cating a strict stereochemical relationship between the receptor site and the pyrethroid for effective insecticidal activity. The active sites probably have a structure, such that upon interaction, pyrethroid molecules must be able to adopt a particular conformation. Another interesting fact is that all known, potent pyrethroid esters have a flexible region in the alcohol moiety.

The results of our study may be interpreted as evidence of whether or not coplanarity between the centers of unsaturation, at two conformational extremes, is consistent with an effective interaction at the pyrethroid binding site. As stated in the biological evaluation section, all of the pyrethroid esters, bearing the "conformational" alcohol moieties synthesized in this study, have no or only very little insecticidal activity. It thus appears that none of the extreme coplanar conformations involving the two aromatic centers of certain pyrethroid alcohols is compatible with the insect binding region.

Recent studies (Plummer and Pincus, 1984) concerned with 2-substituted biphenyl alcohols and 2-substituted heterocyclic analogues of biphenyl pyrethroid alcohols have indicated that insecticidal activity in dependent on the intraring twist angle of the alcohol. More recently (Plummer et al., 1984), studies of pyrethroid insecticides derived from 2,2′-bridged biphenyl-3-ylmethanols indicate that a twist angle of approximately 50° is optimal for biphenyl-related alcohols. These findings suggest that an optimum twist angle exists, and that twist angles less than or more than this angle decrease the insecticidal activity. The study herein supports this proposal in that coplanar aromatic centers (viewed either as linked via a biphenyl linkage or via a phenoxy linkage) are virtually devoid of insecticidal activity. Further studies, in which the two requisite aromatic regions of the alcohol are locked into a rigid framework having defined twist angles, are in progress.

**Registry No.** I, 96706-39-7; II, 96706-40-0; III, 96706-41-1; IV, 96706-42-2; V, 96706-43-3; VI, 96706-44-4; VII, 96706-45-5; 2-dibenzofurancarboxaldehyde, 5397-82-0; 3-dibenzofurancarboxaldehyde, 51818-91-8; 4-dibenzofurancarboxaldehyde, 96706-46-6; 1-dibenzofuranmethanol, 96706-47-7; 2-dibenzofuranmethanol, 86607-82-1; 3-dibenzofuranmethanol, 96706-48-8; 4-dibenzofuranmethanol, 64102-19-8; dibenzofuran, 132-64-9; dibenzofuran lithium salt, 16669-47-9; 1-dibenzofurancarboxylic acid, 54470-37-0; 4-dibenzofurancarboxylic acid, 2786-05-2; chrysanthemic acid chloride, 14297-81-5; sodium cyanide, 143-33-9; 2-dibenzofurancyanomethanol, 96706-49-9; 3-dibenzofurancyanomethanol, 96706-50-2; 4-dibenzofurancyanomethanol, 96706-51-3.

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# Residues of Four Pesticides in Alfalfa Seed and Sprouted Alfalfa Seed following Foliar Applications

Jay C. Maitlen* and J. Eric Halfhill

Plots of alfalfa were treated with the registered rates (1X) and twice the registered rates (2X) of demeton, methidathion, oxydemeton-methyl, and trichlorfon applied as foliar sprays with ground equipment. Mature seed was harvested from all plots, cleaned, subdivided, and a portion of each was sprouted. No detectable residues of the pesticides or their metabolites were found in sprouts prepared from seed harvested from the 1X treatments, nor (with the exception of demeton) in sprouts prepared from seed from the 2X treatments. Sprouts from the 2X demeton treatment contained combined average residues of demeton and its metabolites of 0.03 ppm. Seed from the 1X treated alfalfa contained no detectable residues of methidathion (<0.01 ppm), oxydemeton-methyl (<0.02 ppm), or trichlorfon (<0.02 ppm), but seed from the demeton plots contained residues of 0.02 ppm. Also, residues of demeton and its metabolites (0.24 ppm) and trichlorfon (0.05 ppm) were found in seeds from the 2X treated alfalfa, but no residues of methidathion or oxydemeton-methyl were found.

Sprouted alfalfa seeds have become a popular garnish for salads and sandwiches in the past few years. In California alone, in 1979, it was estimated that about 1.5 million pounds of seed were used in the preparation of sprouts, and it is expected that this use will continue to increase (Hesterman and Teuber, 1979). It is obvious that this amount of seed could not all be produced on "organic" farms, therefore, some of this seed may have come from commercial farms involved in the production of alfalfa

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seed. There is concern that pesticides applied to this alfalfa might result in residues in or on the mature seed that could carry over into the sprouts prepared from this seed. At present, only a few of the pesticides registered for use on alfalfa grown for seed have been evaluated for their residues in seeds and sprouts. Seed harvested from alfalfa that had been treated with registered rates of aldicarb [2-methyl-2(methylthio)propanal, *O*-[(methylamino)carbonyl]oxime], propargite [2-(*p*-*tert*-butylphenoxy) cyclohexyl 2-propynyl sulfite], and chloropyriphos (*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] did not contain detectable residues of these pesticides and neither did the sprouts prepared from this seed (Halfhill and Maitlen, 1983).

This study was conducted to determine whether the registered rate or twice the registered rate of the pesticides demeton (*O*,*O*-diethyl *O*(and *S*)-[2-(ethylthio)ethyl] phosphorothioates], methidathion [*O*,*O*-dimethyl phosphorodithioate *S*-ester with 4-(mercaptomethyl)-2-methoxy-$\Delta^2$-1,3,4-thiadiazolin-5-one], oxydemeton-methyl [*S*-[2-(ethylsulfinyl)ethyl *O*,*O*-dimethyl phosphorothioate], and trichlorfon [dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate] applied to alfalfa would produce residues of these compounds or their toxic metabolites in harvested seed or in sprouts prepared from this seed.

## EXPERIMENTAL SECTION

**Reagents and Apparatus. (a) Stock Standards.** These were prepared separately by dissolving 0.1 g of 99+% demeton thiono, demeton thiol, demeton thiol sulfoxide, demeton thiol sulfone, methidathion, oxydemeton-methyl, oxydemeton-methyl sulfone, or thichlorfon in dichloromethane so that these stock solutions were equivalent to 200 μg/mL. Each stock solution was then diluted as desired.

**(b) Solvent.** Dichloromethane ($MeCl_2$) was technical grade (Van Waters and Rogers) that was redistilled in glass.

**(c) Sodium Sulfate, Anhydrous.** Bakers analyzed reagent grade.

**(d) Gas Chromatograph.** Hewlett Packard Model 5880 equipped with a flame photometric detector operated in the phosphorous mode. For the determination of residues of demeton and its metabolites, oxydemeton-methyl and its sulfone, and methidathion, the GLC column was 61 cm × 4 mm i.d. glass, packed with Chromosorb G HP 80/100 mesh coated with 5% OV 101. For the determination of trichlorfon residues the GLC column was 61 cm × 4 mm i.d., glass, packed with Chromosorb G HP 80/100 mesh coated with 5% Carbowax 20M. The operating parameters for the determination of all pesticides were: column 195 °C; injection port 240 °C; detector 195° C; the nitrogen carrier gas flow rate was 35 mL/min.

**Field Treatment.** Each pesticide treatment was applied to 15 × 80 ft. plots of alfalfa in a commercial alfalfa field at the registered rate (1X) and twice the registered rate (2X). All treatments were foliar sprays applied with a ground applicator, and each was replicated four times. Four plots in each field were left untreated as controls. Table I shows the treatment rates and the number of treatments for each of the pesticides evaluated.

**Sampling Procedure.** The alfalfa was desiccated chemically about 2 weeks prior to seed harvest. At harvest, seed from each plot was threshed separately with a commercial combine. About 3 lbs of the seed from each plot were packaged in a plastic bag and transported immediately to the laboratory where they were cleaned and subdivided. One part of each sample was packaged in a plastic bag and stored in a −10 °C freezer until it was analyzed for pesticide residues. The remaining seed from each sample was sprouted by a published accepted procedure (Hesterman and Teuber, 1979). In sprouting, 30 g of seed were weighed into a 1-quart jar and the top was covered with plastic screen. Twice each day for 5 days, the seeds were flooded 3 times with water and drained. During this time, the jars of sprouting seeds were held in a temperature cabinet at 20 °C in total darkness. The 6th day, the sprouts were placed in the light for 4 h to photosynthesize and then packaged in plastic bags and stored frozen until analyzed.

**Table I. Treatment Rates and the Number of Treatments of Each Pesticide Applied to Alfalfa**

| pesticide applied | treatment rate at each application lb. a.i./acre | no. of applications[a] |
|---|---|---|
| demeton | 0.5[b] | 4 |
| | 1.0 | 4 |
| methidathion | 0.5[b] | 1 |
| | 1.0 | 1 |
| oxydemeton-methyl | 0.5[b] | 4 |
| | 1.0 | 4 |
| trichlorfon | 1.5[b] | 4 |
| | 3.0 | 4 |

[a] Multiple applications were applied at weekly intervals. [b] Indicates the registered rate (1X).

**Analytical Procedures.** Residues of demeton thiono and demeton thiol and its sulfoxide and sulfone metabolites in alfalfa seed and seed sprouts were determined by the modified procedure of Thornton and Anderson (1968). In this method, the residues of the above four compounds were oxidized with potassium permanganate and determined as two compounds, demeton thiono and demeton thiol sulfone. The residues of methidathion were determined by the procedure of Winterlin et al. (1980). A modification of the method of Thornton et al. (1977) was used to quantitate the residues of oxydemeton-methyl and its sulfone metabolite. Also, in this procedure, potassium permanganate was used to oxidize the residues of these two compounds and then they were quantitated as one compound, oxydemeton-methyl sulfone. The residues of trichlorfon were determined by the method of Devine (1973). The modifications to the above referenced procedures were related to the extraction of residues of these pesticides and their respective metabolites from alfalfa seed and alfalfa seed sprouts. These procedures are described in the following paragraphs.

Residues of each of the pesticides and their metabolites were extracted from alfalfa seed by the same procedure, as follows: weigh 40 g of alfalfa seed into a 250-mL conical beaker, add 50 mL of $MeCl_2$, cover the top of the beaker with aluminum foil, and allow the solution to stand overnight in a refrigerator. In the morning, allow the sample solutions to warm to room temperature, transfer it to a quart Waring Blendor jar with 150 mL of $MeCl_2$, add about 30 g (tablespoon) of sodium sulfate ($Na_2SO_4$), and blend the solution at high speed for 4 min. Filter the extract through a funnel plugged with glass wool into and through a second funnel fitted with a 18.5-cm ED grade 513 fluted filter paper and collect the filtered extract in a 250-mL conical beaker. Measure 100 mL of the extract (equivalent to 20 g of seed) into a 125-mL Erlenmeyer flask, place the solution in a 45–50 °C water bath, and evaporate the $MeCl_2$ with the aid of a gentle stream of air. Rinse down the sides of the sample flask with 5 mL of $MeCl_2$, stopper, and hold in a refrigerator until analysis for residues of the investigated pesticide.

**Table II. Recovery of Pesticides from Fortified Alfalfa Seed and Sprouted Alfalfa Seed**

| pesticide added | ppm added | recovery found, % alfalfa seed | recovery found, % alfalfa sprouts |
|---|---|---|---|
| demeton thiono | 0.05 | 76 | 104 |
| | 0.01 | 100 | 127 |
| demeton thiol | 0.05 | 118 | 109 |
| | 0.01 | 110 | 96 |
| demeton thiol | 0.05 | 105 | 103 |
| sulfoxide | 0.01 | 110 | 112 |
| demeton thiol | 0.05 | 107 | 110 |
| sulfone | 0.01 | 105 | 116 |
| methidathion | 0.02 | 102 | 94 |
| | | 87 | 80 |
| oxydemeton-methyl | 0.02 | 108 | 94 |
| | | 103 | 80 |
| oxydemeton-methyl | 0.02 | 107 | 102 |
| sulfone | | 113 | 103 |
| trichlorfon | 0.02 | 109 | 94 |
| | | 106 | 93 |

Similarly, a single extraction procedure was used to extract residues of the pesticides and their metabolites from sprouted alfalfa seed and is as follows: weigh 40 g (fresh weight) of sample into a 1-quart Waring Blendor jar, add 200 mL of $MeCl_2$, and blend for 4 min. Filter the extract solution through a funnel plugged with glass wool into a 250-mL separatory funnel, add about 30 g of $Na_2SO_4$ to the solution, and shake for 1 min. The extract solution is then filtered, measured, and evaporated in the same manner as described for the seed extracts. The resultant residue, equivalent to 20 g of sprouts, is dissolved in 5 mL of $MeCl_2$ and held for analysis as described for seed samples.

The efficiency of the analytical methods was determined by separately fortifying nontreated samples of alfalfa seed and sprouts with known amounts of the pure standards of the previously named pesticides and their metabolites prior to extraction and determining the percent recovery of each (Table II).

Nontreated control samples of alfalfa seed and sprouts were analyzed for residues of the pesticides and their metabolites. None of these samples contained detectable residues of any of the compounds. The lower limit of detection (ppm) for each of the pesticides was as follows: total demeton and its metabolites 0.01; methidathion 0.01; oxydemeton-methyl and its sulfone 0.02; trichlorfon 0.02.

## RESULTS AND DISCUSSION

**Residues in Sprouted Alfalfa Seeds.** As shown in Table III, sprouts prepared from mature seed harvested from alfalfa treated with registered rates (1X) of demeton, methidathion, oxydemeton-methyl, or trichlorfon contained no detectable residues of these pesticides or of their respective metabolites. This occurred even when methidathion, oxydemeton-methyl, and trichlorfon were applied at twice their registered rates (2X). However, when demeton was applied at the 2X rate, combined average residues of demeton thiono, demeton thiol, and its sulfoxide and sulfone were found to be 0.03 ppm in the sprouts.

**Residues in Alfalfa Seed.** Analyses of harvested seed demonstrated that demeton applied to alfalfa at both 1X and 2X rates produced residues of this pesticide and its metabolites in the seed (Table III). The residues found in the seed from the 1X application (0.02 ppm) were not sufficient to produce detectable residues in the sprouts prepared from the seed. However, residues were found when the seeds from the 2X treatment were sprouted. The average combined residues of demeton and its metabolites in these seeds (0.24 ppm) produced average combined residues of 0.03 ppm in the sprouts. These results indicate that if a no residue tolerance for demeton in alfalfa sprouts is established then care should be taken not to exceed the registered application rate. It is possible that an application exceeding the 0.5 lb. a.i./acre or a treatment applied closer to harvest than recommended could produce residues in the seed that could carry over to the sprouts.

**Table III. Residues of Pesticides Found in Alfalfa Seed and Sprouted Alfalfa Seed following the Application of Several Materials to Alfalfa**

| pesticide applied | application rate, lb. ai/acre | interval between last treatment and sampling, days | residues found[a] alfalfa seed | residues found[a] alfalfa sprouts |
|---|---|---|---|---|
| demeton[b] | 0.5 | 35 | 0.02 | [c] |
| | 1.0 | 35 | 0.24 | 0.03 |
| methidathion | 0.5 | 123 | [c] | [c] |
| | 1.0 | 123 | [c] | [c] |
| oxydemeton-methyl[d] | 0.5 | 42 | [c] | [c] |
| | 1.0 | 42 | [c] | [c] |
| trichlorfon | 1.5 | 28 | [c] | [c] |
| | 3.0 | 28 | 0.05 | [c] |

[a] The ppm values shown are the average of four replicate samples. [b] The values shown are the total combined residues of demeton thiono and demeton thiol and its sulfoxide and sulfone. [c] Residues were below the limit of reliable detection, which was 0.01 ppm for demeton and its metabolites and for methidathion and 0.02 ppm for oxydemeton methyl and its sulfone and for trichlorfon. [d] The values shown are the total combined residues of oxydemeton-methyl and its sulfone.

When trichlorfon was applied at the 1X and 2X rate, only the 2X rate produced residues in the harvest seed (0.05 ppm). This residue did not carry over into sprouts prepared from this seed.

When methidathion and oxydemeton-methyl were applied to alfalfa at both the 1X and 2X rates, none of the seed harvested from any of the treated plots contained residues of either pesticide or their respective metabolites.

## CONCLUSIONS

These results demonstrate that when alfalfa grown for the production of seed was treated with registered rates of demeton, methidathion, oxydemeton-methyl, or trichlorfon, the sprouts prepared from the harvested seed contained no detectable residues of these pesticides or their respective metabolites.

## ACKNOWLEDGMENT

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**Registry No.** Demeton, 8065-48-3; methidathion, 950-37-8; oxydemeton-methyl, 301-12-2; trichlorfon, 52-68-6; demetonthiono, 298-03-3; demetonthiol, 126-75-0; demetonthiol sulfoxide, 2496-92-6; demetonthiol sulfone, 2496-91-5; oxydemeton-methyl sulfone, 17040-19-6.

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# Characterization of Arsenic Metabolites in Rice Plant Treated with DSMA (Disodium Methanearsonate)

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In this paper the results of a study of the metabolic products of disodium methanearsonate (DSMA) in hydroponically grown rice plant are reported. Gas chromatography with a multiple ion detection mass spectrometry (GC/MID/MS) and hydride generation–heptane cold trap (HG/HCT) technique was used for species determination of arsenicals. Most of the arsenic in rice plant was present as monomethylarsenic (MM-As) species. Relatively large amounts of transformed products such as inorganic arsenic and dimethyl- and trimethylarsenic species were detected in the nutrient solution through root exudation. Gel permeation and thin-layer chromatographic analyses revealed that the MM-As species accounting for nearly all of the arsenic in root were a mixture of nonconjugated methanearsonate, conjugated MM-As, and reduced methanearsonate (red-MA). The red-MA was further characterized by its being liable to be cleaved into arsenite.

## INTRODUCTION

Organo arsenical pesticides such as methanearsonate monosodium salts (MSMA) and disodium salts (DSMA) are used as a selective preemergent contact herbicide to control weeds in cotton and uncropped land in USA. Methanearsonates (ferric salts) are also used as a fungicide to control sheath blight of rice in Japan.

Root uptake of methanearsonates by plants from nutrient solution is rapid, and the arsenic is translocated into all portion of the plants (Sckerl and Francs, 1969). The degree of absorption through leaves and stem depends upon a rate of application, temperature, and plant species (Arle and Hamilton, 1971; Keeley and Thullen, 1971). Basipetal and acropetal translocation of foliar- and stem-applied methanearsonates (Sckerl and Francs, 1969; Keeley and Thullen, 1971; Rumburg et al., 1960; Duble et al., 1969; Sachs and Michael, 1971) and exudation of the arsenic from roots into rhizosphere (Domir et al., 1976) have been observed.

There is no conclusive evidence that the C–As bond of methanearsonates is cleaved in plants. Methanearsonates are usually found unchanged or in a complex form (Sckerl and Francs, 1969; Duble et al., 1969; Sachs and Michael, 1971). Furthermore, there is no report that methanearsonates are converted into dimethyl- and trimethylarsenic compounds in plants. Recently, Knowles and Benson (1983) suggest in enzymatic study of Johnson grass that methanearsonate is reduced to arsenosomethane ($CH_3As{=}O$) which inhibits the malic enzyme.

The purpose of this study was to determine the metabolic products as arsenic component in rice plant on root-soak-treatment of DSMA.

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## MATERIALS AND METHODS

**Chemicals.** DSMA [99.8% monomethylarsenic (MM-As), 0.2% inorganic arsenic (Inorg-As), <0.1% dimethylarsenic (DM-As), and <0.1% trimethylarsenic (TM-As), determined by the GC/MID/MS–HG/HCT method (see determination of arsenic)] was provided by Kumiai Chemical Ind. Co. Ltd. Sodium arsenite (>99.9% Inorg-As) and sodium arsenate (>99.9% Inorg-As) were purchased from Kanto Chemical Co. DMAA (98.2% DM-As, 1.4% MM-As, 0.4% Inorg-As, and <0.1% TM-As) was purchased by Sigma Chemical Co. TMA=O (96.9% TM-As, 3.1% DM-As, <0.1% MM-As, and <0.1% Inorg-As) was obtained by oxidizing trimethylarsine (TMA, Ventron Co.) with iodine (Odanaka et al., 1983). These arsenicals were diluted with water to prepare 1000 ppm As stock solutions.

**Growth and Treatment.** Rice plants (*Oryza sativa* L. var Nihonbare) were germinated and planted in Kasugai nutrient solution [$NH_4Cl$ 6 mg, $Na_2HPO_4{\cdot}12H_2O$ 3 mg, KCl 4.5 mg, $CaCl_2{\cdot}2H_2O$ 0.6 mg, $MgCl_2{\cdot}6H_2O$ 0.9 mg, $FeSO_4{\cdot}7H_2O$ 1.0 mg/L, pH 5.5] (Kasugai, 1939). Three weeks after the germination, at the two- or three-leaf development stage, the seedlings were transferred to jars (two seedlings per jar) containing a 500 mL of the nutrient solution and DSMA (500 μg as arsenic). The jars wrapped with aluminum film shielding the light were kept in a greenhouse. The growing conditions included natural sunlight with temperatures from 18 to 32 °C. The treated plants were harvested 7 days after being transferred to the DSMA-contained nutrient solution. The roots were thoroughly rinsed with a vigorous flow of water to remove the arsenicals adhering to the root surface. Each plant was sectioned into shoots and roots. The tissue sections were

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