Effect of Epicuticular Waxes of Fruits on the Photodegradation of Fenthion

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After treatment lipophilic pesticides tend to diffuse by penetrating the epicuticular wax of fruits. In this way, solar radiation only acts on pesticide molecules after passing through the waxes. The effect of epicuticular waxes of three fruits (orange, nectarine, and olive) on the photodegradation of fenthion was studied. The waxes affected the photodegradation process of fenthion. The decay rate of fenthion increased in the presence of orange and nectarine waxes, while it decreased when olive wax was used. In all waxes, the transformation of fenthion produced mainly fenthion sulfoxide and low amounts of fenthion sulfone. In orange wax, 50% of the initial fenthion was transformed into unknown compounds. In nectarine wax, fenthion was degraded stoichiometrically into fenthion sulfoxide and fenthion sulfone. In olive wax, the photodegradation of fenthion yielded about 80% of fenthion sulfoxide.

**Keywords:** *Fenthion; photodegradation; fruits; wax*

On the surface of leaves and fruits, a mixture of long chain apolar compounds (hydrocarbons, ketones, esters, alcohols, aldehydes, and free fatty acids) called epicuticular waxes (Bianchi, 1995) is present. Different plants present waxes differing in composition and quantity. Furthermore, the composition and quantity of waxes in the same plant change according to weather and seasonal fluctuations, stage of growth, and fruit maturation. During the application of pesticides, drops of aqueous suspensions containing the active ingredient are deposited on leaves and fruits. After the treatment, the molecules of the pesticides, most of which are made of lipophilic compounds, tend to diffuse by penetrating the epicuticular wax (Riederer and Schreiber, 1995). In this way, solar radiation only acts on pesticide molecules after passing through the waxes. This wax film could act as a sun filter and reduce photodegradative activity. For the pesticides, the differences in the composition of the waxes could lead to different sunlight photodegradation behaviors in the different fruits.

To our knowledge, no specific investigation has been performed to assess the effects of epicuticular waxes of fruits on the sunlight photodegradation of pesticides. This work aims to contribute to the knowledge of this topic through the study of the photodegradation of fenthion, an insecticide commonly used on crops.

EXPERIMENTAL PROCEDURES

Extraction Procedure of Fruit Waxes. Wax extraction was performed as described by McDonald et al. (1993) by fruit immersion in chloroform for 2 min. The quantity of wax on the fruit surface was determined by evaporation of 10 mL of chloroform extract to dryness.

Chemicals. Fenthion was an analytical standard purchased from Ehrenstorfer (Augsburg, Germany); fenoxon was an analytical standard kindly donated by Bayer (Leverkusen, Germany). Metabolites **II**, **III**, **V**, and **VI** (Figure 1) were analytical standards synthesized according to the method by Cabras et al. (1991). Triphenyl phosphate (99%) was used as
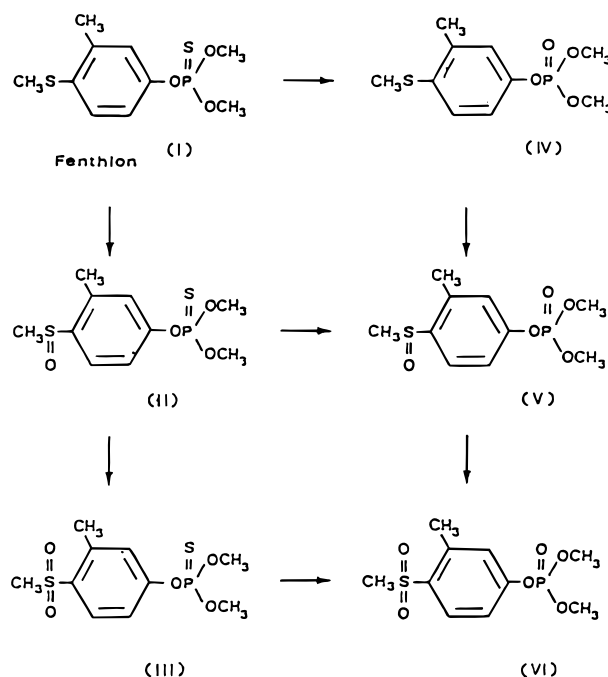
Figure 1. Fenthion (I) and its metabolites and degradation products: fenthion sulfoxide (II), fenthion sulfone (III), fenoxon (IV), fenoxon sulfoxide (V), and fenoxon sulfone (VI).

the internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium). Acetone, chloroform, and ethanol were of HPLC grade, while petroleum ether was a special reagent for pesticide determination (Carlo Erba, Milan, Italy). Stock standard solutions of fenthion and its metabolites (ca. 500 mg/kg each) were prepared in ethanol. Working standard solutions, containing the i.s. at 0.6 mg/kg, were obtained by dilution with an acetone/petroleum ether mixture (1:1 v/v). Two-milliliter portions of these solutions were poured into beakers containing the previously evaporated chloroform extract at the wax concentration present on the fruit surface per cm².

Apparatus and Chromatography. An HRGC Mega 5160 gas chromatograph (Carlo Erba) was employed. It was fitted with an NPD-40 nitrogen–phosphorus detector, an AS 550 autosampler (Carlo Erba), and a split-splitless injector, con-

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Table 1. Half-Lives ($t_{1/2}$) and Correlation Coefficients (r) of Fenthion in Orange, Nectarine, and Olive Wax Extracts after Exposure to Direct Sunlight

fruit	wax ($\mu g/cm^2$)	$t_{1/2}$ (h)	r
control	0	4.8	−0.982
orange	41	3.8	−0.993
	410	2.4	−0.968
nectarine	7	3.9	−0.980
	70	2.4	−0.988
olive	510	11.9	−0.995

nected to an HP 3396A instrument (Hewlett-Packard, Avondale, PA). A Durabond fused silica column (30 m × 0.25 mm inside diameter) (J&W Scientific, Folsom, CA) was employed, with a DB 210 (50% trifluoropropylsilicone and 50% methylsilicone) liquid phase (film thickness of 0.25 μm). The injector and detector were operated at 250 and 260 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 90 °C for 1 min, raised to 160 °C (30 °C/min) and then to 260 °C (10 °C/min), and held for 12 min. Helium was the carrier and makeup gas at 120 and 150 kPa, respectively, while hydrogen and air flows were 110 and 120 kPa, respectively. Calibration graphs for the fenthion and its metabolites were constructed with the i.s. method by measuring peak heights vs concentrations. Good linearities were achieved in the 0−10 mg/kg range, with correlation coefficients between 0.9992 and 0.9995.

Sunlight Photodegradation Experiments. One-milliliter portions of a ca. 5 mg/kg fenthion solution in acetone and a volume of wax extract in chloroform (the appropriate amount to reach the same concentration as on the fruit surface) were poured into beakers (25 mL) and evaporated at ambient temperature (one night in the dark). The beakers were exposed to direct sunlight and removed at prefixed intervals from the sunlight. The residue contained in the beaker was dissolved with 1 mL of an acetone/petroleum ether mixture (1:1 v/v) containing triphenyl phosphate as the internal standard (i.s.) and injected for analysis. The experiments were carried out in three replicates.

RESULTS AND DISCUSSION

Mature fruits were used for the extraction of epicuticular waxes. Waxes extracted from oranges, nectarines, and olives were 41, 7, and 510 $\mu g/cm^2$, respectively. During the photodegradation tests, fenthion was exposed to sunlight in the presence of a film of wax equal to that found on the surface of the fruits. The fenthion decay rate was calculated as pseudo-first-order kinetics, and the half-lives ($t_{1/2}$) and correlation coefficients (r) are reported in Table 1. In control samples exposed to sunlight, fenthion showed a half-life of 4.8 h (Table 1). This value is higher than that calculated in a previous work (Minelli et al., 1996). This difference could be ascribed to the different seasonal conditions in which the experiments were carried out: one in the winter and the other in the summer. Photodegradation kinetics depend on irradiance (Choudhry and Barrie Webster, 1985); therefore, when the experiments are carried out in summer, faster decay rates are observed. Experiments performed with orange and nectarine waxes at 41 and 7 $\mu g/cm^2$, respectively, exposing fenthion to sunlight, showed the same decay rate ($t_{1/2}$ = 3.8 and 3.9 h, respectively), i.e. lower than the value showed by the control samples (4.8 h). In the experiment performed with olive wax, fenthion showed a slow decay rate, ca. 2.5 times lower than the control. Since the quantity of wax on the surface of olives was remarkably higher than that for the other two fruits, we hypothesized that it could filter sunlight more efficiently and determine a lower decay rate. To check this hypothesis, experiments were performed using 10 times as much orange and

Table 2. Residues (mg/kg) of Fenthion and Its Metabolites II and III in Orange, Nectarine, and Olive Wax Extracts after Exposure to Direct Sunlight

time (h)	wax ($\mu g/cm^2$)	fenthion (mg/kg)	fenthion sulfoxide (mg/kg)	fenthion sulfone (mg/kg)	sum (fenthion equiv)
			Control		
0	0	4.49	0	0	4.49
2		3.24	0.61	0	3.82
4		3.16	0.74	0	3.85
6		2.34	0.86	0	3.15
9		1.41	1.25	0	2.59
			Orange Wax		
0	41	4.16	0.22	0.00	4.37
2		3.04	0.92	0.00	3.91
4		1.91	1.74	0.00	3.55
6		1.36	2.20	0.02	3.45
9		0.95	1.58	0.02	2.46
0	410	4.16	0.17	0.00	4.32
2		2.73	0.46	0.03	3.19
4		1.24	1.82	0.05	3.00
6		0.54	2.21	0.07	2.69
9		0.45	1.69	0.07	2.10
			Nectarine Wax		
0	7	4.74	0.20	0.00	4.93
2		3.84	0.42	0.00	4.24
4		2.85	1.28	0.01	4.07
6		1.75	2.43	0.05	4.08
9		1.21	2.93	0.07	4.05
0	70	4.71	0.16	0.00	4.86
2		2.79	1.65	0.00	4.35
4		1.51	3.04	0.05	4.43
6		0.73	3.78	0.10	4.39
9		0.48	4.12	0.10	4.45
			Olive Wax		
0	510	4.97	0.00	0.00	4.97
9		2.82	1.26	0.00	4.01
18		1.73	2.24	0.00	3.85
27		0.82	3.16	0.00	3.81
36		0.57	3.29	0.00	3.68
45		0.38	3.53	0.03	3.75

nectarine wax as was used in the initial experiments. In these experiments, a slight increase in decay rates was observed for both fruit waxes. This indicates that the absorption of solar radiation that is responsible for the photodegradation of fenthion is affected by wax composition and not by film thickness.

The analytical method that was used (Cabras et al., 1993) allowed the simultaneous determination of fenthion and five of its metabolites and degradation products (fenthion sulfoxide, fenthion sulfone, fenoxon, fenoxon sulfoxide, and fenoxon sulfone, Figure 1). Table 2 shows the data relating to residues of fenthion, its transformation products after exposure to sunlight, and the total residue expressed as fenthion. The photodegradation of fenthion in all experiments yielded only two transformation products, fenthion sulfoxide and fenthion sulfone, but their amounts were remarkably affected by the type of wax exposed to sunlight. In control samples, after exposure for 9 h, fenthion yielded only fenthion sulfoxide (1.25 mg/kg) which represented 26% of the initial fenthion. Since 31% of initial fenthion was undegraded, more than 40% of the active ingredient was transformed into other unknown products.

During the solvent evaporation step (one night in the dark) using orange wax at a fruit concentration (41 $\mu g/cm^2$), the transformation of fenthion yielded 0.22 mg/(kg of fenthion sulfoxide). After exposition for 6 h, fenthion sulfoxide reached its highest concentration (2.20 mg/kg), which was 2.5 times higher than the control, and decreased subsequently. This indicates that there are some compounds in orange wax capable of

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of catalyzing the transformation of fenthion into fenthion sulfoxide. This is confirmed by the fact that a small amount of fenthion sulfoxide is produced during the evaporation of the solvent in the dark. Fenthion sulfone was only detected at low concentrations (0.02 mg/kg) after 6 h, and it remained unchanged. At the end of the experiment, the same number of unknown products of fenthion photodegradation was observed as with the control. On increasing the wax concentration 10-fold, an analogous behavior in the formation of fenthion sulfoxide was observed, while the decay rate of fenthion and the quantity of formed fenthion sulfone increased.

The same behavior was observed during the experiments carried out with nectarine wax. Fenthion degradation began at the solvent evaporation step, yielding the same amount of fenthion sulfoxide. After exposure to sunlight, fenthion was almost stoichiometrically transformed into fenthion sulfoxide and fenthion, considering that the average coefficient of variation (CV) was 10%.

In olive wax, the photodegradation of fenthion yields about 80% of fenthion sulfoxide. At the end of the experiment, only when fenthion was 92% degraded, fenthion sulfone was present in low quantities (0.03 mg/kg).

CONCLUSIONS

The presence of wax on the fruit surface affects the photodegradation process of fenthion. The decay rate of the active ingredient and the degradation pathway were both changed. The amount of wax per cm^2 showed a negligible effect on the fenthion decay rate and on the degradation products. The degradative kinetics of fenthion increases in the presence of orange and nectarine waxes, while it decreases when olive wax is used. To explain this different behavior, it can be hypothesized that the radiations from the sun, responsible of the photodegradation of fenthion, are adsorbed by olive waxe; this causes a decrease of their intensity and, therefore, of the speed of photodegradation. In the nectarine and orange waxe, in contrast, the presence of catalysts is responsible for the increased rate of the photodegradation.

In all waxes, the transformation of fenthion produces mainly fenthion sulfoxide and low amounts of fenthion sulfone, but their quantities depend on the type of wax exposed. In orange wax, fenthion sulfoxide forms faster than in the control, and more than 40% of the initial fenthion is transformed into unknown compounds. In nectarine wax, fenthion is degraded stoichiometrically into fenthion sulfoxide and fenthion sulfone. In olive wax, about 80% of the photodegradation of fenthion was into fenthion sulfoxide. The kinetic data obtained in laboratory cannot be directly correlated to those obtained in the field (Cabras et al., 1993; Minelli et al., 1996), and this depends on the fact that residues penetrate also into the cuticle. They have, however, similar trends with half-times of fenthion for oranges being much lower than those for olives. The data on wax can provide information on the photodegradation rate of molecules in different fruits and on the amounts of photoproducts obtained. In this regard, the progress of studies on pesticide photodegradation in the cuticle and in wax will hopefully allow us to make predictions on the photodegradation rate of pesticides in different fruits.

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