Monitoring of Residues of Benomyl on Leaves and Nut Hulls of Black Walnut

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ABSTRACT

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The persistence of residues of benomyl and its degradation products on nut hulls and leaves of black walnut (*Juglans nigra*) trees was determined by cellophane transfer bioassays. In greenhouse studies, residues on leaves were determined following simulated rainfall at four levels applied weekly to incubator-grown walnut seedlings sprayed with benomyl at 0.6 g a.i./L. In field studies, residues on leaves and nut hulls were determined following spray treatments at 0.6, 1.2, and 2.4 g a.i./L. Benomyl at 0.6 g persisted (ED_{100}) on leaves of incubator-grown trees for 9 wk through 25 cm of simulated rainfall. Benomyl at this rate persisted (ED_{50}) on leaves and nut hulls of field-grown trees for 6 wk when rainfall did not exceed about 10 cm/wk. Higher rates of application did not add appreciably to residue persistence.

Additional key words: carbendazim, MBC

Walnut anthracnose develops on leaves and nuts of black walnut (*Juglans nigra* L.), causing premature defoliation, reduced tree growth, and ambered nut meats (1). Benomyl has been used successfully to control this leaf spot disease in Ohio (2) and Illinois (7,9). Sprays in late May and early July are recommended (8) to control ascospore and conidial infections, respectively. Information about fungicide persistence as it relates to the environment would lend some predictability to the timing of spray applications. This study was initiated to determine the persistence of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] on black walnut leaves and nut hulls, with special attention to the effect of rainfall on residue removal.

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MATERIALS AND METHODS

We used a cellophane transfer technique (6) in these tests to monitor topical residue. Five-millimeter cellophane disks (DuPont 215 PD-65) that were not moistureproof were sterilized in boiling water and placed on the fungicide-treated surface of the plant part. A minimal film of water remained between sample and disk. A conidial suspension of *Aspergillus* sp. was seeded onto the cellophane disk surface with a 30-μl capillary tube. Tissue samples were incubated for 48 hr at 24 C in moisturized, sealed plastic containers. Each cellophane disk was then mounted on a glass slide and examined microscopically at ×100 magnification.

Because benomyl and carbendazim (a hydrolytic breakdown product of benomyl) have a fungistatic effect upon germ tube elongation (4), we assessed topical residue by estimating germ tube development microscopically. When germ tubes of 20 or more conidia on a single assay averaged 20 times the spore diameter, the fungus was considered uninhibited. The persistence of fungicidal residues was evaluated by determining the percentage of leaf or hull bioassays showing uninhibited germ tube development.

Eight walnut seedlings approximately 2 mo old were placed in each of three growth chambers (18, 24, or 30 C) with a 12-hr photoperiod and 24 klux light intensity for 1 wk before treatment. The foliage was then sprayed with benomyl (Benlate 50W) at 0.6 g a.i./L to runoff with a 10-L knapsack compressed air sprayer. Each week, rain from a rain simulator (3) at 0.0 cm, 1.25 cm once, 1.25 cm twice, or 2.5 cm was applied to two plants from each incubator at the rate of 0.5 cm/min. Three 9-mm-diameter leaf disks from each plant were collected at random from the most mature leaves after the rainfall treatment. The plants were returned to the growth chambers, and the leaf disks were bioassayed for benomyl residue.

Three groups of ten 2-yr-old black walnut trees in the Illinois Natural History Survey arboretum in Urbana were sprayed with benomyl at 0.6, 1.2, or 2.4 g a.i./L on 5 May, 17 July, or 14 August 1979. Three 9-mm-diameter leaf

disks per plant were collected at random from the oldest, most fully expanded leaves at weekly intervals, and cellophane bioassays were conducted on these leaf samples. Rainfall was monitored weekly at the site.

Nut-bearing walnut trees at Urbana, Fisher, and Carbondale, IL, and Martinsville, IN, were sprayed with benomyl in 1978 with a 50-gal power hydraulic sprayer (John Bean Division, FMC Corp.) at a pressure of 14 kg/cm^2. Two sprays at the concentration of 0.6 g a.i./L were applied to trees at Carbondale and Fisher between 24 May and 12 July, while single sprays at this rate were made at Martinsville and Urbana on 12 and 28 July, respectively. In addition, single sprays at 1.2 or 2.4 g a.i./L were applied on 6 and 12 July at Carbondale and Martinsville, respectively. There were five to eight trees per treatment.

On each collection date, chosen arbitrarily, three nuts were sampled at random from each tree. From individual nuts, three hull cores were cut with a 6-mm cork borer and placed on solidified 2% water agar in a 9-cm petri plate, the hull exterior upwardly exposed. Cellophane bioassays were conducted on these cores. Rainfall data for each testing site were taken from records of nearby U.S. National Weather Service stations.

Table 1. Benomyl persistence on treated leaves of black walnut[a] evaluated weekly by percentage of bioassays in which germ tube development of *Aspergillus* sp. conidia was inhibited

Date treated / Week[b]	Inhibition (%) at application rate[c]			Accumulated rainfall (cm)
	0.6 g/L	1.2 g/L	2.4 g/L	
May 5				
1	100	100	100	1.8
2	97	100	100	1.8
3	100	96	96	2.0
4	100	100	97	2.2
5	76	87	97	2.2
6	62	67	100	8.2
7	50	45	48	8.2
8	32	24	65	9.1
9	10	10	11	31.3
July 17				
1	100	100	100	0.9
2	23	41	54	19.3
3	15	17	26	23.2
August 14				
1	100	92	87	4.8
2	52	74	71	10.4
3	65	50	63	10.4
4	42	54	47	10.4

[a]Two-year-old trees located at Urbana, IL, were treated in 1979.
[b]Each week following treatment, bioassays were conducted on leaf samples.
[c]Each value represents the percentage of bioassays out of a total of 30 conducted per treatment with average germ tube length less than 20 times the spore diameter for 20 or more randomly selected conidia per bioassay.

Table 2. Benomyl persistence on treated nut hulls of black walnut[a] evaluated at various periods by the percentage of bioassays in which germ tube development of *Aspergillus* sp. conidia was inhibited

Location / Days after treatment[b]	Inhibition (%) at application rate[c]			Accumulated rainfall (cm)
	0.6 g/L	1.2 g/L	2.4 g/L	
Carbondale				
25	100[d]	100	100	5.3
47	56	93	100	6.6
80	3	2	2	26.3
Martinsville				
21	100	...[e]	98	10.6
43	85	...	100	16.1
Fisher				
16	100	...	...	5.5
46	17	...	...	14.8
Urbana				
18	98	...	...	7.4
40	22	...	...	14.7

[a]Nut-bearing trees located at Carbondale, Fisher, and Urbana, IL, and Martinsville, IN, were treated in 1978.
[b]Calculated from the last spray application to the time of bioassay evaluation.
[c]Each value represents the percentage of bioassays out of the total conducted per treatment at each collection date with average germ tube length of less than 20 times the spore diameter for 20 or more randomly selected conidia per bioassay.
[d]The foliar spray rate was 1.2 g a.i./L.
[e]Not tested.

RESULTS AND DISCUSSION

In preliminary trials, the length of germ tubes from *Aspergillus* sp. conidia after 48 hr of incubation at 24 C was found to be correlated inversely with the concentrations of benomyl. At 0.0, 1.0, 2.5, 5.0, and 10.0 mg/L, the respective average lengths of the germ tubes were >75, 23, 10, 8, and 5 times the spore diameter.

In growth chamber studies, after a maximum application of 25 cm of artificial rainfall over 9 wk, benomyl at 0.6 g a.i./L remained persistent on the leaves. None of the measurements taken on average germ tube development exceeded 10 times the spore diameter. Neither the incidence of rainfall application nor the effect of temperature altered these results. These tests suggest that some factor other than rainfall is active in degrading or removing the fungicide.

In field studies, benomyl residues on more than 50% of the leaves (ED_{50}) persisted for 6 wk under moderate rainfall (less than 10 cm within 8 wk, trees treated on May 5; Table 1). When rainfall exceeded 18 cm during the week of July 24, residues below ED_{50} were observed within 2 wk (trees treated on July 17). These data indicate that rainstorms exceeding about 6 cm/wk reduced benomyl residue to uninhibitory levels on many leaflets. In the third field trial (trees treated on August 14), there was a weekly increase in bioassays with uninhibited germ tube development despite the lack of additional rainfall. This supports the concept that factors other than rainfall affect degradation. Photodecomposition of carbendazim by ultraviolet light has been suggested as a potential factor affecting its stability (5). At the quantities of benomyl applied, the rate of foliar application did not appreciably affect time of persistence.

Persistence of residues on nut hulls did not differ greatly from persistence on leaves (Table 2). After 40–47 days following an initial application at 0.6 g a.i./L, between 44 and 83% of the bioassays were free from inhibitory surface residue at three test sites. Benomyl residues were more persistent at Martinsville despite a larger accumulated rainfall over the same postreatment period. Sprays at higher application rates on nut hulls at two test sites were more persistent than sprays at 0.6 g a.i./L during the 6- to 7-wk observation period.

Information on fungicide persistence is important in determining the number of applications required to control diseases over a growing season. These data are usually not available for specific crops and their pathogens and rarely evaluate persistence relative to environmental

parameters. Establishing a correlation between environmental factors and fungicide persistence adds an element of prediction to a control program.

This study demonstrated the usefulness of an uncomplicated bioassay technique in determining benomyl persistence on black walnut leaves and nut hulls. Although present recommendations for fungicide application to control walnut anthracnose were based entirely on field trials and knowledge of the events of the pathogen life cycle, these data alone may not be satisfactory to explain failures in disease control. Combining information about the pathogen life cycle and factors influencing chemical persistence will enhance the predictability of the performance of fungicide applications.

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