Efficacy of New Benzimidazole Fungicides Against Sensitive and Benomyl-Resistant *Botrytis cinerea*

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ABSTRACT

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Four alkyl isocyanate homologues of benomyl were synthesized and compared for their toxicities towards benomyl-sensitive (S) and benomyl-resistant (R) isolates of *Botrytis cinerea* using a spore germination test. For the inhibition of germ tube length, methyl, ethyl, and propyl isocyanate homologues of benomyl (MBC-MIC, MBC-EIC, and MBC-PIC, respectively) were as effective against the S isolate as benomyl (MBC-BIC), but they were more effective than MBC-BIC against the R isolate. The hexyl isocyanate homologue of benomyl (MBC-HIC) was less effective than MBC-MIC, MBC-EIC, and MBC-PIC against both isolates. MBC-MIC was equally effective in inhibiting spore germination of both the S and R isolates and was more effective than the other homologues against the S isolate. The sensitivity of spore germination of the S and R isolates to MBC-EIC and MBC-PIC was the reverse of their sensitivity to benomyl, constituting an example of negative cross resistance. For the protection of wounded apples, MBC-EIC, MBC, and benomyl were compared. MBC-EIC was more effective than benomyl and MBC against the R isolate but was slightly less effective than benomyl and MBC against the S isolate. Accordingly, MBC-EIC and possibly MBC-MIC are benzimidazole compounds with potential for use against fungal strains both sensitive and resistant to benomyl.

Benomyl is a widely used fungicide that has been effective worldwide against many diseases of commercially important crops. Clemons and Sisler (5) reported that benomyl decomposed to carbendazim (MBC), and that MBC was also fungitoxic. Later, they reported that MBC would be the toxic material in the plant at sites removed from the point of application of benomyl (6). It was demonstrated that benomyl and MBC interfere with mitosis in fungi (7,9). Much of the research to elucidate the mode of action of benomyl has been conducted with MBC, because MBC was regarded as the principal fungitoxicant.

It was recognized, however, that benomyl was more active than MBC in the inhibition of growth of some fungi, and the differential effect was ascribed to butyl isocyanate (BIC), the other degradation compound of benomyl (10). BIC was reported also to be an inhibitor of cutinase (13). Recently Chiba et al (2) showed for the three isocyanates, BIC, methyl isocyanate (MIC), and hexyl isocyanate (HIC), that HIC was the most active inhibitor of respiration and fermentation of yeast (*Saccharomyces cerevesiae* Meyen ex Hansen).

A serious disadvantage of current commercially used benzimidazole compounds is that their repeated use has resulted in the selection of resistant fungal strains against which benomyl has greatly reduced activity (8,11,12,15,16). On the Niagara Peninsula of Ontario, Canada, *Botrytis cinerea* Pers.: Nocca & Balbis, one of the most important pathogens of grapes, has developed resistance to benomyl (16), and alternative fungicides are needed for crop protection.

The objective of this study was to determine the fungitoxicity of four synthesized alkyl isocyanate homologues of benomyl to both benomyl-resistant (R) and benomyl-sensitive (S) isolates of *Botrytis cinerea*. Also, MBC-EIC, the ethyl isocyanate homologue of benomyl was compared with benomyl and MBC for the protection of wounded apples against R and S isolates of *B. cinerea*. A preliminary report of this study was made in 1987 (4), and an application was filed for a U.S. patent (No. 06/894,029, Substituted Benzimidazole Fungicide, 7 August 1986).

MATERIALS AND METHODS

Compounds tested. Benomyl and MBC were of analytical grade (99% purity) and from E. I. DuPont de Nemours and Co., Inc. Methyl isocyanate (MIC) and hexyl isocyanate (HIC) were from Eastman Kodak Company, and ethyl isocyanate (EIC) and propyl isocyanate (PIC) were from Aldrich Chemical Company Inc.

Benomyl homologues were synthesized by the following procedure. To 1 g of MBC, 20 ml of $CHCl_3$ was added. After being mixed well, 10 ml of the isocyanate of interest was added and stirred to dissolve the MBC at 25 C. After 10 min of additional stirring, 150 ml of hexane was added to precipitate the desired compound. The suspension was filtered through a sintered glass filter, and the precipitate was washed with the appropriate isocyanate containing hexane (10%, v/v), and vacuum dried at 25 C. The yields were in the range of 76–87%. The methyl, ethyl, propyl, and hexyl isocyanate homologues of benomyl were synthesized and are referred to respectively as MBC-MIC, MBC-EIC, MBC-PIC, and MBC-HIC.

The authenticities of the synthesized compounds were confirmed by proton nuclear magnetic resonance (NMR) spectrometry with a Bruker WP-60 FTNMR unit, mass spectrometry (MS) with a Kratos/AEI MS30 unit, and high-performance liquid chromatography (HPLC) with a Hewlett-Packard HP-1090 unit equipped with HP-1040A diode-array UV absorption detector and elemental analysis. HPLC results show no extra peak except a trace of MBC, which is practically impossible to eliminate even with the current advanced knowledge and technology. The analytical results of the elemental analysis are in good agreement with the theoretical values (Table 1). Based on the results of HPLC and elemental analysis, the purities of the synthesized compounds were judged to be as good as those of the benomyl and MBC analytical standards.

Suspension of fungicidal compounds. Because these synthesized fungicidal compounds were of low density and impossible to dissolve at the desired concentrations in water (17), the following procedure was used to prepare a stock suspension of 1,128 μM of each compound. A predetermined amount ranging from 21.56 mg of MBC to 35.92 mg of MBC-HIC was ground with 200 mg of dextrose in an agate mortar, then dispersed in 0.2 ml of an aqueous solution containing 50 mg/ml of Tween 20 and diluted to 100 ml with a dextrose solution (18.04 mg of dextrose per milliliter of water). Each suspension contained the following concentrations: 20 mg/ml of dextrose, 0.1 mg/ml of Tween 20, and 1,128 μM of the compound. This was used as the stock suspension and was diluted sequentially with a factor of 1.78 with an aqueous solution containing 20 mg/ml of dextrose and 0.1 mg/ml of Tween 20,

giving a concentration range of 1,128–0.064 μM for each compound. Immediately after each concentration series was prepared, it was mixed with fungal spores as described below.

Spore suspensions of *Botrytis cinerea*. We used two isolates of *B. cinerea* sensitive (S) to benomyl at 1 μg/ml and two isolates resistant (R) to benomyl. These had been obtained from grapes in 1984, selected as vigorous sporulating cultures from single macroconidia, and maintained on potato-dextrose agar (PDA, Difco), nonamended and amended with benomyl (1 μg/ml, 3.4 μM) for the S and R isolates, respectively. Fresh cultures were grown on PDA in the dark for 3 days and then in fluorescent light (300 μE/m^2/sec) with a 16-hr daily photoperiod for 7–10 days to induce sporulation. The spore suspension (of macroconidia) was prepared by shaking a culture in sterile distilled water, followed by filtration and adjustment to the required spore concentration with sterile distilled water (pH = 6.1 ± 0.4).

Fungitoxicity test. The spore suspension was combined with salts (K citrate and Na citrate) (14) and a freshly prepared concentration series of each compound containing Tween 20 and dextrose. The salts, dextrose, and Tween 20 aided in uniform spore germination and germ tube development. The final concentrations per milliliter were: 3×10^4 spores; 10 mg of dextrose; 0.01 mg of K citrate; 0.01 mg of Na citrate; 0.05 mg of Tween 20, and variable concentrations of the test compound. For each compound, 18 concentrations were used in the range 0.032–564 μM, and compared with a dextrose-salts-Tween 20 control. Suspensions were dispensed as 30 μl drops, with four replicate drops spotted on the inner surface of the lid of an inverted 10-cm-diameter polystyrene petri dish. The other part of the dish covered the drops to minimize evaporation. The germination test was conducted at 21 C in darkness and was terminated after 20 hr with a 30-μl drop of Na azide (2 mg/ml) dissolved in 1:9 glycerol:water. Experiments were repeated once.

For each of the four replicates, 50 spores were examined and considered germinated if the length of the germ tube exceeded the length of the spore. Results from both experiments were averaged. The percentage inhibition of germination was plotted as a probit against the log concentration of the test compound.

A germ tube length test was conducted with spores from the germination test. The lengths of 10 germ tubes were measured for each replicate of both experiments (i.e., 80 observations per isolate per concentration). Germ tubes were selected in an unbiased way as they appeared in the microscope field of view by slowing scanning the germination droplet. The averaged percentage inhibition of germ tube length was plotted as a probit against log concentration of the test compound.

Transformed values showing a linear distribution close to 50% inhibition (EC_{50}) were analyzed using a probit-log concentration program that gave both the EC_{50} value and the slope of the response curve, with 95% confidence limits, for the tests of both germination and germ tube length.

Protection of wounded apples. Cold-stored McIntosh apples were surface-sterilized with Na hypochlorite solution (15 mg/ml) for 3 min, rinsed, and dried. Eighteen apples were arranged in a paper-lined wooden tray (30 × 42 × 8.5 cm) constituting one treatment unit. One such unit was used for each combination of isolate and chemical concentration. Each apple was wounded once with a 4-mm-diameter sterilized nail to a depth of 4 mm. To each freshly made wound was added a 30-μl drop of freshly prepared suspension of either the S or the R isolate of *B. cinerea*, with a concentration of 1×10^4 spores per milliliter. Two hours after inoculation, when the inoculum drop had been absorbed or evaporated, a 30-μl drop of fungicide suspension with Tween 20, salts and dextrose, as used in the germination test, was added. MBC, benomyl, and MBC-EIC were used at concentrations between 18 and 3,170 μM. The treated apples were incubated at 20 C for 7 days and evaluated for botrytis lesions arising from the inoculated wounds. The percentage of fruits infected was calculated for each treatment, and the data were examined by a probit analysis to calculate the EC_{50} and EC_{95} values and their respective 95% confidence limits.

TABLE 1. Percentage of carbon (C), hydrogen (H), and nitrogen (N) by weight in carbendazim (MBC), benomyl, and four benomyl homologues, determined by elemental analysis and compared with theoretical values

	C		H		N	
Compound	Theoret.[a]	Anal.[b]	Theoret.	Anal.	Theoret.	Anal.
MBC	56.54	57.00	4.76	5.01	21.98	22.28
MBC-MIC[c]	53.22	53.35	4.88	5.15	22.57	22.19
MBC-EIC[d]	54.95	55.02	5.35	5.67	21.36	21.63
MBC-PIC[e]	56.51	56.92	5.84	5.91	20.28	21.64
Benomyl	57.92	58.34	6.25	6.59	19.30	19.20
MBC-HIC[f]	60.36	61.77	6.97	7.35	17.60	18.11

[a] Theoretical value.
[b] Analytical value.
[c] Methyl isocyanate.
[d] Ethyl isocyanate.
[e] Propyl isocyanate.
[f] Hexyl isocyanate.

TABLE 2. Concentrations of benzimidazole compounds giving 50% inhibition (EC_{50}) of germination of *Botrytis cinerea* spores sensitive (S) and resistant (R) to benomyl

Compound	Isolate	EC_{50} (μM) and its 95% confidence limits			Resistance level[s]	Slope of response curve and its 95% confidence limits	
MBC[t]	S	316	87–9,620	d[u]		0.23	0.11–0.35 d
	R	>564	–		>1.8	0.17	0.05–0.30 d
MBC-MIC[v]	S	18	15–22	b		1.96	1.23–2.68 ab
	R	17	13–21	b	0.9	1.47	0.76–2.18 ab
MBC-EIC[w]	S	115	65–359	cd		0.44	0.25–0.63 cd
	R	7	4–10	a	0.06	0.49	0.36–0.63 c
MBC-PIC[x]	S	>178	–			0.43	0.16–0.70 cd
	R	55	46–67	c	<0.3	1.19	1.04–1.35 b
MBC-BIC[y]	S	161	87–366	d		0.42	0.33–0.50 c
(benomyl)	R	>317	–		>2.0	0.55	0.35–0.74 c
MBC-HIC[z]	S	138	109–163	d		1.96	1.45–2.46 a
	R	>564	–		>4.1	0.46	0.14–0.78 cd

[s] Resistance level (RL) was obtained by dividing the EC_{50} value for the R isolate by the EC_{50} value for the S isolate.
[t] Carbendazim.
[u] Values in the same column followed by different letters differ significantly ($P = 0.05$) with nonoverlapping 95% confidence limits. Values without confidence limits cannot be compared.
[v] Methyl isocyanate.
[w] Ethyl isocyanate.
[x] Propyl isocyanate.
[y] Butyl isocyanate.
[z] Hexyl isocyanate.

RESULTS

Inhibition of germination. The two sensitive isolates (S1 and S2) responded very similarly in all the fungitoxicity tests. Likewise, the differences between the two resistant isolates (R1 and R2) were no greater than the differences between the results of the first and second experiments, with the same isolate. For simplicity, only the results for isolates S1 and R1 are presented and referred to as S and R, respectively.

For inhibiting spore germination of the S isolate, MBC-MIC was the most fungitoxic, with an EC_{50} concentration of 18 μM, compared with significantly higher values for the other five compounds (Table 2, Fig. 1). However, for inhibiting spore germination of the R isolate, MBC-EIC was the most fungitoxic (EC_{50} 7 μM), followed by MBC-MIC and MBC-PIC. Precise EC_{50} values for MBC, benomyl, and MBC-HIC against the R isolate could not be determined because inhibition of germination was less than 50% even at the highest concentrations of 317 or 564 μM. Observations at higher concentrations could not be made reliably because of visual interference from numerous small crystals of the parent compounds.

The R isolate was also resistant to MBC and MBC-HIC with resistance levels >1.8, but it was equal to the S isolate in sensitivity to MBC-MIC. In contrast, the R isolate was more sensitive to MBC-EIC and MBC-PIC than the S isolate, with resistance levels of 0.06 and <0.3, respectively (Table 2).

The responses of germination inhibition to individual compounds are shown in Figure 1. Despite the transformation of data using probits and log concentrations of compounds, many of the germination responses were not linear over the entire

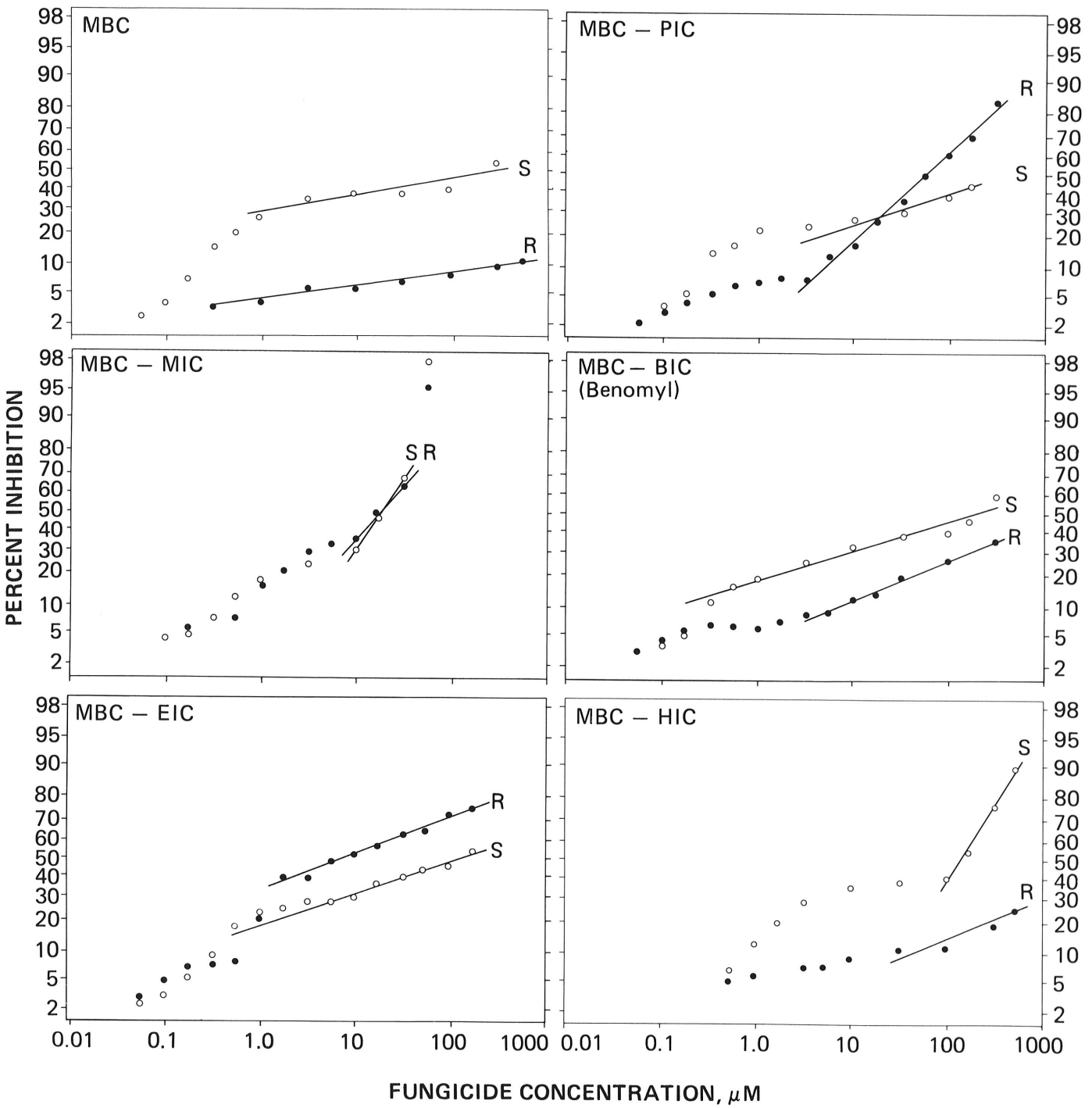

Fig. 1. Response to MBC, MBC-MIC, MBC-EIC, MBC-PIC, MBC-BIC (benomyl), and MBC-HIC of spore germination of *Botrytis cinerea*, sensitive (o) and resistant (•) to benomyl.

concentration range. Several of them, for both the S and R isolates, showed two linear response regions, one at low concentrations in the low inhibition range, and the second at high concentrations in the mid to high inhibition range (Fig. 1). The slopes of the response curves near 50% inhibition were gentle except for the steeper response of the R and S isolates to MBC-MIC and of the S isolate to MBC-HIC (Table 2, Fig. 1).

Inhibition of germ tube length. The six benzimidazole compounds were highly fungitoxic towards the S isolate, with EC_{50} values of 0.14–0.72 μM. Each compound was less fungitoxic to the R isolate than to the S isolate, with EC_{50} concentrations of 0.60–13.17 μM for the R isolate. It was impossible to obtain an EC_{50} value for MBC against the R isolate because germ tube length was reduced by only about 30% at concentrations between 1 and 564 μM (Fig. 2). The levels of resistance for MBC-MIC and MBC-EIC were 4 and 3, respectively, compared with 63 for benomyl and >4,030 for MBC (Table 3). The inhibition of germ tube length increased rapidly with increasing concentrations of the test compounds as illustrated by steep slopes (Fig. 2) and high slope values (regression coefficients), especially for the S isolate (Table 3). The slope values for the R isolate were significantly lower than those for the S isolate in response to benomyl and MBC-HIC.

The transformed data showed a linear response for the S isolate towards all the compounds, although there appeared to be a curvilinear relationship in the lower concentration range that was especially pronounced with MBC-HIC (Fig. 2). These phenomena appeared to be common for the response of the R isolate to all of these compounds (Fig. 2). However, in the case of MBC-HIC, there was a further departure from linearity in the response of the R isolate in the very high concentration range (Fig. 2).

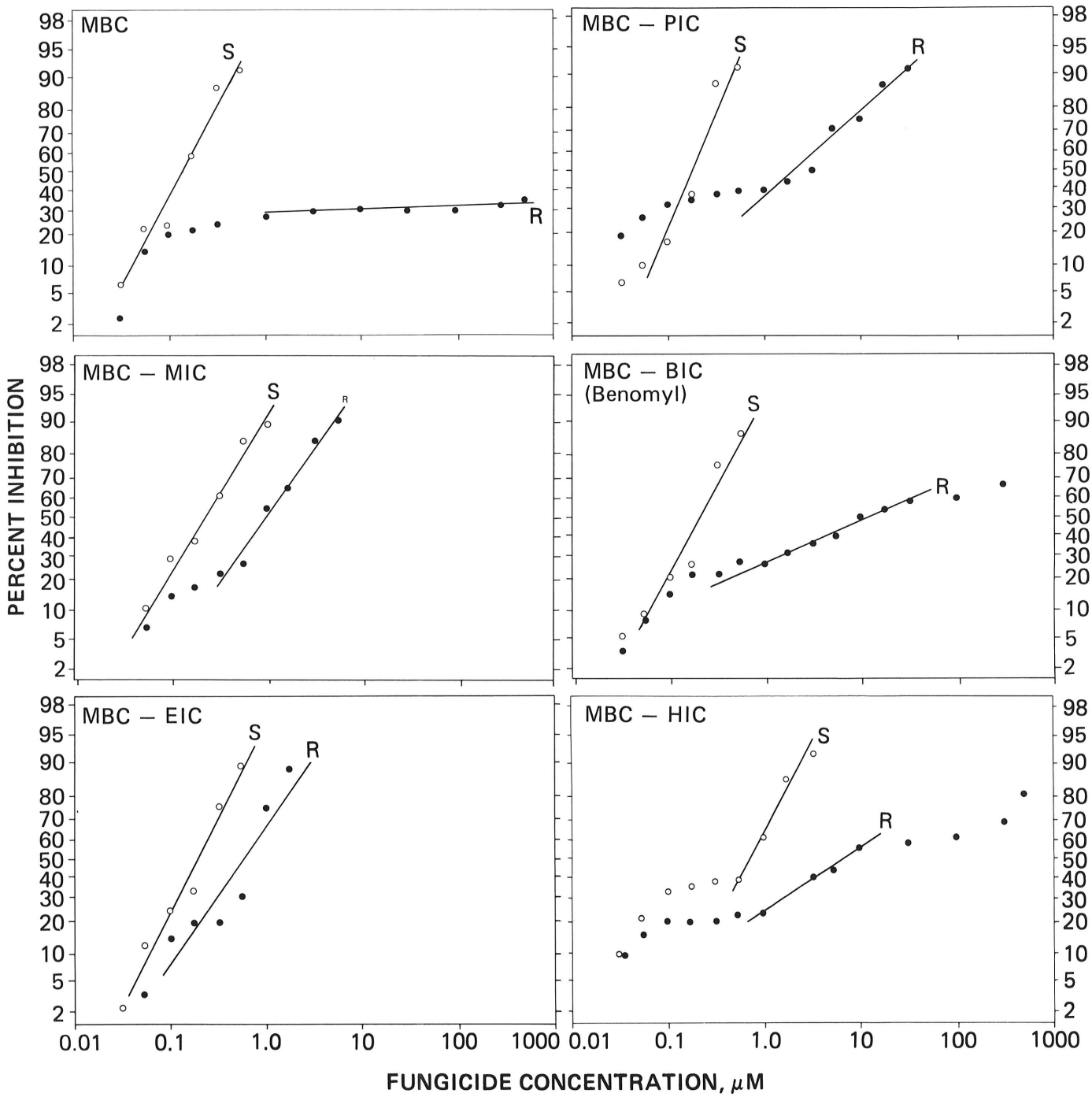

Fig. 2. Response to MBC, MBC-MIC, MBC-EIC, MBC-PIC, MBC-BIC (benomyl), and MBC-HIC of germ tube length of *Botrytis cinerea*, sensitive (o) and resistant (•) to benomyl.

TABLE 3. Concentrations of benzimidazole compounds giving 50% inhibition (EC_{50}) of germ tube length of *Botrytis cinerea* spores sensitive (S) and resistant (R) to benomyl

Compound	Isolate	EC_{50} (μM) and its 95% confidence limits		Resistance level[r]	Slope of response curve and its 95% confidence limits	
MBC[s]	S	0.14	0.11– 0.19 a[t]		2.46	1.66–3.26 ab
	R	>564	–[t]	>4,030	0.08	–[u]
MBC-MIC[v]	S	0.23	0.20– 0.25 b		2.14	1.88–2.41 ab
	R	0.97	0.83– 1.11 c	4	1.82	1.53–2.11 b
MBC-EIC[w]	S	0.20	0.17– 0.22 ab		2.53	2.18–2.88 a
	R	0.60	0.38– 1.13 c	3	1.87	1.01–2.73 abc
MBC-PIC[x]	S	0.18	0.11– 0.30 ab		3.12	1.34–4.90 abc
	R	2.08	1.57– 2.60 d		1.16	0.95–1.38 c
MBC-BIC[y]	S	0.21	0.15– 0.34 ab		2.43	1.36–3.50 abc
(benomyl)	R	13.17	8.78–24.27 e	63	0.57	0.38–0.76 d
MBC-HIC[z]	S	0.72	0.59– 0.83 c		2.44	1.88–2.99 ab
	R	6.22	4.41–10.63 e	9	0.86	0.51–1.21 cd

[r] Resistance level (RL) was obtained by dividing the EC_{50} value for the R isolate by the EC_{50} value for the S isolate.
[s] Carbendazim.
[t] Values in the same column followed by different letters differ significantly ($P = 0.05$) with nonoverlapping 95% confidence limits. Values without confidence limits cannot be compared.
[u] Nonsignificant slope.
[v] Methyl isocyanate.
[w] Ethyl isocyanate.
[x] Propyl isocyanate.
[y] Butyl isocyanate.
[z] Hexyl isocyanate.

TABLE 4. Percentage inhibition by carbendazim (MBC), MBC-EIC[a], and MBC-BIC[b] (benomyl) of wound inoculations of apple fruits with benomyl-sensitive (S) and benomyl-resistant (R) *Botrytis cinerea*

Concentration (μM)	MBC		MBC-EIC		MBC-BIC	
	S	R	S	R	S	R
3,170	...[c]	0[d]	...	...	...	0
1,787	...	0	...	...	...	0
1,000	...	0	...	100	...	0
564	...	0	...	89	...	0
317	...	...	100	67	...	...
178	100	...	94	39	100	...
100	100	...	89	6	100	...
56	89	...	67	11	83	...
32	61	...	50	...	11	...
18	17	...	11	...	11	...
0 (check)	0	0	0	0	0	0

[a] Ethyl isocyanate.
[b] Butyl isocyanate.
[c] Not tested.
[d] 100% infection.

TABLE 5. Concentrations of carbendazim (MBC), MBC-EIC[a], and MBC-BIC[b] (benomyl) (μM) required for protection of apples against benomyl-sensitive (S) and benomyl-resistant (R) *Botrytis cinerea*

Compound	Isolate and resistance level (RL)	EC_{50} (μM)		EC_{95} (μM)	
MBC	S	29	(26–31)[c]	67	(58–84)
	R	>3,170		>3,170	
	RL[d]	>109		>47	
MBC-EIC	S	39	(26–53)	152	(97–435)
	R	224	(139–402)	870	(458–8,270)
	RL	5.7		5.7	
MBC-BIC	S	about 56		about 100	
(benomyl)	R	>3,170		>3,170	
	RL	>57		>32	

[a] Ethyl isocyanate.
[b] Butyl isocyanate.
[c] Mean with its 95% confidence limits in parenthesis.
[d] Resistance level (RL) was obtained by dividing the EC_{50} value of the R isolate by the EC_{50} value of the S isolate, and likewise for the respective EC_{95} values.

The lengths of individual germ tubes of the R isolate varied within fairly narrow limits, in relation to the mean value at each concentration of MBC-HIC. The shortest tubes were 23–53% (mean 33%) shorter than the mean, and the longest tube lengths were 19–56% (mean 39%) longer than the corresponding mean.

Protection of wounded apples. MBC-EIC gave 100% inhibition of infections by the S isolate at a concentration of 317 μM, comparable to the efficacy of benomyl and MBC at 100 μM (Table 4). Against the R isolate, MBC-EIC gave complete protection at 1,000 μM in contrast to the total inefficacy of benomyl and MBC at 3,170 μM.

The concentrations required to give 50 (EC_{50}) and 95% (EC_{95}) protection of inoculated wounds were calculated using probit analysis. For the R isolate, the EC_{50} and EC_{95} values for MBC-EIC were 224 and 870 μM, respectively, compared with no response to MBC and benomyl even at 3,170 μM (Table 5). For the S isolate, the EC_{50} and EC_{95} values for MBC-EIC were slightly higher than those for MBC, but these values were, respectively, slightly lower and slightly higher than the approximate values for benomyl. The resistance level (RL), based on values giving 50% inhibition was 5.7 for MBC-EIC, compared with values of RL >109 for MBC and RL >57 for benomyl.

DISCUSSION

Germ tube length was the more sensitive indicator of fungicidal activity because the concentrations of MBC and of the four isocyanate homologues of benomyl required to give 50% inhibition of germ tube length were less than those required to give 50% inhibition of spore germination. Similarly, in *Venturia inaequalis* (Cke) Wint., germ tube length was more sensitive than spore germination to benomyl, and it was preferred as a criterion for monitoring benomyl resistant spores (15).

In the spore germination study, the R isolate was more sensitive than the S isolate to MBC-EIC and MBC-PIC. This response was inverse to the relative response of S and R isolates to benomyl, and constituted an example of negative cross resistance. The phenomenon was in contrast to the cross resistance of the R isolate to MBC and of MBC-HIC. MBC-MIC was equally toxic to the germination process of both the S and R isolates.

With inhibition of germ tube length as the criterion, the R isolate was also cross resistant to MBC and to the four benomyl homologues. However, for MBC-MIC and MBC-EIC, the resistance levels (RL) were only 4 and 3, respectively, compared

with the much larger RL values of 63 and >4,030 for benomyl and MBC, respectively. Benomyl was more toxic than MBC to germ tube growth of the R isolate, and this was attributed either to the greater intrinsic toxicity of benomyl or, more probably, to the fungitoxicity of butyl isocyanate (BIC), which is released upon the hydrolysis of benomyl to MBC and BIC (10), or possibly, to an interaction effect between these products.

Because benomyl is only slightly soluble in water (17), previous workers prepared stock solutions of benomyl in organic solvents such as acetone and methanol. However, because the rates of decomposition of benomyl to MBC in these solvents are very fast (3), the actual concentrations of benomyl in the sample were probably much lower than intended.

To minimize the decomposition of these new benzimidazole compounds, no organic solvents were used to prepare sample suspensions in this study. Nevertheless, there could have been some decomposition during the preparation of the suspensions, because it took 2 hr ± 10 min to prepare the concentration series of each compound before the mixing with spores and the commencement of incubation at 21 C. The germination test was completed in 20 hr so that the results obtained in both the germination and germ tube length tests should have indicated the biological activities of these compounds fairly accurately.

The departures from linearity of the responses of both the S and R isolates to most of the compounds (Figs. 1 and 2) was not interpreted as evidence of genetically heterogeneous populations. In the response of the R isolate to MBC-HIC, the extremes of individual germ tube lengths were fairly uniform when expressed as percentages of the mean length. This indicated that the R isolate was responding as a single population over the entire concentration range. Furthermore the 95% confidence limits for the EC_{50} values in Tables 2 and 3 showed essentially the same narrow spread for both the S and R isolates, confirming homogeneity of these populations.

The explanation for nonlinearity of response may lie in the mode of action of these benomyl homologues. Nonlinearity may be caused by different modes of action affecting different sites or by the presence of more than one fungitoxic compound. For example, MBC-HIC may hydrolyze to MBC and HIC in the same manner that MBC-BIC (benomyl) hydrolyzes to MBC and BIC (9). Furthermore, HIC was shown to be a more potent inhibitor of yeast respiration and fermentation than BIC and MIC (2). The sequential increases in inhibition may be due, therefore, to an initial response to the parent compound, or to MBC and the isocyanate moiety resulting from the hydrolysis of the parent compound. The additional response at the higher initial concentration of the parent compound, may be a response to increasing concentrations of HIC, the concentration of which will depend on its stability in the aqueous phase. Further studies are in progress to determine the stability and possible modes of action of several of these compounds.

For the protection of wounded apples, benomyl gave 100% protection against the S isolate at 100 μM. This was much lower than the concentration of 862 μM (250 μg/ml) at which benomyl was recommended for commercial use in apple dipping tanks (1). By comparison, MBC-EIC provided 100% protection against the S isolate at 317 μM, and was slightly less effective than MBC and benomyl as judged from the calculated EC_{95} concentrations. However, against the R isolate, MBC-EIC was much more effective than MBC and benomyl, which provided no protection at the highest concentration of 3,170 μM.

The EC_{50} concentrations of MBC-EIC required for the protection of apple wounds against the S and R isolates were respectively, 195 and 373 times greater than the EC_{50} values for inhibition of germ tube length, on the inert polystyrene surface. Under the same conditions, the corresponding EC_{50} values for benomyl and MBC for the S isolate were 267 and 207 times, respectively, showing a factor of approximately the same magnitude.

Under commercial conditions, fungicidal treatment by dipping is practiced only if the apple fruits are being stored for several months at 1 C in common or controlled atmosphere storages. In this experiment, the apples were incubated at 20 C immediately upon inoculation and fungicide treatment, and no simulated cold storage period was used. Because the effects of lower temperatures on the stability and efficacy of alkyl isocyanate homologues of benomyl are not known, it was considered premature to extrapolate these findings to commercial situations. However, it was clearly demonstrated that MBC-EIC was effective against both sensitive and benomyl resistant isolates of *B. cinerea*.

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