High-Performance Liquid Chromatographic Determination of Fenbutatin Oxide and Its Persistence in Peaches and Nectarines

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A new, rapid high-performance liquid chromatographic method for the determination of fenbutatin oxide has been developed and used to study residue persistence in peaches and nectarines. The pesticide was determined in fruits on an ODS 2 column using methanol/HCl 10^{-3} M 93:7 (v:v) plus NaCl (0.3 g/L) as the mobile phase, at the flow of 1.0 mL/min and detected at 205 nm. Under these conditions the detection limit was 0.05 ppm. Recoveries were 90.6–101.3% from peaches and nectarines spiked at 0.05, 1.00, and 5 ppm levels. The persistence of fenbutatain oxide residue on field and greenhouse peaches and nectarines was studied. During the experiment the decline in residue concentration was mainly caused by the dilution effect due to the increase in weight of the fruits. The high stability of this pesticide renders the effects of the environment on the decay rate of the residues negligible.

Fenbutatin oxide is a nonsystemic acaricide with contact and stomach action. It is recommended for the control of the mobile stages of phytophagous mites on a wide variety of crops. In the treated fruit, the residues do not penetrate the skin to any great extent and are found on the outer surface of the skin (FAO/WHO, 1978). In plants, fenbutatin oxide is slowly lost after application. Degradation to inorganic tin occurs through the successive loss of the methylphenylpropyl groups. The principal breakdown product [bis(2-methyl-2-phenylpropyl)tin] oxide] was found to be less than 5% of the parent compound amount applied (FAO/WHO, 1978).

Although the product has been long used, research about the persistent residues in the crops is very scanty (FAO/WHO, 1978). This is probably due to the fact that no easy analytical method exists to carry out routine analysis. In a gas chromatography method the active ingredient (AI) is converted to the chloro derivative in concentrated HCl before analysis (Zweig and Sherma, 1978).

In this work a new high-performance liquid chromatographic (HPLC) method has been developed for the determination of fenbutatin oxide, and it has been used to study its persistence in greenhouse- and field-grown peaches and nectarines.

EXPERIMENTAL PROCEDURES

Materials and Methods. The trial was carried out in a 1000-m^2 greenhouse and in a contiguous field. The varieties employed were Maravilha for peaches and Armking for nectarines. The orchard, planted in 1986, had 6666 trees/ha with a planting space of 1.5 × 1.0 m. A randomized block design was adopted with four replications. Each single plot, spaced 7.5 × 4 m, had 20 trees.

Treatments were carried out on May 6 and 21, 1991, in greenhouse and field, respectively, with Torque, a wettable powder formulation containing 50% AI. Application rates were as recommended by the manufacturer, 50 g of AI/hL and 12.5 hL/ha (single dose, SD) and twice the recommended rate (double dose, DD).

Treatments were applied with an F 320 portable motorized sprayer (Fox Motori, Bologna, Italy).

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Table I. Retention Times[a] of Fenbutatin Oxide with Different Columns and Mobile Phases

	mobile phase				
	acetonitrile/HCl 10^{-3} M (v/v) + NaCl (0.3 g/L)			MeOH/HCl 10^{-3} M (v/v) + NaCl (0.3 g/L)	
column	95:5	90:10	85:15	95:5	90:10
ODS 1	5.53	8.48	12.91	7.02	11.32
ODS 2	11.74	20.26		12.56	29.39

[a] Minutes.

The environment conditions in the greenhouse (temperature and relative humidity) were continuously recorded with an MT 1100 thermohygrograph (SIAP, Bologna, Italy). In the field an S 2000 (SIAP) automatic weather station was employed, which continuously recorded rainfall as well as the above-cited parameters.

Fruits used as control were picked from untreated trees from a separate plot. Samples of 20 fruits were randomly collected from each plot starting about 1 h after spraying and at weekly intervals for a month.

The stones were removed from the fruits, and the pulp was chopped and homogenized with a food cutter.

Chemicals. Acetonitrile and methylene chloride were of HPLC grade (Carlo Erba, Milan, Italy). HCl and NaCl were of reagent grade (Carlo Erba). Water was distilled twice and filtered through a Milli-Q apparatus (Millipore, Molsheim, France). Fenbutatin oxide analytical standard (>99%) was purchased from Ehrenstorfer (Augsburg, Germany). The stock standard solution (≈500 ppm) was prepared by dissolution of the pesticide in methylene chloride and stored at 4 °C; under these conditions it was stable for the entire period of the experiment (2 months). Working standard solutions were prepared by dilution with acetonitrile.

Apparatus and Chromatography. A Varian Model 5020 liquid chromatograph with a UV-100 variable-wavelength detector connected to an HP 1050 autosampler (Hewlett-Packard, Avondale, PA) with a 100-μL injection volume and an HP 3396 A integrator (Hewlett-Packard) were used.

A Spherisorb (Waddinxveen, Netherlands) S_5-ODS 2 (250 × 4.6 mm i.d.) column was employed. The analyses were performed at 205-nm wavelength. The mobile phase for the residue determination in fruits was methanol/HCl 10^{-3} M (93:7 v/v) + NaCl (0.3 g/L) at the flow rate of 1 mL/min.

Extraction Procedure. Twenty-five grams of sample was weighed in a 250-mL screw-capped flask, 50 mL of methylene chloride was added, and the mixture was shaken in a flask shaker

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Table II. Residues[a] of Fenbutatin Oxide on Peaches and Nectarines in the Greenhouse and Field after Treatment with Single and Double Doses

days after treatment	peaches			nectarines		
	fruit wt, g	single dose, ppm	double dose, ppm	fruit wt, g	single dose, ppm	double dose, ppm
			Greenhouse			
0	50 ± 4	1.80 ± 0.44	3.55 ± 0.92	60 ± 6	1.71 ± 0.54	2.92 ± 1.00
8	79 ± 3	1.36 ± 0.35	3.01 ± 0.90	79 ± 3	1.25 ± 0.26	2.14 ± 0.35
15	113 ± 8	1.06 ± 0.42	2.23 ± 0.57	119 ± 9	0.77 ± 0.17	1.85 ± 0.56
22	138 ± 8	0.58 ± 0.33	1.24 ± 0.42	146 ± 7	0.47 ± 0.21	0.90 ± 0.22
29	156 ± 8	0.59 ± 0.12	1.31 ± 0.31	155 ± 9	0.48 ± 0.27	0.86 ± 0.34
			Field			
0	45 ± 4	1.78 ± 0.16	3.20 ± 0.68	52 ± 4	1.09 ± 0.20	2.20 ± 0.40
7	64 ± 3	1.12 ± 0.34	2.34 ± 0.47	78 ± 4	0.88 ± 0.08	1.94 ± 0.18
14	95 ± 3	0.63 ± 0.10	1.10 ± 0.17	112 ± 5	0.75 ± 0.28	1.19 ± 0.19
21	124 ± 6	0.34 ± 0.02	0.90 ± 0.26	122 ± 5	0.55 ± 0.06	1.03 ± 0.37
28	140 ± 15	0.22 ± 0.09	0.71 ± 0.18	126 ± 7	0.58 ± 0.21	0.83 ± 0.35

[a] ppm ± SD.

Table III. Microclimatic Conditions[a] in the Greenhouse and Field during the Period of Residue Evaluation

week	temp, °C			rel humidity, %		
	min	max	mean	min	max	mean
		Greenhouse				
May 6–12	10.5	27.9	19.2	53.4	95.0	74.2
May 13–19	11.1	30.4	20.8	48.9	95.3	72.1
May 20–26	12.6	31.9	22.3	43.1	94.7	68.9
May 27–June 2	13.6	29.6	21.6	43.0	95.6	69.1
		Field				
May 21–27	8.9	25.2	17.1	29.9	98.3	64.1
May 28–June 3	10.5	23.1	16.8	33.4	97.7	65.6
June 4–10	13.0	28.5	20.8	32.1	99.1	65.6
June 11–17	13.8	30.3	22.0	30.4	89.1	59.8

[a] Weekly averages.

(Stuart Scientific) for 20 min. The organic layer was dehydrated with 1 g of anhydrous sodium sulfate. A 2-mL aliquot of organic extract was evaporated to dryness, at room temperature, under a nitrogen stream. The residue was taken up with 1 mL of acetonitrile, and the solution was injected for HPLC analysis.

Recovery Assays. Untreated fruit samples (25 g) were spiked with 0.05, 1.0, and 5.0 ppm of pesticide by adding 100 μL of methylene chloride solutions. Samples were equilibrated for 1 h prior to extraction and subsequently taken through the extraction procedure. The recovery assays were replicated four times.

RESULTS AND DISCUSSION

The determination of fenbutatin oxide was initially tried with normal (NH_2 and CN) and reversed-phase (RP_8 and RP_{18}) columns and different mobile phases (methanol, acetonitrile, methylene chloride, water) but in no case was it possible. A reversed-phase column (ODS) was then used with an acetonitrile/10^{-3} M HCl solution (90:10 v/v) as eluent, but the elution of the pesticide could be achieved only when NaCl (0.3 g/L) was added to this mobile phase. With increasing 10^{-3} M HCl solution percentage, the retention time increases, but the peak sharpness decreases. When the 10^{-3} M HCl solution is greater than 20%, efficiency is very low.

When acetonitrile is replaced with methanol in the mobile phase, retention time increases. Retention time increases also when an ODS 2 column is used instead of an ODS 1 (Table I).

For satisfactory separation and good peak sharpness the eluent ranged from 90 to 85% acetonitrile and from 95 to 90% methanol.

UV spectra in these conditions showed an absorbance maximum at 205 nm, and under optimum conditions the detection limit was 0.05 ppm [according to the criteria of Thier and Zeumer (1987)].

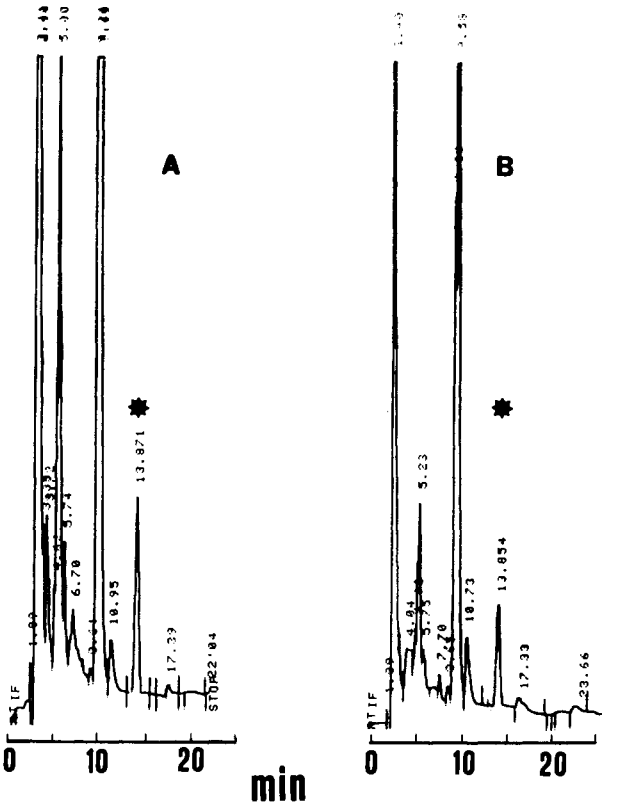

Figure 1. Chromatography of fenbutatin oxide (★) in peaches (A) and nectarines (B) on an ODS 2 column: mobile phase, MeOH/10^{-3} M HCl (93:7 v/v) + NaCl (0.3 g/L); flow rate, 1 mL/min; detection, UV at 205 nm.

A calibration graph was constructed by plotting concentration vs peak height. Good linearity was achieved in the range 0–5 ppm with a correlation coefficient of 0.9997.

For recovery assays the following solvents were tried: *n*-hexane, chloroform, methylene chloride, and benzene. Methylene chloride showed the highest extraction power with a recovery range of 90.6–101.3%. The chromatograms of the extracts obtained from the untreated control samples with this solvent did not give any interfering peaks at the retention time of fenbutatin oxide of 13.9 min when the MeOH/HCl 10^{-3} M (93:7 v/v) plus NaCl (0.3 g/L) mobile phase was used.

The chromatograms of two analytical samples of peaches and nectarines performed under this condition are shown in Figure 1.

The extract contains some peaks with greater retention

times than that of fenbutatin oxide. This fact causes a considerable increase in analysis time for each sample (≈1.5 h).

An experiment to evaluate the behavior of residues in peaches and nectarines cultivated in the field and greenhouse was performed. Fenbutatin oxide residues and mean weight were determined on fruit samples to evaluate the diluting effect on the residue caused by fruit growth during the experimentation.

Experiments carried out in the greenhouse showed (Table II) that mean residues soon after single-dose treatments are similar both in peaches and in nectarines (1.80 and 1.71 ppm). Residues show a tendency to decrease progressively, and at harvest time, about a month later, they decreased by more than a factor 3. If we evaluate the increase in the weight of the fruit, we note that the loss in residue is proportional to the increase in fruit. This points out that the loss of residue is associated only with the dilution effect caused by increase in weight of the fruit. A similar behavior is shown by fruits treated with a double dose (DD).

In the experiments carried out in the field, the mean residues in peaches after single-dose treatment (1.78 ppm) are notably higher than in nectarines (1.09 ppm) (Table II).

The residues show a tendency to decrease progressively, and at harvest time they average, respectively, 0.22 and 0.58 ppm for peaches and nectarines.

The loss in residue in nectarines can be correlated to the dilution effect caused by the increase in fruit weight.

In peaches the residue loss factor is ≈8, compared to a growth dilution factor of ≈3. So in this case the data show a residue loss caused by degradation of the AI. A similar behavior is shown with a DD.

The environmental data reported in Table III show that differences between temperature and humidity mean values between field and greenhouse are remarkably different. Considering the stability to degradation shown by fenbutatin oxide in this experimentation, the degradation rate of this AI may be only slightly affected by environmental aspects.

CONCLUSIONS

The HPLC method developed allows an easy and rapid determination of fenbutatin oxide on peaches and nectarines without using any cleanup.

In this experimentation fenbutatin oxide has shown remarkable stability. For this reason, in treatments carried out 2 weeks before harvest, with a moderate fruit weight increase, the initial residue value should remain practically constant.

In the light of these data, in countries like Italy where the maximum residue limit (MRL) is 0.5 ppm, this limit is easily exceeded even when the preharvest interval of 30 days is respected. It would definitely be exceeded if the treatments were carried out 2 weeks prior to harvest.

Repeated treatments would therefore considerably increase the level of residues, due to the accumulation of such a stable pesticide.

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