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Stability of Selected Pesticide Formulations and Combinations in Aqueous Media

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The stabilities of captan, manocozeb, azinphos-methyl, chlorpyrifos, and phosmet formulations, alone and combined, were studied at the recommended dilution (*X*) for orchard application and at a concentrated dilution (20*X*). Both buffered and unbuffered pesticide suspensions were monitored over a 48-h period. In addition, the effects of the foliar nutrients calcium chloride and sodium borate on pesticide stability and the in vitro effects of chlorpyrifos and azinphos-methyl on the fungitoxic properties of captan and mancozeb were studied. Pesticide stability was not decreased by concentrating or combining with the other chemicals. All unbuffered test suspensions were stable over a 48-h period. When tested alone at the recommended dilution, captan loss averaged 27% after 48 h at pH 9.5. Captan loss averaged about 20% when combined with insecticides under the same conditions. The maximum loss of azinphos-methyl in 48 h was 9%. Phosmet loss, at the recommended dilution, was 70% in 48 h at pH 9.5, while concentrated samples declined by about 10% at this pH. Bioassays indicated that chlorpyrifos may be antagonistic to the fungicidal activity of captan.

Apple growers often combine fungicides, insecticides, foliar nutrients, and surfactants in a single tank mix to reduce spraying time and cost. Low-volume (LV) and ultralow-volume (ULV) sprayers allow growers to apply more concentrated sprays, thus increasing time between tank fill-ups. However, the effects of combining and concentrating these chemicals have not been thoroughly studied. Furthermore, any effects would likely be increased if application of the tank mix were delayed or if the pH of the suspensions were altered.

Several studies have shown dramatic decreases in half-lives of commonly used pesticides with increasing pH (Frank et al., 1983; Freed et al., 1979; Wolfe et al., 1976; McNall, 1974; Heur et al., 1974; Bobb, 1973; Liang and Lichtenstein, 1972). Coli et al. (1984, 1985) found that the addition of technical-grade calcium chloride resulted in significant alkalinization of all test solutions. Sodium borate also increased solution pH (Gradis and Sutton, 1981).

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Although certain materials may increase pH, pesticide efficacy will not necessarily be decreased. Buffering agents may or may not be necessary. Greene et al. (1984) reported that pest control was unaffected by the addition of calcium chloride to apple cover sprays at rates of 27, 54, or 81 kg/ha.

Other studies have examined various chemical combinations for effects on physicochemical and biological properties. Sharples and Kirby (1971) found increased incidence of mildew on oranges when calcium nitrate was added to fungicide sprays in the spring, but no effect was observed for summer sprays. Kirby and Warman (1966) found some effects on suspensibility, wettability, and sinking times of some pesticides when combined with calcium nitrate. They recommended that calcium nitrate be applied as a separate spray. Gradis and Sutton (1981) tested the effects of several insecticides, foliar nutrients, and surfactants on the fungicidal and fungistatic activity of mancozeb [zinc coordination product of manganese ethylenebis(dithiocarbamate)] and captan [*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide]. They reported that the addition of phosmet [phosphorodithioic acid, *S*-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl] *O*,*O*-dimethyl ester] or azinphos-methyl [phosphorodithioic acid, *O*,*O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] ester] reduced the fungistatic and fungicidal activity of mancozeb. Addition of sodium borate, calcium

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nitrate, or azinphos-methyl reduced the fungistatic activity of captan; fungicidal activity was reduced in various combinations that included sodium borate.

Our studies investigated the stability of pesticide formulations, alone and combined, at two concentrations and three pH levels. The effects of two foliar nutrients, calcium chloride and sodium borate, were also examined. Fungicide efficacy was investigated with fungicidal and fungistatic assays.

MATERIALS AND METHODS

Pesticides. Wettable powder (WP) formulations of the fungicides captan (50% WP) and mancozeb (80% WP) and the insecticides azinphos-methyl (50% WP), phosmet (50% WP) and chlorpyrifos [phosphorothioic acid, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) ester] (50% WP) were selected for study, and the formulations were obtained from the manufacturers. Technical-grade calcium chloride (77–80%) and sodium borate (20.5% boron), both foliar-applied nutrients, were included in some tests.

Analytical Methods. A Tracor Model 222 gas chromatograph, equipped with a flame photometric detector operated in the phosphorus mode, was used to measure azinphos-methyl, phosmet, and chlorpyrifos. The same detector, operated in the sulfur mode, was used to measure captan. Two U-shaped glass columns (1.0 m × 2 mm i.d. and 0.75 m × 2 mm i.d.) were packed with 5% OV-210 on Suplecoport (100/120) and 4% SE-30 + 6% QF-1 on Gas Chrom Q (80/100), respectively. Temperatures were as follows: oven, 200 °C; detector, 185 °C; inlet, 200 °C. Gas flows to the detector were 50 mL/min for hydrogen and 80 mL/min for air. Nitrogen was the carrier gas at flow rates of 55–65 mL/min. Retention times were approximately 3 min for captan and 12 min for azinphos-methyl on the OV-210 column and about 2.5 min for captan, 9 min for azinphos-methyl, 7 min for phosmet, and 1.5 min for chlorpyrifos on the SE-30/QF-1 column. Data were quantified by peak height measurements compared to standards of known concentration.

A Waters high-performance liquid chromatography (HPLC) system was also used to measure captan and azinphos-methyl. It consisted of a Lambda-Max Model 481 variable UV/vis detector operated at 254 nm and 0.1 AUFS, a Waters Intelligent Sample Processor (WISP), Model 710B, and a Radial Compression Separation System (RCSS) containing a Radial Pak cartridge (10 cm × 8 mm i.d.) packed with silica (5–10 μm). Methylene chloride at a flow rate of 2.5 mL/min was the mobile phase. Retention times were approximately 2 min for captan and 8 min for azinphos-methyl. Quantitation was by peak height measurement.

Effects of Concentration and Combinations. Deionized water was used to prepare test suspensions of 100 mL in stainless-steel beakers. Formulations were tested at the recommended orchard dilution (240 mg/100 mL for captan and mancozeb; 60 mg/100 mL for azinphos-methyl and chlorpyrifos; 120 mg/100 mL for phosmet) and at a 20 times more concentrated dilution (20*X*). Pesticides were mixed at their equivalent dilutions for all combination treatments. Aliquots were taken within 15 min after mixing; the beakers were then covered with aluminum foil and stored at ambient temperature (20–22 °C). Additional aliquots were withdrawn after 24 and 48 h. Test suspensions were maintained with a magnetic stirrer during sampling. Aliquots of 10 and 200 μL were taken with a Wheaton pipetter and were extracted with 5–10 mL of chloroform. A second dilution was necessary for the 20*X* treatments to give final expected concentrations of 3–12 μg/mL of azinphos-methyl and chlorpyrifos, 6–12 μg/mL of phosmet, and 12–24 μg/mL of captan. The extracts were dehydrated with anhydrous sodium sulfate followed by vortex mixing and settling. Quantitation was by gas chromatography (GC). All tests were replicated at least three times, and all data were mean recoveries from at least two samples. Paired *t*-tests were used to evaluate differences between 0- and 48-h results (Snedecor and Cochran, 1980).

Mancozeb samples were analyzed by the method described by Newsome (1974). However, recoveries were highly variable, and the data are not included in this paper.

Effects of pH. Buffered solutions of pH 4.5, 7.0, and 9.5 were prepared as follows: pH 4.5, with 0.1 M potassium hydrogen phthalate (50 mL) and 0.1 M NaOH (8.7 mL); pH 7.0, with 0.1 M KH_2PO_4 (50 mL) and 0.1 M NaOH (29.1 mL); pH 9.5, with 0.025 M borax (50 mL) and 0.1 M NaOH (8.8 mL). Distilled water was added to each for a final volume of 100 mL. Each experiment consisted of three pesticide treatments (fungicide alone, insecticide alone, fungicide plus insecticide) tested at three pH levels. All treatments were replicated at least three times. Sample preparation and quantitation were the same as previously described; however, an HPLC method was also adapted for use in this part of the study (Carlstrom, 1980). Aliquots of 0.1–1 mL were extracted with methylene chloride. Final concentrations were in the range of 300–800 μg/mL for captan and 75–200 μg/mL for azinphos-methyl. Extracts were centrifuged, mixed with anhydrous sodium sulfate, recentrifuged, and passed through a 0.45-μm filter.

Effects of Foliar Nutrients. The effects of adding technical-grade calcium chloride (2.4 g), sodium borate (2.4 g), or calcium chloride plus sodium borate to azinphos-methyl (0.6 g of 50% WP) plus captan (2.4 g of 50% WP) were tested. Aliquots of 100 mL of deionized water were added to each and the resultant solutions mixed. Aliquots of 200 μL were withdrawn, extracted with 5 mL of chloroform, and mixed with sodium sulfate. An aliquot of 200 μL was withdrawn from the chloroform extract and diluted to 5 mL with chloroform for GC analysis. Suspension pH was recorded at each sampling interval. All tests were replicated four times.

Bioassays. The fungicidal and fungistatic rates of captan and mancozeb were determined by use of the cellophane transfer technique of Neely and Himlick (1966), and the effects of insecticides in combinations with fungicides at these rates were tested. Azinphos-methyl and chlorpyrifos were evaluated separately for fungistatic activity at the recommended field dilution (300 μg/mL). For combination treatments, insecticides were added in proportion to the amount of fungicide based on the recommended field dilutions of the formulations.

The bitter rot fungus, *Glomerella cingulata* (Ston.) Spauld. and Schr., was chosen for the tests. *G. cingulata* was grown on potato dextrose agar at 20–22 °C. Spore suspensions, adjusted to approximately 10^5 conidia/mL in sterile deionized water, were prepared for 4- to 7-day-old cultures.

Antibiotic assay disks (Schleicher and Schuell, No. 740-E) were placed in depressions of Coors white porcelain spot plates and saturated with test pesticide suspensions. Disks (6-mm diameter) were cut from sheets of cellophane (Du Pont PUDO-193) and were sterilized in deionized water. Two cellophane disks were placed on each assay disk, and each was seeded with a drop of the spore suspension from a 25-μL pipet. Plates were stacked in a moist chamber and incubated at 27 °C. Fungicidal and fungistatic tests were performed for each pesticide suspension at 0, 24, and 48 h after preparation.

Table I. Mean Pesticide Recoveries in Unbuffered and Buffered Suspensions after 48 h at the Recommended Field Dilution

treatment	unbuffered	pH 4.5	pH 7.0	pH 9.5
	% Captan[a]			
captan	101	100	97	73**
captan + azinphos-methyl	93	96	99	81**
captan + chlorpyrifos	98	105	93	80**
captan + phosmet		98	103	77*
	% Azinphos-methyl[a]			
azinphos-methyl	100	101	98	91*
azinphos-methyl + captan	99	98	95	95
azinphos-methyl + mancozeb	106			
	% Chlorpyrifos[a]			
chlorpyrifos	100	84	84*	85
chlorpyrifos + captan	102	91	85	89
chlorpyrifos + mancozeb	99			
	% Phosmet[a]			
phosmet		96	92	31**
phosmet + captan		97	100	30**

[a] Data are expressed as percent of 0-h recoveries. Single-asterisk values are significantly different ($0.01 < p < 0.05$, paired t-test) from 0-h recoveries. Double-asterisk values are significantly different ($p < 0.01$, paired t-test) from 0-h recoveries.

Table II. Mean Pesticide Recoveries in Unbuffered and Buffered Suspensions after 48 h at 20 Times the Recommended Dilution

treatment	unbuffered	pH 4.5	pH 7.0	pH 9.5
	% Captan[a]			
captan	101	99	99	99
captan + azinphos-methyl	93	98	103	104
captan + chlorpyrifos	106	108	106	106
captan + phosmet		99	95	97
	% Azinphos-methyl[a]			
azinphos-methyl	98	96	97	95
azinphos-methyl + captan	100	98	102	97
azinphos-methyl + mancozeb	101			
	% Chlorpyrifos[a]			
chlorpyrifos	105	104	105	97
chlorpyrifos + captan	104	101	93	100
chlorpyrifos + mancozeb	106			
	% Phosmet[a]			
phosmet		98	98	89**
phosmet + captan		99	99	94*

[a] Data are expressed as percent of 0-h recoveries. Single-asterisk values are significantly different ($0.01 < p < 0.05$, paired t-test) from 0-h recoveries. Double-asterisk values are significantly different ($p < 0.01$, paired t-test) from 0-h recoveries.

Fungicidal activity was determined by transferring the cellophane disks to potato dextrose agar plates after a 2-h exposure to the pesticide-saturated assay disk. These plates were incubated at 27 °C for 3 days and examined for fungal growth. The test was considered fungicidal if no growth was observed.

Fungistatic activity was determined after 24-h exposure to the pesticide treatments at 27 °C. The cellophane disks were transferred to glass slides and fixed with lactophenol for microscopic examination. If less than 1% of the spores had germinated (germ tube lengths less than half the spore length), the test was considered fungistatic.

All treatments were replicated 12 times, and all fungicidal experiments with captan were repeated three times.

RESULTS AND DISCUSSION

Effects of Concentration and Combinations. The results obtained from stability studies are summarized in Tables I and II. All formulations and combinations tested in unbuffered water were stable over a 48-h period and showed no evidence of decreased stability due to concentrating or combining chemicals.

Effects of pH. Captan was stable over a 48-h period when tested at pH levels of 4.5 or 7.0; however, stability was decreased in all treatments when tested at pH 9.5 (Table I). When tested at 20X, captan was stable at all pH levels (Table II).

The stability of captan was much greater than that previously reported. According to Wolfe et al. (1976), the maximum half-life of captan in water was 710 min at 28 °C, while at levels above pH 8 the half-life was less than 10 min. Frank et al. (1983) reported a half-life of 1 h for technical captan at pH 8.5 in water at 22 °C. In our study, the maximum captan loss after 48 h was about 27% (Table I). When combined with azinphos-methyl, phosmet, or chlorpyrifos at pH 9.5, captan loss was only about 20% in 48 h. These results suggest that captan formulated as a 50% WP is more stable than technical captan in water. Therefore, growers should not experience significant loss of captan in tank mixes after 24 h at near neutral pH.

Maximum loss of azinphos-methyl was 9% after 48 h at pH 9.5 (Table I). Stability was similar to that reported in previous studies. Liang and Lichtenstein (1972) showed that azinphos-methyl was relatively stable in water below pH 10. After 7 days of incubation at pH 10, only 18% of the applied [^{14}C]azinphos-methyl was converted to water-soluble products compared to 97% at pH 11. They also discovered that degradation of azinphos-methyl did not occur over a 12-h period when kept in the dark; but when aqueous solutions were exposed to UV light, rapid degradation ensued. Heur et al. (1974) reported half-lives of azinphos-methyl in aqueous media ranging from 27.9 days (pH 8.6, 25 °C) to 2 days (pH 10.7, 25 °C).

Results with phosmet clearly showed rapid degradation under basic conditions; however, phosmet was stable over a 48-h period at neutral pH or below (Table I). Freed et al. (1979) reported half-lives in water of 7 days (pH 6.1) and 7.1 h (pH 7.4) for phosmet at 20 °C. In the same study, the half-life for 1 ppm phosmet in moist soil at pH 6.2 was 60 days, thus suggesting greater persistence when adsorbed by soil. McNall (1974) reported half-lives of less than 4 h at pH 8.3 and 13 days at pH 4.5 for phosmet. As with captan, the results of this study indicate that formulated phosmet is more stable than the literature suggests. Therefore, as long as suspension pH is neutral or below, delays of 24 h should not affect tank mixes of phosmet.

Data for chlorpyrifos stability in buffered water at the recommended dilution were more variable than for other treatments. There was a significant loss of chlorpyrifos when tested alone at pH 7.0 after 48 h (Table I); however, more replications would be needed to verify this. None of the 20X treatments showed a significant decline (Table II), and none of the combination treatments reduced the stability of chlorpyrifos. The half-life of chlorpyrifos in water has been reported at 53 days at 20 °C and pH 7.4 (Freed et al., 1979). Therefore, chlorpyrifos should be stable in tank mixes, alone or combined with captan or mancozeb, over a 48-h period.

Effects of Foliar Nutrients. There were no significant effects of calcium chloride or sodium borate on the stability of captan or azinphos-methyl. These findings support the recent study by Greene et al. (1984), who found no difference in pest control between tank mixes with and without calcium chloride. Addition of calcium chloride or

Table III. Effects of Calcium Chloride and Sodium Borate on Pesticide Suspension pH at Dilutions 10 Times Recommended Rate

treatment[a]	time after mixing 0 h	24 h	48 h
A + C	7.7	7.4	7.4
A + C + Ca	8.0	7.4	7.5
A + C + S	8.4	8.2	8.2
A + C + Ca + S	7.4	7.2	7.2

[a] Key: A = azinphos-methyl; C = captan; Ca = technical-grade calcium chloride; S = technical-grade sodium borate.

calcium chloride plus sodium borate had little effect on pH (Table III). Coli et al. (1985) reported significant pH increases when calcium chloride was added to tank mixes; however, their pesticide suspensions were at the recommended dilution, while those studied here were 10 times more concentrated. The higher concentration of azinphos-methyl, an acidic material, evidently neutralized much of the alkalinity generated by calcium chloride. Coli et al. (1985) also found that the pH, which increased after addition of calcium chloride, returned to near neutrality within 24 h. We also found a slight drop in pH after 24 h in our tests with calcium chloride (Table III). However, sodium borate alone raised pH, and the higher pH was maintained over the 48-h period. Gradis and Sutton (1981) found reduced activity of captan when combined with sodium borate and speculated that this was due in part to increased pH. The highest pH recorded in our tests was 8.4, and according to our results, it would probably not reduce pesticide concentrations significantly over a 24-h period. In spray tanks with pesticides mixed at the recommended dilutions, initial pH values would likely be higher and the potential for significant hydrolysis would increase.

Bioassays. Concentrations of captan and mancozeb fungistatic to *G. cingulata* were 0.25 and 3.0 μg/mL, respectively. Azinpos-methyl at 300 μg/mL showed fungistatic activity, but chlorpyrifos did not. The fungistatic activities of captan and mancozeb were not affected by the addition of either insecticide. One- and two-day-old suspensions for all treatments maintained fungistatic activity.

Fungicidal concentrations were determined to be 60 and 500 μg/mL for captan and mancozeb, respectively. Fungicidal activity of mancozeb was not affected by the insecticides, and activity was maintained in 48-h suspensions. Captan activity was more variable. Initially, chlorpyrifos at 15 or 300 μg/mL reduced the fungicidal activity of captan (Table IV). Results from 24- and 48-h suspensions were variable. In two of three trials after 24 h there was little effect on fungicidal activity, but in two of three trials in the 48-h test complete loss of fungicidal activity was observed in all treatments.

The results of these fungitoxic studies differ somewhat from those reported by Gradis and Sutton (1981). However, there were some differences in the tests. In fungistatic tests, we added the insecticides at a proportional concentration rather than at the recommended field concentration. Also for the fungicidal tests, we only exposed the spore suspension for 2 h, where they exposed the spore suspension for 3 h. However, both studies showed some loss of fungitoxic activity in some pesticide combinations.

The significance of these results to an orchard situation is difficult to assess. As residues weather, fungitoxic activities will be maintained up to a certain point. If the fungicide–insecticide mixes we studied are applied together and both chemicals weather at about the same rate, the point at which the fungistatic activity is lost should not change significantly. However, the point at which the fundicidal activity of captan is lost may be altered by the presence of chlorpyrifos. This suggests that efficacy in orchards would be reduced; however, the in vitro exposures were for only 2 h. Field tests should be conducted to evaluate the implications of these interactions on disease control.

Table IV. Effects of Azinphos-methyl and Chlorpyrifos on the Fungicidal Activity of Captan against *G. cingulate* as Measured by the Number of Disks (X) with Fungal Growth[a]

treatment	concn, μg/mL	transformed mean $(\overline{X+1})^b$	untransformed mean (X)
captan	60	1.47[a]	1.33
captan + azinphos-methyl	60	1.69[a]	2.00
	15		
captan + azinphos-methyl	60	1.14[a]	0.33
	300		
captan + chlorpyrifos	60	2.56[b]	5.67
	15		
captan + chlorpyrifos	60	3.25[b]	9.67
	300		

[a] Twelve disks/treatment with three replications. [b] Means followed by an identical letter are not different, $p = 0.05$.

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