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# Residues of Croneton and Its Sulfoxide and Sulfone Metabolites in Citrus (*Clementine* Trees) following a Soil Treatment for the Control of *Aphis spiraecola*

Nadav Aharonson,* Ilan Neubauer, Isaac Ishaaya, and Benjamin Raccah

Residues of the insecticide croneton, [2-(ethylthio)methyl]phenyl *N*-methylcarbamate, and its sulfoxide and sulfone metabolites were determined in *Clementine* (*C. reticulata* Blanco) leaves and fruits following soil treatment with 2, 6, and 18 g of active ingredient (AI) per tree. The analytical procedure was improved to separate croneton from its two oxygen analogues. Croneton was extracted with petroleum ether and its sulfoxide and sulfone metabolites with chloroform; all were silylated with bis(trimethylsilyl)trifluoroacetamide and determined by gas–liquid chromatography using the flame photometric detector. The carbamate was found to accumulate in the leaves and mainly in its oxidized forms. Soil treatment with 18 g of AI/tree resulted in a slow accumulation in the leaves during the first 12 days (up to 2 ppm), followed by a much faster rate. After 42 days the total residue level was 12 ppm (0.4 ppm croneton, 5.6 ppm sulfoxide, and 6.2 ppm sulfone). Residues in the leaves were proportional to the dosage applied to the soil. An average level of 4–6 ppm of the carbamate in the leaves provided satisfactory control of the spirea aphid. Similar results were found when the spirea aphids were fed on a synthetic diet containing similar levels of the insecticide.

Croneton (Bay HOX 1901), [2-(ethylthio)methyl]phenyl *N*-methylcarbamate, is a systemic insecticide exhibiting specific action against aphids (Bayer AG, 1974). It was chosen as one of several potential systemic candidates for *Aphis spiraecola* Patch. control in citrus trees by soil application. [*Aphis spiraecola* has recently been named *Aphis citricola* Van der Goot by Dr. D. Hille Lambers (*Entomol. Ber.* **35**, 59 (1975))]. While many insecticides exhibit aphicidal properties, only those providing foliar protection for at least 2–3 weeks were of interest (Bullock, 1972). Investigations of the efficacy of soil treatment with systemic insecticides for citrus insect and mite control have grown in number in recent years (Brooks, 1968; Tashiro et al., 1969; Shaw, 1970; Bullock, 1972; Milne and de Villiers, 1975). However, there are no data available on the systemic action and residues of croneton in citrus trees.

Residues of croneton were determined in recent studies as total carbamate by oxidizing croneton and croneton sulfoxide to the sulfone form (Dräger, 1974) or by thin-layer chromatography of $^{14}$C-carbonyl- and $^{14}$C-ring-labeled croneton (Nye et al., 1976).

The present work reports an improved analytical method for determining separately the residues of croneton and its sulfoxide or its sulfone metabolites (Figure 1) in *Clementine* leaves and fruit. This report is also concerned with the uptake, accumulation, and persistence of croneton or its biologically active metabolites in the trees and their relation to aphid control.

## EXPERIMENTAL SECTION

**Field Treatment.** Croneton 10% granular formulation was applied to the surface of the soil around the base (100 cm diameter) of 4-year-old *Clementine* trees, variety Mikhal, at three rates: 2, 6, and 18 g of AI/tree (160 trees/acre). Overhead irrigation at the rate of 140 L/tree and natural rainfall (0.4 in) provided the equivalent of 4 in. of precipitation following application. Weekly irrigation was continued during the summer.

The insecticide was applied in April to plots of three trees, replicated four times for each of the three dosages. Aphid populations were determined by examining ten young growth terminals per tree (120 terminals per treatment). Heavy infestation was recorded as percent of

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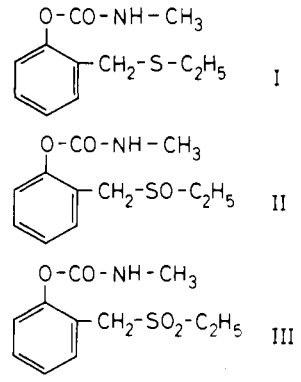


**Figure 1.** Croneton (I) and two of its oxidized biologically active metabolites, sulfoxide (II) and sulfone (III).

terminals containing five aphids or more. The infestation was determined 0, 4, 12, 22, 42, and 126 days after treatment.

**Sampling.** Mature leaves were collected for residue analysis on the same days that the aphid population was determined. Four replicates of 30 g each per treatment were taken on each date. Twenty fruits per treatment were collected on the 126th day. Newly mature leaves (about 4 weeks old) were collected from two replicates on the 22nd and 42nd days. All the samples were stored at about −16 °C until analyzed.

**Chemicals.** Analytical croneton and its sulfoxide and sulfone metabolites were obtained from Bayer AG. The solvents used were analytical grade of acetone, chloroform, and petroleum ether, 40–60 °C. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Supelco Inc.

**Apparatus.** An Omni Mixer with 1-L glass jars and a rotary evaporator (W. Büchi) were used for extraction and concentration of samples. A gas chromatograph, Tracor MT-220 equipped with the Melpar flame photometric detector (FPD) with 394-nm specific filter, was used for determining sulfur-containing compounds. The chromatographic column used was 1.2 m × 4 mm i.d. glass, packed with 2.5% DC-200 and 3.5% QF-1 (w/w) on 80/100 mesh Gas-Chrom Q. Oven temperature was 170 °C, inlet 210 °C, and detector 220 °C, and the nitrogen carrier gas flow was at 100 mL/min.

**Analytical Procedure.** The method used was a modification of that described by Dräger (1974). Citrus leaves (15–20 g) were blended with 300 mL of acetone in an Omni Mixer for 3 min. The extract was filtered through Whatman No. 1 paper and the leaf debris was reextracted with 200 mL of acetone. The extract was reduced on a rotary evaporator to 40 mL; then 80 mL of 0.05 N HCl was added and the solution was left to stand for 20 min to reduce interferences from plant pigments. Croneton was extracted with four portions of 50 mL of petroleum ether, while the sulfoxide and sulfone metabolites were left in the aqueous phase and extracted with three portions of 50 mL of chloroform. The petroleum ether phase was concentrated under vacuum to 4–5 mL. Five milliliters of 20% aqueous $MgSO_4$ and 30 mL of 0.1 N $KMnO_4$ were added to oxidize croneton to its sulfone form, and the solution was left to stand for 15 min. This was followed by four extractions with 50 mL of petroleum ether and three extractions with 50 mL of chloroform. The petroleum ether extract was discarded. The chloroform solutions, one containing the oxidized croneton and the other containing the sulfoxide and sulfone metabolites, were dried over anhydrous $Na_2SO_4$, concentrated to 1.5 mL, and silylated. To each sample was added 200 μL of BSTFA, followed by acetone to bring the volume to 2 mL. The samples were shaken thoroughly and left to stand for at least 30 min, and the resultant silyl derivatives were determined by gas chromatography. The volume of sample after silylation should not be altered.

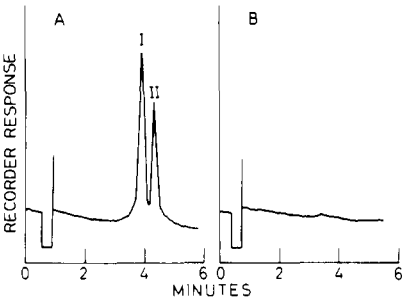


**Figure 2.** Gas chromatograms (FPD) of (A) fortified leaves at 0.13 ppm sulfoxide (I) and 0.1 ppm sulfone (II), (B) untreated *Clementine* leaves.

**Table I. Percent Recovery of Croneton and Its Sulfoxide and Sulfone Metabolites from Fortified *Clementine* Leaves[a]**

| compound | fortification level, ppm | recovery, % |
|---|---|---|
| croneton | 2.0 | 90 |
| | 1.0 | 94 |
| | 0.5 | 92 |
| | 0.1 | 95 |
| croneton sulfoxide | 2.0 | 98 |
| | 1.0 | 97 |
| | 0.5 | 95 |
| | 0.1 | 96 |
| croneton sulfone | 2.0 | 95 |
| | 1.0 | 97 |
| | 0.5 | 100 |
| | 0.1 | 94 |

[a] Limit of detection: 0.06 ppm croneton sulfoxide and 0.04 ppm croneton and croneton sulfone.

In order to prove that the recovered pesticides are carbamates and not the corresponding phenols, we analyzed one sample without silylation using GLC–MS (Varian 2740–Du Pont 21-490 B). The plant extract was purified by two successive TLC runs (Kieselgel, chloroform/acetone, 80:20). The analysis for croneton sulfone and phenol sulfone was performed on an OV-17, 3%, 2 m × 3 mm glass column at 135–160 °C (2 °C/min) and then isothermally at 160 °C. The MS was run at 12 eV. Comparison with authentic croneton sulfone and phenol sulfone indicates, both by retention time and mass spectra (diagnostic peak at $m/e$ 58; Nye et al., 1976), that the plant contains only croneton sulfone.

**Synthetic Diet Feed Study.** Spirea aphids were fed a synthetic diet placed as a drop between two layers of stretched parafilm, a method previously used by Mittler and Pennell (1964) and by Raccah and Tahori (1971). Croneton and its sulfoxide and sulfone analogues were added to the synthetic diet at various concentrations to determine the $LC_{50}$ for each compound. The assays were kept for 48 h at 21 ± 1 °C with a relative humidity of 90% and a photoperiod of 12 h.

## RESULTS AND DISCUSSION

The analytical procedure described herein enabled a quantitative separation of croneton from its sulfoxide and sulfone metabolites. The parent compound was oxidized with potassium permanganate to give croneton sulfone. The two fractions containing either the sulfoxide and sulfone metabolites or the croneton that was converted to the sulfone were silylated and determined by gas chromatography using a sulfur-specific flame photometer

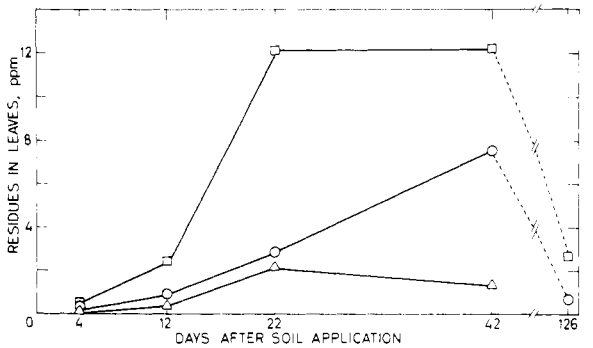


**Figure 3.** Total carbamate residues found in *Clementine* leaves following soil treatment with 2 g (Δ–Δ), 6 g (O–O), and 18 g (□–□) of active ingredients of croneton per tree.

**Table II. Residues[a] (ppm) of Croneton and Its Oxidized Metabolites in *Clementine* Leaves following Soil Treatment with 2, 6, and 18 g of AI Carbamate per Tree**

| days after treatment | | treatment, g of AI/tree | | |
|---|---|---|---|---|
| | | 2 | 6 | 18 |
| 4 | croneton | <0.1 | <0.1 | <0.1 |
| | sulfoxide | 0.1 | 0.1 | 0.4 |
| | sulfone | <0.1 | <0.1 | <0.1 |
| 12 | croneton | <0.1 | <0.1 | <0.1 |
| | sulfoxide | 0.4 | 0.6 | 1.2 |
| | sulfone | <0.1 | 0.3 | 1.2 |
| 22 | croneton | 0.1 | 0.2 | 0.5 |
| | sulfoxide | 1.3 | 1.6 | 8.1 |
| | sulfone | 0.8 | 1.2 | 3.5 |
| 42 | croneton | 0.2 | 0.1 | 0.4 |
| | sulfoxide | 0.4 | 3.9 | 5.6 |
| | sulfone | 0.6 | 3.7 | 6.2 |
| 126 | croneton | | <0.1 | <0.1 |
| | sulfoxide | | 0.3 | 1.2 |
| | sulfone | | 0.3 | 1.5 |

[a] Mean residues from three or four replicates.

detector. Figure 2 shows gas chromatograms of untreated and fortified citrus leaves. Recoveries were over 90% for either croneton or its sulfoxide and sulfone metabolites (Table I).

Total carbamate residues in citrus leaves following soil treatment with three concentrations of croneton are summarized in Figure 3. During the first 12 days after treatment there was a slow increase in the level of the insecticide in the leaves. Between the 12th and 22nd days, a much faster increase in the concentration of this compound was observed.

Accumulation of croneton in the leaves during the first 42 days after treatment showed some similarity to that described by Iwata et al. (1977) for aldicarb.

The conversion of croneton to its two biologically active metabolites is presented in Table II. The level of croneton residues in the leaves was very low throughout the experiment. Croneton was rapidly oxidized to its sulfoxide and sulfone metabolites and the carbamate insecticide accumulated in the leaves in its oxidized forms. During the first 4 days after soil treatment the total amount of the carbamate in the leaves was very low. The delay in the accumulation of this compound in the leaves may have been due to the time needed for the oxidation of the parent compound to its more polar sulfoxide and sulfone metabolites. The mechanism and rate of oxidation in the soil and in the plant are being investigated.

The foliar residue pattern showed higher concentrations of the sulfoxide metabolite during the first 22 days and a gradual increase subsequently in the concentration of croneton sulfone. After 126 days the residues of total carbamate in the leaves were 2.7 ppm for the highest dose and 0.6 ppm for the intermediate one. The residue level in newly mature leaves was somewhat higher (up to 20%) than that found in mature ones. Residues in the fruit, 126 days after treatment, were not detectable (less than 0.01 ppm).

**Table III. Systematic Activity of 10% Granular Croneton Applied against a Field Population of *Aphis spiraecola* to 4-Year-Old *Clementine* Trees**

| soil application with croneton, g of AI/tree | percent of terminals heavily[a] infested at indicated intervals | | | |
|---|---|---|---|---|
| | days after treatment | | | |
| | 4 | 12 | 22 | 126 |
| 2 | 64 a[b] | 88 a | 65 a | 48 a |
| 6 | 33 a | 48 a | 45 b | 37 a |
| 18 | 39 a | 25 b | 3 c | 12 a |
| 0 (control) | 45 a | 91 a | 93 a | 49 a |

[a] More than five aphids/terminal. [b] Means within columns followed by different letters differ significantly at $P = 0.05$, according to Duncan's multiple range test.

**Table IV. Effect of Croneton and Its Metabolites on *Aphis spiraecola* Fed on Synthetic Diet**

| | concn of the insecticide, ppm | percent mortality |
|---|---|---|
| croneton | 0.8-1.3 | 48-54 |
| croneton sulfoxide | 2.0-3.5 | 48-60 |
| croneton sulfone | 2.0-2.5 | 42-67 |

The systemic activity of croneton against a field population of *Aphis spiraecola* on citrus trees is presented in Table III. Soil application of 18 g of AI/tree provided excellent control of the aphid in a heavily infested citrus grove. In the fourth week of the experiment there was a sudden steep decline in the population of the spirea aphid and therefore the maximum residual effectiveness of croneton has not been fully demonstrated. However, based on the residue data obtained from the analysis of the leaves, the effective suppression of the aphid population should probably last for at least 42 days. This assumption is based on data showing that between the 22nd day and the 42nd days after soil application there was still a gradual increase in the residue level of the carbamate in the leaves (Figure 3). Residue data indicate that aphid control might not be very effective during the first 12 days after treatment. This was demonstrated quite clearly in Table III. Croneton, at a rate of 6 g of AI/tree, caused a significant reduction in the aphid population only after 22 days.

Based on biological and chemical measurements it was concluded that an average level of 4–6 ppm of total carbamate in the mature leaves should provide satisfactory control of the spirea aphid on citrus trees. Most of the assays were carried out with mature leaves since it was difficult to collect enough young terminals for analysis.

The toxicity of croneton and its oxydized analogues to the spirea aphid was determined in a nutrient solution and was of the same magnitude as that found on citrus leaves. The $LC_{50}$ for aphids fed on synthetic diet containing croneton or its metabolites are presented in Table IV.

In a second experiment in the fall (November), croneton was applied to the soil in the same citrus grove at a rate of 30 g of AI/tree, as a 10% granular or 50% emulsifiable concentrate formulation diluted with water. The concentration of the insecticide in mature leaves was somewhat higher when applied as a liquid compared to granular formulation. Its concentration in the young terminals was lower than in mature leaves (Table V). Residues in the fruit just before harvest, 25 and 50 days after treatment,

Table V. Effect of the Formulation on Total Uptake of Croneton and Its Metabolites from the Soil by *Clementine* Trees

| croneton formulation | residues in | residues, ppm: days after soil treatment 10 | 25 | 50 |
|---|---|---|---|---|
| 10% granular[a] | mature leaves | 1.9 | | 4.1 |
| | young terminals | 0.3 | 0.6 | |
| | whole fruit | | 0.2 | 0.1 |
| 50% emulsifiable concentrate[a] | mature leaves | 2.4 | | 7.5 |

[a] Equivalent to 30 g of AI/tree.

were 0.2 and 0.1 ppm, respectively, which was much lower than in the leaves.

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# Fate and Efficacy of Acephate after Application to Plants and Insects

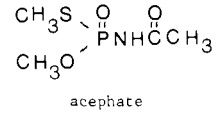
Don L. Bull

A single foliar application of $^{14}$C-labeled acephate was absorbed rapidly by cotton leaves (>50% in 24 h), and unabsorbed residues were essentially depleted in 48 h. The absorbed acephate was metabolized by the leaves to small amounts (ca. 9% of dose) of the insecticide methamidophos and to lesser amounts (<5% combined) of at least four other products. Two of the latter four products were tentatively identified as *O,S*-dimethyl phosphorothioate and *S*-methyl acetylphosphoramidothioate. Absorbed acephate and/or its metabolites were rapidly translocated throughout the plant including the fruit. However, with normal application methods, any such translocation of toxicants that might occur is apparently insufficient to kill pests that feed on new growth or fruit. Acephate was considerably more toxic to third-stage tobacco budworms, *Heliothis virescens* (F.), than to adult boll weevils, *Anthonomous grandis* Boheman, in tests with topical applications to the insects and with bioassays of treated cotton foliage. Some evidence obtained in studies of the absorption and metabolism of $^{14}$C-labeled acephate by the two species suggests that the metabolic conversion of the chemical to methamidophos in tobacco budworms may contribute to the observed differences in susceptibility between species.

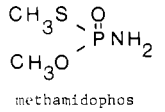
The organophosphorus insecticide acephate (*O,S*-dimethyl acetylphosphoramidothioate) is registered by the Environmental Protection Agency for use in controlling a broad spectrum of arthropod pests of plants. This chemical is recommended for use in cotton production but little is known of its fate after application to plants or of its effects on key pests of that crop. The present report describes the absorption, translocation, and metabolism of acephate in cotton plants and its fate and efficacy after treatment of two important pests of cotton.

MATERIALS

**Chemicals.** Radioactive (two different samples labeled with $^{14}$C at the *S*-methyl position, 3.0 and 4.8 mCi/mmol) and nonradioactive acephate, as well as certain of its theoretical toxic [methamidophos (Monitor), *O,S*-dimethyl phosphoramidothioate] and nontoxic (I, *O,S*-dimethyl phosphorothioate; II, *S*-methyl acetylphosphoramidothioate) metabolites, were provided by Chevron Chemical Co., Richmond, CA. For all tests, $^{14}$C-labeled acephate was diluted with sufficient nonradioactive material to produce a final specific activity of ca. 10 000 cpm/$\mu$g.



acephate



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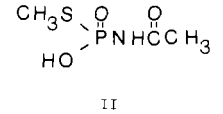

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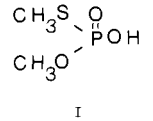

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**Plants and Insects.** Cotton plants used were Stoneville 213 variety grown in the standard way either in the greenhouse or field. Tobacco budworms, *Heliothis virescens* (F.), used in the test were taken from laboratory cultures of (1) an insecticide-susceptible (S) strain maintained through many generations without introduction of new stock and (2) an insecticide-resistant (R) strain tested as the $F_1$ generation from parental stock collected in cotton fields that were under heavy insecticide treatment. Adult boll weevils, *Anthonomus grandis* Boheman, used in the test were obtained from the Robert T. Gast rearing facility at Mississippi State, MS.

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