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Diflubenzuron Surface Residues in Florida Citrus

H. N. Nigg,[1] R. D. Cannizzaro,[2] and J. H. Stamper[1]

[1]University of Florida, IFAS, Citrus Research and Education Center,
700 Experiment Station Road, Lake Alfred, FL 33850 and
[2]Thompson-Hayward Chemical Company, 5200 Speaker Road,
Kansas City, KS 66110

Diflubenzuron (dimilin®) (1-(4-chlorophenyl) 3-(2,6 difluorobenzoyl) urea) is a promising arthropod growth regulator for control of the citrus rust mite (*Phyllocoptruta oleivora* Ashmead) (Bullock and McCoy 1978; McCoy 1978). It has also been used for lepidopterous insects in cotton, soybeans, and forest, as well as in apples, pears, stone fruit, and other horticultural crops. In addition, it is effective against mosquitoes, midges, various weevils and flies, and leafminers (Maas et al. 1980). Diflubenzuron has a listed LD_{50} (14 days) of >400 and 640 mg/kg to the mouse and rat, respectively. It is a marginal eye irritant (Duphar 19?? UKN). It is also of low toxicity to birds and fish (Maas et al. 1980). This compound is only slowly metabolized in insects (Ivie and Wright 1978) compared to more rapid metabolism in mammals (Ivie 1978). Other effects of this compound include chitin synthesis inhibition (Duphar 19?? UKN; Maas et al. 1980) molting process inhibition (McCoy 1978; Bull and Ivie 1980) and sterilization (Wright et al. 1980). Previous studies indicated that diflubenzuron is very persistent on plant surfaces but less so in soil (Bull and Ivie 1978; Maas et al. 1980; Duphar 19?? UKN) and in water (Ivie et al. 1980). Bull and Ivie (1978) found that diflubenzuron levels remained virtually constant on the surface of cotton leaves until rainfall occurred 14 days postspray. Our purpose here was to determine residue levels and seasonal variations in the persistence of diflubenzuron on leaf, fruit, and soil surfaces in Florida citrus.

MATERIALS AND METHODS

Diflubenzuron (25 wettable powder) was applied with a handgun to four randomly selected four-tree Valencia orange blocks at a rate of 10 oz AI/acre at 600 psi. Trees were sprayed to runoff. Sampling of leaves and fruit was according to Iwata et al. (1977). Soil surface samples of Astatula fine sand, vacuumed through a 40-mesh screen, were taken at the tree dripline and midway between trees according to Spencer et al. (1977) as modified by Nigg et al. (1979). Samples selected from each

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four-tree block constituted one replication from a total of four replications.

Diflubenzuron was recovered with two methylene chloride extractions of fruit and leaf washes (Nigg et al. 1979). Recoveries from 1 ng/cm^2 and 10 ng/cm^2 fortifications averaged 90 and 85%, respectively, for leaves, and 81 and 86% for fruit. Diflubenzuron recoveries from fortified soil, extracted elsewhere as described below, averaged 87% (0.05 ppm level) and 91% (50 ppm level).

Leaf and fruit samples were extracted immediately after collection. These extracts were shipped to Thompson-Hayward Chemical Co., Kansas City, KS for analyses. Soil samples were sent without extraction. All samples and extracts were shipped frozen over dry ice, coded and blind, accompanied by fortified soil and extractions from fortified leaf and fruit washes. Recoveries from these fortified samples were reported above.

Soil was analyzed for diflubenzuron and 4-chlorophenyl urea by gently refluxing 20 g of a thoroughly mixed sample in 15 ml water and 150 ml acetonitrile for 30 min. This extract was filtered through Whatman No. 4 filter paper and evaporated to 10 to 15 ml on a rotary flash evaporator at 40°C. The reflex condenser and all other glassware were rinsed with acetonitrile with the rinsings added prior to evaporation. Diflubenzuron was extracted by adding 100 ml deionized water and 10 ml acetonitrile and extracting in a 500 ml separatory funnel with three 50 ml portions of hexane. NaCl was added to break emulsions. The hexane extracts and two 25 ml hexane rinses of the separatory funnel were taken to dryness at 40°C on the evaporator. The remaining aqueous phase was extracted with three 50 ml portions of diethyl ether. These extractions, plus two 25 ml rinses of the separatory funnel, were taken to dryness at 40°C on the evaporator. This ether extraction contains 4-chlorophenyl urea.

Residues from the hexane and diethyl ether extractions were individually dissolved in 100 ml of 3.6 M HCl, boiling chips were added, and the residues reflexed for 12 hr. After cooling, the water was transferred to a separatory funnel, the reflux condenser and flask were rinsed with 25 ml of water, and this rinsate added to the separatory funnel. The combined water was extracted once with 50 ml benzene and the benzene discarded. The water was adjusted to pH >10 with about 30 ml of 12.5 N NaOH and extracted with three 50 ml portions of benzene. Each benzene extract was filtered through 20 g of sodium sulfate into a 500 ml flat bottom flask and the sodium sulfate rinsed with 50 ml of benzene. To the combined benzene extracts was added 0.5 ml of heptafluorobutyric anhydride (HFBA). The flask was then sealed with a glass stopper and placed in an oven at 55°C for 3 hr. After the reaction was complete, the benzene was evaporated to 15 ml at 35°C on the evaporator.

The 15 ml extract was chromatographed on 10 g of Florisil (Fisher 60/100 mesh) topped with 5 g sodium sulfate. The Florisil had been previously washed with methanol, activated at 130°C overnight, and deactivated by adding 5 ml of water to 100 g of Florisil. The 250 mm x 15 mm ID glass column, equipped with a 250 ml reservoir, was plugged with glass wool, the Florisil and sodium sulfate added, and 50 ml of hexane allowed to run to the top of the sodium sulfate. The hexane eluate was discarded. The sample, in 15 ml of benzene, was added and allowed to run to the top of the sodium sulfate. The sample flask was rinsed with 100 ml of 90:10 hexane:ethyl ether and this was added to the top of the column. The benzene and hexane-ethyl ether were collected together, reduced to dryness at 35°C on the evaporator, and transferred to 10 ml of 2,2,4-trimethylpentane for gas chromatographic analysis.

The methylene chloride extractions of fruit and leaf washes were reduced to dryness at 40°C on the evaporator, the residues redissolved in 15 ml water and 150 ml acetonitrile, and then carried through the above procedures.

Samples were analyzed on a Tracor 560 gas chromatograph equipped with a ^{63}Ni electron capture detector. The 1.8 m x 4 mm ID glass column was packed with 5% carbowax on 80/100 mesh Chromosorb W(HP). Operating conditions were: inlet 250°C, oven 185°C, detector 300°C, carrier N_2 rate 45 ml/min, purge 10 ml/min. The minimum detection limit of the anilide derivative was 5 pg.

RESULTS AND DISCUSSION

The data from experiments 1 and 2 appear in Table 1 with dissipation half-lives given in Table 2. The half-lives indicate that diflubenzuron dissipated at very different rates in the two experiments. In the cool, dry period (March to April) of experiment 1, there was essentially no dissipation on leaves and only slow dissipation on fruit. The half-lives during experiment 2 were markedly less for leaves and fruit than those in experiment 1. Soil surface residues disappeared more rapidly than leaf and fruit residues in experiment 1; in experiment 2, soil residue levels were too low and/or erratic for a disappearance rate estimate to be made.

Even though the same application rate was used for both experiments, diflubenzuron levels on leaves and fruit in experiment 2 (hot, wet) were only about one half those in experiment 1 (cool, dry). Soil surface levels in experiment 2 were only about one tenth those in experiment 1.

The major metabolite of diflubenzuron, 4-chlorophenyl urea (Maas et al. 1980), was detected sporadically only in soil and at levels <0.05 ppm.

Table 1. Diflubenzuron residues on surfaces in a Florida Valencia orange grove (10 oz AI/acre).

Experiment 1 (March to April 1978) Day post-treatment	1	3	8	10	15	22	29
Leaves (ug/cm^2)	.17±.02*	.14±.02	.12±.03	.12±.02	.16±.02	.22±.04	.13±.01
Fruit (ug/cm^2)	.40±.04	.29±.05	.33±.03	.32±.02	.29±.02	.27±.03	.32±.05
Soil middle (ppm)	.35±.12	.23±.10	.43±.24	.70±.54	.17±.09	.17±.07	.14±.06
Soil dripline (ppm)	2.68±.73	3.84±.26	1.03±.33	1.82±.39	1.19±.64	1.52±.39	1.07±.30
Experiment 2 (July to August 1978) Day post-treatment	**1**	**3**	**8**		**14**	**22**	**29**
Leaves (ug/cm^2)	.10±.01	.08±.02	.06±.01		.05±.00	.04±.01	.05±.01
Fruit (ug/cm^2)	.18±.06	.19±.01	.18±.03		.14±.02	.08±.01	.07±.01
Soil middle (ppm)	Tr**	Tr	Tr		Tr	Tr	Tr
Soil dripline (ppm)	.10±.02	.08±.04	.54±.47		1.09±.15	.27±.11	.07±.02

*Mean of four replications ± standard error of the mean.
**Trace: <0.05 ppm.

These observations suggest that better control of the citrus rust mite would result from a diflubenzuron application in late spring (cool, dry season) rather than in summer.

Table 2. Diflubenzuron surface residue first-order half-lives ± standard error.

Experiment 1. March to April 1978 (cool, dry)
(T = 20°C, RF = 0 in./day, LW = 7 hr/day)*

	Half-life (days)	Correlation coefficient**
Leaves		-.17
Fruit	118±100	+.47
Soil middle	19±11	+.62
Soil dripline	21±10	+.68

Experiment 2. July to August 1978 (hot, wet)
(T = 29°C, RF = 0.4 in./day, LW = 13 hr/day)*

	Half-life (days)	Correlation coefficient**
Leaves	27±8	+.84
Fruit	18±2	+.96
Soil middle	Levels too low to be detected	
Soil dripline	Levels too erratic to interpret	

*T = Avg. daily temp, RF = Avg. daily rainfall, LW = Avg. daily hrs. leaves wet.
**Correlation coefficient of ln(concentration) with day post-treatment.

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