of flooding akin to the situation in the paddy field markedly retarded sprouting and seedling growth of the weed (Table II).

While isolation and identification of the active principle awaits further investigation, it can be concluded that the plant extract of *O. canum* is a potent allelopathic agent against *C. rotundus*.

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Metaldehyde Residues on and in Citrus Fruits after a Soil Broadcast of a Granular Formulation and after a Spray Application to Citrus Trees

The molluscicide metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetroxocane) is used in California citrus groves to suppress populations of the brown garden snail *Helix aspersa* Müller. After a 56 kg ha^{-1} soil broadcast application of a 7.5% AI granular formulation, the 3-day rind samples showed a maximum residue level of 0.02 ppm of metaldehyde. The 10- and 17-day rind samples and 10-day pulp (edible portion) samples contained <0.01 ppm of metaldehyde. After an unregistered-use spray application, metaldehyde dissipated from unwashed rind with a half-life of 4.6 days during the initial 33 days of the test. Dissipation was somewhat slower during the subsequent 26 days of the test period with a half-life of 14 days. The 10-, 31-, and 59-day pulp samples contained <0.01 ppm of metaldehyde. Residue methodology developed for citrus is given in detail.

The brown garden snail, *Helix aspersa* Müller, is becoming an increasingly serious pest in California citrus orchards as a result of changing orchard management practices. The snails climb into the trees and readily attack maturing fruits. The two most common control materials in use, singly or in combination, are metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetroxocane) and methiocarb. A granular or bran formulation is broadcasted to the soil under the trees when the snails are active, and repeated applications within a growing season are frequently required.

Although minimal residues would be expected on citrus fruits as a result of a soil broadcast, inadequate residue data are available to substantiate the assumption. This study was conducted to fill the data gap for normal use conditions. In addition, a spray application was made to determine how rapidly metaldehyde dissipates from citrus fruits.

The analytical methods for metaldehyde have been reviewed by Selim and Seiber (1973). The method reported by Selim and Seiber (1973) was modified for citrus analysis and allowed for the quantification of metaldehyde residues down to 0.01 ppm.

MATERIALS AND METHODS

Treatment. Each of the three replicate plots for each of two application methods consisted of a row of six mature Valencia orange trees. Applications were made on May 1, 1981, to plots located on the University of California Citrus Research Center, Riverside, CA.

A 200-g aliquot of a 7.5% AI metaldehyde granular formulation was broadcasted under each tree. For 115 trees acre^{-1}, this was 3.8 lb of AI acre^{-1} (4.2 kg of AI ha^{-1}).

A 4 lb of AI metaldehyde gal^{-1} suspension called Slug-N-Snail Special Spray (Cooke Laboratory Products, Pico Rivera, CA 90660) marketed for use on ornamental plants was used to prepare a spray mix which was applied with an oscillating boom spray rig at a rate of 18.8 lb of AI (2000 gal)$^{-1}$ acre^{-1} [21 kg of AI (187 hL^{-1} ha^{-1}]. The entire tree was sprayed to obtain a uniform spray coverage of the tree for residue study purposes. A grower might use 20% of this amount, 3.8 lb of AI (400 gal)$^{-1}$ acre^{-1}, and spray the lower 20% portion of the tree where the snails are likely to reside.

Sampling and Processing. Each sample consisted of 20 fruits collected from the inner four trees of the plot. Samples for rind residue analysis were collected by clipping each fruit and allowing it to drop into a 3-gal jar so as to minimize the disturbance of surface residues. Samples for pulp residue analysis were collected into paper bags and washed prior to peeling to minimize contamination from surface residues. All samples were peeled and chopped on the day of sample collection. One subsample was immediately extracted and analyzed, and the remainder of the sample was frozen until time was available to extract and analyze a second subsample.

Extraction and Cleanup. A sample of 50 g of chopped rind or 100 g of pulp was macerated for 2 min with 150 mL of CH_2Cl_2 in a Waring blender jar. The macerate was decanted onto a 75 mm i.d. filling (powder) funnel with a glass wool plug in the stem and half-filled with anhydrous Na_2SO_4, and the liquid extract was allowed to drain into a filter flask. After gravity filtration ceased, a slight vacuum was used to complete the filtration. The sample was reextracted by macerating for 2 min with 100 mL of CH_2Cl_2. The macerate was decanted onto the filling funnel, and the extract was allowed to gravity filter. The blender jar was rinsed with 50 mL of CH_2Cl_2 and the rinse was used to wash the filling funnel. Vacuum was applied to complete the filtration. The solvent was removed from the combined CH_2Cl_2 extract.

A 22 mm i.d. Kontes Chromaflex column (K-420600) was packed successively with 10 g of activity grade 2 basic alumina, 10 g of activity grade 1.5 neutral alumina, and 30 g of anhydrous Na_2SO_4. The extract was added to the column by using five 5-mL portions of benzene to rinse the flask. The column was successively eluted with 75 mL of benzene and 175 mL of CH_2Cl_2; both eluates were discarded. Metaldehyde was eluted with 250 mL of CH_2Cl_2.

Derivatization. The CH_2Cl_2 was removed and the residue was transferred to a graduated cylinder with benzene. An aliquot (entire sample for the 0.01-ppm level) was transferred to a 15-mL graduated tube. To the tube was added 4 mL of the 2,4-dinitrophenylhydrazine (DNPH) reagent (Selim and Seiber, 1973) and some additional benzene. The tube was capped and periodically shaken during the following hour. The sample was allowed to stand overnight, and the benzene phase was analyzed by gas chromatography (GC) for acetaldehyde 2,4-dinitrophenylhydrazone (acetaldehyde-DNPH).

For the above procedure, acetone solvent and vapor must be rigorously excluded since acetone-DNPH will interfere in the GC analysis. Benzene and CH_2Cl_2 contained trace amounts of what appeared to be acetone and were purified by extracting the solvent with DNPH reagent followed by passage through a column of activity grade 1 basic alumina. The DNPH reagent itself contained background material and was purified by extracting it several times with CH_2Cl_2.

Procedural Recovery. Chopped rind and pulp were fortified with metaldehyde just prior to extraction by blending. Recoveries and standard deviations for three replicate samples were 75 ± 5, 70 ± 2, and 70 ± 4% for chopped rind fortified at 0.010, 0.10, and 1.0 ppm, respectively, and 69 ± 1% for pulp fortified at 0.010 ppm. Rind recoveries were corrected for background interference equivalent to about 0.004 ppm. The interference had the same retention time and double peak characteristics of acetaldehyde-DNPH. Since the column chromatographic cleanup eliminates acetaldehyde from the sample extract, it is speculated that acetaldehyde was generated by acid hydrolysis of plant coextractives which eluted from the column with metaldehyde. The observed double peak characteristics may also have been entirely coincidental and could have arisen from other causes.

Analysis. Metaldehyde was analyzed as acetaldehyde-DNPH by using a Hewlett-Packard Model 5710A gas chromatograph equipped with a nitrogen/phosphorus ionization detector and a 1.8 m × 2 mm i.d. glass column packed with 4% OV-17 on 60–80-mesh Gas-Chrom Q. The nitrogen carrier gas flow rate was 30 mL min^{-1} and the column temperature was 215 °C. Acetaldehyde-DNPH was synthesized by using standard procedures. Since the recrystallized derivative was a nonequilibrium mixture of the syn and anti isomers as a result of selective crystallization, a 100 $\mu g\ mL^{-1}$ standard in benzene solution was placed in contact with a few milliliters of the acidic DNPH reagent to obtain an equilibrium mixture. Then, an aliquot of the benzene phase was removed and diluted for use as a standard solution in the analyses. All residue values were corrected for recoveries as determined from analyses of fortified samples.

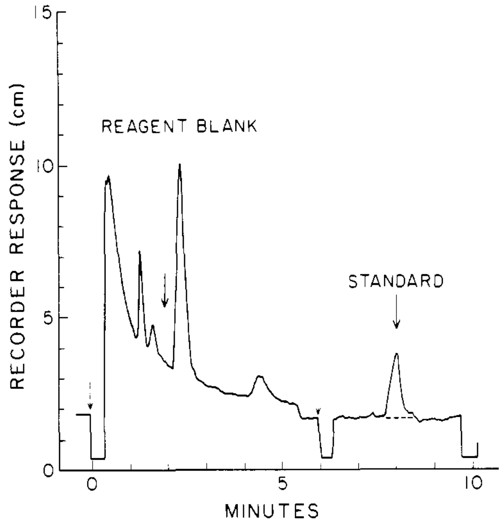

Figure 1. Chromatogram obtained after injection (denoted by dashed arrow) of a reagent blank. The standard peak represents 0.51 ng of acetaldehyde 2,4-dinitrophenylhydrazone which is equivalent to 0.10 ng of metaldehyde. Retention time is 2.1 min.

RESULTS AND DISCUSSION

A 7.5% AI metaldehyde bait formulation is currently recommended for use as a soil broadcast at the rate of 50 lb $acre^{-1}$ for suppressing the brown garden snail in citrus groves (Carman and Pappas, 1980; University of California, 1980). Although a 14-day minimum preharvest waiting interval is specified, no residue data are available to indicate what residue levels might result on fruit after a soil broadcast and how rapidly residues, if present, would dissipate from fruit under field conditions. This study was conducted to answer these two questions.

Since no residues of metaldehyde are legally allowed on citrus, analytical methodology to determine residues down to 0.01 ppm in citrus was developed by modifying the procedure of Selim and Seiber (1973). Figure 1 shows the background peaks resulting from the reagent blank. Figure 2 shows a typical chromatogram of a 0.01-ppm metaldehyde-fortified rind sample; this represents the method used at its limit.

Pretreatment samples contained <0.01 ppm of metaldehyde in the pulp (edible portion of the fruit) and contained background interferences equivalent to ≤0.01 ppm of metaldehyde in and on the rind. After a 50 lb $acre^{-1}$ soil broadcast treatment, the 3-day samples from the three replicate plots showed rind residues of 0.02, 0.01, and 0.01 ppm. The 10- and 17-day samples all showed rind residues of less than 0.01 ppm. The pulp collected from the 10-day sample contained less than 0.01 ppm. Thus, granular applications did not result in significant fruit contamination.

So that data on how rapidly metaldehyde dissipates from fruit could be obtained, a metaldehyde suspension was applied to citrus trees with an oscillating boom spray rig. Figure 3 shows the residue dissipation curve for metaldehyde on and in unwashed fruit rind. The three rep-

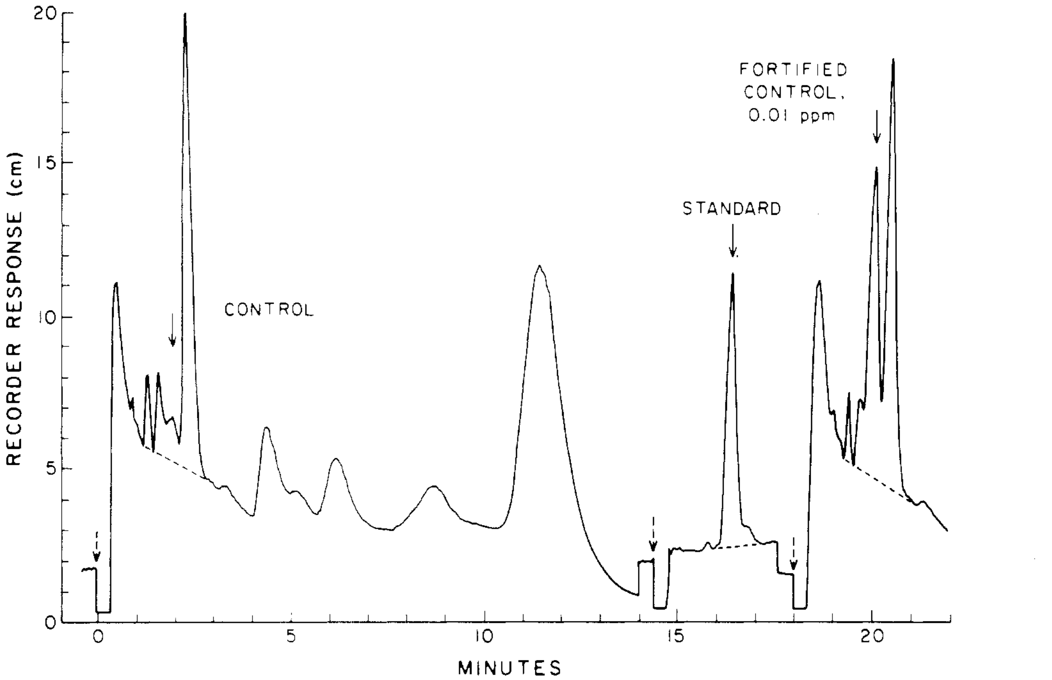

Figure 2. Chromatograms obtained after injection (denoted by dashed arrow) of extract solutions prepared from a control and a 0.01-ppm metaldehyde-fortified control. Injections represent extractives from 40 mg of rind. The standard peak represents 1.8 ng of acetaldehyde 2,4-dinitrophenylhydrazone which is equivalent to 0.35 ng of metaldehyde. Recovery from the fortified control shown was 82%; background in the control was taken into account.

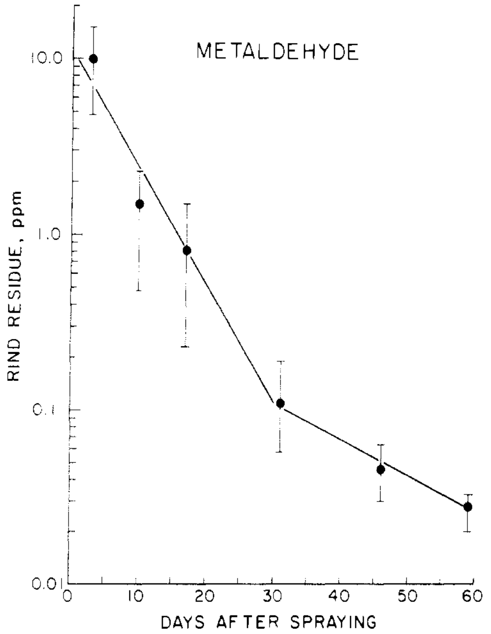

Figure 3. Residues of metaldehyde on and in fruit rind after application of a metaldehyde spray to orange trees. Vertical lines give the spread of values obtained for six analyses, two analyses for each of three replicate plots.

licate plots had 3-day rind residue levels of 5.2, 9.7, and 15 ppm. The spread of residue values shown in Figure 3 for each sampling date reflects field plot and application variations rather than scattered residue analytical data; residues declined in a consistent pattern for each of the three plots. The 10-, 31-, and 59-day samples all contained pulp residues of less than 0.01 ppm of metaldehyde. Since Valencia oranges are 18.7 ± 6.3% rind by weight (Gunther, 1969) and since no residues were present in the pulp, whole-fruit residues are about one-fifth the rind residues. The nonregistered use of metaldehyde sprays to knock down snails from the trees would result in illegal fruit residues.

The dissipation curve shown in Figure 3 can be described during the initial 31 days by the equation ln (ppm of residue) = -0.15(days) + ln (11). The half-life of metaldehyde based on this equation is 4.6 days. Thus, the 14-day recommended waiting interval is equivalent to three half-lives and would allow any residues resulting from a granular application to drop to approximately 12.5% of the initial value.

The initial 33 days of the test period during which metaldehyde disappearance was most rapid was characterized by a maximum daily air temperature between 68 and 95 °F. Traces of rain ($\leq$1 mm) were recorded 14, 15, 19, 26, and 32 days postapplication. The final 26 days of the test period was hotter and had maximum daily air temperaturs between 83 and 107 °F. There were 7 days with temperatures of 100 °F or higher.

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