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CONTROL OF PHOMOPSIS BLIGHT OF

EASTERN REDCEDAR WITH BENOMYL

BY

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in partial fulfillment of the requirements of the
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CONTROL OF PHOMOPSIS BLIGHT OF

EASTERN REDCEDAR WITH BENOMYL

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance. of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser   Date

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CONTROL OF PHOMOPSIS BLIGHT OF

EASTERN REDCEDAR WITH BENOMYL

Abstract

DOUGLAS J. FIEDLER

Control of Phomopsis blight (P. juniperovora) of eastern redcedar
(Juniperus virginiana) with benomyl was investigated using potted
seedlings in a greenhouse. Benomyl sprays and drenches did not
prevent infection. However, three pre-inoculation foliar sprays
(600 ppm a.i.) applied at weekly intervals followed by three post-
inoculation sprays gave significant control of disease progression.
Benomyl drenches of 0, 6.5, 13, 40, 80, 160, 320 and 640 mg/
liter pot resulted in significant blight control with the 40 to
640 mg treatments. Thin layer chromatography coupled with
Penicillium bioassay determined that 3 µg of benomyl (MBC)/g plant
tissue (fresh weight) was the minimal concentration necessary to
limit disease progression.

Fungitoxic activity was also determined biweekly in nursery
grown 2-0 eastern redcedar receiving benomyl applications of 0.6
kg/ha biweekly, 1.4 kg/ha monthly, or 2.8 kg/ha every 6 weeks.
Analysis for systemically transported benomyl (MBC) revealed con-
centrations to be less than the minimal amount necessary for
disease control as indicated by the greenhouse experiments.
However, the percentage of trees with pycnidia and the percentage
of pycnidia with spores were both significantly lower in the
benomyl treatments.

ACKNOWLEDGMENTS

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INTRODUCTION

Phomopsis blight is often a serious disease problem in nurseries

producing junipers and related conifers (38, 43, 49). The causal

fungus, Phomopsis juniperovora Hahn, is widely distributed throughout

the Midwest, New England, and much of the South (17, 18). Although

it is especially damaging in nursery production of eastern redcedar

(Juniperus virginiana L.) and Rocky Mountain junipers (J. scopulorum

Sarg.), the fungus can attack other junipers and species in the genera

Chamaecyparis, Cupressus and Thuja (15, 16, 17, 18). The survival of

infected nursery stock is usually low in outplantings (23, 31).

Schoeneweiss (38) reported that epidemics in ornamental nursery and

landscape plantings in the Midwest have also become a problem in re-

cent years.

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 (19). Only the new

foliage is susceptible to infection (27, 33). The fungus then in-

vades young stem tissue causing blight symptoms which are character-

ized by girdling and death of branches 3 mm or less in diameter (33).

Diseased terminals and branches turn light green, then red brown,

and finally, ashen gray with tiny black pycnidia. Positive diagnosis

of P. juniperovora is based on types of spores produced in these

pycnidia and on growth characteristics of the fungus in culture

(14, 15, 16, 18).

A list of fungicides which have been demonstrated to be effective

for control of Phomopsis blight of junipers was reported by

Peterson (32). In the past the only effective chemical control has been repeated spray applications with mercury fungicides (34, 35). However, their manufacture was curtailed due to environmental concerns. Selection of blight resistant cultivars has been suggested as a practical alternative to chemical control of Phomopsis blight (38).

Results of recent investigations indicate control of Phomopsis blight may be obtained with the systemic fungicide benomyl (13, 26, 43, 49). Otta (26) reported control of Phomopsis blight of 2-0 eastern redcedar in nursery beds with biweekly sprays of benomyl. However, results were for a single season and two subsequent years of testing by Otta (personal communication - Dr. J. D. Otta, Department of Plant Science, South Dakota State University, Brookings, South Dakota, 57007) and this author have yielded no meaningful results because environmental conditions were unfavorable for disease development.

Benomyl is active against many fungi that incite disease on ornamentals, fruits, vegetables and field crops (3). Its exceptional systemic properties (8, 9, 10, 12, 29) and extreme persistence in soils and plant tissues (2, 11, 22, 37, 39) have prompted studies utilizing benomyl drenches for control of some diseases (2, 22, 25, 41, 47). However, the occurrence of benomyl resistance in some fungi (7, 24, 42, 48) is limiting the use of benomyl in disease control efforts.

Preliminary studies conducted with potted eastern redcedar in the greenhouse by Otta (personal communication - Dr. J. D. Otta,

Department of Plant Science, South Dakota State University, Brookings,

South Dakota, 57007) and this author indicate that no control of

Phomopsis blight could be expected with one or two benomyl sprays.

Therefore, work was initiated to determine how field control was

achieved with benomyl in 1973 (26). An understanding of how field

control was achieved might help identify the most economical effec-

tive control, and in turn, might aid in avoiding development of

benomyl resistance in P. juniperovora.

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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) was suspended in water and appropriate quantities of the suspension were added to potato dextrose agar (PDA) to give concentrations of 0.10, 0.25, 0.50, 1.00, 2.50, 5.00 and 10.00 ppm calculated on a w/v active ingredient basis. The PDA was autoclaved after addition of benomyl since Talboys and Davies (45) detected no difference in fungitoxicity between autoclaved and non-autoclaved preparations of benomyl. Twenty-five ml of each benomyl supplemented medium were poured into each of 8 petri plates. A culture of P. juniperovora with no previous exposure to benomyl was used as a source of mycelium and spores to inoculate these plates. Mycelial-agar discs 5 mm in diameter were taken from the periphery of 7-day-old colonies growing on PDA. These discs were inverted and transferred to the center of 4 plates of each concentration of benomyl-amended PDA. In addition, pycnidial spore extrusions from 6-week-old colonies growing on PDA were suspended in sterile water and diluted to 2×10^6 spores/ml. A single 'loopful' of suspension was applied to the center of 4 plates of each concentration of a second series of benomyl-amended PDA plates. All plates were incubated at 24 C with 12 hours of fluorescent lighting daily.

Benomyl spray of potted eastern redcedar: The efficacy of benomyl in controlling Phomopsis blight when applied as a foliar

spray was tested on potted eastern redcedar in a greenhouse. Two-year-old trees 25 to 35 cm in height were transplanted in early November into 13 cm (5 inch) clay pots with a sandy loam soil. Sixteen trees were selected for uniformity of new growth one month after top growth had been initiated. The top 7 cm of eight of these trees were inserted into a 16 x 100 mm pyrex tube during treatments to prevent direct contact with the benomyl sprays. These eight trees were sprayed weekly for 7 weeks with a suspension containing 600 ppm benomyl (Benlate 50 WP) and 0.3 ml of Dupont 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.

All 16 trees were inoculated twice with a P. juniperovora spore suspension. The inoculations were done after the 3rd and 4th spray treatments. The spores were obtained from 6-week-old pure cultures growing on PDA at 24 C with 12 hours of fluorescent lighting daily. An aqueous spore suspension containing 1 x 10^6 spores/ml was sprayed onto the trees with a Medici Universal Multi-Mist aerosol (Scientific Products). The high humidity necessary for infection (33) was obtained by placing a 29 x 32 cm plastic food storage bag over 2 inverted V-shaped wires that had been inserted into each pot. The bags were sealed around the top of each pot with rubber bands. Sunny days were necessary to produce the high humidity levels desired. Shading was provided with cheesecloth whenever it was necessary to prevent the temperature under the bag from exceeding 38 C. The trees were incubated under the plastic bags for 48 hours

following inoculation.

The incubation procedure was repeated weekly for 3 weeks following the second inoculation to provide conditions more conducive to disease progression and pycnidial formation. 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 lesions or dead leaves on the new growth; 1 = death of at least one small terminal bud of new growth; 2 = death of the larger stem of new growth adjacent to the terminal bud; and 3 = death of some tissue of the previous year's growth. An asterisk behind the disease rating indicates pycnidia were present.

Benomyl drench of potted eastern redcedar: The efficacy of benomyl in controlling Phomopsis blight when applied as a soil drench was tested using potted eastern redcedar in a greenhouse. Two-year-old trees 25 to 35 cm in height were transplanted in early November into 13 cm pots using a sandy loam soil. Each pot received 100 ml of water three times a week for the duration of these 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 5 pots per treatment. The treatments began when new growth first appeared and were repeated 4 times at weekly intervals. All trees were inoculated and incubated 5 and 10 days after the last drench using methods previously described. Tissue of each tree was sampled for analysis of benomyl content 0, 7 and 26 days after the

first inoculation. The previous season's growth was sampled at 0 days and new growth at 7 and 26 days. Disease readings were taken at 7 and 26 days.

Extraction of benomyl from the tissue was accomplished by grinding a 0.25 g sample with sand in a mortar and extracting with 3 serial 5 ml aliquots of chloroform. The extracts for each tissue sample were combined and concentrated at 50 C with a rotary flash evaporator. This extract was quanitatively collected in a 6 x 50 mm pyrex culture tube, placed into a 60 C oven, and reduced to 0.2 ml. A 10 μl sample of the final concentrated extract was used for thin layer chromotography (TLC).

Benomyl was detected in the extracts using the TLC and Penicillium bioassay techniques of Peterson and Edgington (28). Data for a standard curve was 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) (6). The chromatographic separation of benomyl and MBC observed by Peterson and Edgington (28) did not occur in my work; 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 weeks after trees initiated new growth and the treatments consisted of 0, 80, 160, and 320 mg benomyl/pot applied twice at weekly intervals. The six trees in each treatment were inoculated 9 and 12 days after the last drench and were incubated under plastic bags as previously described. Plastic bags were replaced for 2 consecutive days each week for 4 weeks following the initial incubation period. 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.

Systemic activity of benomyl under field conditions: Two-year-old eastern redcedar seedlings in nursery beds at Watertown, South Dakota, were used to trace the systemic activity of benomyl. Benomyl (Benlate 50 WP) was applied at three different rates; 0.6 kg/ha biweekly with 15 ml Dupont Spreader-Sticker per 38 l of spray, 1.4 kg/ha monthly, and 2.8 kg/ha every 6 weeks. The treatments were applied with a John Bean 113-1 sprayer attached to a 1.2-m long tractor-mounted boom with nine evenly spaced nozzles. Treatments were replicated 4 times in a randomized complete-block design. Each plot consisted of 12 m of a 0.9 m-wide 6-row nursery bed. These plots received semiweekly sprinkler irrigation May 10 to September 7. Irrigation and natural precipitation totaled approximately 10 cm per month. The beds containing these plots had received four 0.6 kg/ha benomyl sprays during their first growing season. The soil was a sandy loam with approximately 2.5% organic matter.

Benomyl treatments were initiated on April 15, 1976. All replications of each treatment contained 4 subplots with each subplot consisting of approximately ten consecutive seedlings in one of the rows. Each of these subplots was covered with a 17 x 21 cm plastic food storage bag during benomyl applications. Samples of the previous season's growth were taken from trees in the subplots on April 12 and frozen at -20 C for later analysis for benomyl content. Sampling of the current season's growth (new growth) was initiated on May 13 and was repeated every 2 weeks until the seedlings became dormant in the fall. This sampling procedure resulted in seedlings approximately 25% shorter than surrounding seedlings. The tissue from the 4 subplots within each plot was combined and 0.5 g was used in the analysis for benomyl. All samples were frozen for at least one week before analysis for benomyl content.

Tissue samples for benomyl analysis were also taken from the distal 2-3 cm of the upper branches of seedlings not protected from benomyl applications. These samples were taken biweekly from June 22 to September 29 and were taken prior to the benomyl applications when both occurred on the same date. Tissue samples were also taken 1 hour and 1 week after the August 18 benomyl applications.

Effect on secondary inoculum: All diseased trees from previously described spray plots at Watertown were collected on September 29 to test the hypothesis that benomyl inhibits pycnidial and/or spore production by P. juniperovora. Some blight symptoms occurred in all plots by June 22 but new infections after this date occurred in control plots only. Therefore, all diseased trees from the benomyl

treatments and ten comparably degraded trees from each control plot were examined for pycnidia using a 3X binocular scope. Five pycnidia were removed from each tree containing pycnidia. The pycnidia were placed individually into drops of water on microscope slides and examined for spore content. Whenever spores were present one 'loopful' of the spore suspension was transferred to a petri plate containing 25 ml of PDA amended with 300 ppm streptomycin sulfate (PDA-S). Presence of beta spores in the pycnidia was also noted.

RESULTS

In vitro toxicity of benomyl to P. juniperovora: Growth of P.
juniperovora as measured by colony diameters was reduced by about
40% after 6 days on PDA containing 0.1 ppm benomyl (Table 1). Sixty
days after the plates were inoculated 0.1 ppm benomyl had limited
pycnidial formation to within 12 mm of the point of inoculation.
Pycnidia occurred over the entire surface of control plates after
the same incubation period.

Benomyl concentrations of 0.25 ppm or above restricted mycelial
growth to within 1-2 mm of the mycelial-disk inoculum (Table 1).
After 6 days P. juniperovora spores had germinated on benomyl-amended
PDA at all concentrations tested but visible mycelial growth occurred
only in the control and 0.1 ppm benomyl-amended plates. There was
no change in these plates after 60 days incubation.

Benomyl spray of potted eastern redcedar: Three days after the
second inoculation with P. juniperovora all trees had numerous
light brown to purple lesions on any new growth over 1 cm in length.
At 14 and 28 days after inoculation disease progression was significant-
ly greater (P=0.05) on the unsprayed portion than on the sprayed
portion of the eight trees sprayed with benomyl (Table 2). Disease
progression was significantly less (P=0.01) on the sprayed portion of
trees than on the checks (Table 2). The sprayed portion of the trees
treated with benomyl had no increase in disease severity between the
14 and 28 day readings.

Benomyl drench of potted eastern redcedar: All new growth over

TABLE 1. Colony diameters of P. juniperovora after 6 days growth on benomyl-amended PDA.[a]

Benomyl-amended PDA (ppm)	Colony diameters (mm) with initial inoculum of:	
	5 mm mycelial-agar disk	Spores
0	64	41
0.10	39	25
0.25	7-9	0
0.50	7-9	0
1.00	7-9	0
2.50	7-9	0
5.00	7-9	0
10.00	7-9	0

[a]Colony diameter is expressed as the mean of four replications for each concentration.

TABLE 2. _Phomopsis_ _juniperovora_ disease ratings of potted 2-1 eastern redcedar receiving seven benomyl sprays at weekly intervals in the greenhouse.

Treatment	Disease rating - days after inoculation[a]	
	14	28
Sprayed trees		
Benomyl-600 ppm	0.75	0.75
Portion protected from spray	1.75	3.00*
Checks	2.00	3.00*
LSD (P=0.05)	0.88	
(P=0.01)		1.20

[a]Disease rating is expressed as the mean of eight trees which were rated on a 0 to 3 scale: 0 = presence of lesions or dead leaves on the new growth; 1 = death of at least one small terminal bud of new growth; 2 = death of the larger stem of new growth adjacent to the terminal bud; 3 = death of some tissue of the previous year's growth; * = pycnidia were present on all trees.

1 cm in length on all trees receiving the four weekly 0, 1.6, 3.2, 10.0, 20.0, or 40.0 mg/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 3). However, on the same date disease progression was significantly less (P=0.05) than the control in all other treatments (Table 3). After 26 days the disease ratings of the 1.6 and 3.2 mg benomyl drenches did not differ from the control ratings but the 10.0, 20.0 and 40.0 mg drenches were still giving significant (P=0.05) disease control (Table 3).

Mean concentration of fungitoxicant in the previous season's growth at the time of inoculation was approximately double for each increasing benomyl drench rate with the exception of the 40 mg rate (Table 3). However, fungitoxicant concentrations in the trees decreased in all treatments as the experiment progressed.

The concentration of fungitoxicant in foliage of trees within each treatment ranged widely (Table 3). Information on the minimum concentration necessary to prevent disease progression of P. juniperovora was obscured by this wide range of concentrations 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

TABLE 3. _Phomopsis juniperovora_ disease ratings and corresponding concentrations of fungitoxicant in the tissue of benomyl-drenched potted eastern redcedar.

Treatment (mg benomyl/pot)[a]	Disease rating - days after inoculation[b]		Concentration of fungitoxicant-days after inoculation[c]					
			0		7		26	
	7	26	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 repeated 4 times at weekly intervals. Trees were inoculated with _P. juniperovora_ 5 and 10 days after the last drench.

[b]Expressed as the mean of 5 replications. Rating 0 = presence of lesions or dead leaves on the new growth; 1 = death of at least one small terminal bud of new growth; 2 = death of the larger stem of new growth adjacent to the terminal bud; and 3 = death of some tissue of the previous year's growth.

[c]μg fungitoxicant/g tissue (fresh weight) - previous season's growth was used in the tissue analysis at 0 days and new growth at 7 and 26 days.

0.8 µg/g fresh weight, a concentration at least 3 times that necessary to completely suppress growth in benomyl-amended PDA.

Lesions were produced by P. juniperovora on new growth over 1 cm in length on all trees receiving the 0, 80, 160, and 320 mg benomyl drenches. However, the disease in the checks progressed into the 3* category within 20 days after inoculation (Table 4). During the same period disease progression beyond the lesion stage occurred in only 5 of the 24 benomyl treated trees. The fungitoxicant concentrations in the tissue in these 5 trees was 3.0 µg or less/g fresh weight at the time of inoculation and, with one exception, remained below this concentration for the duration of the experiment (Table 4). Disease symptoms on these 5 trees were minor with 1 to 4 small buds killed per tree. 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 4).

A wide variation in concentration of fungitoxicant occurred in trees within each treatment (Table 4). As a result significant (P=0.05) differences between the mean concentrations of fungitoxicant in the new growth of trees receiving the three different benomyl drench rates did not occur until 45 days after inoculation (Table 4).

Systemic activity of benomyl under field conditions: Figure 1 represents the concentrations of fungitoxicant found in the new growth of 2-0 eastern redcedar seedlings that were covered with plastic bags during benomyl foliar applications. Treatment rates of 0.6, 1.4 and

TABLE 4. *Phomopsis juniperovora* disease ratings and fungitoxicant concentrations in new growth tissue of benomyl-drenched potted eastern redcedar.

Treatment (mg benomyl/pot)[a]	Tree No	Disease rating – days after inoculation[b]			Concentration of fungitoxicant – days after inoculation[c]		
		12	20	45	0	20	45
0	1-6	all 1	all 3*	all 3*	0	0	0
80	1	0	0	0	3.5	4.0	0.2
"	2	0	0	0	9.0	8.0	0.2
"	3	1	1	1	3.0	2.0	0.1
"	4	0	0	1	3.5	3.5	0
"	5	0	0	0	4.0	6.0	0.1
"	6	1	1	1	3.0	6.5	0.2
Mean					4.33	5.0	0.13
160	1	0	0	0	3.5	9.0	0.6
"	2	0	0	1	5.5	8.0	0.3
"	3	0	0	0	6.5	5.0	0.3
"	4	0	0	0	4.0	4.5	0.6
"	5	0	1	1	1.6	2.3	0.3
"	6	0	0	0	7.0	10.0	0.8
Mean					4.68	6.47	0.46
320	1	1	2	2	2.0	2.5	0.3
"	2	0	0	0	10.0	10.0	4.0
"	3	1	2	2	1.9	3.0	0.4
"	4	0	0	0	5.6	11.0	1.2
"	5	0	0	0	4.0	4.5	0.8
"	6	0	0	0	2.5	4.5	0.6
Mean					4.33	5.92	1.21
LSD (P=0.05)					2.63	3.14	0.85

TABLE 4. continued

[a]Treatments repeated 2 times at weekly intervals. Trees were inoculated with P. _juniperovora_ 9 and 12 days after the last drench.

[b]Rating: 0 = presence of lesions or dead leaves on the new growth; 1 = death of at least one small terminal bud of new growth; 2 = death of the larger stem of new growth adjacent to the terminal bud; 3 = death of some tissue of the previous year's growth; * = pycnidia were present on all trees.

[c]Expressed as µg fungitoxicant/g tissue (fresh weight).

19
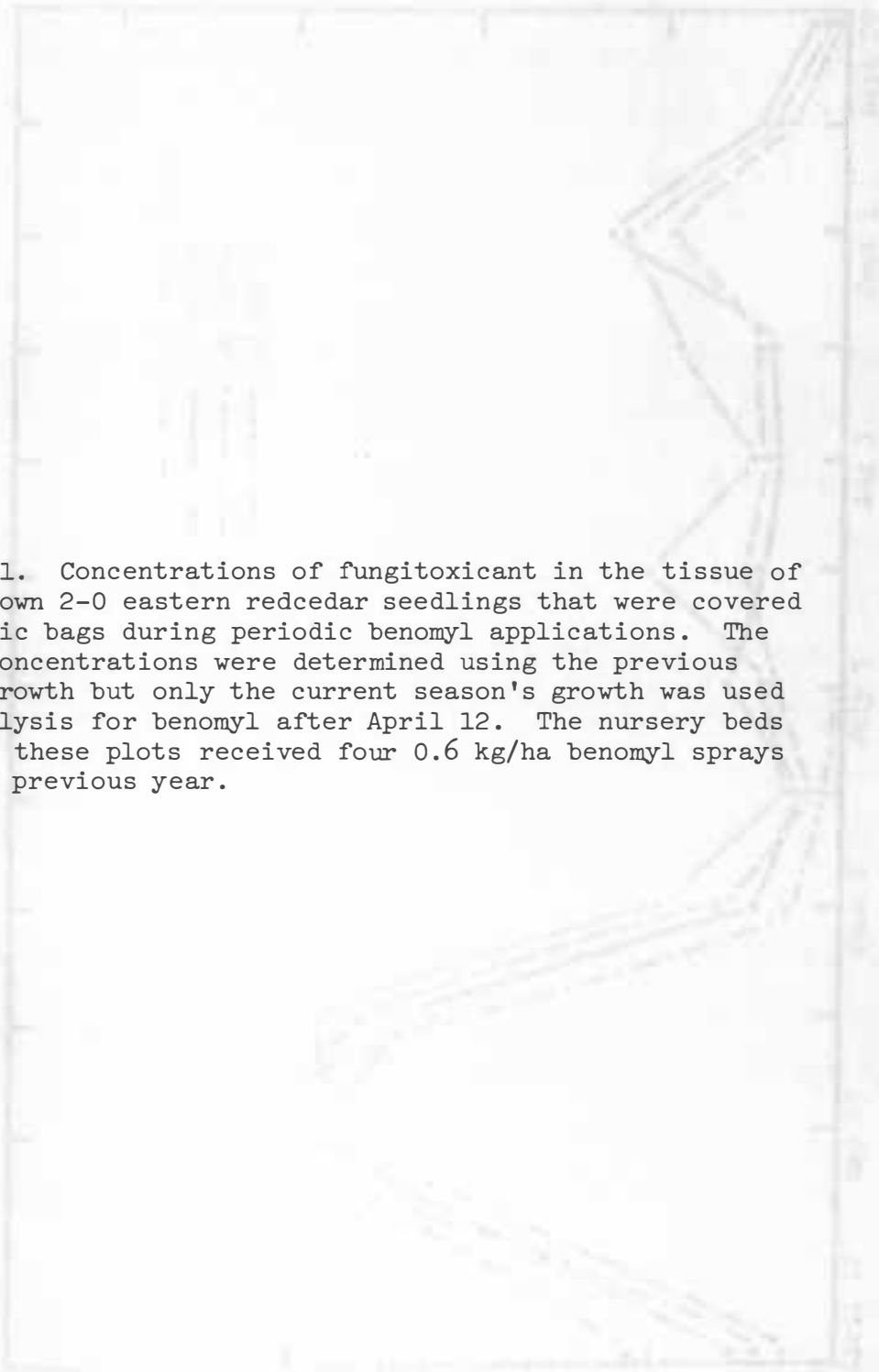
Figure 1. Concentrations of fungitoxicant in the tissue of nursery-grown 2-0 eastern redcedar seedlings that were covered with plastic bags during periodic benomyl applications. The April 12 concentrations were determined using the previous season's growth but only the current season's growth was used in the analysis for benomyl after April 12. The nursery beds containing these plots received four 0.6 kg/ha benomyl sprays during the previous year.

Sampling dates
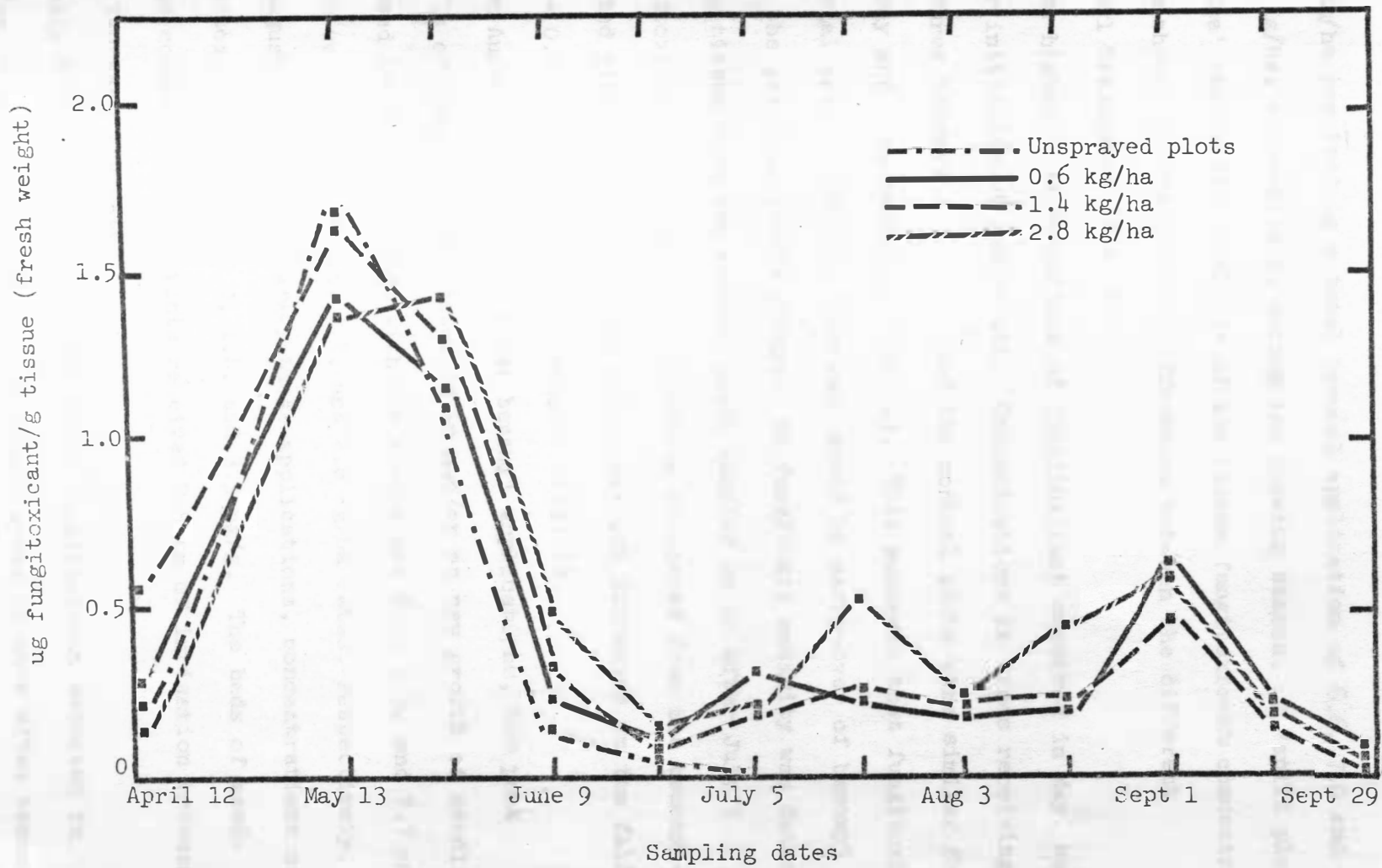
2.8 kg/ha resulted in a total benomyl application of 6.6, 7.0 and 11.2 kg/ha, respectively, during the growing season. A 'split plots in time' statistical analysis of the tissue fungitoxicant concentrations showed no significant differences between the different benomyl treatments (Fig. 1).

The highest concentrations of fungitoxicant occurred in May, soon after initiation of new growth. Concentrations in trees receiving the three benomyl treatments and the control plots were similar for the May and June samplings (Fig. 1). This suggests that fungitoxic material present at this time was caused by carry-over of benomyl from the previous year's sprays. No fungitoxic activity was detected in tissue from the control plots sampled on or after July 5. Fungitoxicant concentrations in tissue collected from the benomyl-treated plots were low during the summer but increased in the fall to 0.5 - 0.7 µg/g tissue (fresh weight) (Fig. 1).

On August 18, one hour after benomyl applications, the peak levels of fungitoxicant detected in and/or on new growth of seedlings exposed to direct contact with the sprays was 6.0, 5.3, and 7.7 µg/g fresh weight for the 0.6, 1.4, and 2.8 kg/ha rates, respectively. On August 25, one week after these applications, concentrations of fungitoxicant fell to 2.0, 1.4, and 3.3 µg/g. The beds of seedlings containing these plots received 2.5 cm of irrigation between the two sampling dates.

Table 5 shows the concentrations of fungitoxicant detected in and/or on the new growth of seedlings 2 weeks or more after benomyl

TABLE 5. Concentration of fungitoxicant (µg/g fresh wt.) in and/or on the distal 2-3 cm of new growth of nursery-grown 2-0 eastern redcedar seedlings receiving the following benomyl applications: 0.6 kg/ha biweekly on each date shown; 1.4 kg/ha on June 17, July 13 and August 18; and 2.8 kg/ha on May 26, July 13 and August 18.[a]

Benomyl treatment	Fungitoxicant concentration in and/or on the tissue[b]							
	June 22	Jul 5	Jul 20	Aug 3	Aug 18	Sept 1	Sept 15	Sept 29
0.6 kg/ha	0.9	0.4	0.7	0.5	0.2	1.1	1.45	6.0
1.4 kg/ha	3.8	0.2	1.8	0	0	0.9	0.1	0.2
2.8 kg/ha	0.1	0	4.0	0	0	2.8	0.3	0.4

[a]Tissue samples were taken prior to benomyl applications when both occurred on the same date.
[b]Mean of 4 replications.

applications. The concentration detected just prior to each bi-weekly 0.6 kg/ha rate ranged from 0.2 to 1.1 µg/g through September 1 (Table 5). The increase in concentration detected after September 1 was probably due to discontinuation of sprinkler irrigation on September 7 and the beginning of seedling dormancy in mid-September. Fungitoxicant was present at very low concentrations or was undetectable in tissue samples taken 2 to 4 weeks after the 1.4 kg/ha applications or 2 to 6 weeks after the 2.8 kg/ha applications (Table 5).

Effect on secondary inoculum: The percent of diseased eastern redcedar with pycnidia and the percent of these pycnidia that contained spores were both significantly lower (P=0.01) in benomyl-treated trees than in the checks (Table 6). Most pycnidia taken from non-treated trees oozed spores abundantly within seconds after contact with water while many pycnidia from benomyl-treated trees had to be crushed to expose spores. Pycnidia containing less than 10 spores were placed in the no spore category.

Growth typical of P. juniperovora, sparse, slow growing mycelium with a dark yellow to orange discoloration of the medium, occurred on each PDA-S plate streaked with a 'loopful' of spore suspension obtained from each pycnidium. Beta spores were observed in only 13 of the 241 pycnidia that contained spores.

TABLE 6. Effect of benomyl treatments on <u>Phomopsis</u> <u>juniperovora</u> spore and pycnidial production in diseased eastern redcedar.[a], [b]

	Treatment (kg benomyl/ha)			
	0	0.6	1.4	2.8
Total number of diseased trees	40	26	39	46
Number of diseased trees with pycnidia	40 a	8 b	17 b	19 b
Total number of pycnidia studied[c]	200	40	85	95
Number of pycnidia with spores	168 a	13 bc	42 b	18 c

[a]Trees were collected from previously described spray plots at Watertown, South Dakota.

[b]Values in rows followed by the same letter do not differ significantly (P=0.01), according to Chi-square statistic for dichotomous data.

[c]5 pycnidia observed per diseased tree with pycnidia.

DISCUSSION

Preliminary studies by Otta (personal communication - Dr. J. D. Otta, Department of Plant Science, South Dakota State University, Brookings, South Dakota, 57007) and this author using potted eastern redcedar indicated that no control of Phomopsis blight is achieved with either one or two pre-inoculation benomyl sprays. However, work reported here shows that a series of pre-inoculation plus post-inoculation sprays results in significant control of the disease. Benomyl did not stop fungal germination and penetration so the observed disease control is probably dependent upon fungitoxic activity within the young leaf and stem tissue. Since no control resulted from two pre-inoculation sprays, several sprays at frequent intervals seem to be necessary to achieve adequate levels of systemic fungitoxicity. A comparison between disease progression in the controls and the unsprayed portions of treated trees demonstrated that systemic translocation of benomyl or its breakdown products within the tree can not be depended upon to protect unsprayed foliage.

Benomyl applied weekly to the point of run-off in the greenhouse experiment was in excess of the biweekly sprays used by Otta (26) to control Phomopsis blight in a nursery. However, this experiment does indicate that field control may have been achieved through systemic fungitoxic activity resulting from benomyl foliar sprays. The spray interval necessary for disease control would probably be affected by factors influencing tissue penetration by the fungicide,

the rate of tree growth, and disease pressure as influenced by existing environmental conditions.

The concentration of fungitoxicant detected in and/or on the new growth of field grown nursery seedlings that were exposed to the 0.6 kg/ha benomyl spray was 6.0 µg/g of tissue when analyzed one hour after the application. The concentration of fungitoxicant detected 2 weeks after each 0.6 kg/ha benomyl spray ranged from 0.2 to 1.1 µg/g during the growing season. This rapid decline in fungitoxicant in and/or on the new growth could be due to sprinkler irrigation washing the fungitoxicant from the foliage and/or lack of translocation to newly forming tissue. The lack of redistribution of benomyl or its breakdown products from older to younger leaves has been noted by others using various plants (12, 40) and is suggestive of movement basically in the apoplastic pathway. Reduced disease control would probably result with a spray interval exceeding 2 weeks.

P. juniperovora incited lesions were observed on all potted trees receiving benomyl drenches even though concentrations of fungitoxicant ranged between 0.4 and 11.0 µg/g in tissue of individual trees. Concentration of systemic fungitoxicant between 0.4 and 0.8 µg/g of tissue gave little disease control. Concentrations ranging from 1.6 to 3.0 µg/g of tissue resulted in the appearance of only minor symptoms consisting of 1 to 4 dead buds per tree. However, progression of P. juniperovora into the young stem tissue was prevented when more than 3 µg of fungitoxicant/g of tissue was detected.

Therefore, 3 µg of fungitoxicant/g of tissue may be the threshold concentration for P. juniperovora inhibition in vivo in eastern redcedar.

Benomyl drenched at rates above 80 mg/pot did not produce significant increases in mean concentrations of fungitoxicant in new-growth of eastern redcedar, but the duration of detectable quantities was increased. Talboys et al., in experiments with Verticillium wilt and potted strawberries (46), also reported that increasing benomyl dose rates had little influence on the level of fungitoxicity attained in the vascular tissues but greatly increased the duration of this fungitoxicity. The long-term effects observed using high dose rates are probably caused by a reservoir of slowly solubilizing benomyl in the soil. The wide range of systemic concentrations occurring in trees receiving the same amount of benomyl was not overcome by increasing the dosage. This variability within treatments could have been caused by differences in root-to-top ratios or differences in rate of growth of the recently transplanted trees.

The highest concentration of systemic fungitoxicant detected in eastern redcedar drenched with benomyl was 11 µg/g fresh weight. The identity of the fungitoxic material was not determined in the work reported here. However, work reported by others (9, 12, 29, 40) with various benomyl-treated plants has shown that the systemically transported fungitoxic compound is mainly methyl benzimidazole-2-yL-carbamate (MBC). Pitblado and Edgington (36) found that

solubility of MBC in an acqueous solution at pH6.5 was 10 μg/ml
with solubility increasing with a decreasing pH. The concentration
of fungitoxic material in the foliage of plants drenched with benomyl
should be expected to be relatively low because of the reported low
solubility of MBC and because movement within plants is indicative
of apoplastic migration (29, 30, 39). This type of movement within
the plant may cause accumulation of fungitoxicant in the tips and
margins of leaves. Therefore, detection of 11 μg of fungitoxicant/g
of tissue does not mean that the fungus is exposed to this concen-
tration. However, Solel et al. (44) found that movement of MBC may
also occur in the symplast but to a lesser extent.

The concentration of systemic fungitoxicant in field grown
nursery seedlings that were not exposed to direct contact with the
benomyl sprays ranged from 0.2 to 1.7 μg/g of tissue during the
growing season. This accumulation of fungitoxicant would be due
to root absorbtion of benomyl or its breakdown products and trans-
location to the foliage. The concentration of systemic fungitoxicant
necessary to obtain disease control in potted eastern redcedar
drenched with benomyl suggests that the systemic concentrations
detected in field grown nursery seedlings would not be adequate to
give disease control.

Benomyl hydrolyzes in aqueous media to form MBC (6) which, due to
its low solubility, has poor soil penetration capabilities (20, 36).
Therefore, soil drenches could result in the fungicide being unavail-
able to many plant root systems. Soil penetration by benomyl can be

increased with the addition of acid surfactants (36). Soil applications of benomyl-acid surfactant combinations have increased the effectiveness of benomyl in the control of Verticillium wilt of cotton (4) and potatoes (1). A single root application of benomyl protected muskmelon from powdery mildew during the growing season (25) and three applications protected strawberries from Verticillium wilt for 5 months (47). The low levels of systemic fungitoxicant detected in the new growth of eastern redcedar at Watertown, South Dakota indicates that an inadequate quantity of fungicide is becoming available to the root systems. This could possibly be corrected by soil incorporation prior to planting in the fall plus infrequent but large doses of benomyl applied as a drench during the first growing season. The 40 mg benomyl/13 cm pot would be equivalent to 27 kg/ha. This treatment level would be economically feasible in a nursery because a bed of eastern red-cedar (0.9m x 122m) typically produces 20,000 seedlings. Therefore, the increase in cost would be only $0.80/1000 seedlings/drench. Application of these large doses of benomyl may give adequate disease protection for the first growing season and a portion of the second. The seedlings are usually sold after the second growing season. The possibility of phytotoxicity to germinating seedlings and disturbances of the soil ecosystem would also have to be determined.

Pycnidia could not be found on many of the Phomopsis blighted seedlings that were receiving applications of benomyl. When pycnidia

were found on diseased trees from benomyl-treated plots, they were
generally few in number when compared to the abundant pycnidia
occurring on diseased trees from untreated plots. Helton (21)
found that benomyl applied as a paint to Cytospora cankers of
golden willow inhibited development of pycnidia and destroyed
undeveloped pycnidia. The antisporulant activity of benomyl
noticed in my work has also been noted by others (5). The control
of Phomopsis blight obtained with benomyl in 1973 (26) may be, in
part, due to this reduction in number of spore-bearing pycnidia.
The resulting reduction of secondary inoculum could possibly
prevent the disease from reaching epidemic proportions. However,
work needs to be done to determine if this occurs due to the systemic
fungitoxicity resulting from foliar penetration of benomyl (MBC) or
from root absorbtion and translocation of benomyl (MBC). This
information could be helpful in determining which method of
application, frequent foliar sprays or infrequent large doses of
benomyl (drenches), has the most potential for effective control
of Phomopsis blight.

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