# Influence of Epicuticular Waxes on the Photolysis of Pirimicarb in the Solid Phase

Filippo M. Pirisi,* Alberto Angioni, Maddalena Cabizza, Paolo Cabras, and Elisabetta Maccioni

Dipartimento di Tossicologia dell'Università di Cagliari, viale Diaz 182, I-09126 Cagliari, Italy

The influence of epicuticular waxes extracted from different fruits on the photodegradation of pirimicarb (**I**) in the solid phase was studied. Waxes were extracted with $CHCl_3$ and $CHCl_3/CH_3OH$ from nectarines (N), oranges ($O_R$), and mandarin oranges (M). All of the waxes affect the qualitative behavior of the photodegradation of **I**: the formation of photoproducts *N*-formylpirimicarb (**II**) and demethylpirimicarb (**III**) was hindered. This influence was found to be independent of the light sources (sunlight or lamp > 290 nm) and of the solvents employed in the extraction of the waxes. The photodegradation rate ($K_{obs}$) of **I** was reduced to a different extent by the presence of waxes, from N and O, and was increased from M (irrespective of the extraction solvent). The photodegradation rates of **II** and **III** were both reduced by all waxes, M included. The waxes extracted with $CHCl_3/CH_3OH$ show a higher inhibition effect on $K_{obs}$ than those with $CHCl_3$. The scales of rate reduction were similar under sunlight and artificial light. Inhibition of the photodegradation rate does not correlate with UV absorbance of waxes or with their content on the surface of the fruits.

**Keywords:** *Pirimicarb; photodegradation; waxes; fruits*

## INTRODUCTION

Mixtures of different apolar organic molecules (free fatty acids, alcohols, aldehydes, ketones, esters, hydrocarbons, etc.) that are present on the surface of leaves and fruits are commonly named "waxes" (Bianchi, 1995). Therefore, if pesticides are sprayed on the fruits (and leaves), depending on their lipophilicity, their molecules diffuse into the waxes and then into the cuticle of the fruits (Riederer and Schreiber, 1995). When fruits are sprayed with a pesticide and then irradiated with light, only the $\lambda$ that penetrates the waxes can hit the pesticide molecules. This hypothesis could account for the discrepancies between the reduced sunlight photodegradation rate of pirimicarb (**I**, Figure 1) on field-sprayed nectarines ($t_{1/2} \approx 10$ days; Cabras et al., 1995) and the rate found in the kinetic study of its photodegradation ($t_{1/2} = 32$ min; Pirisi et al., 1996) if we hypothesize the waxes to act as a light filter. However, we have recently found an increase in the photodegradation rate to sunlight (compared to a blank) of fenthion, when exposed in the presence of waxes extracted from nectarines [*Persica laevis* DC. (N)] and other fruits (Cabras et al., 1997). Moreover in nectarines field-sprayed with **I**, its well-known photoproducts, *N*-formylpirimicarb (**II**) and demethylpirimicarb (**III**), have not been found. In the photodegradation of fenthion, in the presence of waxes N, the photoproduct concentration ratio was found modified with respect to a blank. Therefore, the same wax (N) seems to affect the qualitative behaviors of photodegradation of pirimicarb and fenthion. With this last pesticide, different waxes show an opposite effect on the photodegradation rates.

To try to understand these discrepancies, we here study the photodegradation of **I** and its photocompounds in the presence of the waxes extracted with $CHCl_3$ and $CHCl_3/CH_3OH$ from nectarines (N), oranges [*Citrus sinensis* L. ($O_R$)], and mandarin orangess [*Citrus reticulata* L. (M)]. The study was performed under sunlight and under light from lamps with $\lambda > 290$ nm.

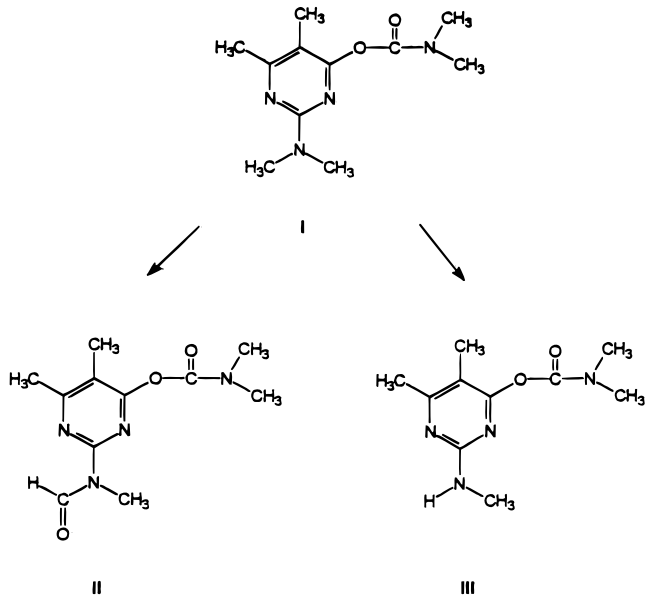


**Figure 1.** Pirimicarb and its photoproducts.

## EXPERIMENTAL PROCEDURES

**Chemicals.** Pirimicarb and its photoproducts were analytical standards (>99%) kindly supplied by ICI Italia (Milan, Italy). Phthalimide (as internal standard, >98.5%) and $J_2$ bisublimate (>98%) were purchased from Fluka (Buchs, Switzerland) and from Carlo Erba (Milan), respectively. Acetonitrile, chloroform, and methyl alcohol were HPLC grade solvents; diethyl ether and petroleum ether (bp 40−60 °C) were

* Author to whom correspondence should be addressed (e-mail pcabras@unica.it).

S0021-8561(97)00529-3 CCC: $15.00 
Published on Web 01/22/1998

**Table 1. Pseudo-First-Order Rate Constants ($K_{obs}$) and $t_{1/2}$ for Photodegradation in the Solid Phase, in the Presence of Waxes and under Different Lights, of Pirimicarb**

| | $CHCl_3$ | | | | $CHCl_3/CH_3OH$ | | | |
|---|---|---|---|---|---|---|---|---|
| fruit | wax ($\mu g/cm^2$) | $K_{obs}$ ($\times 10^{-4}$ $s^{-1}$) | $t_{1/2}$ (min) | W/B[a] | wax ($\mu g/cm^2$) | $K_{obs}$ ($\times 10^{-4}$ $s^{-1}$) | $t_{1/2}$ (min) | W/B[a] |
| | | | | Lamp $\lambda > 290$ nm | | | | |
| blank | 0 | 1.9 | 60 | | 0 | 1.9 | 60 | |
| N | 70 | 0.5 | 222 | 3.7 | 680 | 0.26 | 449 | 7.5 |
| N | | | | | 1360 | 0.34 | 340 | 5.7 |
| N | | | | | 2720 | 0.60 | 207 | 3.46 |
| $O_R$ | 130 | 1.2 | 94 | 1.6 | 130 | 0.54 | 214 | 3.6 |
| M | 66 | 3.2 | 35 | 0.6 | 130 | 3.1 | 37 | 0.6 |
| | | | | Sunlight | | | | |
| blank | 0 | 3.7 | 32 | | 0 | 3.7 | 32 | |
| N | 70 | 0.7 | 168 | 5.3 | 680 | 0.35 | 331 | 10.0 |
| $O_R$ | 130 | 2.5 | 46 | 1.4 | 130 | 1.8 | 64 | 2.0 |
| M | 66 | 4.6 | 25 | 0.8 | 130 | 7.4 | 15 | 0.5 |

[a] W/B = $t_{1/2}$ ratio between wax and blank experiments.

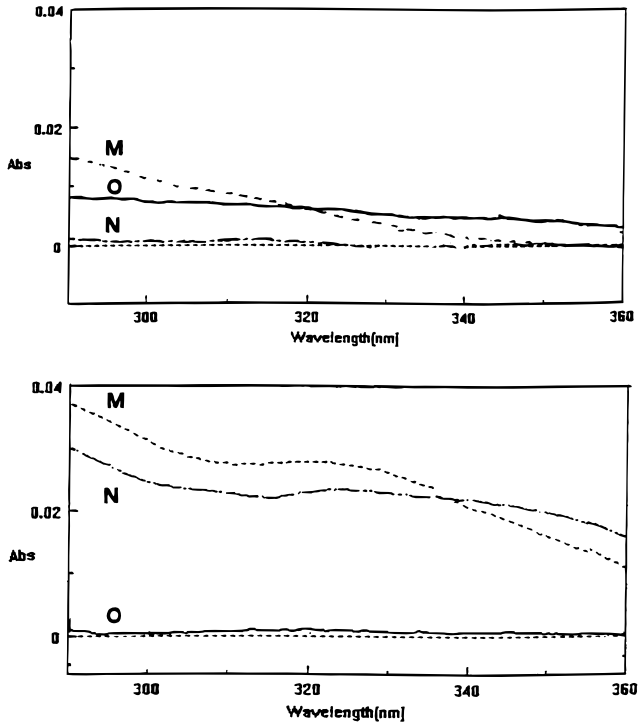


**Figure 2.** UV absorbances of waxes from $CHCl_3$ (top) and $CHCl_3/CH_3OH$ (bottom).

analytical grade reagents (all from Carlo Erba, Milan). Water was bidistilled and purified with a MilliQ apparatus (Millipore, Milan) before use. The phosphate buffer at pH 7 was prepared as described elsewhere (Pirisi et al., 1996). Analytical silica gel plates (thickness = 0.2 mm) were from Merck (Darmstadt, Germany).

**Apparatus.** *High-Pressure Liquid Chromatography.* A Varian 5020 pump (Varian, Palo Alto, CA) provided with an HP 1050 autosampler (Hewlett-Packard, Avondale, CA; loop 100 $\mu$L) was used. The system was connected with a variable-wavelength detector diode array LC-235 ($\lambda = 235$ nm) equipped with an LC-100 reporting integrator (both from Perkin-Elmer, Newark, CT). HPLC columns were $C_8$ Spherisorb, 250 × 4.6 mm i.d., 5 $\mu$m (Waddinxveen, The Netherlands). The mobile phase was $CH_3CN$/pH 7 phosphate buffer at 30:70 (v/v), and the flow rate was 1.0 mL/min.

*UV Spectra.* Spectra of waxes were recorded directly in the extraction solvents at the concentrations found on fruit surfaces (Table 1) by a Varian DM-90 spectrophotometer in the range 290−400 nm (Figure 2).

**Chromatography.** The calculation of the concentration in the chromatograms was made according to the internal standard method (i.s., phthalimide, 5.0 ppm) by plotting the peak height ratio (compound/i.s.) vs concentration. The correlation value of the compounds/i.s. calibration curves was −0.9998. TLC analysis of waxes was made on silica gel plates eluted with a mixture of petroleum ether/diethyl ether (80:20 v/v).

**Extraction Procedure of Fruit Waxes.** Extractions were performed with two different solvents: $CHCl_3$ and a mixture of $CHCl_3/CH_3OH$ (1:1 v/v). Waxes were extracted from the fruits following the method of McDonald (1993). The quantity of wax in the solution was determined by evaporation of 10 mL of extracts. Four fruits of the same ripening degree were measured with a caliper and their surface area determined. The quantity of wax (micrograms per square centimeter) was calculated from these data.

**Light Sources.** A high-pressure mercury lamp (125 W; Helios Italquartz, Milan; $I_\lambda = 3.9 \times 10^{-7}$ $EL^{-1}$ $s^{-1}$ ) with a water-cooled Pyrex jacket was used in laboratory experiments. The natural sunlight experiments were carried out between May and June 1996, at 39° 12′ latitude N and 9° 07′ longitude E from the Greenwich meridian. The average solar actinic irradiance in this period was taken from Choudhry and Webster (1985).

**Irradiation.** In all experiments nonirradiated samples were held in the dark as controls. Each experiment was replicated four times. The samples were prepared as follows. An appropriate aliquot of wax solutions in $CHCl_3$ or $CHCl_3/CH_3OH$ mixture (calculated in such a way as to reach the amount found in the fruit surface) was placed into 2.0-mL borosilicate screw-capped vials; to the mixture, we added 0.5 mL of a solution in $CH_3OH$ of studied compounds to reach the concentration of 2.0 ppm in 1 mL. The solvent was then evaporated with a gentle stream of nitrogen, and the vials were capped and placed into a black cylinder containing the lamp. In the outdoor experiments the vials were exposed directly in a tray. The blanks were prepared from the $CH_3OH$ solutions of compounds in vials lacking in waxes. With the wax N (from $CHCl_3/CH_3OH$) were performed two experiments (lamp > 290 nm) with amounts 2 and 4 times that of the fruit surface.

At selected times one vial was withdrawn, frozen at −25 °C for 10 min, and taken up with 1.0 mL of water containing the internal standard just before the HPLC analysis. The sample was injected without any further preparation.

**Kinetics.** The kinetics were followed by HPLC analysis. The constant rate of disappearance of **I**, **II**, and **III** ($K_{obs}$) was calculated as pseudo first-order rate constants by

$$C = C_0 \, e^{-kt} \qquad (1)$$

## RESULTS AND DISCUSSION

**Quantitative Behavior.** The values of $K_{obs}$ shown in Tables 1 and 2 have a CV ranging between 7.5 and

**Table 2. $K_{obs}$ and $t_{1/2}$ for the Photodegradation of II and III under Lamp $\lambda > 290$ nm in the Presence of Waxes Extracted with $CHCl_3/CH_3OH$**

| | nectarines | | | | mandarin | | | |
|---|---|---|---|---|---|---|---|---|
| compd | wax (mg/cm$^2$) | $k_{obs}$ ($\times 10^{-4}$ s$^{-1}$) | $t_{1/2}$ (min) | W/B[a] | wax (mg/cm$^2$) | $k_{obs}$ ($\times 10^{-4}$ s$^{-1}$) | $t_{1/2}$ (min) | W/B[a] |
| **II**[b] | 0 | 0.15 | 785 | | 0 | 0.15 | 785 | |
| **II** | 680 | 0.04 | 2802 | 3.6 | 130 | 0.02 | 6160 | 7.8 |
| **III**[b] | 0 | 1.7 | 68 | | 0 | 1.7 | 68 | |
| **III** | 680 | 0.37 | 313 | 4.6 | 130 | 0.95 | 121 | 1.8 |

[a] W/B = $t_{1/2}$ ratio between wax and blank experiments. [b] Data from Pirisi et al. (1996).

10.5%. The statistical data were calculated by a computer program (Microsoft Excel 5). The correlation values of $K_{obs}$ ranged between −0.9985 and 0.9650.

As shown in Table 1, the amounts of waxes extracted from the surfaces of fruits were very different according to the extraction capacity of the solvents (Riederer and Schreiber, 1995). Nevertheless, the UV spectra of waxes in the range 290−400 nm (Figure 2) showed reduced and similar absorbances for N, M, and $O_R$ in both extraction solvents.

The waxes from N and $O_R$ (Table 1), compared to a blank, always slow the $K_{obs}$ of the photodegradation of **I**, independent of light sources and the wax amounts calculated on the surface of the fruits. On the contrary, the waxes from M increase the rate of photodegradation.

Indeed, (1) waxes from $CHCl_3/CH_3OH$ are slower by ~2-fold compared to wax from $CHCl_3$; (2) the slower rate cannot be correlated to UV absorbances or to the amount of waxes on the surface of fruits and, in the case of N, is not dependent on the amount of wax employed; and (3) the slower rate seems to be dependent on the nature of the wax. Indeed, both under the lamp > 290 nm and under sunlight, the slowing down occurs in the same order, independent of the extraction solvent, as follows:

$$N > O_R > \text{blank} > M$$

The (slowed) photodegradation rates under sunlight (Table 1) are higher than those under the lamp. This appears to be correct since, during the experiments, the average solar actinic diurnal irradiance [interpolated from the data of Choudhry and Webster (1985)] was 0.99 millieinstein cm$^{-2}$ day$^{-1}$, i.e., about 2.5 times higher than the $I_\lambda$ of the lamp.

**Behavior of Photodegradation.** The irradiation of **I** in the solid phase produces the photocompounds **II** and **III** with a parallel kinetic process (Pirisi et al., 1996). However, in all experiments performed in the presence of waxes, the signals of **II** and **III** have never been recorded in the HPLC chromatograms. In this connection two hypotheses can be proposed:

1. When formed from **I**, compounds **II** and **III** underwent a further kinetic consecutive photodegradation with a much higher rate than that of their formation. In this case the theory of consecutive kinetic process could explain the absence of **II** and **III** in the chromatograms (Frost and Pearson, 1961).

2. The photodegradation of **I** in the presence of waxes follows a different behavior with unknown compounds and **II** and **III** were not produced.

Experiments performed with **II** and **III** irradiated individually under the lamp and in the presence of waxes N and M (extracted with $CHCl_3/CH_3OH$) showed a slowing down in $K_{obs}$, with respect to blank, for both compounds by both waxes (Table 2). The increase in $t_{1/2}$ for **II** was 3.6-fold (N wax) and 7.8-fold (M wax). For **III** these values were 4.6 (N) and 1.8 (M). Therefore, if **I** gives **II** and **III**, they should be detected. Consequently, in these conditions **I** does not give **II** and **III**, and its photodegradation in the presence of all waxes employed in this work follows an unknown behavior to compounds undetectable by HPLC.

## CONCLUSIONS

The experiments performed in the presence of 2 and 4 times the amount of waxes indicate that the inhibition effect on $K_{obs}$ is not dose-dependent. Consequently, the effect of the waxes cannot be the result of a mere light intensity reduction at the pesticide molecules.

The influence on the qualitative photochemical behavior of **I** in the solid phase leading to **II** and **III** (Pirisi et al., 1996) is modified by the waxes. Furthermore, in these conditions, the photodegradation of **I** gave compounds undetectable by HPLC and no signals appear in the chromatograms. Therefore, we are unable to propose a mechanism for the behavior in the presence of waxes. However, since the radical •OH was considered to be the promoter of the formylation and N-demethylation of **I** (Mazellier et al., 1997), the waxes from N and $O_R$ should play the role of "scavengers" of this radical.

Nevertheless, the opposite quantitative effect shown by the wax M on the photodegradation rate of **I** compared to that of **II** and **III** should also indicate different mechanisms in relation to the chemical structure of pesticides and of its photocompounds. This finding seems to be in accordance with the behavior of fenthion (Cabras et al., 1997).

Perhaps, in the wax from M an indirect photodegradation occurs (Choudhry and Webster, 1985), promoted by a chemical photosensitizer that is present only there. Indeed, by TLC, a fraction with $R_f = 0.77$ (Figure 3), attributable to wax esters (Hamilton, 1995), was found only in wax M. This could account for the rate increase. Of course, this speculation cannot be made for the photodegradation mechanism of **II** and **III**, since their photodegradation, $K_{obs}$, is always slower than in the blanks. We are at present carrying out the separation on a semipreparative scale of the fractions of waxes in our laboratories to check their individual influence on the photodegradation rate of **I** and to elucidate their chemical composition.

Photodegradation $t_{1/2}$ values to sunlight found here in the presence of wax N (331 min) are much faster than that in field-sprayed nectarines (≈10 days; Cabras et al., 1995). It is unfortunate that the model system used here cannot be comparable with a fruit, but we suppose such differences should be ascribed to the pesticide's penetrating the cuticles. Therefore, photodegradation trials in the presence of the cuticles separated from the

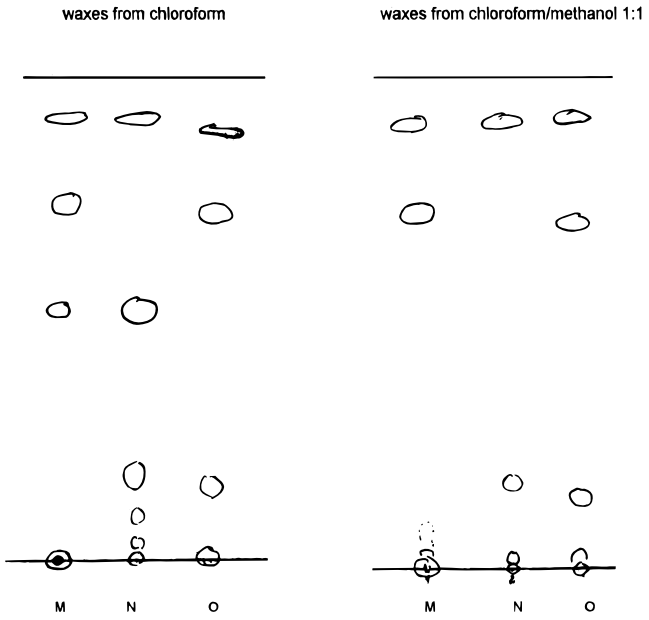


**Figure 3.** TLC of waxes on silica gel. See Experimental Procedures for elution conditions and abbreviations.

fruits should be performed. These trials are in progress in our laboratories. Furthermore, the data found here confirm the trend of waxes N to slow down and to affect both the rate and behavior of pirimicarb photodegradation. Finally, this study and that on fenthion (Cabras et al., 1997) suggest the need to carry out laboratory studies on the photodegradation of pesticides also with waxes. Indeed, this component of fruits and leaves affects the qualitative and quantitative behavior of the fate of the pesticides in the environment.

LITERATURE CITED

Bianchi, G. Plant waxes. In *Waxes: Chemistry, Molecular Biology and Functions*; Hamilton, R. J., Ed.; The Oily Press: Dundee, Scotland, 1995; Vol. 6, pp 175−222.

Cabras, P.; Melis, M.; Cabitza, F.; Cubeddu, M.; Spanedda, L. Persistence of Pirimicarb in Peaches and Nectarines. *J. Agric. Food Chem*. **1995**, *43*, 2279−2282.

Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Minelli, E. V. The effect of epicuticular waxes of fruits on the photodegradation of fenthion. *J. Agric. Food Chem*. **1997**, *45*, 3681−3683.

Choudhry, G. G.; Webster, G. R. B. Protocol guidelines for the investigation of photochemical fate of pesticides in water, air, and soils. *Residue Rev*. **1985**, *96*, 79−136.

Frost, A. A.; Pearson, R. G. *Kinetics and Mechanisms*; Wiley: New York, 1961; pp 166−178.

Hamilton, R. J. Analysis of waxes. In *Waxes: Chemistry, Molecular Biology and Functions*; Hamilton, R. J., Ed.; The Oily Press: Dundee, Scotland, 1995; Vol. 6, pp 311−342.

Mazellier, P.; Jirkovsky, J.; Bolte, M. Degradation of Diuron Photoinduced by Iron(III) in Aqueous Solution. *Pestic. Sci*. **1997**, *49*, 259−267.

McDonald, R. E.; Nordby, H. E.; McCollum, T. G. Epicuticular wax morphology and composition are related to grapefruit chilling injury. *Hortic. Sci*. **1993**, *28*, 311−312.

Pirisi, F. M.; Cabras, P.; Garau, V. L.; Melis, M.; Secchi, E. Photodegradation of pesticides. Photolysis rates and half-life of pirimicarb and its metabolites in reactions in water and in solid phase. *J. Agric. Food Chem*. **1996**, *44*, 2417−2422.

Riederer, M.; Schreiber, L. Waxes. The transport barriers of plant cuticles. In *Waxes: Chemistry, Molecular Biology and Functions*; Hamilton, R. J., Ed.; The Oily Press: Dundee, Scotland, 1995; Vol. 6, pp 131−156.

Received for review June 20, 1997. Revised manuscript received September 17, 1997. Accepted November 1, 1997.

JF970529O