Determination of Buprofezin, Pyridaben, and Tebufenpyrad Residues by Gas Chromatography−Mass-Selective Detection in Clementine Citrus

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A gas chromatography−mass-selective (GC−MS) detection method to determine buprofezin, pyridaben, and tebufenpyrad on the pulp, peel, and whole fruit of clementines is described. The extraction/partition procedure was performed in one step and no cleanup was necessary with the GC−MS in the SIM-mode pesticide determination. Recovery ranged from 75 to 124% with coefficients of variance ranging between 1 and 13%. The limit of determination was 0.01 mg/kg for all pesticides. The field trials showed a similar degradative behavior for all active ingredients (AI), with a great residue decrease during the first week and stability in the second. Just after treatment buprofezin and tebufenpyrad showed lower residues than the maximum residue limit (MRL) fixed in Italy, while pyridaben was below the MRL after a week.

Keywords: *Residues; GC−MS; buprofezin; pyridaben; tebufenpyrad; clementine*

INTRODUCTION

Buprofezin, pyridaben, and tebufenpyrad are nonsystemic acaricide pesticides commonly used in the control of *Tetranychus* and *Panonychus* in citrus fruits (Tomlin, 1997). Buprofezin has been used since the 1980s, while pyridaben and tebufenpyrad have been marketed this decade. Many mono- and multiresidue gas chromatographic methods on buprofezin in vegetables and in the soil are available in the literature. They include the following three steps: extraction with polar solvents, partition with an organic solvent (e.g., hexane), and purification with silica gel or gel permeation. The detectors used were NPD (Valverde et al., 1993; Dejonckheere, 1996), AFID (Nishizawa et al., 1994), ECD (Uchida et al., 1982), and MS−SIM (Valverde et al., 1994). To our knowledge no study has reported on the determination of pyridaben and tebufenpyrad in a vegetable matrix in the scientific literature. Since the fixed legal limits are low (0.1−0.5 mg/kg), a highly sensitive method capable of determining residues of almost 0.01 mg/kg was needed. This paper reports a GC−MS (SIM mode) method without cleanup, for the simultaneous determination of three insecticides and their degradation in clementine fruits.

MATERIALS AND METHODS

Field Trials. The trials were carried out in a citrus grove at Uta (Cagliari, Italy), owned by the Centro Regionale Agrario Spermentale (CRAS), on the clementine SRA 63 cultivar. The grove was planted in 1982 with a plant spacing of 5 × 4 m. A random block design with four replications was used, and every block contained three plants in a single row. Treatments were carried out with an Agrumobar sprayer (Fox Motori F 320, Reggio Emilia, Italy), with the following commercial products: Applaud (25% buprofezin), Nexter (19.8% pyridaben), and Masai (20% tebufenpyrad) at the doses recommended by the manufacturers (respectively 150, 60, and 60 g/hL, with 10−12 hL/ha). The following two experiments were carried out: (1) with pyridaben and (2) with buprofezin and tebufenpyrad.

Twenty-four fruit samples were collected on dry plants before and after the last treatment, and repeated at 6 and 11 days in experiment 1, and for 6 and 14 days in experiment 2. Meteorological data were continuously recorded with an SM 3800 automatic weather station (SIAP, Bologna, Italy).

During the experiments, total rainfall was 44.6 mm, with the most important precipitation of 6.2, 7.0, and 24.8 mm at 2, 12, and 13 days after treatment, respectively, and the maximum and minumum average temperatures were 17.3 and 4.7 °C, respectively.

Reagents. Buprofezin, pyridaben, and tebufenpyrad were analytical standards (>99%) kindly provided by the manufacturer. Triphenyl phosphate (99%) was used as an internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium). Stock standard solutions of the pesticides (~500 mg/kg) were prepared in acetone.

Working standard solutions were obtained by dilution with the extract of untreated whole fruits, peel, and pulp, in acetone/hexane (50/50 v/v) containing the internal standards at 0.03 mg/kg. One milliliter of each solution was evaporated under a gentle nitrogen stream; it was then dissolved with 100 μL of acetone.

Acetone and hexane were HPLC solvents (Carlo Erba, Milano, Italy).

Sample Preparation. Fruit were counted and weighed to determine the average weight. They were then cut with a knife in two equal parts and divided in two batches. The clementine halves from one batch were directly ground and homogenized, while the others were weighed and peeled. The peels were weighed to calculate their percentage contribution to the fruit weight, and subsequently, the peel and the pulp were ground and homogenized separately. In this way it was

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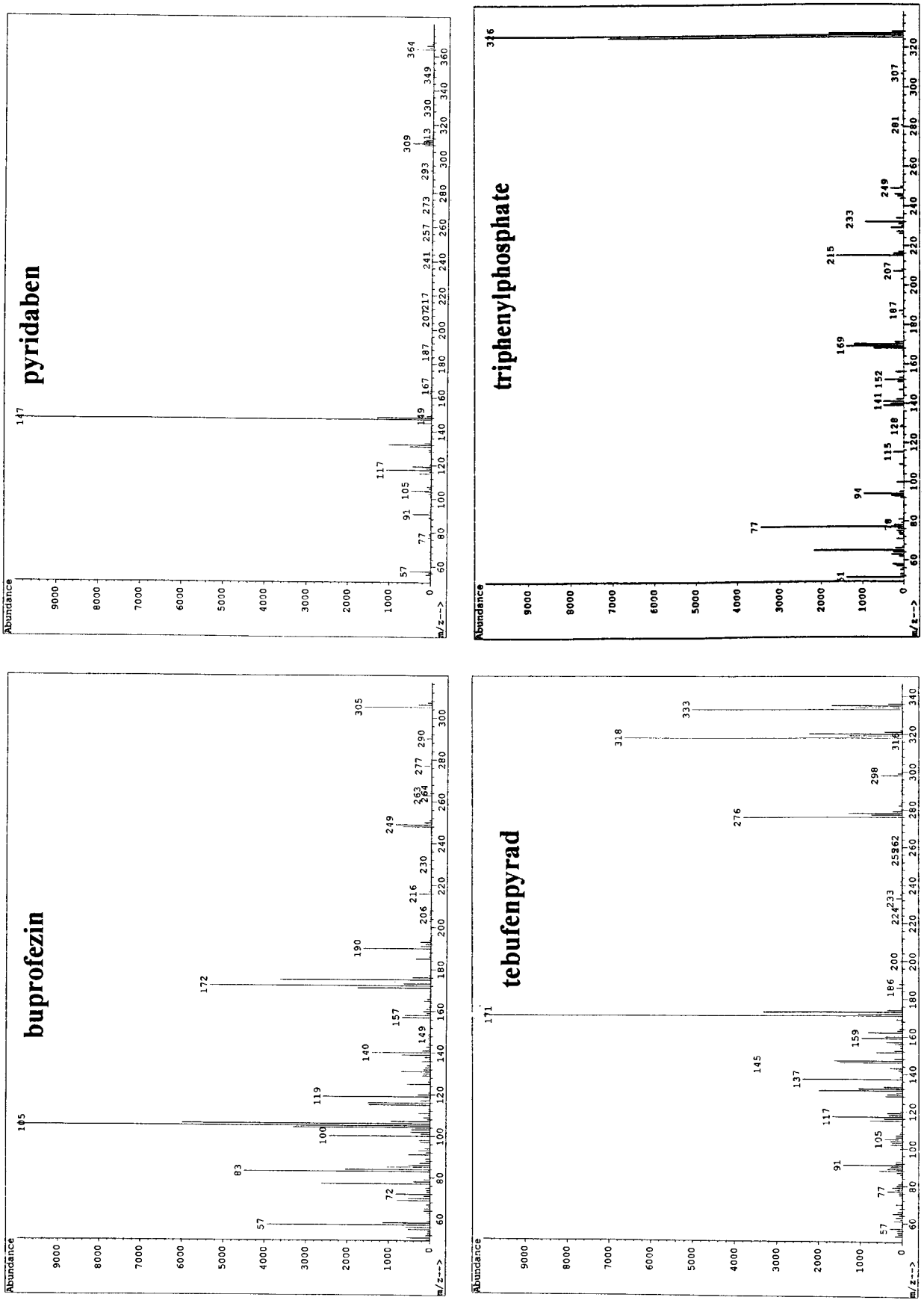

Figure 1. Mass spectra of pesticides.

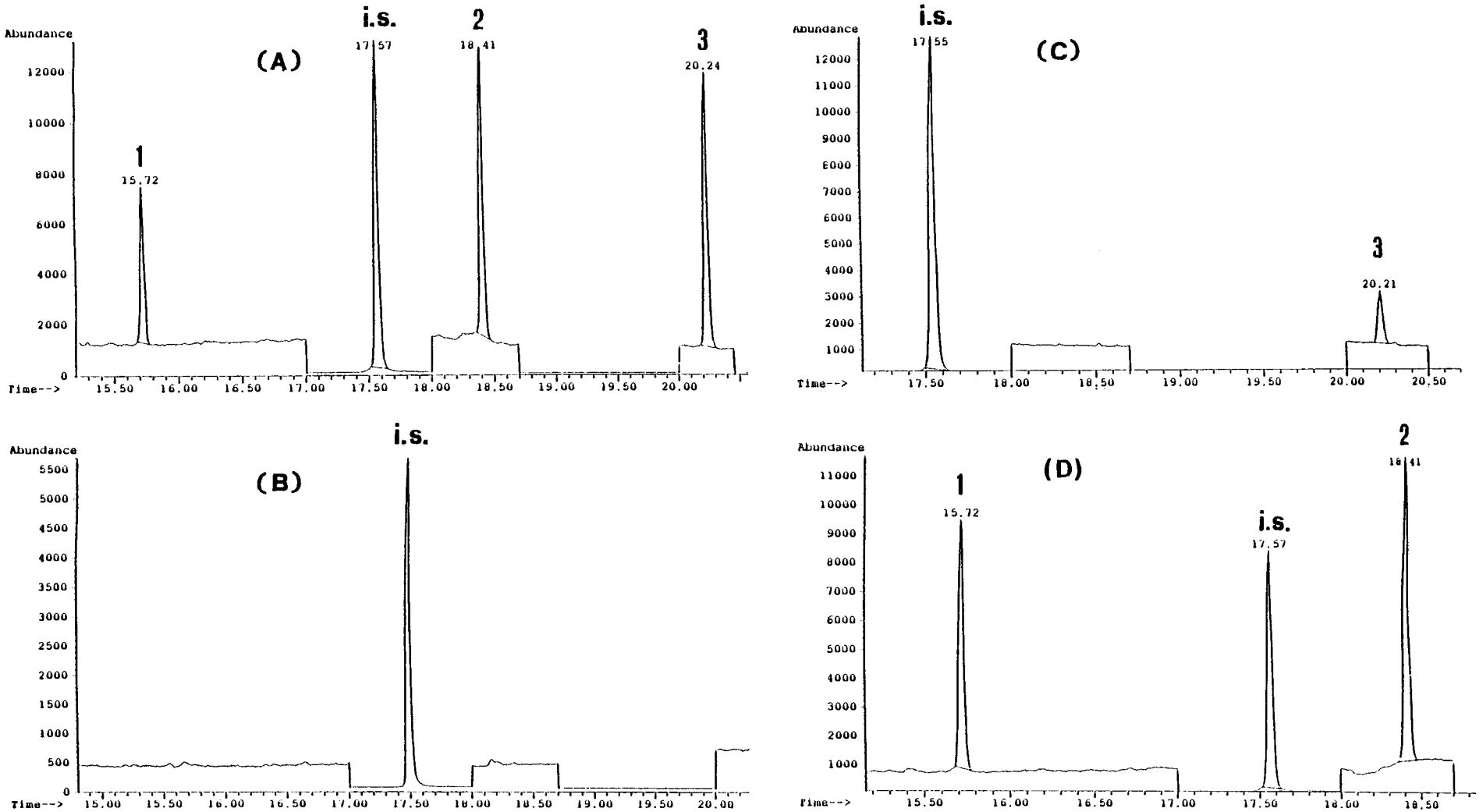

Figure 2. GC−MS chromatograms of pesticides in extracts of untreated peel fortified at ca. 1.0 mg/kg (a), control (b), and samples (c) and (d). For GC conditions see text. Peaks: buprofezin (1), tebufenpyrad (2), and pyridaben (3).

possible to obtain the following three different samples: whole fruit, peel, and pulp.

Extraction Procedure. An aliquot of homogenized sample (10 g for the pulp and whole fruit, and 5 g for the peel) was weighed into a 40 mL screw capped tube; 4 g of sodium chloride and 10 mL of an acetone/hexane mixture (50/50 v/v) containing the i.s. were added, and the tube was agitated for 30 min in a rotatory shaker at 9 rpm. (GFL, Burgwedel, Germany). The phases were allowed to separate and 1 mL of the organic layer was evaporated under a gentle nitrogen stream; it was then dissolved with 100 μL of acetone and injected for analysis.

Recovery assay. Untreated fruit, peel, and pulp samples were fortified with 0.01, 0.1, and 1 mg/kg of pesticide by adding 100 μL of pesticide solution in acetone. Samples were allowed to equilibrate for 30 min prior to extraction and were processed according to the above procedure. At each fortification level, three replicates were analyzed.

Chromatography. An HP-5890 Gas Chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with an HP-5971 GCMS detector (Hewlett-Packard) and a Durabond fused silica liquid-phase DB 5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm) (J&W Scientific, Folsom, CA). The sample (2 μL) was injected in the spitless mode (60 s), the injector temperature was 250 °C, and the oven temperature was programmed as follows: 110 °C raised to 300 °C (15 °C/min) and held for 10 min. Helium was the carrier gas at 0.8 mL/min. Mass spectrometer operating conditions: electron ionization (positive), 65 V; ion source 180 °C; dwell per ion 100 ms; solvent delay 8 min; selected ion monitoring (SIM), *m/z* triphenyl phosphate (i.s.) = 326; buprofezin = 105, 172, 305; pyridaben = 147; tebufenpyrad = 171, 276, 318. *M/z* ions were monitored as follows: buprofezin from 15 to 17 min, triphenyl phosphate from 17 to 18 min, tebufenpyrad from 18 to 18.70 min, and pyridaben from 20 to 20.50 min.

RESULTS AND DISCUSSION

Analytical Methods. The mass spectra of the used insecticide were carried out (Figure 1) and the main ions were chosen for SIM analysis. Since no interference peak was found in the extracts with this method (Figure 2), sample cleanup was not necessary. The comparison between the standards prepared in extract and in solvent showed the effect of the matrix in the calibration curves. This effect has been noted by other authors (Kaufmann, 1997; Fillion et al., 1995) and was attributed to a reduction in the degree of thermal decomposition in the ion source of some pesticides in the presence of the matrix.

To avoid possible errors caused by this matrix effect, standard solutions were prepared in the extracts of the untreated samples. Under the chromatographic conditions described, the calibration graphs (internal standard mode) were constructed by plotting peak area versus concentration. Good linearity was achieved in the range between 0.005 and 1.50 mg/kg with correlation coefficients ranging between 0.9993 and 0.9999.

The extraction partition was done in one step according to Steinwandter (1992). The recovery assay with fortification levels of 0.01, 0.10, and 1.00 mg/kg (Table 1) showed acceptable recoveries (from 75 to 124%) and repeatability (from 1 to 13%). Under the above operating conditions the limit of determination (Thier and Zeumer, 1987) was 0.01 mg/kg for each of the compounds studied.

Field Degradation. Since the average weight of the fruits was constant during the experiment, the residue concentration was not affected by fruit growth. The peel weight ranged between 35 and 40% of the fruit. Table 2 reports the data for the residues in the pulp, peel, and whole fruit. The legal limits used in the discussion are those supported by Italian law and are reported for whole fruit.

Table 1. Recovery of Fungicides from Peel, Pulp, and Fruit Samples

fungicide	fortification level (mg/kg)	recovery (% ± RSD) peel	pulp	fruit
buprofezin	1.00	75 ± 2	95 ± 3	104 ± 4
	0.10	89 ± 2	89 ± 1	85 ± 7
	0.01	113 ± 11	101 ± 6	92 ± 1
pyridaben	1.00	89 ± 9	80 ± 4	75 ± 2
	0.10	88 ± 5	92 ± 3	98 ± 1
	0.01	112 ± 10	102 ± 6	111 ± 4
tebufenpyrad	1.00	78 ± 9	82 ± 3	88 ± 4
	0.10	93 ± 5	90 ± 5	80 ± 7
	0.01	87 ± 13	97 ± 7	124 ± 12

Table 2. Residues of Buprofezin, Pyridaben, and Tebufenpyrad in the Pulp, Peel, and Whole Fruit of Clementines after Treatment

pesticide	days after treatment	residues[a] (mg/kg ± SD) pulp	peel	whole fruit
buprofezin	0	0.17 ± 0.05	0.64 ± 0.07	0.34 ± 0.04
	6	0.02 ± 0.05	0.14 ± 0.04	0.08 ± 0.01
	14	0.01 ± 0.07	0.12 ± 0.02	0.06 ± 0.00
pyridaben	0	0.02 ± 0.01	0.41 ± 0.11	0.14 ± 0.04
	6	n.d.	0.23 ± 0.10	0.08 ± 0.02
	11	n.d.	0.17 ± 0.01	0.06 ± 0.02
tebufenpyrad	0	0.06 ± 0.05	0.36 ± 0.05	0.14 ± 0.03
	6	n.d.	0.10 ± 0.01	0.02 ± 0.01
	14	n.d.	0.08 ± 0.03	0.02 ± 0.01

[a] Residue values are the means of duplicate analysis from three replicates.

Buprofezin. After treatment this pesticide showed a residue of 0.34 mg/kg in the whole fruit. This value is lower than the legal limit of 0.50 mg/kg. The residue in the pulp was 0.17 mg/kg, which was too high to be attributed to contamination during peel separation. This means that the pesticide must have passed through the peel, which is not very thick, and reached the pulp. In the first week buprofezin showed a significant residue decrease from 0.34 to 0.08 mg/kg, in the whole fruit, while during the second week it remained unchanged. Similar results were found in orange trials (FAO/WHO, 1991).

Pyridaben. The residue after the treatment in the whole fruit was 0.14 mg/kg, while the residues in the pulp were negligible. This showed that all residues were on the peel. After one week the whole fruit level was under the legal limit of 0.10 mg/kg. This pesticide did not show any decrease during the following week.

Tebufenpyrad. This active ingredient (AI) showed the same behavior as buprofezin, with a residue value after treatment in the whole fruit of 0.14 mg/kg, which is under the legal limit of 0.5 mg/kg. The degradative behavior was similar to that of the other pesticides studied, with a rapid decrease during the first week and stable residues the second. The rapid decrease of buprofezin and tebufenpyrad in the first week could be due to the rain washing (6.2 mm after 2 days after treatment). We cannot attribute the residue decrease to pesticide solubilization in water, because between the second and third sampling there were two greater precipitations (7.0 and 24.8 mm), but the residues were constant. At treatment time, dust could be on the fruit; therefore pesticides would settled both on the wax of the fruit surface and on the dust. The pesticide settled on the fruit surface tend to spread by penetrating the epicuticular wax (Rieder and Schreiber, 1995), which

would not allowed their solubilization in water. During the rain, the dust is removed from the fruit together with its residues. The subsequent rains do not decrease residues because on the fruit the dust has been washed away by the first rain.

The residues were mainly concentrated in the peel. The residue in the whole fruit at harvest time was 0.02 mg/kg.

CONCLUSIONS

For the determination of these three pesticides an easy and rapid method was used, consisting of only one step before injection in the gas chromatograph. The extraction procedure allowed acceptable accuracy and repeatability. The determination limit was 0.01 mg/kg for all pesticides. Field trials showed a similar degradative behavior for all AI's, with a significant decrease during the first week and substantial stability in the second. Just after the treatment buprofezin and tebufenpyrad showed residues under the legal limit, while pyridaben was under the legal limit after a week.

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