with the S, O, and CH_3, bringing CH_3 close to S and resulting in rearrangement.

The necessary strong Lewis acid will in most cases probably be free Al^{+3}, as shown here. The volatilization of all OH^- can be expected to produce a number of structures in the clay crystal analogous to lattice defects such as those found in metals. As the clay is heated to extreme temperatures, probably in the range of 1300° to 1400° C. in the case of kaolinite, a process similar to the annealing of a metal can be expected to take place, during which these defects should gradually be eliminated, and after which the clay becomes a glassy mass which should possess no catalytic or absorptive properties and no internal surface. The number of strong Lewis acid sites is probably not large, but their strength is indicated by the pK_a of Barden clay and bentonite, less than 1.52, although the pH of the slurry is greater than 4.

This type of reaction can be expected to occur to a greater extent in the case of Barden clay than most other clays, since natural Barden clay has a much greater crystallinity than many others. The "lattice defect," when it occurs, thus must migrate further before it encounters its opposite member, eliminating itself. The more amorphous Attaclay and bentonite will eliminate the lattice defect more readily.

To some extent, the cationic exchange capacity of a clay is a measure of the regularity of its crystal structure. A low cationic exchange capacity is to be expected to be associated with a regular structure, with few sites at which ions may exchange. In the natural clay, a low cation exchange capacity should be concommitant with few sites for chemisorbed water, which tends to mean less catalytic decomposition by a hydrolysis mechanism. In clay heated at 950° C. for 24 hours, the very regularity of the original crystal which produces the low cationic exchange capacity will give rise to a long migration distance for the lattice defect, which will encourage the molecular rearrangement.

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HERBICIDE DEGRADATION

Microbial Degradation of Selected Herbicides in Soil

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EPTC, amitrole, amiben, and ipazine are degraded by the microflora of soil. $C^{14}O_2$ was not released microbiologically from soil receiving tagged propazine, atrazine, or simazine. The susceptibility of chlorobenzoates to decomposition is related to the number of chlorines on the aromatic ring. A technique for determining the ability of a specific soil population acting on one aromatic herbicide to destroy a structurally related compound is described, and the method is used to show that a 2,4-D–metabolizing microflora of soil is inactive on MCPA and 2,4,5-T and that benzoate-metabolizing microorganisms are inactive on monochlorobenzoates. The resistance of dichlorophenols to microbial destruction is associated with the presence of a chlorine in the position meta to the phenolic hydroxyl. Seed inoculation with a 4-(2,4-DB)–utilizing *Flavobacterium* protected alfalfa in sterile soil amended with the herbicide, but little protection was observed in nonsterile soil.

Despite an increasing concern with the persistence and residual effects of herbicides and the dominant position occupied by microbiological agencies in environmental detoxication, little information is available on the chemicals which are metabolized and inactivated by the soil inhabitants. The present communication summarizes a series of studies designed to determine the role of soil microorganisms in the degradation of herbicidal compounds. The substances investigated include thiolcarbamates, triazines, chlorinated benzoates, chlorophenols, and phenoxy compounds.

Unequivocal evidence for the participation of the subterranean microflora in the degradation of thiolcarbamate, benzoate, and phenylacetate herbicides has yet to be established, and microorganisms active upon these pesticides have not been obtained in monoculture. Amitrole, however, appears to be broken down microbiologically, and increases in soil temperature and organic matter level enhance the decomposition (*13*). The microbiological contribution to the detoxication of the triazine herbicides remains uncertain. Destruction of the triazines has been attributed, in part at least, to microbiological agencies (*5, 15*). Other

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investigators observed a very rapid evolution of $C^{14}O_2$ from soil receiving ring-labeled simazine (*12*), and the isolation of a number of gram-negative bacteria which can use simazine as the sole nitrogen source for growth has been reported (*7*).

In the present study, the microbial degradation of herbicides was determined by measuring the release of $C^{14}O_2$ from labeled herbicides applied to soil, by spectrophotometric measurement of cleavage of the aromatic ring, and by plant bioassay. In addition, the possibility of protecting susceptible plants developing in herbicide-treated soils by means of seed inoculation with bacteria effective in inactivating the pesticide was explored.

Procedure

EPTC Decomposition. To determine whether soil microorganisms are active in the degradation of thiolcarbamates, a sample of moist Honeoye silt loam (pH 6.5) containing 100 p.p.m. of EPTC (99.7% ethyl *N,N*-di-*n*-propylthiol carbamate) was incubated in sealed containers at 25° C. After 7 months, three series of additions were made to 125-ml. bottles containing 18 ml. of sterile medium: series A was treated with 3.0 ml. of distilled water; series B received 1.0 gram of treated soil and 2.0 ml. of an EPTC solution to give a final concentration of 225 p.p.m. of the chemical; and series C received the herbicide solution plus 1.0 gram of treated soil that had been sterilized by autoclaving. The test medium contained 2.5 grams of $(NH_4)_2SO_4$, 0.4 gram of K_2HPO_4, 0.1 gram of KH_2PO_4, 0.1 gram of $MgSO_4.7H_2O$, 0.05 gram of $CaCl_2$, 0.015 gram of $FeSO_4.7H_2O$, 0.1 mg. of Na_2MoO_4, and 0.2 gram of yeast extract in 1.0 liter of distilled water. The bottles were incubated at 25° C., and at periodic intervals, the contents of four bottles from each series were mixed with 50 grams of the 0- to 1-mm. fraction of air-dried, Honeoye silt loam. The soil was planted immediately to spring oats (Garry variety), and the root lengths of the seedlings were measured after 6 days.

Isotopic Methods. A possible role for microorganisms in the degradation of the following herbicides was studied by measuring the release of CO_2–C^{14} from soil treated with the tagged pesticide: simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine], atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), propazine [2-chloro-4,6-bis(isopropylamino)-*s*-triazine], ipazine (2-chloro-4-diethylamino - 6 - isopropylamino - *s*-triazine), all uniformly labeled in the ring; carboxyl-labeled amiben (3-amino-2,5-dichlorobenzoic acid) and fenac (2,3,6-trichlorophenylacetic acid); and amitrole - 5 - C^{14}(3 - amino - 1,2,4-triazole). Each treated soil sample was incubated in a 1500-ml. jar, at the base of which was inserted a small cup containing 25 ml. of 20% NaOH solution. The soil was placed immediately about the alkali-containing vessel, and the bottle was sealed with a rubber stopper through which was inserted a glass tube that extended into the alkali. The tube permitted regular sampling of the alkali without disturbing the contents of the bottle. The carbonate was precipitated as $BaCO_3$, plated, and counted (*8*).

One series of containers received 200 grams of nonsterilized Honeoye silt loam, while the second set received a like amount of soil that had been autoclaved for 2 hours. After the soils had become dry, 40 ml. of water and 5.0 μc. of the labeled herbicides were added to each jar. The specific activities of simazine, atrazine, ipazine, propazine, amitrole, amiben, and fenac were 5.0, 20.0, 16.7, 2.2, 12.5, 5.4, and 2.5 μc. per mg., respectively.

In experiments with EPTC, the $BaCO_3$ precipitate was washed three times with benzene to remove residual herbicide. The carbonate precipitates, obtained in experiments with the other herbicides, were washed 3 times with distilled water prior to plating. The counting gas was a mixture of 98.7% helium and 1.3% butane.

Spectrophotometric Methods. The spectrophotometric procedure used to ascertain the decomposition of aromatic herbicides and related compounds was that of Whiteside and Alexander (*16*), except that 2.0 grams of Dunkirk silt loam was added to 100 ml. of the solution, the benzoate substrate was supplied at concentrations of 2 to 15 mg. of chemical per flask, and the wavelength for determining benzoate degradation was 245 mμ. All incubations were at 25° C. The concentration of the benzoates was calculated from the absorbancy of the supernatant liquid.

Inoculation Experiments. The microorganism used for seed inoculation was a *Flavobacterium* sp. active in the degradation of 4-(2,4-dichlorophenoxy)butyric acid, 4-(2,4-DB) (*4*). The bacterial cells were harvested after 5 days of growth in a 4-(2,4-DB) medium (*11*), washed twice, and suspended in 0.02*M* phosphate buffer, pH 7.0. Seeds of DuPuits alfalfa were inoculated with a heavy bacterial suspension and the seeds planted $^1/_8$ inch below the surface of Honeoye soil and Williamson silt loam (pH 5.5) contained in pots 7 inches high and 5 inches in diameter, 1.0 kg. of soil per pot. Prior to potting, some of the soil samples were autoclaved and then subsequently received, where appropriate, sufficient 4-(2,4-DB) in aqueous solution to give a final concentration of 10 p.p.m. The pots were maintained at 25° C. with no attempt to maintain sterility, and the plant development was recorded at the end of 6 days.

Results and Discussion

EPTC Decomposition. By means of the oat bioassay technique, indirect evidence was obtained that the soil microflora is active in EPTC degradation. The root lengths of oat plants treated with the herbicide-containing medium inoculated with nonsterile soil (series B) were not significantly different (at the 5% confidence level) from the root lengths of plants exposed to the same medium receiving sterile soil (series C) after 0 and 6 days of incubation. The oat development was significantly better in series B than in series C after incubation periods of 12, 24, and 48 days. Further, although the roots of series B grew more poorly than those receiving a water-medium amendment (series A) at 0, 6, and 12 days, there was no statistically significant difference between the two treatments on the 24th and 48th days. Consequently, despite the 7-week persistence of phytotoxicity in the medium receiving sterile soil, the phytotoxicity had dissipated in the presence of the active microflora of unheated soil. The extent of degradation was not evident, but the molecule had clearly been modified microbiologically to an extent sufficient to destroy the inhibitory influences.

To enrich further the microflora active in the degradation, 4.0 grams of Honeoye silt loam, treated 7 months earlier with EPTC, was added to 50 ml. of the inorganic salt solution supplemented with 100 p.p.m. of both EPTC and yeast extract. The flasks were sealed and incubated on a rotary shaker at 30° C. and 10-ml. aliquots subcultured weekly into fresh medium. After six such serial transfers, the microbial population was tested for its ability to release $C^{14}O_2$ from EPTC labeled at the C_1 position of the ethyl group. For this purpose, a sealed, two compartmented vessel that permitted free gaseous exchange between the two portions was used. One compartment contained 50 ml. of the inorganic medium supplemented with 100 p.p.m. of EPTC, 100 p.p.m. of yeast extract, and 5.0 μc. of EPTC–C^{14} (6.8 μc. per mg.), and the second contained 20 ml. of 20% NaOH. One set of reaction vessels received 10 ml. of the enrichment culture, and a second set was maintained as uninoculated controls. After a 4-week incubation period at 30° C., the CO_2 trapped in alkali was precipitated as $BaCO_3$, and the precipitate plated and counted. The CO_2 released in 4 weeks from the soil receiving the enrichment had 306 c.p.m. of C^{14}, while the uninoculated sample exhibited only 52 c.p.m. after correction for background.

The data demonstrate that the thiolcarbamate is metabolized microbiologically, but the rate of CO_2 release from the ethyl moiety of the molecule is slow despite the more rapid detoxication demonstrated above for the parent pesticide. The difference between the slow release of $C^{14}O_2$ from labeled EPTC and the more rapid microbial inactivation of the herbicide, as revealed by plant bioassay, suggests detoxication without appreciable mineralization of the ethyl moiety of the molecule.

Inasmuch as compounds structurally related to EPTC seem to have a marked effect on a wide spectrum of organisms, a study was undertaken to determine the influence of a number of thiolcarbamates upon the growth of a *Bacillus* sp.

After sterilization by passage through fritted glass filters, the test compounds were added to nutrient broth (Difco) to final concentrations of 1 to 1000 p.p.m. The inoculated cultures were incubated at 30° C. on a rotary shaker, and turbidity measurements were made to ascertain the rate of growth. Levels of 5.0 p.p.m. of *n*-propyl diethyl, *tert*-butyl ethyl *n*-butyl, and *n*-butyl di-*n*-butyl thiolcarbamates were found to be toxic, whereas 10.0 p.p.m. of EPTC, *n*-amyl diethyl, ethyl di-*n*-butyl, ethyl ethyl *n*-butyl, propyl ethyl *n*-butyl, *n*-propyl di-*n*-propyl, ethyl di-allyl, ethyl *n*-propyl allyl, allyl di-*n*-propyl, *tert*-butyl di-*n*-propyl, and 2-chloroallyl di-*n*-propyl thiolcarbamates were required for a toxic action to be noted.

Table I. Cumulative Release of $C^{14}O_2$ Resulting from Applications of Tagged Herbicides to Honeoye Silt Loam

Herbicide	Radioactivity in Evolved CO_2, C.P.M.[a]				
	1 week	2 weeks	4 weeks	8 weeks	16 weeks
Amitrole	185,000	110,000	195,000	...	200,000
Amiben	4,500	6,500	11,500	...	74,000
Fenac	1,160	1,500	1,750	2,500	1,100
Simazine	410	350	580	410	940
Ipazine	...	1,050	950	1,150	90,000
Atrazine	0	125	30	0	400
Propazine	0	10	20	0	0

[a] Corrected for C^{14} in $BaCO_3$ derived from sterile soil controls.

Because these studies were performed with liquid cultures, in which toxicities are probably more marked than in soil, it would appear that concentrations far higher than those used in practice are required to affect soil bacteria. In agreement with the suggested lack of significant influence on microorganisms in soil are the findings that 50 p.p.m. of EPTC exerted a negligible effect upon the rate of conversion of 50 p.p.m. of added ammonium nitrogen to nitrate in Honeoye silt loam and that the rate of O_2 consumption in the soil amended with ethanol (10 μmoles of ethanol per 4 grams of soil) was not materially reduced in the presence of 100 p.p.m. of EPTC, although 500 p.p.m. of the herbicide reduced the rate by more than 50%.

Decomposition of Amitrole, Fenac, and Triazines. A possible role of soil microorganisms in degrading several herbicides was ascertained by measurement of the release of $C^{14}O_2$ from labeled pesticides applied to soil. Autoclaved soil was used as a control to detect possible nonbiological mineralization of the test compound. The data in Table I demonstrate that amitrole is readily attacked microbiologically, essentially the same quantity of tagged CO_2 being found after 1 as after 16 weeks; the $BaCO_3$ obtained from the sterile, amitrole-amended soil never exhibited more than 380 c.p.m. above background. The data with fenac and simazine are anomalous, since only a small amount of the C^{14} was liberated as CO_2, and the quantity did not increase with time of incubation as commonly occurs with resistant substrates undergoing microbial decomposition. It may be that the small quantity of tagged CO_2 arose from the degradation of labeled substances contaminating the herbicide preparations rather than from the herbicides themselves. Even after 16 weeks, no CO_2 was released from propazine (Table I). These results are in agreement with the prolonged persistence of this compound or its toxic derivatives in the field. Atrazine also was resistant to microbial destruction, and only by the 16th week was there a rise in activity in the carbonate precipitate. The increase in radioactivity in the trapped CO_2 from the 8th to the 16th week was similar in magnitude for simazine and atrazine and may reflect the first production of CO_2, the final product of herbicide dissimilation. Of the triazines, ipazine alone appeared to be attacked to a significant extent, yet more than 2 months elapsed before mineralization was detectable.

The data suggesting an apparent function of the microflora in the degradation of amitrole are in agreement with the results of Ashton (*2*) and also conform with the rapid inactivation in soil as estimated by plant bioassay (*6*). On the other hand, compounds which exhibit a prolonged residual herbicidal activity are likely to be resistant to microbial destruction, although resistant compounds do not necessarily have an extended duration of activity in soil. Thus, the finding by Sheets and coworkers (*14*, *15*) that the toxicity in simazine-, ipazine-, atrazine-, and propazine-amended soil was retained, even 15 months after application, tends to support the present observations that the *s*-triazine herbicides are not readily susceptible to microbial action. The extraordinarily rapid breakdown reported for simazine (*12*) is difficult to explain, however, unless the $C^{14}O_2$ released originated not from the applied herbicide but rather from labeled impurities in the pesticide preparation. Nevertheless, in view of the results of Kaufman, Kearney, and Sheets (*10*), it is likely that the soil microflora is capable of metabolizing triazine compounds, but undoubtedly at a slow rate.

Degradation of Benzoates, Phenols, and Phenoxy Compounds. As results of three separate experiments on the degradation of chlorobenzoates in soil were essentially identical, only illustrative data are presented. All the monochlorobenzoates were quickly destroyed as measured by decrease in ultraviolet absorbancy, and no residual absorption remained in the test solutions after 3 weeks or less with any of these compounds. Benzoate itself was decomposed in a shorter time than the chloro compounds (Figure 1). It would appear that the active agent in destruction is microbiological, since no ring rupture could be detected in 1 month when the three monochlorobenzoate - soil suspensions contained 100 p.p.m. of $HgCl_2$. The 2-, 3-, and 4-fluorobenzoates were oxidized in less than 2 weeks, but no destruction of the first two was detected in the first 7 days. By contrast, the light absorption of the following compounds was not significantly reduced by the soil microflora in 60 days: 2,4-, 3,4-, and 2,5-dichlorobenzoic acids; 2,3,4-, 2,3,5-, 2,3,6,- and 2,4,5-trichlorobenzoic acids; 2,3,4,5-tetrachlorobenzoic acid; 2,4-dichloro-3-nitrobenzoic acid; and 3 - amino - 2,5 - dichlorobenzoic acid (amiben). The benzoic acid ring itself was destroyed in less than 3 days under identical circumstances. Despite the resistance of the aromatic nucleus of amiben to microbial attack, the carboxyl is cleaved biologically at a slow but easily detectable rate (Table I). These data can be interpreted as reflecting either a dissimilar rate of amiben decomposition in soil as contrasted with soil suspensions, a not unlikely hypothesis, or a removal of the carboxyl group with no ring cleavage.

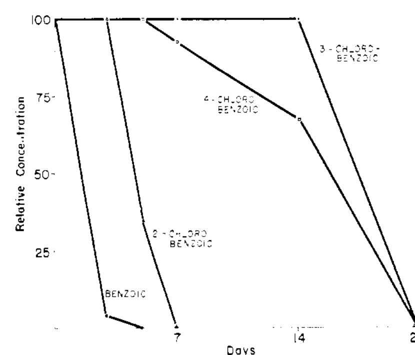

Figure 1. Decomposition of monochlorobenzoic acids in soil suspension

The results demonstrate that the number of chlorines on the aromatic ring determines the susceptibility of the benzoates to microbial degradation. This is in contrast with the phenol and phenoxy compounds, in which the position rather than the number of

Table II. Effect of Seed Inoculation with *Flavobacterium* sp. on Growth of Alfalfa in 4-(2,4-DB)–Treated Soil

Treatment	Alfalfa Development: Honeoye s. l. Sterile	Honeoye s. l. Nonsterile	Williamson s. l. Sterile	Williamson s. l. Nonsterile
None	++++[a]	++++	++++	+++
4-(2,4-DB)	−	−	−	−
4-(2,4-DB) and *Flavobacterium* sp.	++++	+	+	+
4-(2,4-DB) and *Flavobacterium* sp.[b]	++++	+	++++	+

[a] ++++. Plant development comparable to that in sterile soil receiving neither herbicide nor microbial inoculant. +++. Greater than 75% emergence, compared to controls, and some phytotoxicity. +. Marked phytotoxicity, poor alfalfa top and root growth, and less then 25% of plants emerged, compared with untreated soil. −. No alfalfa emergence and no seed germination.

[b] Herbicide and bacteria applied to soil 3 weeks before planting date. In remaining treatments, 4-(2,4-DB) and inoculant applied at planting time.

halogens governs susceptibility or resistance to decomposition (*1*, *4*). In agreement with these findings are the observations, obtained by determination of chloride release in amiben-treated soil, that only small quantities of the herbicide are mineralized during a 5-month period (*9*).

The spectrophotometric technique provides a suitable and very simple procedure to determine whether the same microflora decomposing one aromatic pesticide or intermediates in its destruction will metabolize structurally related compounds or postulated intermediates. For example, an enrichment culture active on 2,4-dichlorophenoxyacetic acid (2,4-D) was established by the addition of 2% (w./v.) of soil into the inorganic salts solution containing 40 p.p.m. of 2,4-D. A second increment of 2,4-D to give a final concentration of 40 p.p.m. of the herbicide was added when the initial amount was destroyed, and an identical third increment was added when the ultraviolet light absorption of the second had disappeared. When the third addition was metabolized, sufficient 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-chlorophenoxyacetic acid (4-CPA), 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2,4-dichlorophenol were added to separate flasks to a final concentration of 50 p.p.m., and the absorbancy was measured at regular intervals at 288, 279, 279, and 283 mμ, respectively. In such conditions, the absorbancy of 4-CPA and 2,4-dichlorophenol had largely disappeared within 23 days and 17 days, respectively, while that of MCPA and 2,4,5-T remained unchanged even at the end of 52 days. The rate of destruction of 4-CPA by the enrichment was somewhat slower than that with 2,4-D, however. Although it is possible that it was not the organisms of the 2,4-D enrichment culture but rather another organism carried over from the original soil inoculum that was metabolizing 4-CPA and 2,4-dichlorophenol, the persistence of 2,4,5-T and MCPA in the presence of the adapted population demonstrates that the microflora of this soil which is responsible for the degradation of 2,4-D has no activity on 2,4,5-T and MCPA. This is in contrast with the results of Audus (*3*).

The spectrophotometric procedure was used to ascertain whether the addition to the benzoic acid molecule of a single halogen modified the availability of the substrate to such an extent that the benzoate-utilizing species could no longer attack the altered compound. A soil–benzoic acid–inorganic salts mixture was incubated until spectrophotometric determinations revealed that all of the benzoic acid had been metabolized, at which time 2.0 ml. of the benzoate-destroying enrichment culture was transferred to flasks of the inorganic salts medium supplemented with 10.0 mg. of benzoic, 2-chloro-, 3-chloro-, or 4-chlorobenzoic acids. Under such conditions, the benzoate disappeared in a day, while no disappearance of the monochlorobenzoates was detected in 22 days, results indicating that the species metabolizing benzoic acid are inactive or show little activity on the monochlorobenzoates.

It was reported previously that some but not all chlorophenols were oxidized by the soil microflora (*1*). By means of the spectrophotometric procedure, 2,6-dichlorophenol was observed to be destroyed readily, while the absorbancy associated with 2,3-, 3,4-, and 3,5-dichlorophenol persisted for long periods. The susceptibility of all the dichlorophenols has been tested, the 2,4- and 2,5-dichlorophenol having been previously examined, and the patterns of dichlorophenol resistance to ring cleavage support the concept advanced earlier (*1*, *4*)–namely, that the soil population is unable to degrade rapidly aromatic molecules which contain a halogen in a position meta to the phenolic hydroxyl.

Seed Inoculation with *Flavobacterium*. The extent of alfalfa seedling emergence and development in pots receiving 4-(2,4-DB) and an inoculum of a 4-(2,4-DB)–degrading bacterium was assessed 6 days after the inoculated seed was planted. The differences between treatments were marked, and only qualitative observations (Table II) were therefore made. At a level of 10 p.p.m. of 4-(2,4-DB), there was no evidence of alfalfa growth in either sterile or nonsterile soil.

The results summarized in Table II show that seed inoculation gave good protection to the plants in sterile Honeoye silt loam, but the inoculant had only a slight detoxifying action in the nonsterile soil. In the Williamson silt loam, the plant protection afforded by the inoculant from herbicide injury was marginal, in either the sterile or nonsterile soils. If, however, *Flavobacterium* sp. was allowed to develop for 3 weeks in the sterile Williamson soil prior to sowing, the resulting population did bring about a detoxication of the soil surrounding the roots to an extent adequate to permit normal plant development. Nevertheless, in no instance did the inoculated organism become sufficiently well established in the presence of the indigenous population to bring about more than a slight degree of plant protection.

The inability to eliminate the phytotoxicity of 4-(2,4-DB)–treated soil by seed inoculation with the herbicide-degrading bacterium may have resulted from the use of 4-(2,4-DB) concentrations considerably in excess of the quantity required for alfalfa suppression, and significant environmental detoxication in nonsterile soil may possibly be achieved with herbicide levels recommended for field application. However, since the *Flavobacterium* has no apparent major ecological advantage at the levels of 4-(2,4-DB) applied to soil for weed control, success in establishing susceptible plants in herbicide-treated soil by means of seed inoculation must await better means of introducing and establishing a specific beneficial microorganism in natural ecosystems.

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FOLIAR APPLICATION

Effects of Chloroform and Surfactants on Permeability of Apricot Leaf Cuticle

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A penetration test with plant leaf cuticle membranes was used to measure the effect of surfactants (anionic, cationic, and nonionic) on the permeability of leaf cuticle to a slowly penetrating compound (sucrose) and a rapidly penetrating compound (N-isopropyl-α-chloroacetamide). Mixing surfactants with these compounds altered their penetration little, if at all, and even prior soaking of the cuticle disks in 1% solutions of the surfactants or sodium hydroxide increased the penetration of the α-chloroacetamide slightly in only one case. Prior soaking of the cuticle disks in chloroform, however, increased the permeability of leaf cuticle to the acetamide markedly. It is concluded that although cuticle permeability can be modified, the surfactants currently used commercially in pesticide formulations do not exploit the opportunity to alter the permeability of hydrated leaf cuticle.

The successful marketing of agricultural chemicals administered by foliar application depends on effective formulation. Subsequent to the requirements for storage stability, dispersion, and surface coverage, a requirement for optimal interaction with the plant surface is reached. For herbicides, defoliants, and systemic pesticides, maximum penetration of the toxicant is desired, whereas for surface protectants, poor penetration can minimize pesticide dilution, phytotoxicity, and residue problems.

Surfactants are widely used in pesticide formulating since enhancement of herbicidal effectiveness by surfactants has been amply demonstrated (*1*, *3*), but the reason for the effect is not clear. In an extensive comparison of surfactant effects, Jansen, Gentner, and Shaw (*9*) observed three types of surfactant-herbicide interaction. Enhancement of herbicidal activity was most common, but in some cases the surfactants were ineffective, and in others suppressed activity. In a study of the phytotoxicity of surfactants, Furmidge (*5–7*) suggests that penetration of the wax and cutin layers of the leaf is a prerequisite, and Freed and coworkers (*4*, *8*) emphasize the specificity of surfactant-herbicide interaction required for maximum effectiveness.

In this work, a previously described (*2*) penetration test was used to determine if surfactants altered the permeability of leaf cuticle.

Experimental

The methods used have been described in detail (*2*). Apricot (*Prunus armeniaca* L.) leaves were procured locally and 1000 upper apricot leaf cuticle disks produced. Approximately 750 were expended in this work.

The radioactive compounds were reserves of those used in the earlier work (*2*).

The surfactants used, described in Table I, included anionic, cationic, and nonionic types. A comparison of their effects on surface tension (Table I) shows no large differences in this property. Anionics included an alkyl sulfonate, an alkyl sulfate, and an alkyl benzene sulfonate, and the cationics were represented by the quaternary ammonium compound. The nonionics are the surfactants of choice for commercial pesticide use because of their low phytotoxicity (*7*), and the five tested included a commercial alkylaryl polyoxyethylene glycol formulation (Colloidal Products X-77); Sterox NJ (nonylphenyl polyoxyethylene ether) which Monsanto sells for pesticide formulation and use; and Sterox SK (dodecyl polyoxyethylene thioether) which has shown promise in USDA greenhouse evaluations (*9*).

The tests were made with two C^{14}-labeled compounds, sucrose being representative of slowly penetrating compounds, and *N*-isopropyl-α-chloroacet-

Table I. Surfactants Used and Surface Tension of 0.1% Solutions

Surfactant	Manufacturer[a]	Trade Name	Dynes/Cm. R. T.[b]
Sodium *n*-dodecane sulfonate	1		26.1
Sodium *n*-dodecylbenzene sulfonate	1		33.9
n-Dodecyl polyoxyethylene ether	1		34.0
Sodium dodecyl sulfate	2	Sipon WD	33.3
Cetyltrimethylammonium bromide	3	T-5650	31.0
Blend of alkylaryl polyoxyethylene glycols, free fatty acids, 2-propanol	4	X-77	30.0
Dodecyl polyoxyethylene thioether	1	Sterox SK	29.1
Dodecylphenyl polyoxyethylene ether	1	Sterox DJ	29.7
Nonylphenyl polyoxyethylene ether	1	Sterox NJ	32.0

[a] 1. Monsanto Co., St. Louis, Mo. 2. Alcolac Chemical Corp., Baltimore, Md. 3. Distillation Products Industries, Rochester, N. Y. 4. Colloidal Products Corp., Sausalito, Calif.
[b] Data supplied by T. B. Hilton, Inorganic Division, Monsanto Co.