Dissipation of Teflubenzuron and Triflumuron Residues in Field-Sprayed and Cold-Stored Pears

Pipina G. Aplada-Sarlis and George E. Miliadis*

Benaki Phytopathological Institute, 7 Ekalis Street, Kifissia 14561, Greece

Nicholas G. Tsiropoulos

Department of Theoretical and Applied Sciences, University of Thessaly, Pedion Areos, Volos 38334, Greece

Dissipation of residues of benzoylurea insecticides teflubenzuron (TFB) and triflumuron (TFM) under field conditions was evaluated on a pear orchard in Greece. Residues were determined by UV−HPLC analysis, with a detection limit of 0.030 mg/kg for both pesticides. TFB residues in pears were found to persist for 2 weeks and decline thereafter with 48% of the initial deposit remaining 42 days after the last application. TFM residues were found to decline following first-order kinetics and with a half-life of 39(±7) days. Residues of both pesticides found in pears collected at harvest maturity were lower than the maximum residue limits (MRLs) set by individual countries. Dissipation of TFB and TFM in cold-stored pears was also evaluated. TFB residues were very persistent for the whole storage period, whereas TFM residues did not dissipate for 6 weeks and then showed a constant decline; 7% of the initial concentration remained at the end of the storage period of 29 weeks.

Keywords: *Teflubenzuron; triflumuron; pears; dissipation; residues; storage*

INTRODUCTION

Teflubenzuron (TFB) and triflumuron (TFM) are the common names of 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea and 1-(2-chlorobenzoyl)-3-(4-trifluoromethoxyphenyl)urea, two benzoylurea insecticides acting by contact and ingestion as insect growth regulators inhibiting chitin synthesis and so interfering with the formation of the insect cuticle. They are recommended for the control of Lepidoptera, Phyllidae, Diptera, and Coleoptera on a variety of crops including fruit trees (British Crop Protection Council and The Royal Society of Chemistry, 1994).

Application of TFB and TFM in Greece is common on pome fruit trees, mainly apple and pear trees. The preharvest interval (PHI), that is, the number of days from the final application to harvest, for pears is 30 days for TFM and 60 days for TFB. No maximum residue limits (MRLs) have been set by the European Union or by FAO for these compounds in fruit crops, including pears. However, the importance of pears in the diet requires data concerning dietary intake of benzoylurea insecticide residues and therefore data for evaluating the behavior of TFB and TFM residues in this crop. There are no papers in the literature concerning the dissipation behavior of TFB and TFM in pears or other fruit.

The objective of this study was therefore to obtain data on the dissipation behavior of TFB and TFM in pears, under field conditions and during storage of pears in refrigerated rooms.

* Corresponding author (e-mail bpipest@otenet.gr; telephone ++301 8077587; fax ++301 8077506).

EXPERIMENTAL PROCEDURES

Chemicals. *(a)* Analytical standards of TFB (certified purity > 97.4%) and TFM (certified purity > 99%) were obtained from Cyanamid (Princeton, NJ) and Bayer (Mannheim, Germany), respectively. The stock solutions (1000 ppm of each insecticide) as well as the calibration and the spiking standard solutions were prepared in methanol.

(b) Nomolt 15SC, containing 15% w/v TFB, and Alsystin 25WP, containing 25% w/w TFM, were the formulations used for field applications.

(c) All solvents were purchased from Lab-Scan (Dublin, Ireland). Methanol was of HPLC grade, and all others were of pesticide residue grade.

(d) The anhydrous sodium sulfate was of proanalysis grade (Merck).

(e) The SPE cartridges were Isolute containing 500 mg of unbonded silica and were purchased from IST Ltd. (Mid Glamorgan, U.K.).

Field Experiment. The experimental site was a pear orchard, of the Kristalli variety, located near Volos in central Greece, which was square planted in 1964. The trees were vase shaped, spaced 10 m from each other, and managed during the experiment by routine horticultural practices. The experimental design comprised three equivalent plots, each of four trees. One of the experimental plots was sprayed with the TFM formulation, one was sprayed with the TFB formulation, and the other one was left untreated to be used as control. Two applications were carried out with each pesticide on July 6 and July 27, 1997, using a pressurized hand gun sprayer at high volume to run off. The aqueous solution of TFM formulation was applied at 20 g of active ingredient (ai)/100 L of water and the aqueous suspension of TFB at 10.5 g of ai/100 L of water. These are the labeled maximum recommended doses for pear trees. During the whole experiment the average minimum/maximum daily air temperatures were 19.9/28.4 °C, the average relative humidity was 51%, the average solar radiation was 6.2 kWh m^{-2} day^{-1}, and the total rainfall was 5.4 mm.

10.1021/jf980846a CCC: $18.00
Published on Web 06/03/1999

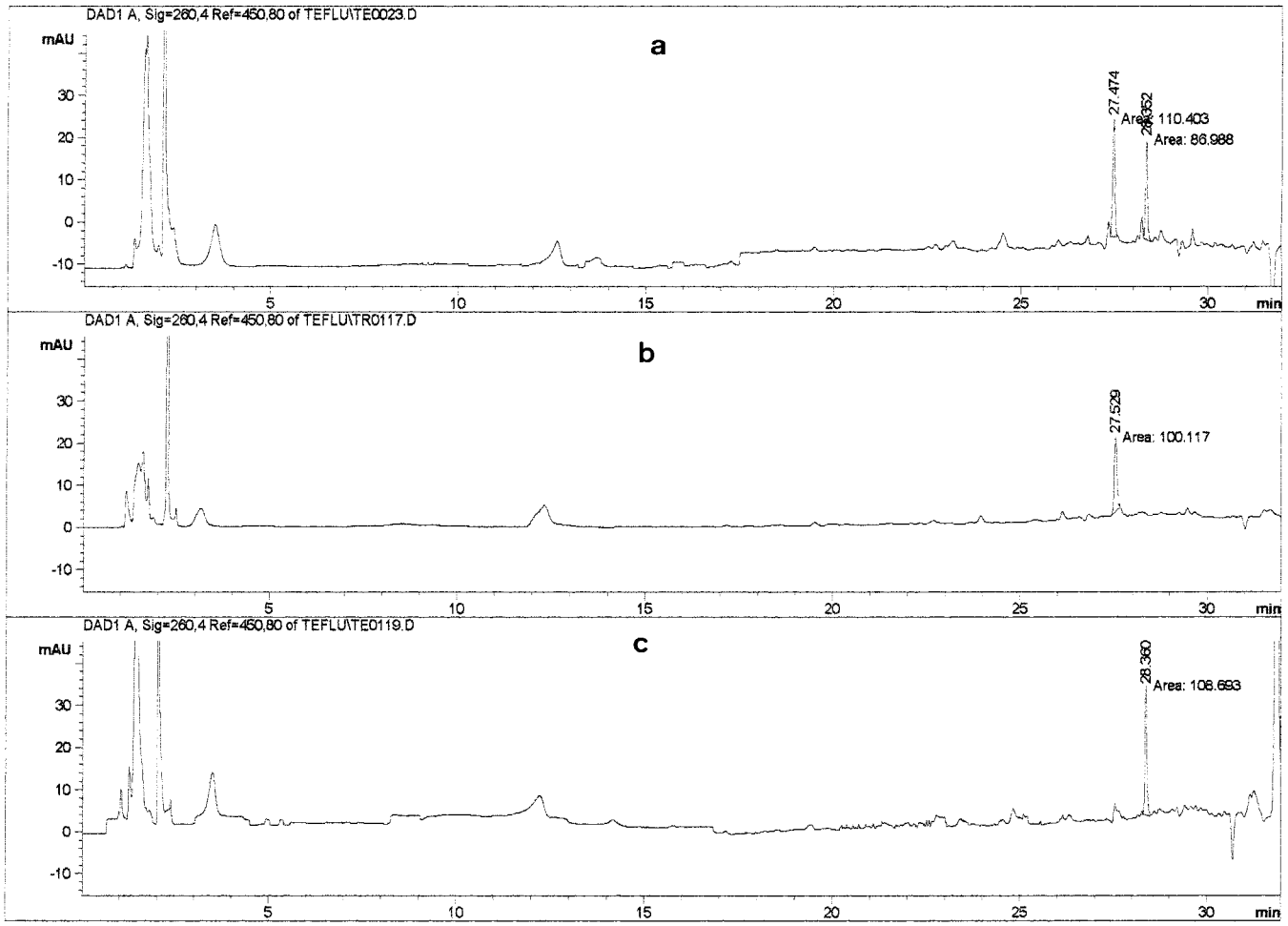

Figure 1. Chromatograms of (a) control pear fortified at 0.15 mg/kg with TFM (27.5 min) and TFB (28.4 min), (b) field sample treated with TFM, and (c) field sample treated with TFB.

Sampling. Sampling was performed according to FAO/WHO recommendations (FAO/WHO, 1986) by randomly collecting a total of 24 pears from several positions in all trees from each plot. The overall sample weight from each plot and for each sampling ranged from 2 to 3 kg. The samples were forwarded to the laboratory, where the pears were chopped and blended after removal of stems and stalks. The homogenized material was subdivided into 50 g aliquots as analytical replicates and stored in individual bags at −20 °C until extraction.

For each experimental plot, samples were collected just before the last application time (−0 days samples) and at 0 (1 h postspray), 2, 4, 7, 10, 14, 19, 23, 27, 34, and 42 days after the last application to study the dissipation of each pesticide. The harvest maturity (fruits ripe for immediate consumption) took place at 30−37 days following the last application.

Cold Storage. For studying the dissipation rates of TFM and TFB in cold-stored pears seven 24-fruit samples from each plot were collected 23 days after the last treatment (at horticultural maturity) for long-term storage. The samples were packed on site and forwarded for cold storage for 3, 6, 9, 14, 19, 24, and 29 weeks at 0 ± 1 °C and ∼95% relative humidity. Stored fruit were sampled and homogenized the same as fresh fruit.

Residue Analysis. *(a) Extraction and Cleanup Procedure.* All samples were analyzed in triplicate for TFB and TFM residues by ethyl acetate/sodium sulfate extraction (Ministry of Public Health, Welfare and Sport, 1996) and solid-phase extraction cleanup on silica cartridges (Tsiropoulos et al., 1999). Briefly, 50 g of homogenized sample was blended in an Omni mixer at 6000 rpm for 3 min with 100 mL of ethyl acetate and 50 g of anhydrous sodium sulfate; 50 mL of the filtrated extract was evaporated to dryness. The residue was dissolved in 5 mL of hexane, and 1 mL of the extract was passed through the silica cartridge. TFB and TFM were eluted by 2 mL of 2-propanol/dichloromethane (1:9), dried under a gentle nitrogen stream, redissolved in 1 mL of methanol, and filtered through a 0.2 μm filter in a vial ready for HPLC analysis.

(b) HPLC Determination. A Hewlett-Packard model HP 1090, series II, liquid chromatograph, equipped with a UV diode array detector and an ODS Hypersil C18 column 250 × 2.1 mm, 5μm particle size, was used for the determination of the two compounds. The following operating conditions were used: mobile phase, 10% v/v methanol in water for 5 min, increased to 64% at 20 min, then to 100% methanol at 25 min, and held there for 5 min; flow rate, 0.3 mL/min; injection volume, 20 μL; detector wavelength, 260 nm; and column temperature, 42 °C. Under these conditions the retention times were 27.5 and 28.4 min for TFM and TFB, respectively. Residues determined are referred to the whole fruit including the peel.

RESULTS AND DISCUSSION

Method Efficiency. The described method of analysis of pear samples for TFB and TFM residues is relatively simple, and the cleanup of the extract is sufficient, as can be seen from the chromatograms in Figure 1. The detector response calibration curve in the investigated ranges of 1−200 ng for TFB and 7.5−200 ng for TFM was found linear. The regression equations for the calibration curves, at 12 concentration levels,

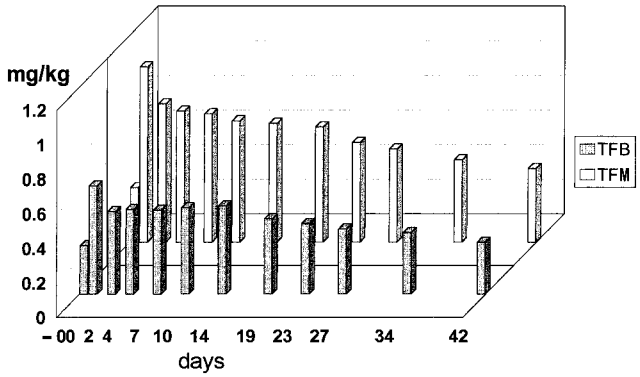

Figure 2. Residues of TFB and TFM (milligrams per kilogram) in field-treated pears at various intervals after the last application (0 days). −0 days represent residues remaining on pears from previous application. Residue values are means of three analytical replicates with SD ≤ 11.2% for TFB and SD ≤ 9.6% for TFM.

Table 1. Mean Recovery (n = 3) and RSD of TFB and TFM at Various Spiking Levels in Pears

concn (mg/kg)	recovery (%)		RSD	
	TFB	TFM	TFB	TFM
0.05	107	108	10	10
0.075	86	105	6.6	4.0
0.15	92	94	7.8	7.6
0.25	93	103	1.7	4.4
0.75	92	96	9.4	4.7
2.5	92	100	8.5	9.0
5.0	89	103	7.4	6.5

were $y = -3.84 + 2.85x$ for TFB and $y = 1.06 + 3.38x$ for TFM, with correlation coefficients of 0.999 for TFB and 0.9999 for TFM. Residue concentration in the samples was calculated from the measured area of each compound peak in the sample's chromatogram using the calibration curve equation (external standard method).

The efficiency of the method was evaluated by spiking of homogenized control samples (untreated pears) at various concentration levels with TFB and TFM spiking standard solutions. Spiked samples were extracted and processed according to the above-described procedure. Table 1 presents the results of the recovery study. All recovery values in this table are acceptable for residue determinations (Greve, 1984). The method's detection limit, according to the U.S. EPA (1984), is the product $s \times t_{st}$, where s is the worst case standard deviation at the lowest validation level and t_{st} the Student t value, which at the 99% confidence level and for two degrees of freedom is 6.96. The detection limit was found to be 0.030 mg/kg for both pesticides.

Dissipation of Residues in Field Pears. No TFB or TFM residues were detected in any control pear samples that were collected at various sampling dates. TFB and TFM residues found in pear samples at various time intervals following the last application are presented in Figure 2. Residues in this figure are the mean values of three analytical replicates, and the variabilities about these means, measured by relative standard deviaion (RSD), were 1.4−11.2% for TFB and 0.5−9.6% for TFM. Residues of TFB and TFM remaining from the first application (−0 days) were 0.27 ± 0.02 and 0.31 ± 0.01 mg/kg, respectively, whereas initial deposits immediately after application (0 days) were 0.63 ± 0.02 and 1.01 ± 0.05 mg/kg, respectively. A significant loss of residues was observed for both pesticides within 2 days following application. These losses are approximately 24% for TFB and 21% for TFM and may be attributed to postapplication volatilization (Majewsky, 1991). Thereafter, TFM residues declined with time, whereas TFB residues did not dissipate for 14 days after application and declined thereafter. At 42 days following application, 48 and 42% of the initial deposit were found on the pears for TFB and TFM, respectively, indicating a persistence of these molecules on the fruit tissue. The residues' decline may be attributed primarily to growth dilution between application and sampling, as well as to volatilization that occurs during the first days following application, removal by weathering, heat decomposition, sunlight UV radiation, or other complex conditions (Spynu, 1989).

Table 2. TFM and TFB Residues[a] in Pears after Various Intervals in Cold Storage

time (weeks)	TFM	TFB
0	0.58 ± 0.02	0.40 ± 0.02
3	0.59 ± 0.04	0.36 ± 0.01
6	0.61 ± 0.03	0.36 ± 0.03
9	0.40 ± 0.00	0.37 ± 0.01
14	0.22 ± 0.02	0.34 ± 0.00
19	0.088 ± 0.01	0.39 ± 0.01
24	0.050 ± 0.003	0.33 ± 0.02
29	0.039 ± 0.001	0.38 ± 0.02

[a] In milligrams per kilogram; mean ± SD of three analytical replicates for each sample.

The decline of TFM residues with time was found to be described mathematically by a pseudo-first-order rate equation. The regression line equation for the concentration (C) related to time (t) was $\log C = -0.0588 - 0.0077t$ ($n = 11$) with a correlation coefficient $r = 0.971$, which shows a high correlation at $p = 0.01$. The half-life ($t_{1/2}$) of TFM in pears under field conditions was evaluated, from the regression lines, to be 39 ± 7 days.

Residue levels of both pesticides can be compared only with individual countries' MRLs, due to the lack of MRLs set by the European Union or the FAO/WHO. For example, France has set 1 mg/kg for TFM and 0.5 mg/kg for TFB in pears (Index Phytosanitaire, 1998). Residues of both pesticides found in ripe pears collected at harvest maturity (34 days after last application) were 0.47 ± 0.02 for TFM and 0.36 ± 0.01 for TFB, values lower than the MRL values set by France.

The effect of peeling on reducing TFM and TFB residues was also estimated by analyzing some of the pear samples before and after peeling. It was found that ∼60% of the residues of both pesticides were removed by peeling, indicating that the remaining residues have penetrated into tissues deeper than the outer skin.

Dissipation of Residues in Cold-Stored Pears. Residues of TFM and TFB found in pears after various storage intervals are given in Table 2. TFM residues remained relatively stable for the first 6 weeks but then declined over time; TFM concentration at the end of the 29 weeks of storage was found to be ∼7% of the initial concentration found at the beginning of the storage process. The dissipation rate for the last 23 weeks followed a pseudo-first-order rate equation, $\log C = 0.084 - 0.0549t$ ($n = 6$), with $r = 0.989$. The half-life ($t_{1/2}$) of TFM in pears under cold storage conditions was evaluated from the regression line to be 11 ± 1 weeks. Residues of TFB remained stable for the whole storage period (29 weeks), indicating high stability of the pesticide's molecule under cold storage conditions.

ACKNOWLEDGMENT

We thank Mrs. K. Bourou, Mrs. G. Giannopoliti, and Ms. K. Polymerou for processing samples.

LITERATURE CITED

British Crop Protection Council and The Royal Society of Chemistry. *The Pesticide Manual*, 10th ed.; BCPC and RSC: London, U.K., 1994.

FAO/WHO. Recommended method of sampling for the determination of pesticide residues. *Codex Alimentarius Commission of the Joint FAO/WHO Food Standards Programme*, 2nd ed.; Food and Agricultural Organization/World Health Organization: Rome, Italy, 1986; Vol. XIII, Part VI.

Greve, P. A. Good Laboratory Practice in Pesticide Residue Analysis. In *Pesticide Residue Analysis*; Ambrus, A., Greenhalgh, R., Eds; Proceedings of a Joint WHO/FAO Course; WHO/FAO: Rome, Italy, 1984; p 281.

Index Phytosanitaire. *Association de Coordination Technique Agricole*, 34th ed.; Paris, France, 1998; pp 107−109.

Majewski, M. S. Sources, Movement, and Fate of Airborne Pesticides. In *Pesticides Chemistry*; Frehse, H., Ed.; Proceedings of the 7th International Congress Pesticide Chemistry (IUPAC); VCH: Weinheim, Germany, 1991; p 307.

Ministry of Public Health, Welfare and Sport. *Analytical Methods for Pesticides Residues in Foodstuffs*; Van Zoonen, P., Ed.; Bilthoven, The Netherlands, 1996; Part I, p 4.

Spynu, E. I. Predicting Pesticides Residues to Reduce Food Contamination. *Rev. Environ. Contam. Toxicol.* **1989**, *109*, 89−107.

Tsiropoulos, N. G.; Aplada-Sarlis, P.; Miliadis, G. E. Determination of Benzoylurea Insecticides in Apples and Pears by Solid-Phase Extraction Cleanup and Liquid Chromatography with UV Detection. *J. Assoc. Off. Anal. Chem.* **1999**, *82*, 213−216.

U.S. EPA. *Fed. Regist.* **1984**, *49* (209), 198.

Received for review July 30, 1998. Revised manuscript received April 7, 1999. Accepted April 14, 1999.

JF980846A