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Effect of the Epicuticular Waxes of Fruits and Vegetables on the Photodegradation of Rotenone

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The effect of epicuticular waxes extracted from fruits (apple, nectarine, pear, and plum) and vegetables (tomato and eggplant) on the photodegradation of rotenone was studied. The waxes affected the decay rate and the degradation pathway of this botanical insecticide. Tomato, nectarine, and plum waxes decreased the photodegradation rate compared to controls, whereas apple and pear waxes increased it. Rotenone irradiated under sunlight without waxes gave seven photoproducts; in contrast, in the presence of waxes it changed its behavior, leading to different pathways according to the wax employed. The main photoproduct formed was 12aβ-rotenolone.

KEYWORDS: Rotenone; epicuticular wax; photodegradation; fruit; vegetable

INTRODUCTION

The amount of nonsystemic pesticides, once deposited on fruits, decreases mainly by evaporation, thermodegradation, codistillation, or photodegradation (*1*). Sunlight is the main agent responsible for botanical pesticides' degradation. After treatment, pesticides spread into the epicuticular waxes present on fruits and vegetables (*2*). Epicuticular waxes are a mixture of long-chain compounds such as hydrocarbons, ketones, alcohols, aldehydes, and free and esterified fatty acids (*3*). Their composition and quantity vary in the different vegetable species and in the same species may differ according to fruit maturation, stage of growth, weather, and environmental conditions.

As shown in previous papers (*4*−*6*) epicuticular waxes can affect the photodegradation rate and the photoproducts that are formed.

Pesticides' decay rates can be increased, decreased, or unchanged by waxes; the wax composition of the different fruits and vegetables affects both the photodegradation and the nature of photoproducts by directing degradation to one pathway or another, whereas the amounts of wax do not interfere with the photodegradation (*4*).

Rotenone is a botanical insecticide obtained from the roots of many tropical plants of the genera *Derris*, *Tephrosia*, and *Lonchocharpus* and is employed for protection of many horticultural and fruit crops. The determination of residues on some crops (lettuce, tomato, and olive) showed that the half-life ($t_{1/2}$) of rotenone was 0.9, 3.6, and 4.0 days, respectively (*7*, *8*).

In a previous paper it was reported that at 50 °C rotenone was not affected by evaporation and thermodegradation (*8*). Because photodegradation is one of the main factors of natural product degradation, one possible cause of these different decay rates can be due to the variability of epicuticular waxes. Cheng et al. (*9*) studied rotenone photodegradation in different solvent solutions and in the solid state; they identified the main photoproducts obtained after exposure to sunlight (**Figure 1**). To our knowledge, no specific investigation has been performed to assess the effects of epicuticular waxes of fruits and vegetables on the decay rate and photodegradation products of this insecticide.

The aim of this work is the evaluation of the effects of epicuticular waxes extracted from four types of fruits (plum, nectarine, apple, and pear) and two vegetables (tomato and eggplant) on the photodegradation rate of rotenone and their influence on the nature of the photocompounds. In this work an HPLC method for the determination of rotenone and its photoproducts was developed.

EXPERIMENTAL PROCEDURES

Extraction Procedure of Epicuticular Waxes. Fruits (plums, nectarines, apples, and pears) and vegetables (tomatoes and eggplants) were harvested at commercial ripening. Wax extraction was performed as described by McDonald et al. (*10*) by immersion of the fruits and vegetables in chloroform. The quantity of wax (micrograms per sqaure centimer) on the fruit surface was determined by evaporation of 10 mL of chloroform extract to dryness and by calculating the surface area of the fruits. A fruit's surface was calculated by wrapping the fruit with aluminum paper: the paper used was then weighed. Thus, knowing the weight of 1 cm^2 of the aluminum paper used, it was easy to calculate the surface.

Chemicals. Rotenone was an analytical standard purchased from Sigma (purity = 95−98%). Photoproducts 12aα-rotenolone (M3b), 6′,7′-epoxyrotenone (M2), and dehydrorotenone (M4) were standards kindly donated by Prof. J. Casida (University of California at Berkeley). The 12aβ-rotenolone (M3), not available as a commercial standard, was obtained from rotenone by synthesis according to the method of Crombie and Godin (*11*). Chloroform (Carlo Erba), acetonitrile (Merck), and water were of HPLC grade. Stock standard solutions of rotenone and its photoproducts (∼1000 mg/kg each) were prepared in acetonitrile. Working standard solutions were obtained by dilution with an aceto-

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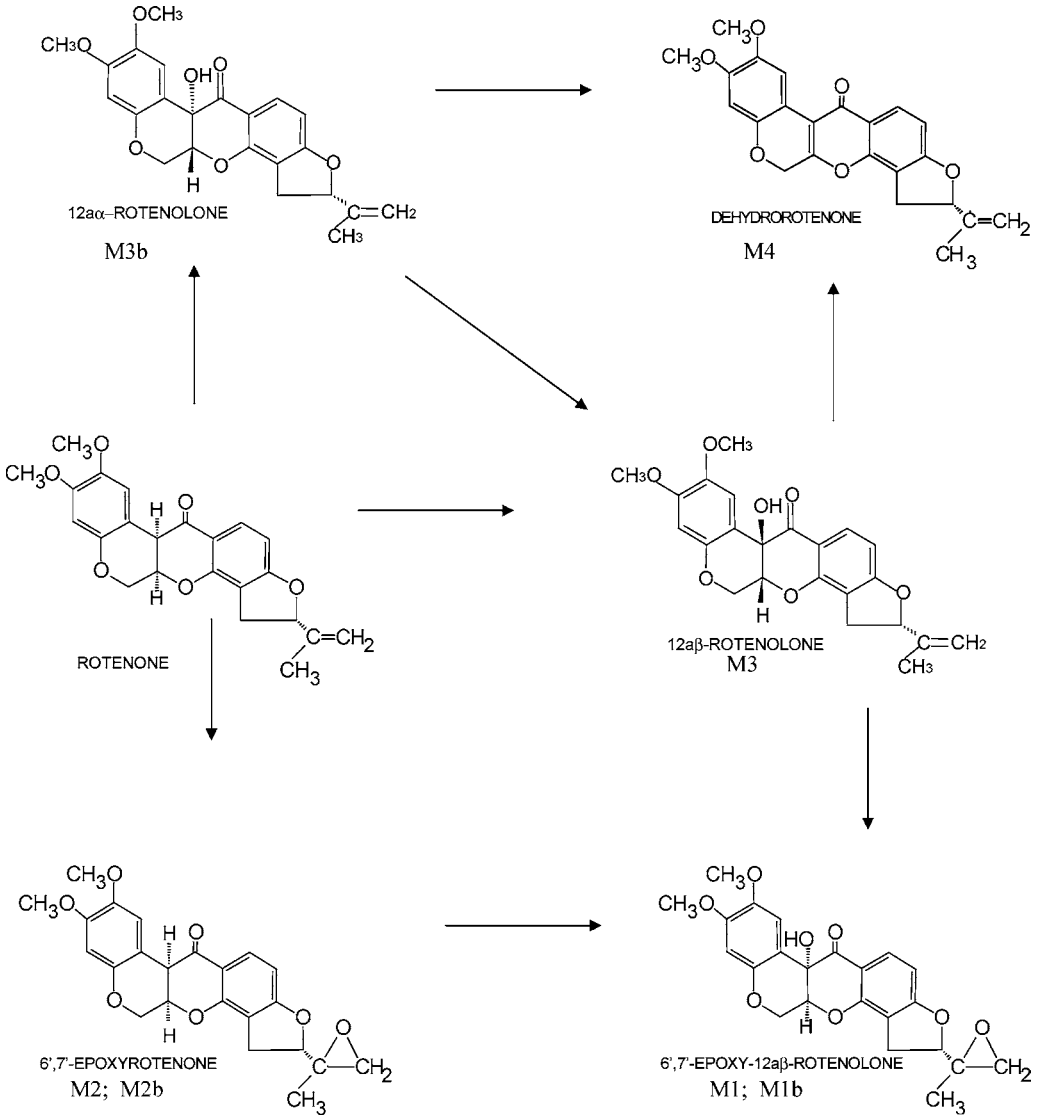

Figure 1. Major products of rotenone photodegradation.

nitrile/water mixture (1:1 v/v). M1 and M1b, not available as commercial standards, were determined as rotenone, whereas M2b was determined as M2.

Apparatus and Chromatography. *HPLC Analysis.* An Agilent Technologies (Waldbronn, Germany) model 1100 liquid chromatograph was used, fitted with a diode array detector (DAD), UV 6000 LP (ThermoQuest, San Jose, CA). A Waters Spherisorb S5 ODS 2 (250 × 4.6 mm, 5 μm) column was employed. A linear gradient was used for the separation of rotenone and its photoproducts: initial mobile phase, acetonitrile/water (50:50; v/v), reaching 85:15 (v/v) in 14 min. Before each injection, the LC system had to be stabilized for 8 min with an acetonitrile/water mobile phase (50:50; v/v). The injection volume was 20 μL, and the flow rate was 1 mL/min. The analysis was performed at a wavelength of 295 nm according to a maximum reported in the spectrum.

LC-MS Analysis. An HPLC system (Shimadzu, Milan, Italy) equipped with an SPD M10 Avp DAD detector, a SIL 10 AD vp auto injector, a LC 10 AD binary pump, coupled on line with a MS 2010 mass spectrometer (Shimadzu), was employed. UV and MS data were acquired and processed using Shimadzu LC-MS solution software. The column used was a 150 × 2.1 mm i.d., 3.5 μm, Waters Symmetry C18. The injection volume was 20 μL, and the flow rate was 0.4 mL/min. UV detection was by absorbance at 295 nm. MS conditions were as follows: APCI (±) source; probe, 400 °C; CDL, 270 °C; block, 300 °C; flow gas, N_2 at 2.5 L/min; probe voltage, 4 kV.

Sunlight Photodegradation Experiments. Portions of rotenone solution in acetonitrile and a volume of wax extract in chloroform (the appropriate volume to reach the same concentration as on the fruit and vegetable surface) were poured into Petri dishes of 5 cm diameter (~20 cm^2) and evaporated at ambient temperature (one night in the dark at 25 °C) to have a uniform film on the surface. The dishes were exposed to direct sunlight at 39° 14′ latitude north and 3° 20′ longitude west from the Rome Monte Mario meridian and removed from the sunlight at prefixed intervals (15 min, 30 min, 1 h, and 3 h). The samples were irradiated in August between 10:00 a.m. and 1:00 p.m. During this trial the average daily solar radiation, recorded with an AD-2 automatic weather station SILIMET, (Modena, Italy) was 4398 W/m^2. The residue contained in the dishes was dissolved with 10 mL of an acetonitrile/water mixture and injected for analysis. The experiments were carried out in three replicates.

Statistical Analysis. Analysis of variance (ANOVA) was performed by MSTAT-C software (1991). Mean comparisons were performed by Tukey's test at $P \leq 0.05$, when appropriate.

RESULTS AND DISCUSSION

Photoproduct Analysis. *HPLC Analysis*. The separation of rotenone and its photoproducts was obtained by HPLC with an ODS 2 column with a gradient of acetonitrile/water (50:50; v/v), reaching 85:15 (v/v) in 14 min. These conditions allowed a complete separation of the photoproducts (**Figure 2**). The chromatogram (**Figure 2**) shows two unknown photoproducts with retention times (t_R) of 5.39 min (M1) and 5.51 min (M1b),

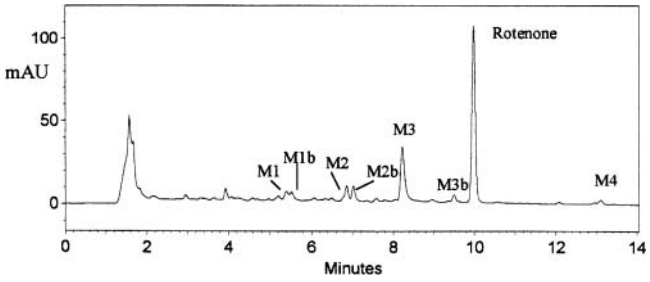

Figure 2. HPLC chromatogram of rotenone and its photoproducts in control.

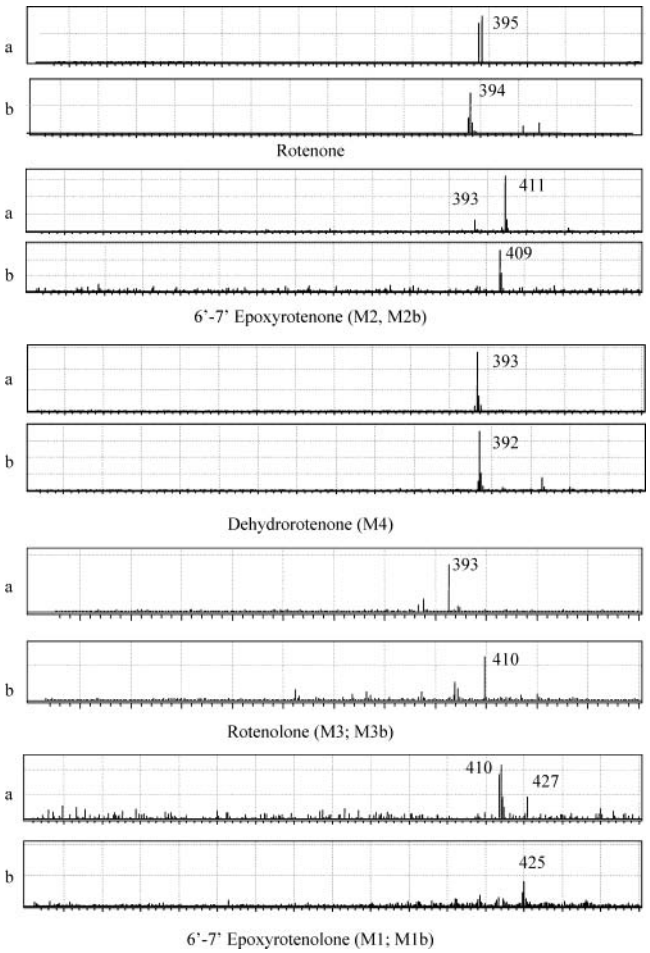

Figure 3. APCI (**a**, positive; **b**, negative) mass spectra of rotenone and its metabolites.

6′,7′-epoxyrotenone (M2) at 6.85 min, an unknown peak (M2b) with the same UV spectrum as M2 at 7.07 min, 12aβ-rotenolone (M3) at 8.22 min, 12aα-rotenolone (M3b) at 9.49 min, and dehydrorotenone (M4) at 13.09 min. Photoproducts M2, M3, M3b, and M4 were confirmed by comparison of their retention times and UV spectra with one of the original standards. A good linearity was obtained in the range of 0.01−10 mg/kg for rotenone and its photoproducts with correlation coefficients between 0.9997 and 0.9999.

LC-MS Analysis. LC-MS analyses were performed by using APCI, in both positive and negative mode. **Figure 3** shows the ions produced by single compounds in the two ionization modes. The behaviors of rotenone and its metabolites were similar. Rotenone and dehydrorotenone did not show [M + 1] ions, as could be expected in APCI positive mode; they showed [M-1] in negative mode due to the high availability of delocalization of the negative charge. α- and β-rotenolone showed in APCI positive mode the loss of water yielding the ion [M + 1(−18)] *m*/*z* 393 and in negative mode the ion [M$^-$] *m*/*z* 410. 6′-7′-Epoxyrotenone (M2) gave *m*/*z* 411 in positive mode and *m*/*z* 409 in negative mode. This behavior was identical for the peak M2b. The peak M1 showed an ion at *m*/*z* 427 in positive mode and at *m*/*z* 425 in negative mode. Moreover, an increase in the probe voltage from 4.0 to 4.5 kV led to a fragmentation of the ion at *m*/*z* 427, giving an ion with *m*/*z* 410 corresponding to a loss of water [M + 1(−18)]. The MS behavior of this compound suggests that it may be a 6′,7′-epoxyrotenolone with MW of 426 amu.

From the data obtained by DAD HPLC and LC-MS and the data from the literature (*9*) it can be assumed that the peak M2b is an isomer of 6′,7′-epoxyrotenone (M2) and that the peaks M1 and M1b are, indeed, two isomers of 6′,7′-epoxyrotenolone.

Table 1. Half-Life ($t_{1/2}$) and Correlation Coefficient (*r*) of Rotenone in Fruit and Vegetable Wax Extracts after Exposure to Direct Sunlight

fruit/vegetable	wax (μg/cm^2)	$t_{1/2}$ (min)	*r*
control without wax	0	38a	–0.960
eggplant	42	44a	–0.922
tomato	124	69b	–0.911
apple	705	22c	–0.982
nectarine	317	51ab	–0.916
pear	737	25c	–0.981
plum	180	59b	–0.922

Photodegradative Kinetics. During the evaporation of chloroform in the dishes (in the dark at room temperature), rotenone began degrading and forming M3. At the time of the exposure to sunlight all samples contained M3 (~0.05 μg/cm^2). The rotenone decay rate was calculated as a pseudo-first-order kinetic. **Table 1** shows half-lives and correlation coefficients of rotenone and wax quantity on the fruit surfaces (micrograms per square centimeter).

The half-life of rotenone irradiated without waxes was on average 38 min. The decay rate of rotenone was not increased by eggplant wax ($t_{1/2}$ = 44 min); it was slower in the presence of nectarine wax ($t_{1/2}$ = 51 min), plum wax ($t_{1/2}$ = 59 min), and tomato wax ($t_{1/2}$ = 69 min), whereas it was faster in the presence of apple ($t_{1/2}$ = 22 min) and pear waxes ($t_{1/2}$ = 25 min). It is remarkable that apple and pear waxes, which increased the decay rate of rotenone, were more abundant on the fruit surfaces (over 700 μg/cm^2).

The different behaviors of rotenone in the presence of various waxes have been previously observed for other pesticides (*4*−*6*). Some compounds present in the waxes probably absorb solar radiations, which are responsible for photodegradation, and may act as a filter responsible of slowing the decay rate. On the other hand, when the decay speed of rotenone is increased, some compounds present in the waxes probably acted as catalysts of photodegradation.

Influence of Wax on the Nature of Photocompounds. Rotenone irradiated under sunlight without waxes (control) formed seven major photoproducts (M1−4), three of which were isomers. Rotenone irradiated in the presence of tomato wax gave the same photoproducts as in the control, whereas with the other waxes formed only some of them (**Figures 4** and **5**). Data are reported in micrograms per square centimeter (**Tables 2** and **3**). Using the ratio of surface to weight determined in fruits and vegetables (eggplant, 1.17 cm^2/g; tomato, 1.80 cm^2/g; apple, 0.99 cm^2/g; nectarine, 0.94 cm^2/g; pear, 1.30 cm^2/g; plum, 1.10 cm^2/g) they can be easily converted to milligrams per kilogram.

Eggplant Waxes. In the presence of eggplant wax photoproducts identical to the control were formed with the exception of

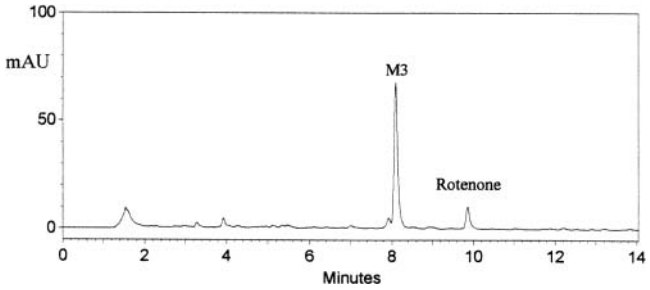

Figure 4. HPLC chromatogram of rotenone and its photoproducts obtained after exposure to sunlight in nectarine waxes.

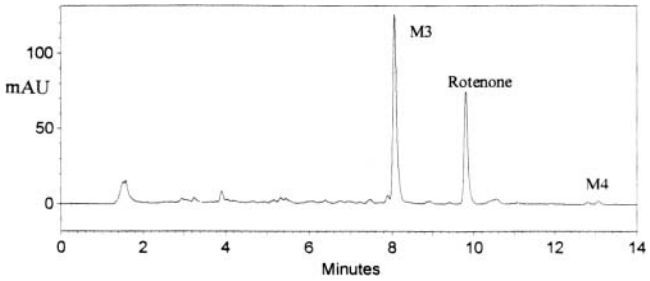

Figure 5. HPLC chromatogram of rotenone and its photoproducts obtained after exposure to sunlight in plum waxes.

Table 2. Residues (Micrograms per Square Centimeter ± SD) of Rotenone and Its Photoproducts in Vegetable Wax Extracts after Exposure to Direct Sunlight

time (min)	M1 + M1b	M2 + M2b	M3	M3b	rotenone	M4
			Control without Wax			
0	nd[a]	nd	0,07 ± 0.00	nd	3.26 ± 0.16	nd
15	0.09 ± 0.00	0.33 ± 0.04	0.57 ± 0.32	0.06 ± 0.00	1.78 ± 0.12	0.06 ± 0.01
30	0.13 ± 0.01	0.41 ± 0.02	0.43 ± 0.05	0.05 ± 0.01	1.30 ± 0.26	0.05 ± 0.01
60	0.13 ± 0.02	0.15 ± 0.01	0.41 ± 0.22	nd	0.39 ± 0.18	0.03 ± 0.00
180	0.09 ± 0.00	nd	0.10 ± 0.01	nd	0.10 ± 0.05	nd
			Eggplant Wax			
0	nd	nd	0.05 ± 0.01	0.02 ± 0.03	4.55 ± 0.07	nd
15	nd	0.13 ± 0.01	0.36 ± 0.01	0.02 ± 0.00	1.51 ± 0.28	0.04 ± 0.00
30	nd	0.14 ± 0.01	0.39 ± 0.04	0.02 ± 0.00	0.96 ± 0.14	0.04 ± 0.00
60	nd	nd	0.34 ± 0.01	nd	0.55 ± 0.08	0.01 ± 0.01
180	nd	nd	0.22 ± 0.07	nd	0.16 ± 0.08	nd
			Tomato Wax			
0	nd	nd	0.05 ± 0.00	nd	4.35 ± 0.02	nd
15	0.07 ± 0.02	0.10 ± 0.00	0.40 ± 0.22	0.01 ± 0.00	3.00 ± 0.15	0.04 ± 0.04
30	0.07 ± 0.01	0.10 ± 0.01	0.65 ± 0.05	0.04 ± 0.01	1.35 ± 0.31	0.09 ± 0.02
60	0.07 ± 0.00	0.10 ± 0.00	0.65 ± 0.04	0.01 ± 0.00	1.05 ± 0.35	0.03 ± 0.01
180	0.07 ± 0.00	0.10 ± 0.01	0.55 ± 0.06	nd	0.60 ± 0.12	0.03 ± 0.01

[a] nd < 0.01.

M1 and M1b (6′,7′-epoxyrotenolone). Photoproducts M2 and M2b (6′,7′-epoxyrotenone) reached their highest concentration in 30 min and then decreased to complete disappearance. M3 (12aβ-rotenolone) was the most abundant photoproduct; it reached its highest concentration in 30 min (0.39 μg/cm^2) and then decreased slowly. The concentration of its isomer M3b (12aα-rotenolone) was, on the contrary, very small and, after 30 min, disappeared completely. M4 (dehydrorotenone) reached its highest concentration between 15 and 30 min and then disappeared completely. Rotenone and M3 were the only compounds present at the end of the experiment.

Tomato Waxes. Rotenone irradiated in the presence of tomato waxes produced seven main photoproducts (M1−4). Photoproducts M1 and M1b (6′,7′-epoxyrotenolone) were produced only in rotenone irradiated without waxes (control) and with tomato waxes, but in this case their level was constant during all experiments. Photoproducts M2 and M2b reached 0.10 μg/cm^2 in 15 min and remained constant up to the end of the experiment, whereas in the control they reached their highest concentration (0.41 μg/cm^2) in 30 min and then decreased, disappearing after 180 min. M3 was the most abundant photoproduct; it reached its highest concentration (0.65 μg/cm^2) between 30 and 60 min and then decreased slowly. Its isomer M3b was produced at very low level, reaching its highest concentration in 30 min and disappearing completely. M4 was produced at a very low level, but it was present until the end of the experiment (0.03 μg/cm^2).

Table 3. Residues (Micrograms per Square Centimeter ± SD) of Rotenone and Its Photoproducts in Fruit Wax Extracts after Exposure to Direct Sunlight

time (min)	M1 + M1b	M2 + M2b	M3	M3b	rotenone	M4
			Apple Wax			
0	nd	nd	0.04 ± 0.01	0.53 ± 0.06	4.22 ± 0.08	nd
15	nd	nd	0.52 ± 0.02	0.18 ± 0.02	1.18 ± 0.08	0.07 ± 0.01
30	nd	nd	0.66 ± 0.03	0.09 ± 0.01	0.60 ± 0.09	0.07 ± 0.01
60	nd	nd	0.59 ± 0.02	0.02 ± 0.01	0.24 ± 0.02	0.05 ± 0.00
180	nd	nd	0.15 ± 0.01	nd	0.01 ± 0.00	nd
			Nectarine Wax			
0	nd	nd	0.02 ± 0.00	nd	2.36 ± 0.22	nd
15	nd	nd	0.58 ± 0.10	nd	0.97 ± 0.12	nd
30	nd	nd	0.60 ± 0.04	nd	0.64 ± 0.06	nd
60	nd	nd	0.50 ± 0.06	nd	0.54 ± 0.05	nd
180	nd	nd	0.22 ± 0.00	nd	0.15 ± 0.01	nd
			Pear Wax			
0	nd	nd	0.05 ± 0.00	nd	3.98 ± 0.09	nd
15	nd	nd	0.48 ± 0.06	nd	1.61 ± 0.08	0.05 ± 0.01
30	nd	nd	0.85 ± 0.01	nd	0.67 ± 0.24	0.08 ± 0.01
60	nd	nd	0.90 ± 0.02	nd	0.31 ± 0.05	0.08 ± 0.00
180	nd	nd	0.52 ± 0.08	nd	0.02 ± 0.01	0.04 ± 0.01
			Plum Wax			
0	nd	nd	0.05 ± 0.01	nd	2.64 ± 0.13	nd
15	nd	nd	0.49 ± 0.07	nd	1.67 ± 0.04	nd
30	nd	nd	0.46 ± 0.07	nd	0.85 ± 0.04	0.04 ± 0.00
60	nd	nd	0.44 ± 0.06	nd	0.63 ± 0.02	nd
180	nd	nd	0.28 ± 0.02	nd	0.26 ± 0.04	nd

[a] nd < 0.01.

Apple Waxes. In the presence of apple wax only three photoproducts were produced, M3, M3b, and M4. M3 was the most abundant photoproduct, and as in the sample irradiated in the presence of tomato waxes, it reached its highest concentration (0.66 μg/cm^2) after 30 min and then decreased. M3b was produced in the presence of apple wax before exposure to sunlight (0.53 μg/cm^2 at time zero); after exposure to sunlight, it decreased, disappearing completely. M4 was produced at very low levels, and at the end of the experiment it was not detectable.

Pear Waxes. In the presence of pear wax only two photoproducts (M3 and M4) were produced. M3 was the most abundant photoproduct; it reached its highest concentration (0.90 μg/cm^2) after 60 min and then decreased slowly. M4 showed low levels, with a behavior similar to that of the sample irradiated in the presence of other fruit and vegetable waxes.

Nectarine and Plum Waxes. M3 was the only photoproduct formed when rotenone was irradiated in the presence of nectarine and plum waxes, with the only exception at the 30 min time point at which, in the presence of plum wax, M4 was formed at very low level (0.04 μg/cm^2). M3 reached the highest concentration between 15 and 30 min.

Conclusions. The data reported by this study show that the presence of waxes on fruit and vegetable surfaces affects both the decay rate of rotenone and its degradation pathway. It was observed that nectarine and plum waxes decrease the photo-

degradation rate compared to controls, whereas apple and pear waxes increase it. Rotenone irradiated under sunlight without wax (control) gave seven photoproducts. With different waxes, in particular, rotenone formed identical photoproproducts, but only in the presence of tomato wax were all photoproducts formed. In contrast, in the presence of the other waxes, rotenone formed only some of the seven photoproducts: in the presence of eggplant wax five photoproducts were formed; in the presence of apple wax, three photoproducts; in the presence of pear wax, two photoproducts; and in the presence of nectarine and plum wax, only one photoproduct was produced. M3 was the main photoproduct produced in all samples with and without waxes. In the presence of pear wax it reached its highest concentration of 0.90 μg/cm^2, which represented 22% of the initial rotenone. In contrast, M3 reached its lowest amount produced with eggplant wax, 0.40 μg/cm^2, which represented 9% of the initial rotenone. Except for tomato wax, which does not seem to affect the production of photoproducts, all of the other waxes changed the behavior of rotenone photodegradation, leading to different pathways.

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