Effect of Environmental and Biological Factors on Persistence of Malathion Applied as Ultra-Low-Volume or Emulsifiable Concentrate to Cotton Plants

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A comparison of the persistence of malathion applied to cotton leaves as a water-diluted emulsifiable concentrate or as an ultra-low-volume formulation showed that the ULV persisted longer than the emulsifiable concentrate with a half-life value of 4.6 and 2 days, respectively. Studies with glass surfaces indicated that faster evaporation of the EC was, in part, responsible for the differential in persistence of the two formulations, particularly at 50° C. The EC formulation penetrated the leaf surface faster and a greater percentage of malathion was found in the internal fraction of the leaf compared with the ULV formulation. Metabolism on the leaf surface did not appear to contribute to the difference in the persistence of both formulations, although a small amount of the monocarboxylic acid of malathion was found on the surface of plants treated with both formulations. In the internal fraction, the dicarboxylic acid metabolite of malathion was the major decomposition product.

One of the newest methods of applying toxic chemicals for the control of insect pests is the ultra-low-volume application of technical insecticides. Messenger (1964) and Skoog *et al.* (1965) demonstrated that undiluted malathion applied at a rate of 8 fluid ounces per acre was effective in control of several species of grasshoppers. Cleveland *et al.* (1966) stated that the application of ultra-low-volume technical malathion at 8, 12, and 16 fluid ounces per acre was as effective against the boll weevil, *Anthonomus grandis* Boheman, as the standard application of methyl parathion at the rate of 0.4 pound per acre in 2 gallons of water.

Adair *et al.* (1967) compared a number of emulsifiable concentrate water-diluted insecticides with ultra-low-volume formulations, and reported that the ultra-low-volume formulation performed as well as or better than the emulsifiable concentrate in the control of several cotton insects. Brazzel (1967) reported that ultra-low-volume malathion applied in droplet sizes ranging from 100 to 200 microns drifted less than the emulsifiable concentrate water-diluted formulation. He also reported obtaining two to four times greater recovery of the ultra-low-volume formulation than the water-diluted emulsifiable concentrate.

Several workers have indicated that the disappearance rate of technical malathion is slower than the same dose applied as an emulsifiable concentrate dilute formulation (MacCuaig, 1966; Wheeler *et al.*, 1967).

A study was undertaken to substantiate differences in the rate of disappearance of ultra-low-volume or emulsifiable concentrate malathion, diethyl mercaptosuccinate, *S*-ester with *O,O*-dimethyl phosphorodithioate, when applied to cotton (Delta Pine Smoothleaf) and to attempt to explain differences in the results obtained.

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MATERIALS AND METHODS

The two formulations of malathion used in this study were: technical malathion—95% active ingredient obtained from American Cyanamid Co., Princeton, N. J.—and emulsifiable concentration of malathion—57% active ingredient—commercial grade, diluted with water (1 to 4). For all greenhouse studies, the Universal aerosol spray kit (Nutritional Biochemicals Corp.) was used to apply small amounts of the insecticide on plants or glass plates. In one test, leaves were dipped in a 2.5% water-diluted emulsifiable concentrate. Three replicates were run on plants except in the dipping method, where only one replicate was run. Two replicates were run on glass plates.

Leaf samples were collected at random from malathion-sprayed plants and untreated plants which were kept under the same conditions in the greenhouse. The zero-time samples were collected as soon after spraying as the surface of the plants had dried. Subsequent samples were taken after 1, 3, 6, and 9 days. Extraction of the residues usually was started within an hour after collection of samples.

Fifty-gram leaf samples were used for malathion residue determinations. External residues were obtained by washing the treated leaves three times in 50-ml. portions of carbon tetrachloride. For internal residue analysis, material was prepared by macerating washed leaves in 100 ml. of distilled water in a Waring Blendor for 15 minutes. The brei was strained through cheesecloth into 1-quart, airtight containers. The internal extract was acidified to pH 2 to 3 with concentrated hydrochloric acid to extract the acidic metabolites, and then 100 ml. of carbon tetrachloride was added. Samples were next placed on a mechanical shaker for 15 minutes. The layers were separated, and the carbon tetrachloride layer was filtered through glass wool and retained for the determination of malathion present.

Malathion Determination. A method described by Norris *et al.* (1954) for the determination of malathion in formulations was adapted for this study. This method is based on alkaline decomposition of malathion to so-

dium dimethyl phosphorodithioate and conversion to a yellow copper complex. Malathion and either of the half esters *S*-[(1-carboxy-2-carbethoxy) ethyl] *O,O*-dimethyl phosphorodithioate or *S*-[(2-carboxy-1-carbethoxy) ethyl] *O,O*-dimethyl phosphorodithioate will respond to this procedure (Mattson and Sedlak, 1960). In this method, an aliquot of the carbon tetrachloride layer containing about 0.25 to 2.5 mg. of malathion in 100 ml. of carbon tetrachloride was used. The amount of malathion was determined by reference to a standard graph prepared by running 0- to 25-ml. portions of a standard solution (0. 1 mg. of malathion per ml. of solvent) through the same procedure.

C^{14}-Labeled Malathion Studies. In later studies, C^{14}-malathion (Nuclear-Chicago Corp.), labeled at the 2 and 3 position of the succinic acid moiety (specific activity 2.87 mc. per mmole) was used. A semiautomatic microapplicator provided with a 0.05-ml. glass syringe was used to apply the radioactive technical malathion on cotton leaves. The radioactive emulsifiable concentrate malathion was prepared from technical C^{14}-malathion by emulsifying with a drop of Triton X 152 a measured amount of the technical form in water at a 1 to 4 ratio of malathion to water. Fifty milligrams of the emulsified water-diluted malathion was spread on the upper surface of the cotton leaf by means of a micropipet. All plants were held in the greenhouse, where the day temperature ranged from 32° to 38° C. At 0, 1, 3, and 12 days a leaf picked at random was removed from four treated plants for malathion radioactivity determinations. Two replicates were run and the results averaged.

External extracts were obtained by rinsing the leaves three times with carbon tetrachloride, and internal residues were obtained by macerating rinsed leaves in a mortar with carbon tetrachloride and Florisil added to aid in grinding. The homogenate was transferred quantitatively to a flask, and was shaken for 15 minutes with carbon tetrachloride. The residue was also extracted with acetone which was handled separately. Prechromatographic purification procedures were adapted from Menn *et al.* (1960). Briefly, crude extracts were spotted on filter paper and were then separated by ascending development with acetonitrile for a distance of about 2 inches. The acetonitrile was able to separate malathion, malaoxon, the mono ester, and the diacid selectively from interfering lipides which remained at the origin. The malathion and its metabolites were then extracted from the filter paper with acetone. The acetone extract was concentrated to a small volume for chromatographic analysis.

Thin-layer plates were prepared as described by Stahl (1964) using silica gel G. The concentrated C^{14}-malathion eluates were applied to plates and developed with butanol–acetic acid–water (10:1:10) or hexane–acetone (4 to 1). The second system gave better separation with less interference from plant extracts. Malathion, malaoxon, the mono ester, and dicarboxylic acid metabolites of malathion were cochromatographed. The plates were removed when the solvent front had moved about 10 cm., and were allowed to dry. The plates were then placed in contact with Kodak Blue Brand x-ray film and exposed for 10 days. The developed films were then matched with the thin-layer chromatograms to locate the spots and determine R_f values. The nonradioactive metabolites were identified by color development as described by Walker and Beroza (1963). Spots on thin-layer plates, thus located, were removed and transferred to 20-ml. plastic vials. Fifteen milliliters of Liquofluor (purchased from Nuclear-Chicago Corp.) scintillation solution was added to each vial and the radioactivity was determined with a Packard Tri-Carb Model 314-X scintillation spectrometer. Each sample was counted for two 1-minute periods, and the results were averaged. The results were corrected for background and quenching, which was accomplished by recounting samples after the addition of an internal standard.

Deposits on a Glass Surface. To study the effect of several environmental factors, Petri dishes were sprayed with malathion as either a technical or emulsifiable diluted form of both formulations on the glass plates. Some sprayed plates were held under field or greenhouse conditions. Other plates were held in temperature cabinets at 30°, 50°, or 70° C. and some were held at room temperature (approximately 27° C.). Plates were analyzed at 0, 1, 5, and 12 hours after treatment.

In order to study the effect of ultraviolet radiation on the disappearance rate of the two formulations, Chromato-Vue (Ultra Violet Products, Inc.) was used. The ultraviolet energy was provided by two high intensity tubes, one with a filter transmitting long waves (3660 A.) and the other transmitting short waves (2537 A.). The intensity of these lights was found to be 272.8 μw. cm.$^{-2}$ at 23 cm. Sprayed plates were held 23 cm. from the light. Other plates were held under a fluorescent light while controls were held in a dark chamber. Readings were taken at zero time and after 1 week of radiation. The room temperature was held at 27° C. during the test; however, the temperature under the ultraviolet light was found to be 10° C. higher than the other treatments.

Samples of four plates were taken after various periods of time to the laboratory for malathion residue determination. Four plates were washed thoroughly with carbon tetrachloride, and the solutions were combined together in one glass-stoppered flask and kept in a cold chamber until malathion could be estimated. A small aliquot was removed before malathion determination, condensed, and spotted on a thin-layer plate as previously described to determine if any degradation products were present.

RESULTS AND DISCUSSION

Owing to the application procedure used, the initial deposit of the two malathion formulations differed widely. The ULV formulation had an initial deposit which ranged between 1.38 and 1.62 mg. per leaf, while the water-diluted malathion EC applied only on the upper surface had initial deposits which averaged 2.32 to 3.55 mg. per leaf. The water-diluted malathion EC, which was applied by dipping the leaf, had an initial deposit of 1.17 mg. per leaf.

In all cases in this study, the ULV formulation persisted longer than the EC formulation (Tables I and II). The ULV formulation disappeared from the leaf surface gradually, while the malathion EC residues on the leaf surface showed the more typical degradation and persistence curve similar to that reported for various pesticides by Gunther and Blinn (1955). They expressed the opinion

that the degradation behavior is due to the disappearance of the extracuticular and cuticular residue by weathering.

The half-life value colorimetrically determined for ULV malathion averaged 5.5 days, while it averaged 2.3 days for the EC applied to the upper leaf surface. Leaves dipped in the water-diluted formulation gave a half-life value of 1.8 days. The small difference in the half-life values of the EC applied on both or only the upper surface may be due to the greater surface area involved for evaporation or absorption in the case of the dipping technique. The results of the malathion EC are in agreement with results reported by Koivistoinen (1961), who found that the half-life values on several vegetables were approximately 1 day. In contrast, Blinn *et al.* (1959) found that the half-life residue of malathion on orange peels was greater than 32 days. They suggested that the malathion remained because of its absorption into the oils of the orange peel.

The level of ULV malathion in the internal fraction increased slowly while the water-diluted formulation rapidly increased, reaching a peak in 6 days (Table I). These results indicate that the EC formulation penetrated the leaf faster and that a greater percentage of the dose penetrated the leaf cuticle than the ULV formulation. The total residues, surface and internal, of the EC formulation decreased much faster than the ULV, possibly because of faster evaporation of the EC formulation. To check this hypothesis, glass plates were sprayed with both formulations and held under various conditions.

The results indicate that temperature is a major factor resulting in the loss of malathion from smooth surfaces. In all cases, the ULV formulation persisted longer than the EC. However, the degree of temperature was important. At a relatively low temperature, 30° C., and at room temperature, both formulations persisted about equally (Table II). At a high temperature of 70° C., both formulations rapidly disappeared. At a moderate temperature of 50° C., however, a difference in the persistence of these two formulas occurred (Table II). The ULV persisted longer than the EC form. No degradation products of malathion from the glass plates were found. The results indicate that temperature in the 40° to 60° C. range could account, at least in part, for the disappearance of the EC formulation.

There are few reports in the literature concerning the role of evaporation as a factor which constantly affects the pesticide residue. In this respect, Thomas (1956) stated that evaporation of P^{32}-Schradan from leaf surfaces during the first 2 days after application occurred at only one fifth the rate of that from glass slides. Matsumura (1960) related differences of evaporation between plant surfaces and glass to the fact that malathion is absorbed on the plant surface, so the evaporation of the compound would be substantially smaller than from the surface of glass under similar conditions. In the present work, the diluted malathion EC exists as a thin film over the leaf which covers much more surface area than the ULV, which exists as small discrete droplets, so it is expected that it will be subjected to more evaporation than the ULV formulation.

Plates exposed for 1 week to both long and short waves of ultraviolet light showed a decrease in malathion for both ULV and EC as compared with those kept in the dark (Table III). The ultraviolet light also resulted in an increased amount of the polar material in both formulations. The amount of degradation products appeared to be about the same in both formulations and thus could not account for the differences in persistence of the two formulations. There was a difference in the recovery of malathion from the two formulations. The temperature

Table I. Comparison of Surface and Internal Residues of ULV or Water-Diluted EC Formulations of Malathion

	Malathion Remaining after Indicated Days									
	0		1		3		6		9	
Formulation	Mg.	%	Mg.	%	Mg.	%	Mg.	%	Mg.	%
ULV										
Surface	14.7	93.0	13.2	88.0	11.3	75.9	7.7	44.6	5.1	34.3
Internal	0.27	1.8	0.32	2.2	0.47	3.1	0.84	5.6	0.90[a]	5.5[a]
EC[b]										
Surface	28.8	98.0	13.4	79.8	10.5	38.2	5.7	20.0	2.4	9.0
Internal	0.55	2.0	1.4	4.9	2.0	6.5	2.4	7.7	0.74[a]	2.1[a]
EC dipping[a,c]										
Surface	11.50	97.90	8.68	73.90	1.50	12.80	0.10	0.85	0	0
Internal	0.24	2.10	0.90	7.67	1.00	8.50	...	...	...	...

[a] One replication, rest are an average of three replicates.
[b] Malathion applied to upper leaf surface.
[c] Malathion applied by dipping in a 2.5% water-diluted formulation.

Table II. Effect of Temperature on Persistence of Malathion When Applied as ULV or Water-Diluted EC to Glass Plates

	Mg. of Malathion per Plate after Indicated Hours									
	0		1		5		12		24	
Temp., °C.	ULV	EC	ULV	EC	ULV	EC	ULV	EC	ULV	EC
Room temp. approx. 27	6.25	5.75	6.25	5.75	6.25	5.75	6.25	5.75	5.75	5.00
30	6.25	5.75	6.25	5.75	6.25	5.53	6.25	4.95	4.90	4.50
50	6.25	5.75	5.53	3.75	5.00	4.60	5.00	3.75	4.93	2.25
70	6.25	5.75	0	0	0	0	0	0	0	0

Table III. Comparison of Persistence of ULV or Water-Diluted EC Formulation of Malathion Applied to Glass Surface and Held under Different Kinds of Radiation

	Malathion after 1 Week Indicated Treatment							
	0 Time		Dark		Fluorescent Light		U. V. Light	
Formulation	Mg.	%	Mg.	%	Mg.	%	Mg.	%
ULV	21.4	100	21.0	98.1	18.25	85.3	11.44	53.5
EC	37.5	100	36.0	96.0	28.16	75.1	6.80	18.1

under the ultraviolet light was 10° C. higher than the other treatments; thus this difference between the two formulations may be caused by temperature and evaporation rather than decomposition by the ultraviolet light.

El-Refai (1960) found that ultraviolet light irradiation caused chemical changes in malathion in vitro. However, Koivistoinen (1961) found that the elimination of ultraviolet rays from the sunlight had very little retarding effect on the losses of malathion from plants growing in field trials. Both of these conclusions are supported by this work, since degradation occurred under ultraviolet light, but the quantity of degradation products was small.

Visible light did not increase the disappearance rate of malathion as only slight differences between the two formulations as well as those in the dark are shown (Table III). These results differ from those of Koivistoinen (1961), who reported that visible light affected the disappearance of malathion EC depending on the plant material involved. His results may have been owing to temperature effects, since the nature of the surface affects the amount of light absorbed and, thus, the temperature.

During this study, rain removed both formulations equally well from glass plates. Smith *et al.* (1955) in their washing trials showed removal of 84 to 100% of the malathion residues from various plant products.

Rate of Disappearance of C^{14}-Labeled Malathion. The previously described experiments showed that the ULV malathion could persist longer than that of the EC formulation when applied to cotton plants. It had been suggested that the disappearance of malathion EC was the result of the formation of decomposition products in plants at a faster rate than the ULV formulation. It had also been suggested that possibly enzymes on the surface of the plant degraded the EC at a faster rate, since the diluted malathion EC covers much more surface area of the plant than the ULV formulation (Nolan, 1967). For this reason, an experiment with C^{14}-malathion was conducted to compare the disappearance behavior as well as the metabolic products of both technical C^{14}-malathion and the water-diluted C^{14}-malathion EC.

Results obtained by using C^{14}-malathion confirmed what was first found with the use of the colorimetric method, that the water-diluted C^{14}-malathion EC disappeared at a faster rate than the technical C^{14}-malathion (Figure 1*A*). After 12 days from application, there was only 0.87% of the initial radioactivity on the surface of leaves which received C^{14}-malathion EC, while after the same period of time radioactivity counts revealed that 21.8% of the initial radioactive malathion was still on the surface of those plants treated with ULV technical C^{14}-malathion. Half-life values were 3.8 and 1.2 days for ULV and C^{14}-malathion EC, respectively.

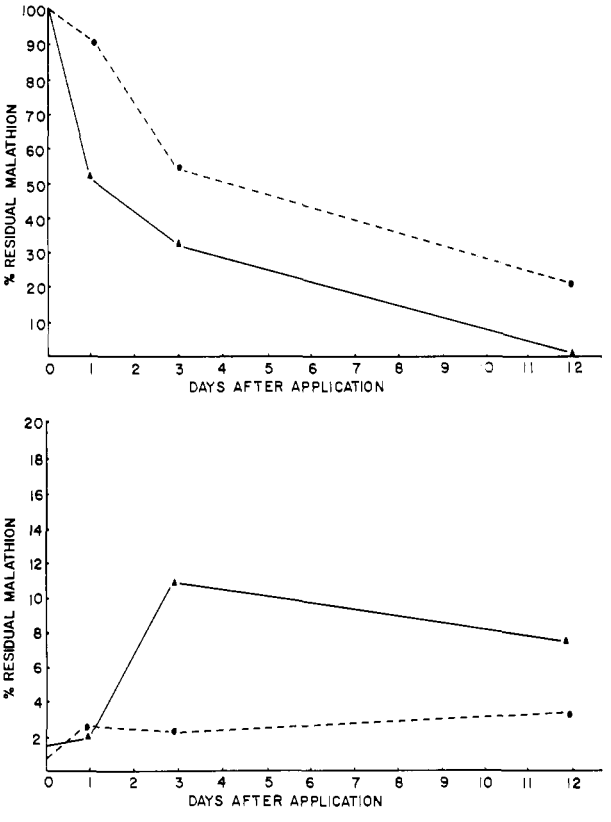

Figure 1. Residues of C^{14}-malathion applied to the upper leaf surface of cotton as a ULV or EC formulation and held in greenhouse

A. Residues from leaf surface
B. Internal leaf residues
- - - C^{14}-malathion ULV
——— C^{14}-malathion EC

Analysis of the radioactive materials from the leaf surface of both formulations and spotted on thin-layer plates indicated the presence of a very small amount of a monocarboxylic acid metabolite of malathion. No other decomposition products of malathion were found.

Internally, only the C^{14}-malathion is evident at zero days. A gradual buildup of radioactivity in the internal fraction occurred with ULV C^{14}-malathion (Figure 1*B*), while the C^{14}-malathion EC increased rapidly in the internal fraction between 1 and 3 days. Autoradiograms of thin-layer plates of the internal CCL_4 fraction showed an increase in the amount of malathion and one decomposition product which was identical with the R_f of malathion–dicarboxylic acid in two systems (Figure 2). The acetone extracts showed a small amount of malathion not extracted by the CCL_4 possibly because of insufficient rinsing. Tomizawa and Sato (1962) found that the major

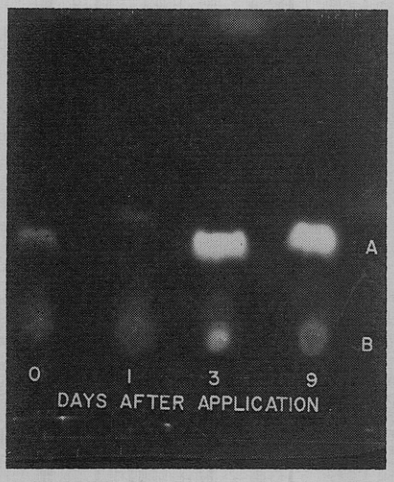

Figure 2. Thin-layer autoradiograph of internal C^{14}-malathion EC fraction showing increase in malathion (*A*) at 3 days and presence of small amount of malathion dicarboxylic acid (*B*)

Silica Gel G developed with hexane–acetone (4:1)

metabolite of rice plants was the dicarboxylic acid, while the monocarboxylic acid was present in lesser amounts.

Radioactive counts continued to appear at all holding intervals, decreasing gradually. At 12 days from application, 3.2 and 7.4% of the initial radioactivity was found in the internal fraction of plants treated with C^{14}-malathion ULV and EC, respectively.

One important route by which a lipide-soluble pesticide disappears from the exterior of the plant is by absorption into the cuticle, where the outer layer, at least, is of a lipophilic nature like the pesticide itself. Surfactants, like emulsions, act primarily by virtue of their combined polar and apolar properties in the same molecule, rendering compatible two phases which are otherwise incompatible. From the cuticle, the chemical may penetrate farther into the cells of the plant. As in the case of the colorimetric method, the C^{14}-malathion showed that the water-diluted EC formulation penetrated faster and appeared in the internal fraction in greater amounts than the ULV formulation.

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