Root Dip Treatments for Controlling Blueberry Stem Blight Caused by *Botryosphaeria dothidea* in Container-Grown Nursery Plants

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ABSTRACT

Cline, W. O., and Milholland, R. D. 1992. Root dip treatments for controlling blueberry stem blight caused by *Botryosphaeria dothidea* in container-grown nursery plants. Plant Dis. 76:136-138.

Dipping highbush blueberry roots into a benomyl-kaolin clay slurry at concentrations of 2,000–3,000 μg/ml resulted in protection against stem blight development caused by *Botryosphaeria dothidea*. Reduction in lesion development was greatest when kaolin clay was used as the slurry rather than Terra-sorb. Phytotoxicity was observed in plants treated with rates higher than 3,000 μg/ml.

Blueberry stem blight, caused by *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & de Not. (syn. *B. ribis* Gross. & Duggar), is the primary cause for failure of 1- and 2-yr-old plantings in North Carolina. Both highbush blueberry (*Vaccinium corymbosum* L.) and rabbiteye blueberry (*V. ashei* Reade) are susceptible. Symptoms include rapid wilting with browning or reddening of leaves on individual branches, often followed by the death of the entire plant as the fungus spreads downward through vascular tissue to the base of the plant (3,8). Naturally occurring field infections usually can be traced to a wound as the point of initial infection (2,4). Milholland (6) reported that while infection can occur on nonwounded stems, wound penetration is required for vascular infection and typical disease development.

The most recent survey in North Carolina indicates a rise in stem blight incidence from 9% in 1959 to 23% in 1985. Increases in mechanized harvest, mechanized pruning, and a shift to more susceptible cultivars have been cited as contributing factors (4). Under field conditions, mortality is highest in young bushes with plant death decreasing as plant age increases (2). Ballington and Krewer (1) recommend the use of 2-yr-old nursery plants over rooted cuttings to enhance survival.

This research was supported in part by the North Carolina Agricultural Research Service. In addition, portions of this research were supported by the Southeastern Blueberry Council, Inc. of North Carolina.

The use of trade names does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.

Accepted for publication 24 June 1991 (submitted for electronic processing).

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Benomyl has in vitro activity against mycelial growth of *B. dothidea* at concentrations as low as 0.001 ppm, with complete suppression of mycelial growth at 1.0 ppm (R. D. Milholland, *unpublished*). In preliminary studies, benomyl was superior to both penconazole and thiophanate-methyl when used at equivalent rates in a root dip to control *B. dothidea*; benomyl rates from 2,000 to 8,000 μg/ml were effective with some phytotoxicity occurring at rates higher than 3,000 μg/ml (W. O. Cline and R. D. Milholland, *unpublished*).

This study was initiated to develop a fungicidal root dip treatment that would limit disease expression on blueberry nursery plants infected by the stem blight fungus and for use in the nursery as a general preventive treatment on cultivars known to be susceptible to stem blight.

MATERIALS AND METHODS

Dormant 1-yr-old bare-rooted cuttings of Bluechip, a cultivar susceptible to stem blight, were used. The test compared two base substrates for the root dip slurries: kaolin clay, marketed as Carotex (Byrd Distributing, Inc., Raleigh, NC), and Terra-sorb, an acrylamide copolymer, potassium acrylate (Industrial Services Intl., Bradenton, FL). Kaolin clay was used at 454 g per 546 ml of water and Terra-sorb at 7.75 g per liter of water. All benomyl (Benlate 50WP) concentrations are expressed in micrograms per milliliter of active ingredient. Benomyl suspensions were prepared at 0, 2,000, 3,000, 4,000, and 8,000 μg/ml by first mixing the appropriate concentration of benomyl with water, then adding kaolin clay or Terra-sorb and mixing by stirring to a homogenous consistency. Plants were dipped in each substrate × benomyl treatment by thoroughly immersing the root system and stem up to the soil line on each rooted cutting or plants were placed in pots on top of a 100-ml volume of the dip substrate that had been poured in rather than being dipped. Both techniques supplied approximately 100 ml of substrate to each plant. Plants were immediately potted in 15 cm diameter × 15 cm deep tar paper pots in a peat/sand mixture (1:1, v/v) and placed in beds in a factorial design. The experiment was arranged in framed outdoor ground beds built on a 40-cm-deep bed of sand with 50% overhead shade from a permanent lath structure. Four replications with two stems per replication were prepared for each run; the experiment was repeated in two concurrent runs beginning in April and May 1987, respectively, and data from both were combined. Plants were fertilized every 3 mo using Osmocote 19-6-12 and with a solution of Peter's 20-20-20 fertilizer (W. R. Grace & Co., Fogelsville, PA) as needed.

Plants were inoculated 3 and 6 mo after treatment. Inoculations were carried out using a highly virulent isolate (BD-17) of *B. dothidea* from blueberry (2). Cultures were initiated from single spores and grown on oatmeal agar consisting of 50 g of ground oatmeal and 15 g of Difco Bitek agar per liter of water (OMA) for 2 wk under 24 hr/day fluorescent lighting at 22–24 C. Inoculations were made on attached, succulent to semihardened stems, approximately 3 mo old, from the current season's growth. Aerial hyphae from a 1-cm^2 area of a 2-wk-old OMA culture were introduced onto the cut end of each stem through wounds made by excising the upper portion of stems (approximately 3- to 6-mm-diameter cut surface) (2). Plants were placed immediately in an insulated walk-in chamber (W. A. Brown & Son, Inc., Salisbury, NC) modified to deliver 100% relative humidity (RH) at 25–32 C for 72 hr, then moved to the test location and randomized. Two stems were inoculated on each plant; lesions were measured in millimeters at 1, 2, 3, and 11 mo after inoculation by measuring surface-visible necrotic discoloration extending downward from the point of inoculation. At 11 mo, five lesions each were chosen at random to compare 3- and 6-mo treatments where both were treated with 3,000 μg/ml of benomyl in kaolin clay. These stems were dipped in 70% ethanol and flame-sterilized, after which 1- to 3-mm wedges were notched out of the leading edge of necrotic areas and plated on OMA for determination of pathogen survival.

RESULTS AND DISCUSSION

Mean squares from analysis of variance calculations indicate highly significant ($P = 0.001$) differences attributable to benomyl rate, dip technique, and inoculation date (Table 1). Differences increased over time in the interaction between benomyl rate and dip technique, whereas the interaction between benomyl rate and inoculation date was significant across all rating periods. Experimental runs differed significantly at 11 mo after inoculation with average lesion lengths for runs one and two of 104 and 88 mm, respectively. Run one included a longer initial warm-weather infection period compared with run two (April–October vs. May–October), which may explain this difference.

Benomyl rates of 2,000 and 3,000 μg/ml were the most effective at limiting disease development after 11 mo (Table 2). Kaolin clay was the best substrate material ($P = 0.05$) with differences attributable to substrate becoming greater over the course of time. In all cases, plants treated with benomyl had significantly shorter lesion lengths than nontreated plants. When kaolin clay was used, lesion lengths increased as the concentration of benomyl increased above 3,000 μg/ml; at concentrations higher than 3,000 μg/ml, plants were stunted and chlorotic. Benomyl uptake by the plant might have decreased when the concentration of benomyl was raised above a certain point, perhaps because of some phytotoxic reaction to an excess of the fungicide. If this occurred, it could account for the unexplained increase in lesion lengths observed with higher rates.

When averaged across all benomyl rates, immersing plants in benomyl suspensions provided better control than treatments in which the slurry was poured into the half-filled pot before placing the plant in the potting medium. Immersing roots in 3,000 μg/ml of benomyl-kaolin clay slurry provided the best protection from stem blight development. However, in the 2,000 and 3,000 μg/ml treatments, totally immersing plants in the benomyl solution resulted in only slightly shorter lesion lengths than did pouring the solution into the half-filled pot before placing the bare-rooted plant; these differences were not significant and, in some cases, reversed in the course of 11 mo (Table 3). The percent survival of plants set or immersed in kaolin clay alone was 50 and 62, respectively. All benomyl treatments provided 100% survival after 11 mo. In one treatment, Terra-sorb alone provided 100% survival when used as an immersion, whereas plants set (not immersed) in Terra-sorb alone had a 62% survival rate.

Significant differences were apparent between plants inoculated 3 mo and those inoculated 6 mo after benomyl treatment (Table 4). Measurements of lesion length after 11 mo and percent survival were significantly different between inoculation dates for all treatments regardless of substrate or dip techniques. Only data from the 2,000 μg/ml benomyl rate are presented as an example. Higher initial lesion ratings were recorded for the 6-mo inoculation date, indicating a partial loss of fungicide efficacy before inoculation. Lesions also advanced more rapidly in the 6-mo inoculation treatments, indicating far less residual protection over the 11-mo rating period. Attempts to reisolate *B. dothidea* after

Table 1. Analysis of variance results for factorial treatment effects and interactions

Factor	df	Mean square			
		1 mo	2 mo	3 mo	11 mo
Run	1	1,570	120	56	20,018*
Substrate (S)	1	193	423	3,270	13,794*
Benomyl rate (B)	4	28,249**[a]	24,691**	27,658**	27,731**
S × B	4	2,218*	1,081	1,818	1,572
Dip technique (D)	1	16,776**	9,331*	3,491	9,105
D × S	1	286	145	114	286
D × B	4	2,001	4,371*	6,710*	14,933**
D × S × B	4	1,423	3,410*	2,700	3,758
Inoculation date (I)	1	9,890**	196,812*	214,296**	499,675**
I × S	1	487	470	69	955
I × B	4	5,483**	3,954*	7,205**	12,381*
I × S × B	4	1,501	2,673	2,312	4,730
I × D	1	330	4,410	10,362*	18,225*
I × D × S	1	34	4,805	9,212*	3,131
I × D × B	4	2,873*	992	1,130	4,397
I × D × S × B	4	688	1,534	2,417	4,203
Error	279	932	1,298	1,519	3,007
Coefficient of variance		73.92	51.71	52.03	56.91

[a] Significance levels from two-way analysis of variance. $P > F = 0.05$(*) and 0.001(**).

Table 2. Effect of benomyl rates and dip substrate on development of stem blight of Bluechip highbush blueberry caused by *Botryosphaeria dothidea*[a]

Benomyl (μg/ml)	Substrate	Lesion length (mm) at intervals after inoculation			
		1 mo	2 mo	3 mo	11 mo
0	Kaolin clay	99[b]	89	93	99
2,000	Kaolin clay	8	11	12	16
3,000	Kaolin clay	6	8	11	14
4,000	Kaolin clay	46	69	78	48
8,000	Kaolin clay	17	38	37	67
0	Terra-sorb	74	85	98	121
2,000	Terra-sorb	27	33	35	42
3,000	Terra-sorb	30	38	39	43
4,000	Terra-sorb	32	43	51	64
8,000	Terra-sorb	17	26	36	56

[a] Only main effects for benomyl rate × substrate are shown. Plants were inoculated 3 mo after dip treatment.
[b] Each value represents the mean of 16 observations rounded to the nearest whole number.

Table 3. Effect of benomyl rates, substrate, and dip techniques on development of stem blight on the highbush blueberry cultivar Bluechip caused by *Botryosphaeria dothidea*[a]

Benomyl (μg/ml)	Substrate	Dipping technique[b]	Lesion length (mm)	
			1 mo	11 mo
0	Kaolin clay	Set	102[c]	95
0	Kaolin clay	Immersed	97	102
2,000	Kaolin clay	Set	11	15
2,000	Kaolin clay	Immersed	6	17
3,000	Kaolin clay	Set	9	23
3,000	Kaolin clay	Immersed	3	4
0	Terra-sorb	Set	76	111
0	Terra-sorb	Immersed	72	132
2,000	Terra-sorb	Set	28	39
2,000	Terra-sorb	Immersed	26	45
3,000	Terra-sorb	Set	32	49
3,000	Terra-sorb	Immersed	28	36

[a] Plants were inoculated 3 mo after treatment.
[b] Immersed = entire root ball submerged in substrate up to soil line. Set = root ball placed on top of 100 ml of substrate in pots without dipping.
[c] Each value represents the mean of eight observations rounded to the nearest whole number.

Table 4. Effect of benomyl and dip substrate on stem blight development caused by *Botryosphaeria dothidea* inoculated 3 and 6 mo after treatment across all benomyl rates

Substrate	Time of benomyl application (mo)	Lesion length (mm)		Survival (%)
		1 mo	11 mo	
Kaolin	3[a]	6[b]	17	100
Kaolin	6	19	98	62
Terra-sorb	3	26	45	100
Terra-sorb	6	44	132	38

[a] Months from benomyl treatment to inoculation.
[b] Each value represents the mean of eight observations rounded to the nearest whole number.

11 mo from plants the roots of which were immersed in 3,000 μg/ml of benomyl-kaolin clay 3 mo before inoculation were unsuccessful. By contrast, the fungus was reisolated from 100% of inoculations from the same treatment combination when a 6-mo dip-to-inoculation interval was used.

Although a number of treatments permitted 100% survival of inoculated test plants, lesion lengths varied widely. As stated, benomyl was most effective when used at a rate of 2,000–3,000 μg/ml of active ingredient; these rates of benomyl also were effective in limiting stem canker development caused by *B. corticis* (Demaree & M. S. Wilcox) Arx & E. Müller (7). Higher rates resulted in phytotoxicity and longer lesion lengths. Dipping plants in a substrate rather than set-in application was assumed at the outset of this test to be the better method, but there were no differences among the most efficacious treatments. Significant differences did occur when main effects of the dipping technique were combined across all other treatments, indicating that dipping generally provided a more uniform source of benomyl for uptake by plants. Benomyl root dips using these techniques are apparently effective only for a period of 3 to less than 6 mo. Infections occurring after that time period will progress in spite of these treatments.

Based on our results, it appears that benomyl root dips may be useful in limiting *B. dothidea* development but may not protect plants fully throughout a long growing season. In field studies, Creswell and Milholland (4) were able to collect spores of *B. dothidea* year-round with the exception of a few weeks in winter, which would seem to circumvent a control measure that is effective for only 3–5 mo, however, maximum infection of wounded plants exposed to natural inoculum occurred in June. If protective dip-treating could be timed to coincide with this period of highest disease probability, a significant reduction in the number of infected plants could be achieved.

Future efforts will concentrate on determining the usefulness of these dip treatments under field conditions. Benomyl retention in the root zone may be enhanced by the lower amount of leaching expected to occur in the field as opposed to container nursery bed regimes of daily overhead watering. Another possibility for extending the protected period would be the use of a clay base, which has a higher ionic capacity. This might be a way to retain benomyl within the dip solution, provided that ionic binding does not deactivate benomyl completely (5).

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