Effect of Oil Emulsions on the Uptake of Benomyl and Thiabendazole in Relation to Control of Verticillium Wilt of Cotton

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ABSTRACT

Two commercially available paraffinic oils, Orchex N 795 (P795) and Orchex 696 (P696) augmented the uptake and translocation of benomyl and thiabendazole in cotton plants. P795 and P696 oils were more effective than the naphthenic oil, Orchex 792 (N792). The isoparaffinic oil Humble 3408 (IP3408) was ineffective in augmenting the uptake and translocation of benomyl or thiabendazole. P795 or P696 oils at 20% concn were more effective in augmenting translocation of benomyl than at lower concns, but the higher oil concns were phytotoxic. When benomyl was made water-soluble with HCl (pH 1.7 - 2.0), paraffinic oils also augmented its uptake. When ^{14}C-thiabendazole plus P795 oil was applied to lower parts of the stem, a significantly larger quantity of radioactivity was detected in the upper stem and petioles of the cotton plant than when ^{14}C-TBZ was applied in water containing the wetting agent Triton X-100. When cotton plants were inoculated with *Verticillium albo-atrum* after thiabendazole or benomyl (20 mg/plant) was applied to the stem with the paraffinic oils, disease onset was delayed or prevented. Neither chemical applied in an aqueous suspension delayed or prevented disease.

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Application to soil of the systemic fungicide benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate, and thiabendazole, 2-(4-thiazolyl)benzimidazole (TBZ), experimentally controlled Verticillium wilt caused by a microsclerotial isolate of *Verticillium albo-atrum* Reinke & Berth. on cotton (*Gossypium hirsutum* L.) in the greenhouse (8, 14, 15). In the field where root growth was much more extensive than in pots, a yield increase due to partial control of *Verticillium* occurred only at high dosages of benomyl (9, 10, 11, 13, 17).

Since foliar or stem application of a systemic fungicide at a critical stage of disease stress might be more effective at lower dosages than soil application, research on this appeared to be necessary. However, in our studies methyl 2-benzimidazole carbamate (MBC) from benomyl (21) was not taken up from foliage of cotton plants in sufficient quantity to control Verticillium wilt, except when benomyl was acidified and made water soluble (4, 5, 12).

Since uptake of the herbicide atrazine, 2-chloro-4-ethylamine-6-isopropyl amine-S-triazine, was augmented by a paraffinic oil emulsion (1, 22), it seemed likely that uptake and translocation of benomyl might also be augmented by oils. Several other recent reports also indicate that certain oils increased the penetration of systemic fungicides in plants (18, 19, 23, 24). The effect of oils on the uptake of systemic fungicides was also noted in a recent review (11). In another paper from this laboratory, the augmentation of uptake of MBC by oil was shown by use of ^{14}C-MBC (26).

We will report here that the uptake of thiabendazole (TBZ) or MBC (from benomyl) through stem tissue of cotton plants as measured by a leaf or stem disk bioassay method was substantially increased by mixing paraffinic

oils with the fungicides before application, and that the degree of disease control was increased. A portion of this work was briefly presented (12) previously.

MATERIALS AND METHODS.—Cotton plants (cultivar 'SJ-1') were grown in sand or in a mixture of peat moss and sand (1:1, v/v) in 10.2-cm (4-inch) diam plastic pots and fertilized weekly with a nutrient solution (15). Plants were 3-6 wk of age when treated. Plants were inoculated by the stem-puncture method (8) at a point below the treated area (generally at the cotyledonary node), or by root drench (50 ml of a spore suspension at 10^6 spores/ml) applied to young roots on the periphery of the root ball (8). Treatments were replicated four times and each experiment was repeated three or more times.

Chemicals in oil were prepared by suspending benomyl (as Benlate® 50% WP) or TBZ (as Mertect® 60% WP) in the oil emulsion. To obtain methyl 2-benzimidazole carbamate hydrochloride (MBC·HCl) (4, 5), 10 g technical benomyl was suspended in 100 ml acetone and 300 ml of 0.34 N HCl was added and the mixture heated to 70 C in a water bath until the material was completely dissolved (1-2 h). The solution contained the active chemical MBC·HCl and possibly other derivatives of benomyl. In some tests the chemical in the oil-in-water emulsion (20 mg in 0.5 - 2.0 ml) was painted on each stem with a small camel's-hair brush. Control treatments included a suspension of chemical in water, the oil emulsion alone, or water alone. In greenhouse experiments, the emulsion containing the test chemical was prevented from contaminating the soil by wrapping an absorbent paper around the lower part of the stem and by placing paper towels on the soil surface.

Physical characteristics of oils used on plants are discussed by de Ong (7), and by Calpouzos (6). The paraffinic oil Orchex N 795® (P795) contained 96% unsulfonated residue (UR) and had a 70-sec viscosity, the paraffinic oil Orchex 696® (P696) 96% UR, and had a 60-sec viscosity, the isoparaffinic oil Humble 3408® (IP3408) 98% UR and had a 34-sec viscosity, and the naphthenic oil Orchex 792® (N792) 82% UR and had a 77-sec viscosity. P795 and N792 contained T-Mulz AO_2® (Thompson Hayward, Kansas City, Kansas), and IP3408 contained T-DET N-4® (Thompson Hayward) emulsifiers at concns of 2% (w/w). Letter coding for the oils: N = naphthenic, P = paraffinic, and IP = isoparaffinic. IP3408 was reported to be an efficacious carrier for the herbicides chloropropham and terbacil (2).

The presence of a translocated fungitoxicant in the upper part of the plant was detected by a potato dextrose-streptomycin agar diffusion bioassay utilizing *Verticillium albo-atrum* as the test fungus (8). The area of a zone of inhibition (ZI) around the bioassayed leaf or stem disk (mm^2 minus the area of the plant material) was a relative indication of the amount of fungitoxicant in the plant material. Bioassays of tissue above the area of application were made 5-7 days after treatment.

Translocation of ^{14}C-TBZ in oil-in-water emulsion.—Eight μg of ^{14}C-TBZ uniformly labeled in the benzene ring were dissolved in 3.2 ml benzene and used as a stock solution. The sample proved to be homogeneous when assayed by thin-layer chromatography (25).

An 80-μliter aliquot of the stock solution (containing 0.2 μCi of ^{14}C-TBZ) was evaporated to dryness at 50 C. Nonlabeled TBZ suspension (0.2 ml) containing 2,500 μg/ml TBZ and 0.1% Triton X-100 (isooctylphenyl polyethoxy ethanol), and 10 μliter P795 oil were added to the labeled TBZ residue and the suspension was thoroughly mixed. An area 5 cm in length circling the stem of a 5-wk-old cotton plant (10-15 cm above the soil level) was delimited with lanolin rings. The labeled TBZ suspension was applied to the surface of the plant in small portions to prevent runoff, and the applied solution was allowed to dry at room temp. Two plants were harvested at 5 and 10 days after treatment.

The roots of treated plants were washed with tap water and the secondary roots removed. The plants were sectioned into 5-cm segments above the tip of the tap root. A sample (1 cm in length) of each segment was separated into bark and xylem tissues. Sections (1 cm long) of each petiole were also removed about 5 cm from the base. The samples were minced and transferred to scintillation vials containing 10 ml of Bray's solution (3). The radioactivity was determined by use of the Packard Tri-Carb Liquid Scintillation Spectrometer Model 3002.

RESULTS.—Benomyl (20 mg) was mixed with P795 oil (0.5 ml) and applied to the lower stem of each treated 6-wk-old cotton plant in the greenhouse experiment. When leaf disks from fully expanded leaves above the treated area were bioassayed about 10 days later, a ZI of fungal growth was detected; but when comparable plants were treated with benomyl (20 mg) in water or with oil alone, no ZI was detected. Some of the leaves on oil-treated plants wilted. Since on some plants the tip leaves became oil-soaked and eventually necrotic, wilting and necrosis appeared to be due to translocation of the oil. Since the undiluted oil was phytotoxic, 10% and 30% oil-in-water emulsions were tested. At these concns the degree of phytotoxicity was considerably reduced but not entirely diminished. An example of a typical experiment is given in Table 1. On plants inoculated by the stem puncture method, no wilt symptoms occurred 5 days after treatment with benomyl (20 mg/plant) and P795 oil (30% emulsion); whereas, on those treated with benomyl in water, symptoms of Verticillium wilt appeared. The large ZI value only from benomyl + oil-treated plants indicated uptake and translocation of MBC from the treated area of stems (Table 1).

In other experiments in which the effect of different concns of P795 oil added to benomyl were compared, the incidence (%) of Verticillium wilt was 0 at 20% and 30% oil but at 10% and 1% the incidence was 25 and 100%, respectively. In another experiment, TBZ was also most effective when applied in a 20 and 30% oil emulsion, but the mixture was phytotoxic at the 30% oil concn.

There was some variability between experiments in the effect of different concns of oil. In one test in which TBZ or benomyl was applied to stems in 10% P795, the fungicides were detected in the leaves by bioassay and the incidence of Verticillium wilt was reduced (Table 2).

In a field experiment at Shafter, California, the paraffinic oils P795 and P696, the naphthenic oil N792, and the isoparaffinic oil IP3408 were applied with benomyl (20 mg benomyl in 5 ml of oil emulsion) to the stems of separate groups of ten 6-wk-old plants. At 7- and 13-day intervals after treatment, leaf disks collected from nontreated fully expanded leaves near the top of the

TABLE 1. The effect of oil (30% Orchex N795) on the uptake of benomyl applied to cotton stems (20 mg/plant), and on the incidence of Verticillium wilt of cotton 22 days after stem puncture inoculation

Treatment		Verticillium wilt incidence (%)	Plant height (cm)	Bioassay[a] ZI (mm^2)	
Chemical	Inoculation			4 days	25 days
None	−	0	54	0	0
None	+	100	44	0	0
Oil	+	100	35	0	0
Benomyl in water	+	100	37	0	0
Benomyl in oil	+	0	41	330	0

[a]Leaf disks were from fully expanded leaves above the treated area on the stem. ZI = area (mm^2) of zone of inhibition of fungal growth beyond edges of the leaf disk on bioassay plates.

TABLE 2. Control of Verticillium wilt by application of benomyl and thiabendazole (20 mg in 5.0 ml of 10% 795 oil) to stems of cotton plants 5 days prior to stem puncture inoculation

Treatment		Postinoculation (%) Verticillium wilt		Vascular discoloration[a] after 14 days			Plant height (cm)
Chemical	Inoculation	6 days	14 days	node 1	node 2	node 3	
No treatment	0	0	0	0	0	0	72
No treatment	+	75	100	3.2	3.2	1.7	59
Oil	+	75	100	3.8	3.5	2.5	60
Benomyl + oil	+	0	0	2.5	2.5	0.6	69
Thiabendazole + oil	+	0	0	2.8	2.0	0.6	69

[a]Vascular discoloration readings (0 = no xylem discolorization; 5 = xylem entirely brown) were recorded for cross sections of the stems above the cotyledonary node (node 1).

plant, were bioassayed. The ZI values in Table 3 indicated that the paraffinic oils P696 and P795 augmented uptake and translocation more than the naphthenic N792 oil. The ZI values also increased with increased oil concns from 10 to 20%. IP3408 was relatively ineffective. When a comparable test was conducted in nearby plots 6 wk later, the results were similar to those shown in Table 3.

In a nearby plot, leaves of cotton plants in several rows were sprayed with each of the same benomyl + oil emulsions (1,000 ppm benomyl in 20% oil). The upper leaves of the plants were protected from the spray with large polyethylene bags and bioassayed 7 and 13 days later. There was no detectable ZI around any of the nontreated leaf disks collected from plants which had received the foliar spray. This indicated that there was little or no translocation upward from leaves and that application to stems was likely to be more efficient than application to leaves.

In a greenhouse experiment, acidified benomyl (2,500 μg/ml) with and without P795 oil, was applied three times at 3-day intervals to the foliage of 5-wk-old plants. The roots of the treated plants were inoculated by drenching the soil with a spore suspension. Both treatments controlled the disease, but in bioassays of the upper stems of acidified benomyl + oil-treated plants, there was a higher ZI (avg. 2,064 mm) than on those treated with acidified benomyl alone (avg. 837 mm). When oil was applied with the acidic formulation of benomyl, it was slightly phytotoxic to the foliage, but the height of the plants was not affected.

When ^{14}C-TBZ in P795 oil (5%) was applied to cotton stems, considerably higher amounts of radioactivity was detected in plant tissue above the point of application than when ^{14}C-TBZ was applied in water. The amount of radioactivity in stems decreased with increased height of the sampling area above the treated region (Table 4). Five days after treatment, ^{14}C-TBZ in oil had translocated to the petioles but ^{14}C-TBZ in water had not. Ten days after treatment with ^{14}C-TBZ in oil, radioactivity detected in the petioles was markedly higher than in plants treated with ^{14}C-TBZ in water (Table 4). There was essentially no translocation downward from the area of application.

DISCUSSION.—The mechanism of the effect of oils on the uptake of fungicides is as yet obscure, but oils appear to be taken up through stomates or fissures in the cuticle (16). Saunders and Lonnecker (20) believed that oils soften or solubilize the cutin layer. They reported that a 70-sec oil deposited on a leaf surface could not be recovered after 7 days whereas oil deposited on aluminum foil was recovered almost completely, indicating that oil was taken up by the plant. Our observation that oil appeared in tip leaves following application to the lower stem indicated that the oil was translocated in the plant.

Despite the increase in effectiveness of benomyl and TBZ by the use of oil, this method has not been capable of inducing enough uptake of either fungicide to control

TABLE 3. Effect of different oils on uptake and translocation of benomyl and thiabendazole (20 mg/plant) when applied to stems in the field

Oil adjuvant	Concn (%)	Post-treatment biossay (ZI)[a]			
		Benomyl		Thiabendazole	
		7 days	13 days	7 days	13 days
None (water)		0	0	0	0
P795	10	104	45	13	0
P795	20	296	182	63	13
P696	10	126	13	0	0
P696	20	402	151	104	28
N792	10	28	13		
N792	20	104	28		
IP 3408	10	0	0	0	0
IP 3408	20	13	0	0	0

[a]Leaf disks were taken from fully expanded leaves above the treated area on the stem. ZI = area (mm^2) of zone of inhibition of fungal growth beyond edges of the leaf disk on bioassay plates.

TABLE 4. Effect of P795 oil (5% water emulsion) on translocation of ^{14}C-thiabendazole in cotton plants

Sampled 5-cm segments of plant tissue from which radioactivity was measured	Counts per minute/cm segment of tissue					
	^{14}C-thiabendazole			^{14}C-thiabendazole + oil		
	Bark	Xylem	Petiole	Bark	Xylem	Petiole
Five days after treatment						
0 - 5 Root zone	0	0		0	0	
5 - 10 Root zone	0	0		0	0	
10 - 15	0	0		0	0	
15 - 20 treated zone	1,583	196	0	1,492	651	
20 - 25	11	52	0	34	107	3
25 - 30	0	17	0	18	51	18
30 - 35	0	12	0	8	36	7
35 - 40	0	5	0	0	17	5
40 - 45	0	0	0	0	30	
Ten days after treatment						
0 - 5 Root zone	0	0		1	0	
5 - 10 Root zone	1	0		7	0	
10 - 15	3	0		0	0	
15 - 20 treated zone	1,620	340	0	1,507	641	
20 - 25	76	130	17	118	244	15
25 - 30	27	100	28	44	157	51
30 - 35	17	103	26	31	159	70
35 - 40	8	27	35	15	61	60
40 - 45	0	8	0	6	27	15

Verticillium wilt in field trials (10). Since infection of cotton by *Verticillium albo-atrum* in the field could occur at any time during the growing season, control of the disease may require that the fungicide be present in the xylem tissue continuously from June until August. In our work only large dosages 22-44 kg benomyl/ha (20-40 lb benomyl/acre) applied in soil were capable of inducing a degree of control sufficient to increase the yield of cotton (10).

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