouse benches for 48 hr following inoculation. The incubation procedure was repeated

0031-949X/80/01004605/$03.00/0

weekly for 3 wk following the second inoculation to provide favorable conditions for disease progression and formation of pycnidia. Disease readings were taken 14 and 28 days after the second inoculation.

A disease rating system was established based on severity of disease progression: 0 = presence of small lesions or a few dead needles on the new growth; 1 = death of the tip of new growth on at least one small branch; 2 = disease progression into the stem of the new growth; and 3 = disease progression into tissue of the previous year's growth. A dagger (†) behind the disease rating indicates pycnidia were produced by *P. juniperovora* on the diseased tissue.

Benomyl drench of potted eastern redcedar. The efficacy of benomyl applied as a soil drench for control of Phomopsis blight was tested using potted *J. virginiana* in a greenhouse. Two-year-old trees 25–35 cm in height were transplanted in early November into 13-cm diameter pots containing equal volumes of a sandy loam soil. Each pot received 100 ml of water three times a week for the duration of the experiments. Benomyl treatments of 0, 1.6, 3.2, 10.0, 20.0, and 40.0 mg/pot were applied in 100 ml of water to the soil surface of each of five pots per treatment. The treatments began when new growth first appeared and were repeated four times at weekly intervals. All trees were inoculated and incubated (as previously described) 5 and 10 days after the last drench. One centimeter portions of new growth were collected from branch tips of each tree for analysis of benomyl content 0, 7, and 26 days after the first inoculation. Disease readings were taken at 7 and 26 days after inoculation.

Extraction of benomyl from the tissue samples was accomplished by grinding a 0.25-g sample with sand in a mortar and extracting with three serial 5-ml aliquots of chloroform. All extracts from each tissue sample were combined and concentrated at 50 C in a rotary flash evaporator. The concentrated extract was quantitatively transferred into a 6 × 50 mm pyrex culture tube, placed in an oven at 60 C, and reduced to 0.2 ml. A 10-μl sample of the final concentrated extract was used for thin-layer chromatography (TLC).

Benomyl was detected in the extracts by using the TLC and *Penicillium* bioassay techniques of Peterson and Edgington (11). Data for a standard curve were obtained by measuring diameters of inhibition zones produced on developed TLC plates by 5, 10, 25, 50, and 100 ng of benomyl dissolved in chloroform. A 10-ng internal benomyl standard was developed with tissue extract preparations on all TLC plates. No attempt was made to determine the chemical nature of the fungitoxic material in the tissue extracts. For the purpose of this paper these tests will be referred to as "benomyl assays," although it is recognized that the fungitoxic material is likely to have been a benomyl breakdown product such as methyl benzimidazole-2-yl-carbamate (MBC) (2). The chromatographic separation of benomyl and MBC observed by Peterson and Edgington (11) was not detected in this study; only one zone of inhibition with R_f 0.72 resulted from either benomyl standards or extracts from benomyl-drenched trees.

A second benomyl drench experiment was conducted as previously described with the following exceptions: treatments began 4 wk after trees initiated new growth and the treatments consisted of 0, 80, 160, and 320 mg benomyl/per pot applied twice at weekly intervals. Six trees in each treatment were inoculated 9 and 12 days after the last drench and were incubated under plastic bags as previously described, except that plastic bags were replaced for 48 hr each week for 4 wk following the initial incubation. A 0.25-g sample of new shoot growth was taken from each tree for benomyl analysis at 0, 20, and 45 days after the first inoculation. Disease readings were taken at 12, 20, and 45 days after inoculation.

Chemical control of *Phomopsis juniperovora* in the field. Three-year-old *J. virginiana* in nursery beds at the Big Sioux State Conifer Nursery, Watertown, SD, were used to test the efficacy of four different fungicides for controlling seedling blight incited by *P. juniperovora*. The following fungicidal treatments (rates were a.i.) were applied in 190 L of water per hectare: 0.6 kg benomyl, 0.9 kg captafol, 0.6 kg chlorothalonil, 0.5 kg triphenyl tin hydroxide, and a water control. Benomyl and captafol were applied with 70 ml of spreader sticker per hectare. Plots were 12 m long in a 0.9-m-wide six-row nursery bed. Each treatment was replicated four times in a randomized complete-block design. Fungicides were applied with a John Bean 113-L sprayer attached to a 1.2 m tractor-mounted boom with nine evenly spaced nozzles.

All treatments were initiated on 12 May 1977. Benomyl treatments were repeated every 2 wk through 8 July and weekly thereafter through 6 September. All other treatments were applied weekly over the same period.

Juniper branches (5.0–7.5 cm long) infected with *P. juniperovora* and bearing spore producing pycnidia were collected from diseased trees in the field in the fall of 1976 and stored at 4 C until used. Individual branches were taped to 12.5-cm stakes and fourteen were evenly distributed in each plot on May 4 to provide uniform inoculum sources. All replications of the benomyl treatment contained four subplots of approximately 10 adjacent seedlings in a row. Subplots were covered with 17 × 21-cm plastic bags during benomyl applications.

Visual ratings on spread of infection from the inoculum sources were made on 8 June, 9 July, and 21 July. The percentage of trees infected with *P. juniperovora* in each plot was determined in late September by counting both infected and healthy trees in a 60 × 60-cm area around each inoculum stake. Further evaluations were limited to the control and the benomyl treatments. Twenty diseased trees were collected from each replication and stored at 4 C. Each tree was rated for percent diseased foliage and for the length (mm) of stem tissue bearing *P. juniperovora* pycnidia. Diseased trees were incubated at 23 C and 100% RH for 24 hr. Ten pycnidia were removed from each tree and examined microscopically for the presence of spores.

Benomyl activity on or in *J. virginiana* tissue under field conditions. Trees in benomyl plots that were covered with plastic bags during the application of benomyl were used to determine the amount of benomyl in or on new growth as a result of root absorption. Tissue samples consisting of 1–2 cm of the youngest growth on the upper branches of seedlings were collected from the subplots weekly and were combined into one sample for each replication on each sampling date. Similar samples were collected from trees in the main plots of the control and benomyl treatments just prior to, immediately after, and 1 wk after the spray treatments from 12 May through 8 July. The samplings just prior to spraying and 1 wk after spraying became synonomous with the initiation of weekly benomyl sprays on 8 July. All samples were placed in plastic bags and frozen at −20 C for at least 1 wk before analysis for benomyl content. Benomyl assays were conducted as previously described.

Period of *J. virginiana* susceptibility to *P. juniperovora*. Paired plots were established in *J. virginiana* beds during the second growing season at the Big Sioux Conifer Nursery, Watertown, SD.

TABLE 1. Disease ratings of potted *Juniperus virginiana* grown two seasons in the nursery and one season in the greenhouse, sprayed seven times at weekly intervals with benomyl, and inoculated with *Phomopsis juniperovora*

Treatment	Postinoculation disease rating[a,b]	
	14 days	28 days
Sprayed trees		
Benomyl-600 μg/L	0.75	0.75
Portion protected from spray	1.75	3.00†[c]
Water control	2.00	3.00†
LSD ($P = 0.05$)	0.88	
($P = 0.01$)		1.20

[a] Rating: 0 = presence of lesions or a few dead leaves on the new growth; 1 = death of the tip of new growth on at least one small branch; 2 = disease progression into the stem of the new growth; and 3 = disease progression into tissue of the previous year's growth.
[b] Each value represents the mean of eight trees.
[c] † = pycnidia were present on all trees.

Each plot consisted of a 30 × 30-cm area of the nursery bed and was at least 2 m from other plots in the bed. One *J. virginiana* branch (5.0–7.5 cm long) infected with and bearing pycnidia of *P. juniperovora* was taped to a 12.5-cm pot stake and placed into the middle of one of the paired plots. The second plot was left as a control. Treatments were replicated 12 times for each period studied. The stakes were removed from the plots after a minimum accumulation of 2.5 cm of moisture (rainfall and sprinkler irrigation). A new set of plots was established on the date the infection stakes were removed from the first set of plots. This procedure was repeated throughout the growing season and resulted in six sets of paired plots. All plots were observed weekly for Phomopsis blight and scored as + or − on the basis of occurrence of infected trees. Diseased trees were removed from the plots each week to prevent buildup of secondary infection. Random samples were collected for isolation on PDA to confirm presence of *P. juniperovora*.

RESULTS

In vitro toxicity of benomyl to *P. juniperovora*. Growth of *P. juniperovora* as measured by colony diameters was reduced by approximately 40% after 6 days on PDA containing 0.1 mg benomyl per liter. Sixty days after the plates were inoculated, 0.1 mg benomyl per liter had restricted growth and pycnidium formation to within 12 mm of the point of inoculation. Pycnidia were formed over the entire surface of control plates within the same incubation period. Benomyl concentrations equal to or greater than 0.25 mg/L restricted mycelial growth to within 1–2 mm of the mycelial-disk inoculum. After 6 days, *P. juniperovora* spores had germinated on benomyl-amended PDA at all concentrations tested, but mycelial growth was visible only in the control and 0.1 mg/L benomyl-amended plates. There was no change in these plates after 60 days of incubation. The minimum concentration of benomyl that totally suppressed *P. juniperovora* growth was between 0.10 and 0.25 mg/L.

Benomyl spray of potted eastern redcedar. Three days after the second inoculation with *P. juniperovora* all trees had numerous light brown to purple lesions approximately 1 mm in diameter on all new growth over 1 cm in length. Disease progression on the controls and on the unsprayed portion of benomyl treated trees was significantly greater than on the benomyl sprayed portion of trees (Table 1). There was no difference in final disease severity between control trees and portions of benomyl treated trees protected from the benomyl spray. The sprayed portion of the trees treated with benomyl had no increase in disease severity between the 14- and 28-day readings.

Benomyl drenches of potted eastern redcedar All new growth over 1 cm in length on all trees receiving four weekly 0. 1.6, 3.2, 10.0, 20.0, or 40.0 mg per pot benomyl drenches had numerous lesions 6 days after the first inoculation with a *P. juniperovora* spore suspension. The light brown to purple lesions were approximately 1 mm in diameter. Disease progression in trees receiving four 1.6-mg applications of benomyl was similar to that in the control 7 days after inoculation (Table 2). However, on the same date disease progression in all other treatments was significantly less ($P = 0.05$) than in the control (Table 2). After 26 days the disease ratings of the 1.6 and 3.2 mg benomyl drenches did not differ from the control, but the 10.0, 20.0, and 40.0 mg drenches significantly ($P = 0.05$) reduced disease ratings (Table 2).

Mean concentration of fungitoxicant in the previous season's growth at the time of inoculation was approximately double for

TABLE 2. Phomopsis blight severity ratings and concentrations of fungitoxicant in the tissue of benomyl-drenched potted *Juniperus virginiana*

Treatment (mg benomyl/pot)[a]	Postinoculation disease rating[b]		Postinoculation concentration of fungitoxicant[c]					
			0 days		7 days		26 days	
	7 days	26 days	mean	range	mean	range	mean	range
0	1.4	3.0	0	0	0	0	0	0
1.6	1.2	3.0	0.7	0.4–1.2	0.7	0.4–1.2	0.6	0.4–0.8
3.2	0.4	3.0	1.5	0.5–4.8	0.8	0.5–1.6	0.5	0.3–0.7
10.0	0.2	1.2	2.6	0.8–5.8	1.0	0.8–1.2	0.8	0.5–1.1
20.0	0.2	0.4	4.9	2.8–5.8	2.2	0.8–3.2	1.1	0.8–1.6
40.0	0.6	0.6	5.3	3.2–8.4	2.7	0.8–5.6	1.0	0.6–1.4
LSD ($P = 0.05$)	0.75	0.61	2.16		1.36		0.40	

[a] Treatments applied four times at weekly intervals. Trees were inoculated with *P. juniperovora* 5 and 10 days after the last drench.
[b] Rating: 0 = presence of lesions or a few dead leaves on new growth; 1 = death of the tip of new growth on at least one small branch; 2 = disease progression into the stem of new growth; and 3 = disease progression into tissue of previous year's growth. Ratings are the average of five replications.
[c] Previous season's growth was used in the tissue analysis at 0 days and new growth at 7 and 26 days; expressed as μg fungitoxicant/g tissue (fresh weight) detected in tissue extracts by using the thin-layer chromatography and *Penicillium* bioassay techniques (11).

TABLE 3. Phomopsis blight severity ratings and fungitoxicant concentrations in new growth tissue of benomyl-drenched, potted *Juniperus virginiana*[a]

Treatment (mg benomyl/pot)[b]	Postinoculation disease rating[c]			Postinoculation concentration of fungitoxicant[d]					
				0 days		20 days		45 days	
	12 days	20 days	45 days	mean	range	mean	range	mean	range
0	1	3†	3†	0	0	0	0	0	0
80	0.4	0.4	1.0	4.3	3.0–9.0	5.0	2.0–8.0	0.1	1–0.2
160	0	0.2	0.4	4.7	1.6–7.0	6.5	2.3–10.0	0.5	0.3–0.8
320	0.4	0.7	0.7	4.3	1.9–10.0	5.9	2.5–11.0	1.2	0.3–4.0
LSD ($P = 0.05$)				2.6		3.1		0.9	
($P = 0.01$)	0.5	1.1	1.2						

[a] All values are the average of four replications.
[b] Treatments repeated two times at weekly intervals. Trees were inoculated with *P. juniperovora* 9 and 12 days after the last drench.
[c] Rating: 0 = presence of lesions or a few dead leaves on the new growth; 1 = death of the tip of new growth on at least one small branch; 2 = disease progression into the stem of the new growth; 3 = disease progression into tissue of the previous year's growth; † = pycnidia present on all trees.
[d] Expressed as micrograms of fungitoxicant per gram of tissue (fresh weight) detected in tissue extracts by the thin-layer chromatography and *Penicillium* bioassay techniques of Peterson and Edgington (11).

each increasing benomyl drench rate with the exception of the 40 mg rate (Table 2); however, fungitoxicant concentrations in the trees decreased in all treatments as the experiment progressed.

Information on the minimum concentration necessary to prevent disease progression of *P. juniperovora* was obscured by the large variability in concentrations between trees (Table 2), and the rapidly decreasing concentration of fungitoxicant in the trees; however, in the 1.6 and 3.2 mg drench rates, fungal growth was not arrested by tissue concentrations of fungitoxicant as high as 0.8 μg/g fresh weight, a concentration at least three times that necessary to completely suppress growth in benomyl-amended PDA.

In the second benomyl drench experiment lesions were again produced by *P. juniperovora* on all new growth over 1 cm in length (Table 3). The disease ratings in the checks progressed into the 3* category within 20 days after inoculation. During the same period disease progression beyond the lesion stage occurred in only five of the 24 benomyl treated trees. The fungitoxicant concentrations in the tissue in these five trees was 3.0 μg or less per gram fresh weight at the time of inoculation and, with one exception, remained below this concentration for the duration of the experiment. Disease symptoms on these five trees were minor with one to four small buds killed per tree. Concentrations of fungitoxicant in tissue of individual trees was as high as 10 μg/g of fresh weight and still did not prevent inital infection and the formation of small necrotic lesions. There was very little disease progression in the benomyl-drenched trees between 20 and 45 days after inoculation even though the level of fungitoxicant in the tissue dropped markedly (Table 3). A wide variation in concentration of fungitoxicant occurred in trees within each treatment.

Chemical control of *P. juniperovora* in the field. Significant control of Phomopsis blight under heavy infection pressure was achieved in the field in *J. virginiana* (during the third growing season) with triphenyl tin hydroxide, captafol, and benomyl (Table 4). However, the infection levels in the captafol and triphenyl tin hydroxide plots were economically unacceptable. Disease severity on individual infected trees, the percent infected trees bearing pycnidia, the amount of diseased tissue bearing pycnidia, the percent pycnidia with spores, and the spread of infection from inoculum sources, all were significantly less in the benomyl

TABLE 4. Effects of fungicide sprays on the development of Phomopsis blight caused by *Phomopsis juniperovora* on *Juniperus virginiana* grown 3 yr in the nursery at Watertown, SD in 1977

Treatment and rates[a]	Disease incidence[b]							
	Visual ratings[c]			Trees infected (%)[d]	Disease severity[e]	Trees with pycnidia (%)	Tissue per tree w/ pycnidia (mm)	Pycnidia with spores (%)
	9 Jun	8 Jul	21 Jul					
Control	0.8	3.0	3.9	91	80	100.0	81	41.5
Chlorothalonil - 0.6 kg/ha	0.9	2.8	3.5	85	...	...	...	...
Triphenyl tin hydroxide - 0.5 kg/ha	0.7	2.5	3.0	73	...	...	...	...
Captafol - 0.9 kg/ha	0.8	2.0	2.9	67	...	...	...	...
Benlate - 0.6 kg/ha	0.6	0.9	2.4	25	34	25	3	5.6
LSD ($P = 0.01$)	NS	0.90	0.42	17.0	10.7	8.8	23.6	4.8

[a] All treatments initiated on 12 May 1977. Benomyl treatments were repeated every 2 wk through 8 July and weekly thereafter through 6 September. All other treatments were applied weekly over the same period.
[b] Mean values for four replications.
[c] Rating scale of 0–4.0 = no infection, 1 = all infection within 15 cm of infection stake, 2 = new infection between 15 and 30 cm from infection stake, 3 = new infection more than 30 cm from infection stake, 4 = new infection more than 30 cm from infection stake plus severe browning and/or death of trees.
[d] Readings taken on 26 September, 1977.
[e] Expressed as percent of foliage that was diseased.

TABLE 5. Benomyl concentrations in or on the new growth of *Juniperus virginiana* in a nursery sprayed with benomyl in 1977[a]

Date of sampling and/or spraying[b]	Trees exposed to spray[c]		Trees protected from spray[d]
	Benomyl present before spraying (μg/g of tissue)	Benomyl present after spraying (μg/g of tissue)	Benomyl present in tissue (μg/g of tissue)
12 May	0	6.6 ± 2.5	
19 May	1.6 ± 0.6		0.1 ± 0.08
26 May	0.7 ± 0.5	8.3 ± 2.3	0.3 ± 0.02
2 June	1.0 ± 0.0		0.0
9 June	0.3 ± 0.2	5.4 ± 2.0	0.2 ± 0.14
17 June	0.6 ± 0.4		0.0
23 June	0	10.5 ± 2.4	0.20 ± 0.12
30 June	1.4 ± 0.9		0.50 ± 0.33
8 July	0.7 ± 0.5	3.6 ± 1.1	0.50 ± 0.28
16 July	0.4 ± 0.3	5.6 ± 2.2	0.40 ± 0.31
21 July	0	8.7 ± 2.2	0.30 ± 0.23
28 July	2.4 ± 1.0	20.0 ± 2.3	0.0
5 August	1.7 ± 0.8	12.6 ± 1.7	0.0
11 August	2.9 ± 0.4	28.5 ± 4.5	0.0
22 August	3.5 ± 2.0	14.4 ± 3.0	0.0
29 August	3.8 ± 0.8	23.1 ± 4.1	0.0
6 September	6.6 ± 2.1	36.4 ± 5.9	0.0

[a] All values represent the mean of four replications.
[b] Samples were collected just prior to, immediately after, and 1 wk after 0.6 kg/ha benomyl sprays through 8 July. Weekly benomyl sprays were initiated on 8 July which made the samplings just prior to spraying and 1 wk after spraying synonymous.
[c] Sprays consisted of 0.6 kg of benomyl plus 70 ml of spreader sticker in 190 L of water per hectare.
[d] Trees were covered with a plastic bag secured at the base of the tree during the spraying operation. Bags were removed after the liquid evaporated from adjacent trees.

treatment compared to the control.

Systemic activity of benomyl under field conditions. Fungitoxicant concentration in trees protected from direct foliage contact with the benomyl sprays was very low during the entire sampling period (Table 5). The highest concentration detected was 0.5 μg/g of tissue.

The fungitoxicant concentration upon or within foliage of trees sprayed with benomyl fluctuated widely between sampling periods (Table 5). The highest concentrations were detected immediately after spraying, as would be expected. The lowest levels were detected 2 wk after benomyl spraying. Weekly benomyl sprays were initiated on 16 July and large increases in fungitoxicant concentration were detected in the 28 July through 6 September samples. No new infection was observed in the benomyl treated plots after 21 July.

Period of *J. virginiana* susceptibility to *P. juniperovora*. *J. virginiana* in the nursery bed were susceptible to infection by *P. juniperovora* throughout the growing season (Table 6). No disease was induced by inoculum placed in the plots during the period 21 August–4 September. During this period trees had begun to enter dormancy, so very little new growth (susceptible tissue) existed in the plots.

DISCUSSION

This study demonstrates that a series of preinoculation plus postinoculation benomyl sprays results in significant Phomopsis blight control. The failure of benomyl to inhibit *P. juniperovora* spore germination in vitro and the development of small necrotic lesions on benomyl treated needles or stems of *J. virginiana* suggest that control of Phomopsis blight is dependent upon fungitoxic activity against mycelium growing within the young leaf and stem tissue. Foliar sprays at frequent intervals achieved the levels of fungitoxicity in the tissue necessary to prevent disease development. Drench experiments in the greenhouse indicate that 3.0 μg of fungitoxic material per gram of tissue is necessary to prevent Phomopsis blight development.

The failure to prevent Phomopsis blight on portions of benomyl sprayed trees protected from the spray indicates that very little systemic redistribution of foliar absorbed fungitoxic material occurs. This suggests that new growth is probably not protected from infection until it comes into direct contact with benomyl spray droplets.

Greenhouse results with benomyl drenches demonstrated that *J. virginiana* roots have the capacity to absorb benomyl from the soil and to translocate fungitoxic material to new foliage. Our field results, however, indicate that only very low levels of fungitoxic materials were absorbed by the roots and translocated to new growth of *J. virginiana* after repeated spray applications which resulted in a total deposition of 7.8 kg of benomyl per hectare. These results indicate the need for frequent benomyl applications to protect the new growth of *J. virginiana* from Phomopsis blight.

TABLE 6. Occurrence of Phomopsis blight in *Juniperus virginiana* in a nursery bed during various periods of the growing season[a]

	Infected Plots (%)[b]		
	Inoculated	Control	Moisture (CM)[c]
2 May–26 May	50	0	3.0
26 May–12 June	75	0	5.3
12 June–26 June	50	0	5.8
26 June–23 July	83	0	7.5
23 July–21 August	75	0	7.0
21 August–4 September	0	0	1.1

[a]Inoculum consisted of a 2- to 3-cm branch of *J. virginiana* bearing pycnidia of *P. juniperovora*, taped to a 12.5-cm stake.
[b]All values based on the percent of 12 replications.
[c]Includes both rainfall and water applied by sprinkler irrigation.

Control efforts at the Big Sioux Conifer Nursery cannot be relaxed during the growing season because the trees are produced under a system that promotes maximum growth during a short growing season. Our infection plot studies demonstrated that tissue susceptible to *P. juniperovora* was present from initiation of growth in the spring until dormancy began in the fall.

Of the chemicals tested, only benomyl provided control at a level that could be recommended for commercial operations. Control apparently was achieved primarily through the antisporulant activity of benomyl on *P. juniperovora*, similar to that previously reported for other fungi (1,14,18).

Our current recommendation for control of Phomopsis blight of *J. virginiana* in nursery beds is to spray weekly with beomyl during the entire growing season and to rogue all plants with any dead foliage every 7–10 days. This recommendation has been followed for the last 4 yr at the Big Sioux Conifer Nursery where our research plots were located. No Phomopsis blight was observed in production beds in 1977, even though the nursery has a history of numerous Phomopsis blight epidemics and conditions at the nursery produced a blight epidemic in our untreated inoculated controls.

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