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COMPARATIVE FUNGICIDAL ACTIVITY OF BENOMYL AND ITS BREAKDOWN PRODUCT METHYL 2-BENZIMIDAZOLECARBAMATE (MBC) ON CITRUS

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Abstract. **When exposed to heat or moisture the fungicide benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) begins to decompose into one or more compounds depending upon conditions. Two of these compounds, methyl 2-benzimidazolecarbamate (MBC) and 3-butyl-2,4dioxol(1,2-a)-s-triazinobenzimidazole (STB) are to be expected to form under the conditions that benomyl is handled by the Florida citrus industry.**

Suspensions of benomyl held under conditions simulating those typical of commercial practices were found to be less effective than freshly prepared benomyl against benzimidazole sensitive Penicillium digitatum. **Laboratory prepared MBC was found to be 10-15 times less effective against a mildly resistant strain of** P. italicum.

Since it was introduced to the Florida citrus industry, about 7 years ago, the fungicide 1-(butylcarbamolyl)-2-benzimidazolecarbamate (benomyl) has become the fungicide of choice of the majority of Florida's packinghouses. A recent survey, by the author, of 30 Orange, Lake and Polk county (unpublished) packinghouses found that 18 were using benomyl. These represented 18 million of the 27 million cartons packed by these houses (12).

One reason that benomyl has reached this level of use, displacing thiabendazole (TBZ), is that benomyl is available at a much lower cost. One service company offers a treatment at a level that will give the quantities recommended for decay control (19, 20, 21, 26), at about one half the cost of TBZ. In addition, benomyl is available to the packinghouse as an end use product (20) that the house may use themselves, whereas TBZ is not available in a similar form at the present time (11, 27).

In Figure 1 the decomposition pathways of benomyl are illustrated. When subjected to heat or moisture, Uv radiation and some solvents benomyl decomposes via pathway *a* to 1-(butylcarbamoyl)-2-benzimidazole carbamate (MBC) (4, 6, 7, 9, 10, 28), with *n*-butyl isocyanate (BIC) as a by-product (7, 28). At neutral pH or when dry benomyl is heated, about 90% becomes MBC and the balance 3-butyl-2,4-dioxo(1,2-*a*)-*s*-triazinobenzimidazole (STB) (5, 28), pathway *b*. In alkaline conditions STB is formed, pathway *c*, and under very alkaline conditions, pH 12 or higher, 1-(2-benzimidazolyl)-3-*n*-butyl urea (BBU) is formed, pathway *d* (5, 28). Under strong alkaline conditions both MBC and BBU slowly decompose further to 2-aminobenzimidazole (2-ABZ) (9, 17) pathways *c* and *f*.

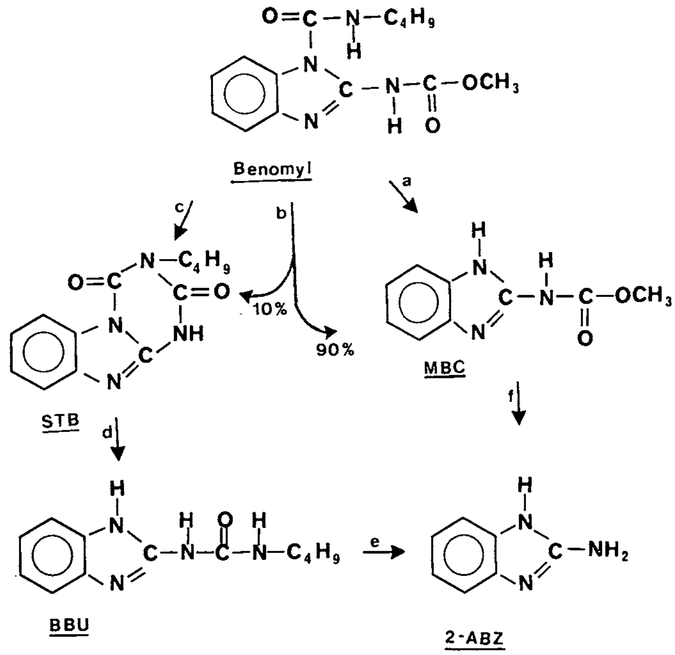

Fig. 1. Decomposition pathways of benomyl.

Under commercial conditions benomyl mixes are usually complex mixtures that do not result in pure reactions (3, 7). Instead, the result will be a mixture of benomyl and its decomposition products (Table 1) principle of which will be MBC. (4, 6, 7, 8, 16, 17, 28).

The reaction rate of benomyl is related to the concentration of the suspension (9, 17, 18) and may be quite rapid (7), leaving very little benomyl to be applied to the fruit. Table 2.

The fungicidal efficacy of the breakdown products of benomyl becomes of primary importance as it is apparent that, outside the laboratory, little benomyl, as such, may ever reach the fruit. That MBC is fungitoxic has been

Table 1. Percent conversion of benomyl in aqueous suspension to MBC and STB at several pH (9, 17).

		Suspension concentration			
		5000 ppm			250 ppm
Product	Time	pH 8	pH 9	pH 10	pH 8
STB[w]	2.5 days	*[z]	6.7	24.8	* (36 days)[y]
STB[x]	13 days	*[z]	16.3	43.8	9.8
MBC[x]	" "	41.2	34.8	24.0	76.6
Benomyl[x]	" "	58.8	49.0	31.5	13.6

[z]* = STB too little to measure.
[y]STB not present in measurable quantities after 36 days.
[w]In commercial citrus storage wax.
[x]In buffer solution.

Table 2. Percentage of benomyl remaining versus hours after mixing. (9, 18).

Vehicle	Concentration	Percent Benomyl remaining				
		3 hrs	8 hrs	16 hrs	24 hrs	7 days
Buffer pH 7.6	25,000 ppm	88	87	86	85	70
Packout Wax pH 7.6	25,000 ppm	87	82	74	67	—
Packout Wax pH 10.5	1,000 ppm	45	24	6	2	—

established by several workers (8, 9, 10), but they have found it to be considerably less effective than benomyl. Clemons and Sisler found MBC to be from 4 to 30 times less effective than benomyl against some organisms and equal to benomyl against others (8), but none of the organisms tested are of economic importance on citrus. Eckert et al. (9, 10) tested MBC against citrus disease organisms (*Penicillium*) but only reported on sporulation control, and did not address the question of decay control, which is of major import to the Florida citrus industry. (2, 3, 19, 21, 25).

Materials and Methods

In order to evaluate the effect of benomyl's instability on its use in the Florida citrus industry, it is necessary to define the conditions to which it is subjected. In addition to surveying the environment of benomyl in packinghouses, the behavior of MBC, as compared to benomyl, and the behavior of benomyl under conditions simulating those found in the field is important.

The evaluation of benomyl under Florida conditions was broken into three parts; a survey of conditions, a trial of benomyl vs MBC and a simulated commercial trial.

Survey

Florida commercial packinghouses were surveyed to determine the conditions benomyl was being subjected to prior to use. Packinghouse personel were interviewed, application rates were determined, samples taken for pH measurement and the volume of benomyl preparation was determined to estimate the time of exposure to the vehicle. No effort was made to determine which operations were the most common or typical, but a variety was sought to illustrate the range of conditions that may be found.

Benomyl vs. MBC

Approximately 1800 valencia oranges were innoculated by scratching (1 cm long by 1 mm wide by 1 mm deep) then painting the scratch with a suspension of *Penicillium italicum* spores that had been previously tested a slightly resistant to TBZ (growth on agar containing 10 ppm TBZ but no growth on 20 ppm). The fruit was incubated at 70°F and 85% relative humidity for 24 hours before treatment.

Meanwhile technical benomyl was dispersed in 0.5 Normal Hydrochloric Acid (HCl) and held under agitation for 72 hrs. The resultant suspension was filtered then the filtrate washed with 0.1N HCl followed by distilled water washes. The filtrate was then dried and the MBC content determined by Uv spectrophotometry.

The fruit was then treated with wax containing TBZ at 500 and 1000 ppm; benomyl at 200 and 500 ppm; and MBC at 133, 329, 1317 and 3292 ppm which is equivalent to 200, 500, 2000 and 5000 ppm benomyl respectively. The fruit was then held at 58°F and 85% relative humidity for 12 days then examined.

Simulated Commercial Test

Suspensions of commercially available benomyl (11, 26, 27) at 5000 ppm ai. were made up at several pH. These suspensions were held for 60 days then diluted to the equivalent of 600 ppm benomyl and used to treat grapefruit that had been previously innoculated with a benomyl sensitive strain of *P. digitatum.*

Each grapefruit received 5 punctures 3 mm deep then the fruit were briefly immersed in a suspension of the spores. The fruit was then held 16 hrs at 70°F, randomized into 9 lots of 20 fruit each, then, treated by flooding for 2 minutes with each treatment.

The treated fruit was held for an additional 6 days at the same conditions before examining.

Results and Discussion

Survey

Benomyl was found to be applied either in water or in shipping wax. When applied in water it was either first made up into a concentrate which was subsequently diluted for a drencher or spray, or it was prepared as a single strength suspension in a drencher or spray. The pH of the vehicle, water or shipping wax, was the primary factor in the suspensions pH. This could be modified by the use of other additives such as Dow-Hex, chlorine or wetting agents used in preparing the suspension.

All benomyl applications in Florida are individual variations, by supplier or packinghouse, of a single product, 50% wettable powder (26), which is the only one having both state and federal registrations for Florida (11, 27).

Citrus waxes used in Florida were found to have pH's between 8.0 and 10.3 which, with the exception of the two waxes at 8.0 and 8.1, favors the production of some STB. Some of the waxes used (Table 3) have a pH high enough to strongly favor the production of STB, as it is formed in quantity at pH 10, and at pH 10.5 and above STB becomes soluble and the rate of formation increases greatly (5).

Most packinghouses were found to have a tank that will hold between 150 and 800 gallons of shipping wax so that they do not have to replenish their supply more than once a week. If the tank has been supplied by the wax supplier it may be large enough to hold from 2 to 4 weeks supply, thus reducing the number of deliveries that they have to make. Usually the tanks are refilled when they are down to about one fourth of their capacity, resulting in some of the benomyl being exposed to the wax for extended periods. The time of exposure increases greatly towards the end of the season when the volume of fruit processed per week often drops and the rate of wax consumption drops.

When benomyl is dispersed in water for use as a prewax spray it is used in one week. When used in a drencher it is used until the solution is too dirty to use, which depends upon the opinion of the operator and may be as long as 6 weeks. If a concentrate is prepared for dilution it may last an entire season.

Benomyl vs MBC

The resistant strain of *P. italicum* was chosen to give a greater differentiation in control if such a difference exists.

Table 3. Commercial environments of Benomyl in Florida.

Source[z]	Trade Name[y] or Description	Volume [x]	Time Exposed[w]	pH[v]
WAX				
Amer. Mach	Sealbrite 65	150 to 800 gal	7 to 30 days	10.1
" "	" 561	" "	" "	9.7
Brogdex	Britex 555 JT	" "	" "	9.2
FMC	Sta-Fresh 465	" "	" "	9.7
Fresh Mark	Fresh Wax 31C-R2	" "	" "	10.3
" "	" " S	" "	" "	10.1
Pennwalt	Citrus Lustr WT-6-FY	" "	" "	8.7
	" " 209	" "	" "	8.1
	" " 267	" "	" "	8.0
WATER				
Packinghouse A	Metering Concentrate	55 gal	30-90 days	6.5
	Diluted Concentrate	25 gal	4-96 hrs	7.1
	Drencher Solution	600 gal	20-30 days	8.9
Packinghouse B	Drencher Concentrate	100 gal	60-270 days	5.0
	Drencher Solution	600 gal	20-30 days	6.3
Packinghouse C	Tank Mix	150 gal	2-3 days	7.0

[z]Company names indicate packout wax manufactured for use in Florida.
[y]Designates specific Benlate environment for identification.
[x]Typical quantities found in Florida packinghouses.
[w]Typical length of time benomyl may be in contact, "wetted" by wax or water before use.
[v]Analysis of samples taken from packinghouses in Oct. 1980.

Since one gram of benomyl decomposes to 0.67 grams of MBC the lower levels were used that corresponded to some commercially used and experimental levels of benomyl (2). The MBC quantities in Table 4 are followed by the amount of benomyl that would decompose to that amount of MBC.

Table 4. Fungicidal activity of Benomyl vs. MBC.

Treatment[z]	Number		Percent	
	Fruit	Rots	Decay	Control[y]
Checks (Wax only)	190	129	67.9	0 a
Benomyl-200 ppm	191	107	56.0	17.5 b
Benomyl-500 ppm	188	91	48.4	28.7 c
MBC-133 (200)	189	136	72.0	0 a
MBC-329 (500)	187	132	70.6	0 a
MBC-1317 (2000)	190	107	56.3	17.1 b
MBC-3292 (5000)	185	105	56.8	16.3 b
TBZ-500	189	134	70.9	0 a
TBZ-1000	189	129	68.3	0 a

[z]Figures in parenthesis following MBC concentrations is the amount of benomyl that will degrade to that quantity of MBC.
[y]Percent control as compared to the checks taken as 100. Values not followed by the same letter differ significantly at the 95% level of confidence.

Under the conditions of the test, MBC was found to be 10 to 15 times less effective than benomyl. The efficacy of the fungicides may have been reduced by encorporating them into shipping wax (2, 3) but this should have been the same for all treatments.

Simulated Commercial Test

When compared to fresh benomyl, all treatments resulted in lower decay control. As the pH increased, the efficacy of the suspension decreases. The pH 10 sample was apparently contaminated at preparation and a fermentation resulted in a pH of 5.5 at the first check 30 days after preparation. This was adjusted to pH 10 at that time but the "damage" had apparently been done and the benomyl had changed to MBC.

Table 5. Activity of benomyl suspensions after 60 days at various pH.

Treatment[z]	Innoculations	Takes[y]	Control[x,v]
Checks	100	86	0 a
pH 4	100	35	59.3 cd
pH 6	100	39	54.7 c
pH 7	100	40	53.5 c
pH 8	100	49	43.0 b
pH 10[w]	100	38	55.8 c
pH 12	105	50	44.6 b
Fresh Benomyl 600 ppm	100	30	65.1 de
TBZ 1000 ppm	100	29	66.3 e

[z]Checks were flooded for 2 mins with plain water; all other treatments were for two minutes with the treatment as described in the text.
[y]Number of innoculation points that showed decay.
[x]Expressed as a percent using the checks as 0%.
[w]Bacterial growth in suspension caused pH to rise to 5.5 in first thirty days, pH readjusted to 10 which remained stable for balance of time.
[v]Values not followed by the same letter differed significantly at the 95% level of confidence.

That benomyl is unstable is well reported in the literature and is recognized by the Federal government of the United States in setting residue standards for this fungicide (1). The principle breakdown product of benomyl, MBC, is demonstrated to be less effective than the fresh fungicide and due to the time and exposure conditions of benomyl, not only MBC will be formed but also STB should be expected.

Since neither MBC nor STB has as great an efficacy as benomyl, their are at least two main areas of concern that are interrelated.

The first is that since most packinghouses are using the minimum amount of fungicide that they must to meet state legal requirements, decomposition to less of a fungicide with lower efficacy would mean substandard decay control. The other area of concern is that by applying sublethal doses of fungicides the buildup of fungicide resistant strains of molds may develop (14, 15, 22, 25, 29). The conditions found may, in some cases, favor the production of STB which has not been demonstrated to have any fungicidal activity. This kind of application may even result in strains of mold that are stimulated by benzimidazole fungicides (24). Once resistant molds are established in the packinghouse, they are very difficult to control (13, 15, 23, 25, 29).

Since benomyl is a systemic fungicide (8, 9, 10, 16) whereas TBZ (16) and MBC (8, 9, 10) are not it is important to get the maximum amount of undegraded fungicide onto the fruit. This may be accomplished by applying benomyl in a vehicle that is no more alkaline than pH 8.5 and keeping the amount prepared for application to no more than the amount that may be used up in one day. Since the half life of benomyl, at use strength, is 7 hrs (7), an alternate would be to prepare a strong (3%) suspension that could be then metered into a small reservoir for dilution to use strength. Table 2 indicates that using this up rapidly would give more benomyl at the fruit. Part of the reason for the slower degradation of benomyl in concentrates is probably the build up of BIC concentration which slows down the degradation of benomyl (7). By making up no more than a weeks supply of concentrate at one time, the amount of benomyl available for decay control will be greatly increased.

Benomyl is an excellent fungicide, if used properly, but the trend in the Florida citrus industry is to abuse it in application and handling. If this fungicide is to be handled in a marginal manner it may be wise to increase the amount used in order to maintain good decay control.

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THE ISOLATION OF PENICILLIUM DIGITATUM SACC. STRAINS TOLERANT TO 2-AB, SOPP, TBZ AND BENOMYL

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Abstract. **Sporeload exposure plates and isolates from decayed fruits from citrus packinghouses are routinely used in our laboratory to isolate cultures of green molds and to determine their sensitivity to commercial fungicides. A large body of data has been accumulated that demonstrates the widespread occurrence of** Penicillium **strains that are individually tolerant to either 2-AB (2-amino butane), SOPP (sodium orthophenylphenate) or the benzimidazoles (thiabendazole and benomyl). Doubly resistant strains have also been reported by several investigators. However, we have, for the first time, isolated strains of** Penicillium digitatum **with tolerance to all three groups of fungicides. These multiple tolerant strains have been found to be sensitive to**