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FUNGICIDE COMBINATIONS AS A REPLACEMENT FOR BIPHENYL ON CITRUS

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Abstract. **Combinations of sodium orthophenylphenate (SOPP) and thiabendazole (TBZ), when applied in shipping wax to citrus, have reduced sporulation in Penicillium digitatum and P. italicurn at much lower levels than has been demonstrated as required for either fungicide when used alone. Export shipping and marketing conditions were simulated during which this effect was noted. Laboratory tests in vitro on normal and TBZ resistant strains of P. digitatum confirms this synergistic effect. This combination is suggested as a replacement for biphenyl as a postharvest treatment for citrus.**

Of the fungicides presently available for postharvest use on citrus, biphenyl is unique in that it is not applied directly to the fruit as a solution or suspension prior to packing. Instead pads impregnated with the chemical are placed in the carton at the time of packing where the biphenyl subsequently vaporizes to act as a fumigant (2, 3, 6, 7, 8, 9). Although in wide use in the citrus industry since its introduction over 40 years ago (3, 9) it has several drawbacks.

1. The "chemical" odor is objectional to some consumers (2).
2. For maximum efficacy the pads should be placed so that they will be as close as possible to each fruit in the carton (2). This is accomplished by placing the pads between layers of fruit in the carton (8, ll), thus making an additional operation for the packer.
3. There is often a tendency for the pads to stick together which results in some cartons receiving more than the normal two pads per carton (8).
4. The residue of biphenyl in the peel continues to increase with time and some shippers have had fruit in foreign markets with residues over the legal tolerance (2, 9, 10, 11).
5. Biphenyl's greatest value has been in inhibiting the sporulation of *Penicillium* molds to reduce the soiling of adjacent fruit. Since this is accomplished by the vapor, and some varieties absorb biphenyl vapor strongly, its action varies with the variety treated (2, 3, 9).
6. Under some circumstances biphenyl has been observed to cause or increase peel injury (7).

Desirable qualities in a replacement for biphenyl would include: 1) ease of application; 2) consistant activity within normal application ranges; 3) non-injury of fruit; 4) sporulation control; 5) wide range of activity; and 6) reasonable cost. Of the fungicides presently accepted in the United States (8, 12), none meet these requirements totally. 2-aminobutane (2-AB) and sodium o-phenylphenate (SOPP) are simple to apply but 2-AB is expensive and has a limited range (8). SOPP can cause injury to the peel of the fruit when high residues are the result of a wash or drench (2, 5) and even when 25 ppm was applied in wax, although no injuries resulted, sporulation was not controled (1).

Eckert and Kolbezen (3) demonstrated that thiabendazole (TBZ) would control sporulation, to some extent, at levels of 6 to 9 ppm on fruit but that the high concentrations required in the wax formulations used resulted in nozzle plugging. They also found considerable variation in residues from fruit to fruit in a single treatment variable. Benomyl, being also insoluble, would be subject to the same limitations.

In 1970 the authors began investigations on methods to reduce the variance in residues on individual fruits when applying high concentrations of TBZ in wax. Noting that when SOPP was present in the wax greater sporulation control was obtained than when it was absent, we initiated investigations of this phenomenon. These were conducted in three areas, as in *vitro* evaluation, *in vivo* laboratory tests, and in simulated shipping tests which were conducted through 1977.

Materials and Methods

For the *in vitro* tests, petri dishes were prepared with various levels of SOPP and TBZ in the agar. TBZ at 0, 1, 5, and 10 ppm in combination with SOPP[3] at 0, 5, 10, and 15 ppm, giving a total of 16 different combinations.

These plates were then inoculated by streaking with a suspension of *P. digitatum* spores. The first set of plates were prepared, in duplicate, with a normal or non-resistant strain of the mold. A second set was prepared, also in duplicate, with spores of a resistant strain that had been grown on agar containing TBZ at 20 ppm.

After 72 hours the plates were examined and scored on the basis of growth and spore production as compared to the plain agar (control). Scores were given on a scale of 0 to 10 each for growth and sporulation so that each plate received 2 scores. Thus a plate with a score of 2-5 would be about 2/10ths inhibited as to growth and 5/10ths inhibited in sporulation as compared to the control (O-O). Plates with no growth at all would be 10-10.

Upon examination of the plates inoculated with the normal strain, the control plates were completely covered with mold having abundant spores. There was no growth at all on any of the other plates (score IO-IO). The plates innoculated with the resistant strain all had some growth and the scores are reported in Table 1.

One *in vivo* laboratory evaluation was conducted by waxing, in the laboratory, 'Valencia' oranges with commercial citrus wax containing SOPP[4] at 0, 1, and 2% and TBZ at 0, 1300, 2000, 4000, 5000, 10,000 or 20,000 ppm to give ratios of 0, 0.5:1, 1:1, 2:1, 5: 1, 10:1 and 15:1. Ten fruit of each treatment were syringe inoculated with one ml of a suspension (2 million spores per ml) of blue and green citrus mold spores

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[3]Where **SOPP concentrations (ppm or %) are given, we have followed the established custom of expressing this as the tetrahydrate (Dowicide A) rather than the anhydrous form (5).**

[4]Residues **from the application of SOPP are reported as** *o*-**phenylphenol (OPP). United States Code of Federal Regulations (CFR) Title 40 part 180.129.**

Table 1. Growth and sporulation of TBZ resistant *Penicillium digitatum* on agar.[z, y]

SOPP Concentration[x]	TBZ Concentration 0	1	5	10
	Score			
0	0-0	0- 1	1- 5	2- 6
5	1-4	4-10	4-10	4-10
10	2-5	5-10	5-10	5-10
15	3-6	6-10	5-10	7-10

[z]Agar formula: 19.5 gm Potato Dextrose agar, Difco; 2.5 gm Yeast Extract; 1.75 gm Proteose Peptone No. 3; 20 gm Dextrose; 0.5 gm KH_2PO_4; 1 gm $MgSO_4$; 20 gm Dehydrated agar; Deionized water Q.S. 1 liter.
[y]See text for scoring.
[x]See footnote 3 in main text.

Table 3. Sporulation of inoculated 'Valencias'.

Variable	Treatment	Storage Conditions Temp.	Relative Humidity	% Sporulation at ___ Days[z] 8	10	18	118
1a	Control	45°F	100%	0	0	5	95
1b	SOPP-TBZ[y]	45°F	100%	0	0	0	5
2a	Control	58°F	89%	80	100	100	—[x]
2b	SOPP-TBZ[y]	58°F	89%	20	40	60	—[x]
3a	Control	82°F	71%	100	100	100	—[x]
3b	SOPP-TBZ	82°F	71%	40	70	80	—[x]

[z]Sporulation expressed as a percent of total estimated possible.
[y]Fungicide residue: 1.48 pp TBZ; 8.28 ppm OPP. See footnote 4 of text.
[x]These variables abandoned at the end of 18 days.

(P. italicurn and *P. digitatum).* The inoculated fruit were then held 19 days at 55°F. The fruit were then evaluated for sporulation control which was expressed as a percent reduction when compared to the control. At the time of waxing, samples of each treatment variable were analyzed for fungicide residues. The average of duplicate samples are included with the test results in Table 2.

In another laboratory trial 350 'Valencia' oranges were divided into two lots, half were waxed with a non-fungicidal wax (control) and half with a wax containing 2% SOPP and 3500 ppm TBZ. Samples were analyzed for OPP and TBZ residue. The remaining fruit were syringe inoculated as above. Each treatment was then divided into three lots of 50 fruit each and held in storage under differing conditions as the decay progressed. These fruit were examined periodically and scored for total sporulation as a percent of the total possible. These results are reported in Table 3.

Concurrently with the above experiments, trials were conducted to evaluate the activity of SOPP-TBZ combinations under commercial conditions. For comparison with biphenyl in sporulation control, 2% SOPP with 3500 ppm TBZ was decided upon, as preliminary trials determined that it was impractical to apply higher combined concentration in the equipment then in commercial use.

In each of these tests, conducted between 1970 and 1977, fruit of a single grower lot was treated in a commercial packinghouse during normal operation with the exception of the wax treatment. Cartons were then packed with syringe innoculated fruit distributed throughout the carton. These cartons were then held under conditions to simulate shipping to an export market. These tests are summarized in Table 4.

Results and Discussion

In the *in vitro* tests the presence of either TBZ or SOPP at all levels completely controlled normal *P. digitatum* which, considering the activity of these two fungicides, could be expected (2, 3, 5, 6, 8, 10, 11), but with the TBZ resistant strain, growth occurred on all plates. Some inhibition of growth and sporulation was exhibited by either fungicide alone, but neither one was able to prevent the mold from forming spores. From Table 1 it can be seen that in combination, even at the lowest levels tried (TBZ 1 ppm and SOPP 5 ppm) there was a greater inhibition of growth than by either 10 ppm TBZ or 15 ppm SOPP and all combinations gave total inhibition of sporulation.

When fruit was waxed, inoculated and held in the laboratory this effect continued (Table 2), but not to as great an extent as on agar. On fruit treated with SOPP at 1% and 2% the spore formation by the mold was as great as on fruit without any fungicide. TBZ did reduce the quantity of spores formed, but each of the fruit of those variables still had a spore load more than 50% as heavy as the control. In those treatments receiving a combination of SOPP and TBZ surface mycelial growth was greatly reduced, although growth continued below the surface. Of the 10 fruit in each of Variables 5 through 10, from 2 to 8 fruit had no mycelia visible on the surface.

In the storage test summarized in Table 3 it is apparent that, even though the growth and sporulation of penicillium molds are affected by the temperature of storage, the reduction by combined SOPP and TBZ at commercially practical levels will reduce spore formation.. This effect is especially apparent during long term storage at low temperature.

Table 2. Sporulation control by TBZ/SOPP combinations on laboratory waxed 'Valencias'.

Variable	% SOPP[z]	TBZ ppm	Ratio SOPP:TBZ	Calculated OPP[x]	Residue[y] TBZ	Surface[w] Growth	Sporulation[y] Control
1	0	0	--	0	0	10	0
2		5,000		0	2.0	10	35
3	0	10,000	—	0	3.7	10	40
4	1	0	—	3.8	0	10	0
5	1	20,000	0.5:1	3.8	7.6	2	99
6	1	10,000	1:1	3.8	3.7	5	100
7	2	5,000	2:1	3.8	2.0	4	95
8		4,000	5:1	7.5	1.5	4	95
9	2	2,000	10:1	7.5	0.7	7	90
10	2	1,300	15:1	7.5	0.5	10	85
11	2	0	—	7.5	0	10	0

[z]See footnote 3 of text.
[y]See text for explanation.
[x]See footnote 4 of text.
[w]Number of fruit of 10 inoculated having mycelial growth visable on surface.

Table 4. Simulated export tests.

Test	Fruit	Size[z]	Treatment	Residue[y] OPP	Residue[y] TBZ	Storage[x]	No.[w] Inoc.	% Soiled[v]	% Decay[u]	Sporulation[t] Control
1	Navels	113	Biphenyl	—	—	21d (48°)	10	—[s]	34.0[r]	60%
			TBZ 3500	—	1.3	& 7d (68°)	10	—[s]	7.1[r]	25%
			SOPP 2%	—[s]	—			—[s]	25.0[r]	0
			SOPP-TBZ[q]	—	1.8		10	—[s]	8.6[r]	90%
2	Lemons	140	Biphenyl	4.8	—	21d (55")	10	41.0	13.1[r]	85%
			SOPP 2%		—	& 7d (70°)	10	84.2	12.5[r]	0
			SOPP-TBZ[q]	5.4	1.8		10	38.9	0.4[r]	25%[p]
3	Lemons	140	Biphenyl	—	—	21d (55°)	10	18.0	0.2	70%
			SOPP-TBZ[q]	—[s]	1.9	& 7d (70°)	15	24.0	0.2	60%
4	Grapefruit	36	Control	—	—	19d (52°)	5	38.0	0	0
			SOPP-TBZ[q]	—[s]	—[s]			9.0	0	95%
5	Lemons	140	Biphenyl	—	—	21d (54°)	20	34.4	0.5	60%
			TBZ 7800	—			20	39.4	4.4	47%
			SOPP-TBZ[q]	[illegible]	8.33.3		20	24.2	0.8	67%
6	Valencias	88	Biphenyl	6.0	2.9—	14d (50")	15	55.2	1.0	25%
			SOPP-TBZ[q]				15	2.4	0.3	96%

[z]Number of fruit per California-Arizona carton.
[y]Expressed as parts per million.
[x]Time in days at temperature in degrees F. i.e. 21d (48°) & 7d (68°) is 21 days at 48°F followed by 7 days at 68°F.
[w]Number of syringe inoculated fruit packed in each carton.
[v]Sound fruit soiled by spores, would need washing before sale.
[u]Does not include syringe inoculated fruit.
[t]Estimation based on potential sporulation.
[s]This analysis not made on this test.
[r]Includes the decay of 30 scratch inoculated fruit per carton, see text.
[p]Possible that test was contaminated with TBZ resistant mold, see text.
[q]SOPP concentration 2% with 3500 ppm TBZ in wax.

In the simulated export tests summarized in Table 4, we found that a combination of SOPP and TBZ gave consistently better control than either fungicide alone. When compared with biphenyl the combination treatment gave, with one exception, equal or greater control of sporulation and in every case resulted in less commercial loss of fruit due to soiling and decay.

In the first two tests reported in Table 4, fruit that had been scratch inoculated (1 cm long by 1 mm deep then painted with a spore suspension) were included to evaluate the decay control of each treatment. In test 2 biphenyl gave markedly better sporulation control than the combination of SOPP and TBZ, this may be due to inadvertently having used TBZ resistant mold. The spore suspension used in this trial was prepared using moldy fruit from a packinghouse that was subsequently shown to be having problems with resistant mold (4). Since this was not suspected at the time no tests were made to confirm this assumption.

Lemons consistently had better results with biphenyl which was to be expected since they do not absorb the vapors of biphenyl from the atmosphere as greatly as other varieties, thus leaving more vapor to control sporulation (2, 9).

In combination, SOPP and TBZ would also be expected to have an effect greater than that of either fungicide alone, and this has been shown by some workers (I 1) in the case of decay control. Sporulation control has not been demonstrated by SOPP at levels below those that cause phytotoxicity in citrus (1, 2, 5), nor has TBZ been demonstrated to give adequate reduction of sporulation by normal *Penicillium sp.* strains at residue levels below 6 ppm and as high as 9 ppm does not give complete control (3). In the trials we conducted sporulation control was obtained on fruit having much lower residue levels (1 to 3 ppm TBZ with 5 to 7 ppm OPP). Since the joint action of these 2 fungicides is greater than the sum of their effects when acting independently, the combination of SOPP and TBZ has been demonstrated to be a synergism.

A major advantage of this synergistic combination is that at the levels required for effectiveness, it would not be necessary to exceed the restriction placed by some foreign markets. Whereby the combined residue of multiple fungicides cannot exceed 100% of their combined tolerances (12).

As a replacement for biphenyl the combination of SOPP and TBZ meets many of the criteria for the theoretical ideal material. It is: 1) easy to apply, any packinghouse presently using water wax requires no modification to use it; 2) active within a wide range of residues which allows for the normal variations of commercial operation; 3) non-injury of the fruit; 4) effective; 5) active against most postharvest diseases of citrus; and 6) should be relatively inexpensive to use.

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