Effect of Insecticides, Nutrients, and Adjuvants on In Vitro Fungistatic and Fungicidal Activity of Captan and Mancozeb

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ABSTRACT

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The in vitro effect of phosmet, methomyl, azinphosmethyl, calcium nitrate, sodium borate, and a spreader-sticker on the fungicidal activity of captan and mancozeb was investigated by using a cellophane transfer technique. Spore germination and germ tube length of conidia of *Botryosphaeria dothidea* and *Glomerella cingulata* were used to measure fungitoxic properties of the chemicals. The fungistatic and fungicidal activity of mancozeb was reduced by the addition of phosmet and azinphosmethyl, and that of captan was reduced by the addition of sodium borate, phosmet, or azinphosmethyl.

Additional key words: apple (*Malus sylvestris*), *Colletotrichum gloeosporioides, Dothiorella* sp.

Apples (*Malus sylvestris* Mill.) are affected by numerous diseases, insect, mite, weed, and rodent pests, and physiologic disorders. Control of diseases and insects on fruit and foliage is based on the use of fungicides and insecticides in a calendar spray program (10). These materials are usually applied as a tank mix 10–14 times a season. Foliar nutrient sprays are recommended for the control of physiologic disorders such as bitter pit (3,7,12), cracking (3), and internal cork (7); and although not recommended, nutrients are often combined with the pesticides in a single spray application (T. B. Sutton and G. C. Rock, *unpublished data*). In addition, surfactants or spreader-stickers are added to the tank mix to increase wettability and spread.

Information on the efficacy of fungicides combined with insecticides, surfactants, and nutrients is limited. Using exocarp disks cut from apples treated with combinations of fungicides, insecticides, and surfactants, Chung (2) found no effect of insecticides on the ability of folpet to produce inhibition zones on agar covered with spores of *Glomerella cingulata*. Kirby and Warman (4) investigated the physicochemical effects of calcium nitrate on several fungicides by observing wetting power and sedimentation rates. They concluded that calcium nitrate should be used as a separate spray when reduced volumes of water are used.

In orchard studies, Sharples and Kirby (11) found that the deposition of captan and dinocap was not affected by the addition of calcium nitrate. When used only as a summer spray on apple trees, calcium nitrate had no effect on mildew incidence, but when used with captan and dinocap in the spring and summer sprays, mildew incidence increased. Apple scab control was not affected.

Our study investigated the influence of various combinations of insecticides, nutrients, and a spreader-sticker on the activity of mancozeb and captan against *Botryosphaeria dothidea* and *G. cingulata*.

MATERIALS AND METHODS

Chemicals. We tested captan 50% WP, mancozeb 80% WP, azinphosmethyl 50% WP, phosmet (Prolate) 70% WP, methomyl 90% WP, calcium nitrate (15.5% N, 19% soluble calcium), sodium borate (20.5% boron, Solubor), and modified phthalic glycerol alkyd resin (77%, Triton

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B-1956). Captan and mancozeb are fungicides; azinphosmethyl, phosmet, and methomyl are insecticides; calcium nitrate and sodium borate are nutrients; and the phthalic glycerol alkyd resin is a spreader-sticker.

In all tests that included a fungicide, the fungicide suspension was prepared first and the other chemicals added. Unless otherwise stated, chemicals in treatment combinations were used at the equivalent recommended orchard rate (10). All suspensions were prepared in deionized water and agitated by a magnetic stirrer throughout each test.

Isolates. *Colletotrichum gloeosporioides* Penz., imperfect stage of *G. cingulata* (Stonem) Spauld and V. Schr., and the *Dothiorella* stage of *B. dothidea* G. and D. (syn. *B. ribis*), were used. The fungi were grown on potato-dextrose agar and oatmeal agar, respectively, at 25 C under ultraviolet light. Conidia were obtained from 7- to 10-day-old *G. cingulata* cultures and 2- to 3-wk-old *B. dothidea* cultures by flooding the plate surface with deionized water. The spore concentration was adjusted to 50,000–70,000 spores per milliliter.

Fungicidal and fungistatic assay. The cellophane transfer technique of Neely and Himelick (9) was used to detect in vitro fungicidal and fungistatic activity. Antibiotic assay disks (Schliecher and Schuell, No. 740-E) were placed in Coors white porcelain spot plates (112×92 mm, with 12 depressions 5 mm deep) and saturated with the test suspension until a meniscus formed between the disk and the side of the depression. Three 5-mm cellophane disks, punched from sheets of DuPont PUDO-193 cellophane and autoclaved in deionized water, were placed on each assay disk and seeded with the spore suspension by using a capillary tube. The plates were stacked in a moist chamber and incubated at 28 C. Fungistatic activity was determined after 24 hr. The cellophane disks were transferred to glass slides, stained with aniline blue plus lactophenol, and observed microscopically for germination. A spore was considered germinated if the germ tube length was equal to or longer than the spore width (1). Dunnett's procedure (13) was used to detect any significant difference between the control mean and each treatment mean.

In the fungistatic tests, results were recorded as fungistatic or nonfungistatic. A chemical was considered fungistatic when less than 1% of the spores germinated or the germ tube length was less than half the spore length. In these tests, the activity of the fungicide alone was used as the control.

Fungicidal activity was determined by transferring the cellophane disks to petri plates of potato-dextrose agar plus streptomycin (30 μg/ml) after 3-hr incubation on assay disks saturated with the test suspension. The suspension was considered fungicidal if no growth occurred in 72 hr at 28 C.

All fungistatic and fungicidal treatments were replicated 12 times and each experiment was repeated at least three times.

pH effects. The pH of each chemical suspension was recorded. We determined the effect of pH on fungicide activity by adjusting the acidity of the captan and mancozeb concentrations with 0.01 M HCl or 0.01 M NaOH to pHs comparable to those of the mixtures tested. Fungistatic and fungicidal activity was then determined as described. Conidia of *B. dothidea* and *G. cingulata* were germinated in deionized water adjusted to a pH range from 4 to 9, to determine the effect of pH on spore germination.

RESULTS

Fungistatic and fungicidal tests. None of the nonfungicides or combinations of them were fungistatic to *B. dothidea* and *G. cingulata*, but seven of the 10 treatments significantly reduced germ tube length (Table 1). Only calcium nitrate, methomyl, and Triton B-1956 had no significant effect on germ tube length. The combination of azinphosmethyl, calcium nitrate, and sodium borate resulted in the greatest reduction in the germ tube length of both fungi. Azinphosmethyl and phosmet caused greater reduction in germ tube lengths of *G. cingulata* than of *B. dothidea*.

Several treatments caused change from fungistatic to nonfungistatic (Table 2). When used in concentrations fungistatic to *B. dothidea* (6 μg/ml) and *G. cingulata* (8 μg/ml), mancozeb became nonfungistatic with the addition of either phosmet or azinphosmethyl. Concentrations of captan fungistatic to *B. dothidea* (4 μg/ml) and *G. cingulata* (1 μg/ml) were nonfungistatic with the addition of

Table 1. Effect of chemical additives on the germ tube length of conidia of *Botryosphaeria dothidea* and *Glomerella cingulata*

Treatment and rate[a] (μg/ml a.i.)	pH	Mean germ tube length (μm)[b]	
		B. dothidea	*G. cingulata*
Deionized water control	7.2	500	345
Calcium nitrate (130)	6.8	500	316
Sodium borate (50)	8.8	234*	137*
Methomyl (540)	7.4	500	368
Phosmet (840)	4.4	324*	47*
Azinphosmethyl (300)	6.1	272*	36*
Modified phthalic glycerol alkyd resin (236)	6.4	500	362
Calcium nitrate, sodium borate	8.3	63*	88*
Phosmet, calcium nitrate, sodium borate	8.3	206*	106*
Azinphosmethyl, calcium nitrate, sodium borate	8.3	23*	21*
Methomyl, calcium nitrate, sodium borate	8.5	39*	71*

[a] Rates are the same alone and in combination treatments. All concentrations are the recommended field rates (10).
[b] Germ tube lengths >500 μm could not be accurately measured and are reported as 500 μm. * = germ tube length is significantly different from the control ($P = 0.01$).

Table 2. Effect of chemical additives on rates of mancozeb and captan fungistatic to species of *B. dothidea* and *G. cingulata*

Treatment[b]	Fungicide activity[a]			
	B. dothidea		*G. cingulata*	
	Mancozeb 6 μg/ml	Captan 4 μg/ml	Mancozeb 8 μg/ml	Captan 1 μg/ml
Fungicides alone	FS	FS	FS	FS
Fungicide plus				
Phosmet	157	113	44	96
Phosmet, calcium nitrate, sodium borate	FS	107	FS	78
Azinphosmethyl	39	51	31	68
Azinphosmethyl, calcium nitrate, sodium borate	FS	FS	FS	FS
Methomyl	FS	FS	FS	FS
Methomyl, calcium nitrate, sodium borate	FS	44	FS	33
Calcium nitrate, sodium borate	FS	54	FS	128
Calcium nitrate	FS	FS	FS	FS
Sodium borate	FS	134	FS	166
Modified phthalic glycerol alkyd resin	FS	FS	FS	FS

[a] Expressed as fungistatic (FS) or in germ tube length (μm).
[b] All additives were at the recommended orchard rate (10).

phosmet or azinphosmethyl or of calcium nitrate and sodium borate.

The concentration of captan that was fungicidal to *G. cingulata* (50 μg/ml) was not fungicidal with the addition of phosmet, calcium nitrate, and sodium borate; calcium nitrate and sodium borate; azinphosmethyl, calcium nitrate, and sodium borate; or sodium borate. The concentration of mancozeb that was fungicidal to *G. cingulata* (600 μg/ml) was not fungicidal with the addition of phosmet; phosmet, calcium nitrate, and sodium borate; azinphosmethyl, calcium nitrate and sodium borate; or azinphosmethyl. Concentrations fungicidal to *B. dothidea* (captan, 100 μg/ml; mancozeb, 100 μg/ml) were not affected by any additive tested.

Effect of pH. The pH of suspensions of the chemicals and combinations ranged from phosmet at pH 4.4 to sodium borate at pH 8.8 (Table 1). When combined with the various concentrations of captan and mancozeb, phosmet and azinphosmethyl reduced the pH of the suspension; in any combination, sodium borate increased the pH.

Spores of both fungi germinated, and germ tube lengths were comparable to those of controls in a pH range from 4 to 9. When adjusted to this same range of pH, sodium borate, phosmet, and azinphosmethyl did not show any change in activity. The activity of captan was reduced when adjusted to a more basic pH (6–8.3). Mancozeb was not affected by pH adjustment. Fungicidal concentrations of both fungicides were not reduced by adjustment.

DISCUSSION

The results of this study indicate that addition of phosmet or azinphosmethyl to mancozeb can cause significant reduction in its activity against both *B. dothidea* and *G. cingulata*. In addition, combining sodium borate, phosmet, or azinphosmethyl with captan caused reduction in its activity against both fungi. Mancozeb seems to be the most seriously affected fungicide when combined with other materials. When used alone, mancozeb at 600 μg/ml is fungicidal against *G. cingulata*, but when combined with phosmet or azinphosmethyl, 1,000 and 800 μg/ml, respectively, are necessary to obtain a fungicidal response.

The reduced activity of captan when combined with sodium borate may be due in part to a pH effect. The loss of activity by captan in alkaline solutions has been reported (6), and combinations with sodium borate were alkaline. The reduced activity of mancozeb and captan when combined with phosmet or azinphosmethyl does not appear to be related to pH. Suspensions of both insecticides are acidic (pH 4.5–6), and adjustments to the pH of fungistatic concentrations of mancozeb and captan to this range had no effect on fungicide activity. The phosphate radical of phosmet or azinphosmethyl may chelate with the mancozeb and cause it to flocculate, or in this test, a physical flocculation may have occurred that caused clumping of the mancozeb and subsequent loss of activity.

Kirby and Warman (4) found that the suspensibility of some fungicide-insecticide mixtures is poor, and that the addition of calcium nitrate caused further detrimental effects. We always mixed and tested materials at the equivalent dilute orchard rate. However, many growers apply spray materials at two to 33 times that rate. With this method of application, the materials are concentrated in the spray tank and, because they remain concentrated longer, any physicochemical or pH effects would be intensified.

The biological significance of these findings is difficult to assess under orchard conditions. Although rates of fungicides in the fungistatic tests were considerably less than those used in orchards, they may be analogous to concentrations on fruit and foliage after deposition and subsequent degradation. Spray deposits are not uniform throughout the tree and may differ considerably even on the same leaf or fruit (5). Thus, at any one time, deposits in certain portions of the tree could exceed the recommended rates, while other deposits could be considerably less than recommended. As the fungicide begins to degrade, concentrations could become very low (8), and on rewetting, suspensions of fungicides and additives on the leaf or fruit surface could be within the range of those we tested. Any factor that reduces fungicide activity could become particularly important as disease management programs are developed in which spray programs are tailored for specific diseases and inoculum levels.

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