Influence of Oils on the Uptake and Translocation of Methyl 2-Benzimidazolecarbamate in Cotton

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ABSTRACT

The hydrochloride salt of ^{14}C-methyl 2-benzimidazolecarbamate (^{14}C-MBC·HCl) penetrated the bark of the second internode of cotton plants, translocated upward, and accumulated gradually in the leaf blades. Orchex N795 and Orchex N792 oils at 10 or 20% concn enhanced the uptake of ^{14}C-MBC or ^{14}C-MBC·HCl in cotton. ^{14}C-MBC·HCl was taken up more readily than ^{14}C-MBC. A rapid sensitive method for testing the effect of adjuvants on the uptake and translocation of ^{14}C-MBC or other ^{14}C-labeled fungicides is described.

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A number of systemic benzimidazole compounds have been tested for the control of Verticillium wilt of cotton (4, 8, 9, 12) and other wilt and systemic diseases (3, 5, 16). Methyl 2-benzimidazolecarbamate (MBC) was selected in this investigation, not only because of its inherent fungitoxic and systemic properties in plants, but also because other benzimidazole fungicides (benomyl and Topsin M) are chemically transformed to MBC in solution and in plants (18, 19), and their protective action against disease could therefore be partially attributed to MBC.

The overall performance of a systemic fungicide in controlling a disease could be influenced by the adjuvant used with it, or by its formulation. Buchenauer and Erwin (4) reported that acidic solutions of benomyl, MBC, or thiabendazole (TBZ) sprayed on the foliage and stems of cotton plants were more effective in controlling Verticillium wilt than the corresponding neutral compounds. Erwin et al. (7) applied benomyl or MBC·HCl, with and without a paraffinic oil, to the lower part of the stem of cotton plants and by measuring uptake and translocation with a leaf disk bioassay found that oil enhanced uptake of both.

Nonphytotoxic oils enhance the uptake of certain herbicides (1, 2, 17) and fungicides (6, 7, 15, 20). To test the influence of oils and to obtain quantitative data on the extent of uptake and translocation of MBC in cotton, we treated the second internode of 3-4-wk-old cotton plants with ^{14}C-MBC or ^{14}C-MBC·HCl, each incorporated with various oils at certain concns, and followed the translocation of ^{14}C to the stems, petioles, and leaves. An abstract describing part of this work has been published (21).

MATERIALS AND METHODS.—Benzimidazole carbamic acid-^{14}C-methyl ester (ring-2-^{14}C) (^{14}C-MBC) was purchased from ICN Chemical and Radioisotope Division, Irvine, California. When the labeled material was assayed by thin-layer chromatography and scanned with Vanguard Automatic Chromatogram Scanner, a single intense peak was detected which had an R_f value comparable to that of authentic MBC.

Conversion of ^{14}C-MBC to ^{14}C-MBC·HCl.—A known amount of ^{14}C-MBC was transferred to a round-bottom flask and 2 ml 0.3 N HCl was added. The suspension was heated in a 70 C water bath for 2 h and the solution evaporated to dryness under vacuum. The residue (^{14}C-MBC·HCl) was dissolved in a known volume of distilled water.

Plant material and method of application of test solutions.—Cotton plants (cultivar 'SJ-1') were grown in a greenhouse for 3-4 wk. Plants of a uniform height and morphology were selected and placed in a controlled environment chamber [16-h day cycle (232 lux, 29 C), and 8-h night (18 C)]. Relative humidity (RH) was between 25 and 50%. A portion of the second internode (generally 5 cm in length) was marked and 50 μliters of a test solution was applied to the surface with a microsyringe and allowed to dry. A test solution contained a certain amount of the labeled fungicide, 0.1% Triton X-100 (isooctyl phenyl polyoxy ethanol), a certain concn of the test oil, and distilled water to make up a standard volume. The amount of label applied to each plant was standardized in each experiment but varied slightly from one experiment to another (generally 0.5 to 1 μCi of ^{14}C was applied to each plant). Three oils were tested: the paraffinic oil Orchex N795® (P795), the naphthenic oil Orchex N792® (N792), the isoparaffinic oil Humble 3408® (IP3408). P795 and P792 contained T-Mulz AO_2® and IP3408 contained T-Det N-4® (Thompson Hayward (2% w/w). All oils were received from Esso Research and Engineering Company, Baytown, Texas. Oil concns in the test solutions were varied between 1 and 22%.

Following application of the test solutions plants were placed in the controlled environment chamber for a specific period of time after which they were harvested and each plant divided into root (R); first internode which was further divided into bark (B_f) and stele (S_f); second or treated internode which was also divided into bark (T_b) and stele (T_s), leaf blades (L), petioles (P) and stem above the treated internode (StA). In some experiments, only the leaf blades were harvested and analyzed. Plant parts were placed in paper cups, and dried in an oven at 80 C for 24 h. Plant tissue was broken up into 1- to 2-mm pieces or pulverized (leaf-blade tissue) and a 0.5-g portion was analyzed for radioactivity by a modified oxygen combustion technique (21).

RESULTS.—Cotton plants were treated with test solutions containing ^{14}C-MBC·HCl or ^{14}C-MBC·HCl incorporated with 22% P795 oil. At 4, 7, or 11 days after treatment two plants of each group were harvested,

TABLE 1. Distribution[a] of ^{14}C-MBC·HCl in cotton plants at various times after treatment of stems with and without P795 oil[b] (22%)

Plant part[c]	4 days after treatment		7 days after treatment		11 days after treatment	
	MBC·HCl (%)	MBC·HCl + Oil (%)	MBC·HCl (%)	MBC·HCl + Oil (%)	MBC·HCl (%)	MBC·HCl + Oil (%)
R	0.00	0.33	0.10	0.05	0.05	0.05
B_F	0.10	0.17	0.38	0.28	0.72	0.36
S_F	0.03	0.04	0.38	0.41	0.35	0.25
T_B	70.10	65.40	67.50	57.90	60.00	32.00
T_S	8.00	8.30	8.90	7.40	7.40	9.40
St_A	9.00	11.80	7.70	11.50	9.40	8.90
P	3.50	3.00	3.00	3.40	2.00	2.30
L	9.40	11.30	12.20	17.30	20.30	47.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

[a]Each value represents the counts per minute (c/m) per plant part divided by the total c/m experimentally recovered from all parts of the plant × 100.

[b]P795 is Orchex 795 paraffinic oil.

[c]R = root; B_F = bark of the first internode below the treated internode; S_F = stele of the first internode; T_B = bark of treated internode; T_S = stele of treated internode; St_A = stem above the treated internode; P = leaf petioles; L = leaf blades.

divided into various morphological parts and analyzed for ^{14}C. The proportion of ^{14}C (counts per minute) detected in a particular plant part was expressed as a percent of the total radioactivity (c/m) which was experimentally recovered from all parts of the plant. Less than 1% of the label was detected in the first internode and the root of each plant at all times after treatment (Table 1). Most of the radioactivity was detected in the treated bark. The proportion of ^{14}C in the petioles, in the stem above the treated internode, or in the treated stele was almost constant at all days after treatment, but the proportion of ^{14}C detected in the leaf blades of ^{14}C-MBC·HCl-treated plants increased as the time after treatment increased. Quantitatively, the proportion of ^{14}C detected in the leaf blades was over twice as high in the oil-treated plants as in plants treated with ^{14}C-MBC·HCl alone. The reduction in the proportion of ^{14}C in the bark of oil-treated plants indicated that the oil enhanced transport of MBC.

In other experiments, cotton plants were treated with solutions of ^{14}C-MBC or ^{14}C-MBC·HCl each incorporated with 0, 1, 5, 10, or 20% concns of P795, N792, or IP3408. The leaf blades were harvested 14 days after treatment and the radioactivity was determined. For all three oils, the radioactivity in the leaves increased as the concn of oil increased between 1 and 10% (Table 2). Increasing the concn of oil to 20% either resulted in increase of ^{14}C in the leaves (^{14}C-MBC·HCl incorporated with P795 oil or ^{14}C-MBC with IP3408 oil) or slightly reduced it (^{14}C-MBC·HCl incorporated with N792 or IP3408 oils, or ^{14}C-MBC with N792 oil), compared to 10% concn (Table 2). The radioactivity in the leaves of plants treated with ^{14}C-MBC alone (control) was generally lower than that in the leaves of plants treated with ^{14}C-MBC plus oils at all concns (except in plants treated with IP3408 or N792 oils at 1% concn). Radioactivity in the leaves of plants treated with ^{14}C-MBC·HCl alone (control) was higher than that in leaves of plants treated wtih ^{14}C-MBC·HCl plus IP3408 at all concns, or those treated with 1% P795 or N792 oils; and was approximately equal to the radioactivity in the leaves of plants treated with 5% of either oil. IP3408 at 20% concn slightly increased the uptake of ^{14}C-MBC (Table 2). Ten percent of P795 or N792, therefore, appeared to be optimum for maximum uptake of ^{14}C-MBC or ^{14}C-MBC·HCl. It was not possible to compare the uptake of ^{14}C-MBC·HCl with that of ^{14}C-MBC because each experiment was conducted at a different time, and the amount of label applied to the plants in each experiment was slightly different in each case (Table 2).

In another experiment, equivalent amounts of ^{14}C-

TABLE 2. Amount of radioactivity in the leaves of cotton plants 14 days after treatment of stems with ^{14}C-MBC or ^{14}C-MBC·HCl each incorporated with various concns of the oils P795, N792, or IP3408

Oil[a]	Concn (%)	c/m/g dry weight × 10^{-2} Expt. A ^{14}C-MBC[b]	Expt. B ^{14}C-MBC·HCl[b]
None	0	61[c]	125[c]
P795 paraffinic oil	1	99	55
	5	216	118
	10	231	176
	20	174	196
N792 naphthenic oil	1	51	89
	5	77	162
	10	327	210
	20	311	174
IP3408 isoparaffinic oil	1	42	22
	5	61	66
	10	63	81
	20	73	70

[a]The oils are Orchex 795 and 792 and Humble 3408.

[b]The data are from two experiments carried out under comparable conditions, but at different times.

[c]Each value is an average of two replications.

MBC or ^{14}C-MBC·HCl each incorporated with 0 or 10% P795 oil were applied to cotton plants. Four plants were used for each treatment. The leaves of three plants in each treatment were left intact and analyzed for ^{14}C 12 days after treatment. A variation of this method was tested by excising the leaves of the fourth plant (in each treatment) at the time of treatment, leaving only the two uppermost expanding leaves which were used for analysis 12 days after treatment.

The radioactivity detected in the leaves of intact plants or in the two leaves of the partially leaf-excised-plants (Table 3) was highest when plants were treated with ^{14}C-MBC·HCl incorporated with 10% P795 oil. ^{14}C-MBC·HCl alone was taken up more readily than ^{14}C-MBC. P795 oil increased the uptake of either form of the fungicide (MBC or MBC·HCl). In all treatments, the radioactivity in the leaves of partially leaf-excised-plants was much higher than that in the leaves of comparable intact plants (Table 3).

TABLE 3. Radioactivity per gram of leaf tissue of cotton plants 12 days after treatment of stems with equivalent amounts of ^{14}C-MBC or ^{14}C-MBC·HCl incorporated with the oil P795 at 10% concn

Treatment	Plant with excised leaves[x] (cpm/g dry wt)	Plants with intact leaves[y] (Avg cpm/g dry wt)
^{14}C-MBC·HCl + 10% P795	109,305	9,620 a
^{14}C-MBC·HCl	21,690	4,173 b
^{14}C-MBC + 10% P795[z]	20,820	3,180 bc
^{14}C-MBC	5,550	1,200 c

[x]At the time of treatment, all leaves of one plant were excised except the two uppermost expanded leaves which were used for analysis.

[y]Values followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

[z]P795 is Orchex 795.

DISCUSSION.—^{14}C-labeled MBC·HCl applied to the second internode of the cotton plant, facilitated the detection of the upward or downward movement of the fungicide from the site of application. ^{14}C-MBC·HCl penetrated the bark to the xylem, and like other systemic fungicides (11), it translocated upward possibly by the transpiration stream and gradually accumulated in the leaf blades. ^{14}C-MBC·HCl did not move downward to the lower bark, or to the roots, of the cotton plant. These findings were in agreement with those of Peterson and Edgington (13) who reported that MBC passively translocated upward from the roots of benomyl-treated bean plants through the xylem and accumulated in the margins of the leaf blades. They explained the movement and distribution of the fungicide within the plant on the basis of physical forces (14).

The paraffinic oil P795 at 22% concn did not alter the general distribution pattern of ^{14}C-MBC·HCl from the site of treatment, but apparently increased its accumulation in the leaf blades particularly at 7 or 11 days after treatment. The amount of label in the treated stele, in the stem above the treated internode, or in the petioles of oil-treated plants was constant and comparable to that of the control (plants treated with the fungicide only) at all times after treatment. This indicated that, once the fungicide had penetrated the bark to the xylem, it was swept upward at a constant rate to the leaf blades, and that the higher accumulation in the leaf blades of oil-treated plants was due to an enhancement of the uptake of the fungicide through the bark. Oils could influence the physical characteristics of the cutin, thus rendering the plant surface more permeable to pesticides (17).

Different oils had different effects on the extent of uptake and translocation of ^{14}C-MBC or its hydrochloride salt. The isoparaffinic oil IP3408, 98% unsulfonated residue (U.R.) and viscosity 34 sec did not enhance the uptake of either form of the fungicide (MBC or MBC·HCl) but rather reduced it. The paraffinic oil P795 (96% U.R. and viscosity 70 sec) or the naphthenic oil N792 (82% U.R., and viscosity 77 sec) at 10 or 20% concn enhanced the uptake and the accumulation of the fungicide in the leaf blades. Hull (10) in his review article noted that oils having viscosity over 70 sec and U.R. over 75% were more effective in improving the control of annual grasses in maize when incorporated with the herbicide atrazine.

The methods described above for the study of the effect of oils on the uptake and translocation of ^{14}C-MBC in cotton can be adapted to the study of the effect of other adjuvants on the uptake and translocation of ^{14}C-labeled systemic fungicides in general. Analysis of only the leaf blades eliminated the necessity for the analysis of the entire plant. Analysis of only the leaf blades depended, however, on the accuracy of measuring and applying equivalent amounts of radioactivity to plants of uniform morphology. The uptake of the labeled fungicide also depended, among other factors, on the concn of the fungicide and on the surface area of the treated part of the plant. These factors were standardized in this study.

Further improvement of this method was achieved by excising all except two expanding leaves at the time of treatment. When the radioactivity was analyzed, there was a higher count per minute per gram of leaf tissue, compared to plants with the entire complement of leaves. This increased the sensitivity of the method. It also reduced the possible error due to sampling since the analytical combustion procedure (A.I. Zaki and D. C. Erwin, *unpublished*) did not permit the use of a sample larger than 0.5 g.

The application of the latter method clearly showed that ^{14}C-MBC·HCl was taken up more readily by the cotton plant than ^{14}C-MBC, thus confirming the results of Buchenauer and Erwin (4). P795 oil at 10% concn equally enhanced the uptake of either form of the fungicide. Quantitatively, the amount of ^{14}C in the leaves of treated plants increased in this order: ^{14}C-MBC $<$ ^{14}C-MBC plus oil $\leqslant$ ^{14}C-MBC·HCl $<$ ^{14}C-MBC· HCl plus oil.

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