

## Photoprotection by Plant Extracts: A New Ecological Means To Reduce Pesticide Photodegradation

BORIS EYHERAGUIBEL,<sup>†,§</sup> CLAIRE RICHARD,<sup>†,§</sup> GÉRARD LEDOIGT,<sup>‡,¶</sup> AND  
ALEXANDRA TER HALLE<sup>\*,†,§</sup>

<sup>†</sup>Laboratoire de Photochimie Moléculaire et Macromoléculaire, Clermont Université, Université Blaise Pascal, B.P. 10448, F-63000 Clermont-Ferrand, France, <sup>§</sup>CNRS, UMR 6505, LPMM, F-63177 Aubiere, France, <sup>‡</sup>Physique et Physiologie Intégratives de l'Arbre Fruitier et Forestier, Clermont Université, Université Blaise Pascal, B.P. 10448, F-63000 Clermont-Ferrand, France, and <sup>¶</sup>INRA, UMRA 547, PIAF, F-63100 Clermont-Ferrand, France

A pesticide's reactivity toward light at the leaf surface after crop treatment is rarely considered, although such degradation reactions directly affect the pesticide's effectiveness. To overcome these limitations, the use of plant pigments was proposed as a new class of photoprotecting agent. The photoprotecting properties of seven plant pigments were tested under controlled conditions over herbicide sulcotrione. Grape wine extracts were tested over a panel of pesticides from distinct chemical families. The addition of plant extracts almost systematically reduced the pesticide's photoreactivity. The grape wine extracts improve at least by 38% the half-life of photolysis of almost all of the active ingredients tested, except for the herbicide triclopyr. Fustic tree extract increases by 82% the photostability of the herbicide sulcotrione. Plant extracts mainly act as sunscreens; that is, the photostabilization of the active ingredient is due to the competitive energy absorption of UV photon. The use of natural plant extracts is a promising strategy to limit pesticide photodegradation. It is a way to develop sustainable and innovative technology for the plant protection industry, being beneficial from both economic and ecological points of view.

**KEYWORDS:** Photoprotection; photodegradation; plant extract; pesticide; anthocyanins

### INTRODUCTION

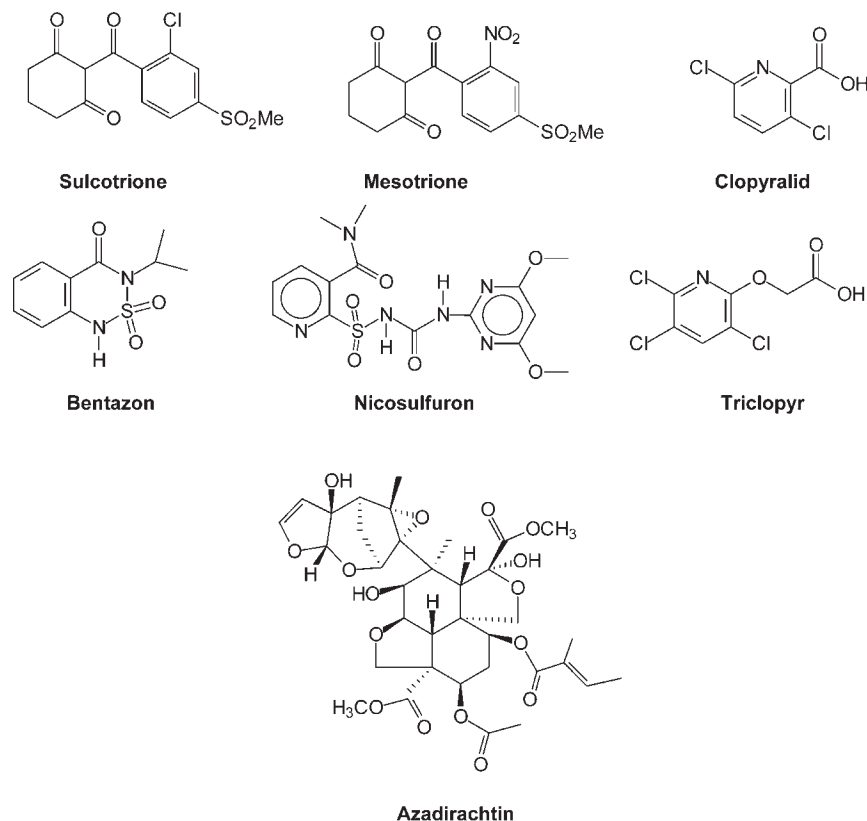
Acknowledgment of the Precautionary Principle has provided a conceptual and legal framework for strengthened evaluation criteria of pesticides (1). As a consequence, pesticides are already some of the most thoroughly evaluated molecules on the market. Furthermore, there has been an effort to develop new pesticides with improved properties that provide significantly reduced usage rates and better selectivity. However, in the past decades only a few new modes of action or novel chemical families have been developed. For instance, 10 new modes of herbicide action were commercialized between 1970 and 1985, whereas only 1 new mode of action (HPPD inhibitors) has been introduced since 1991 (2, 3). Alternatively, development of new formulations offers an economically attractive means for improvement of pesticide efficacy and selectivity by strongly modifying the interactions between pest, pesticide, and crop. In some cases, an optimal adjuvant combination can reduce the effective pesticide dose by as much as 10-fold (4), which obviously represents important progress with regard to environmental impact. Increasing attention is being paid to develop safer and more environmentally friendly pesticide adjuvants (5) such as biological adjuvants based on plant products (6) or waste plant products (7).

After field treatment, volatilization, wash-off, photodegradation, and thermal degradation of the pesticide compete with its transport

to the biological target site, limiting the pesticide's effectiveness. Whereas volatilization and wash-off are estimated and controlled by formulation adjustments (8), photodegradation is often overlooked. Although it was noted about 30 years ago that "a meaningful proportion of pesticides are not stable to sunlight on crops after field application" (9), few photochemical studies on vegetation were subsequently performed (10, 11). The use of sunscreen adjuvants to overcome photodegradation has also presented limitations so far (12). Some are phytotoxic, whereas others are hazardous to the environment (13). Other alternatives, such as the use of organoclays (14), reduce pesticide bioavailability. As plants protect themselves from the damaging effect of sunlight by producing pigments, we proposed the use of these pigments as a new class of photoprotecting agent for phytosanitary formulations (15).

The plant pigments used here are raw hydroalcoholic extracts. Raw extracts are complex mixtures; they contain a large proportion of polyphenols, among which the red anthocyanins have been widely studied. The role of anthocyanins in plant resistance to UV-B stressors relies on three different mechanisms. First, anthocyanins serve as optical filters to reduce photoinhibition (decreased quantum efficiency of photosynthesis when leaves receive more light energy than can be used for photosynthesis) (16). Second, anthocyanins act as sunscreens against damaging UV-B radiation (17). Finally, anthocyanins mitigate photooxidative injury in leaves by efficiently scavenging free radicals and reactive oxygen species (18). Apart from the economic benefit of

\*Author to whom correspondence should be addressed (phone 00 33 4 73 40 71 76; e-mail Alexandra.ter-halle@univ-bpclermont.fr).



**Figure 1.** Structures of the studied active ingredients.

using raw plant extracts rather than purified anthocyanins, the raw mixture also allows intermolecular association, called copigmentation. This enhances anthocyanin stability and also provides a hyperchromic effect (19).

To estimate and characterize the photoprotecting properties of the plant pigments, the photochemical degradation of various pesticides (**Figure 1**) was evaluated under controlled conditions with grape wine extract.

## MATERIALS AND METHODS

**Chemicals.** All solvents and chemicals were used as received. The active ingredients (ai) sulcotrione, mesotrione, bentazon, triclopyr, clopyralid, nicosulfuron, and azadirachtin were analytical standards purchased from Riedel de Haën (Pestanal, Saint-Quentin Fallavier, France). The commercial formulations were obtained from a regular agricultural shop. The formulations used in this study were Mikado (sulcotrione, 300 g L<sup>-1</sup>), Callisto (mesotrione, 100 g L<sup>-1</sup>), Basamais (bentazon, 480 g L<sup>-1</sup>), Garlon Pro (triclopyr, 240 g L<sup>-1</sup>, and clopyralid, 60 g L<sup>-1</sup>), Milagro (nicosulfuron, 40 g L<sup>-1</sup>), and NeemAzal-T/S (azadirachtin A, 1%). Carnauba gray wax (melting point = 82–84 °C) was purchased from Prolabo (VWR, Fontenay-sous-Bois, France). Plant pigments were kindly provided by Couleur de Plant (Rochefort, France) for the *Sorghum bicolor*, *Haematoxylum campechianum*, *Rubia tinctorum*, *Morus tinctoria*, *Isatis tinctoria*, and *Coreopsis tinctoria* hydroalcoholic extracts. *Vitis vinifera* hydroalcoholic extract was provided by Grap'Sud (Cruviers-Lascours, France, lot 08010). Solvents were obtained from Riedel de Haën (methylene chloride, gradient grade, and methanol, HPLC grade). Formic acid (99%), potassium dihydrogenophosphate (99.5%), and disodium hydrogenophosphate (99%) were obtained from Prolabo. Water was purified using a Millipore Milli-Q system (Millipore aQ, resistivity = 18 MΩcm, DOC < 0.1 mg L<sup>-1</sup>).

**Characterization of the Plant Pigments.** Total phenolic contents were determined colorimetrically using the Folin–Ciocalteu reagent, as described by Emmons et al. (20). UV–vis absorption spectra of pigment solution (0.1% in water) were recorded using a Cary 300 Varian spectrophotometer.

**Photochemical Experiments.** Photochemical experiments were conducted in a Suntest CPS photosimulator (Atlas). The procedure for wax film preparation and irradiation has been described previously (21). For every active ingredient, the resulting concentration at the film surface was set within the agricultural recommended rate ranges: sulcotrione, 300 g ha<sup>-1</sup>; mesotrione, 150 g ha<sup>-1</sup>; bentazon, 1200 g ha<sup>-1</sup>; triclopyr, 240 g ha<sup>-1</sup>; clopyralid, 60 g ha<sup>-1</sup>; nicosulfuron, 20 g ha<sup>-1</sup>; azadirachtin, 1200 g ha<sup>-1</sup>. Prior to photolysis experiments, tests were run in the dark; for all of the active ingredients tested in the time scale of the experiment there was no loss. This indicates that there is no thermal degradation, volatilization, or wax penetration of any active ingredient. The loss of active ingredient measured upon irradiation is thus attributed to photodegradation only. The photoprotection experiments were carried out using the same procedure and by adding plant extracts. The ratio of the amount of plant extract over the amount of active ingredient (*R*) was set within the range of 1.5–9. All active ingredient decays followed pseudo-first-order kinetics within the 0–50% of conversion extent. Pseudo-first-order rate constants and half-lives are expressed with errors representing the 95% confidence level.

**HPLC Analyses.** Monitoring of pesticides was determined using a Waters Alliance HPLC system consisting of a separation module 2695 and a Dual λ absorbance detector 2487. A 25 μL aliquot was injected in an Agilent Zorbax SB-CN column of 250 mm length and 4.6 mm i.d.; flow rate = 1 mL min<sup>-1</sup>. The detection wavelength and eluent system were set according to relative properties of each active ingredient. For sulcotrione, mesotrione, bentazon, clopyralid, and triclopyr the detection wavelength was set at 231, 270, 300, 280, and 293 nm, respectively. The mobile phase consisted of 40% acetonitrile and 60% water acidified at pH 2.5 with 3% of formic acid. Azadirachtin was analyzed using a Waters Acquity UPLC system consisting of a binary solvent manager, sample manager, and PDA detector under the following conditions. A 0.7 μL aliquot was injected in a Waters BEH C18 column of 50 mm length and 2.1 mm i.d.; flow rate = 0.5 mL min<sup>-1</sup>. The detection wavelength was set at 217 nm. The mobile phase consisted of 25% acetonitrile, 25% methanol, and 50% water.

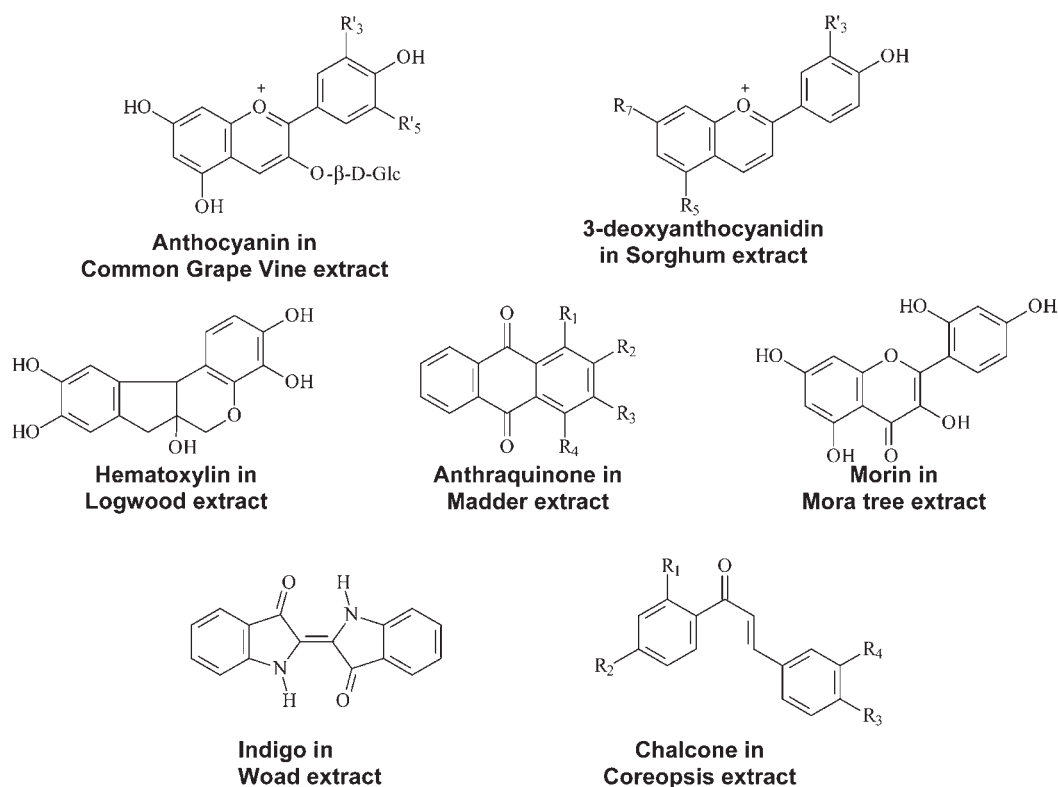
## RESULTS AND DISCUSSION

**Characterization of the Plant Extracts.** Seven plant extracts were characterized and tested for their ability to protect the active

**Table 1.** Characterization of the Plant Extracts (pH and Polyphenol Content)

common name	Latin name	main constituent(s) identified in the extracts	pH <sup>a</sup>	polyphenol content <sup>b</sup> (%)
grape wine	<i>Vitis vinifera</i>	anthocyanin	4.3	66
sorghum	<i>Sorghum bicolor</i>	3-deoxyanthocyanidin	7.0	41
logwood	<i>Haematoxylum campechianum</i>	hematoxylin	6.5	87
madder	<i>Rubia tinctorum</i>	anthraquinone	6.4	8
fustictree	<i>Morus tinctoria</i>	morin and malcurin	5.7	75
woad	<i>Isatis tinctoria</i>	indigo	9.5	4
coreopsis	<i>Coreopsis tinctoria</i>	chalcone	5.2	8

<sup>a</sup> pH of a solution of the plant extract at 100 mg L<sup>-1</sup>. <sup>b</sup> Total polyphenolic content in acid gallic equivalent determined by the Folin–Ciocalteu method in aqueous solution (0.1% m/v).

**Figure 2.** Structures of the main constituents of the plant extracts tested.**Table 2.** Effect of Grape Wine Extract on Pesticide Photodegradation<sup>a</sup>

active ingredient	half-life of photolysis	half-life of photolysis with photoprotection	gain in stability (%)
sulcotrione (300 g ha <sup>-1</sup> )	4 h ± 10 min	6 h 40 min ± 1 h	38
mesotrione (150 g ha <sup>-1</sup> )	36 min ± 1 min	2 h 10 min ± 2 min	72
bentazon (1200 g ha <sup>-1</sup> )	29 h 10 min ± 50 min	58 h 20 min ± 2 h	50
clopyralid (60 g ha <sup>-1</sup> )	30 h ± 4 h	53 h 30 min ± 5 h	45
triclopyr (240 g ha <sup>-1</sup> )	10 h 40 min ± 7 min	10 h 30 min ± 5 min	0
azadirachtin (1200 g ha <sup>-1</sup> )	16 h ± 2 h 30 min	28 h 20 min ± 2 h 30 min	43

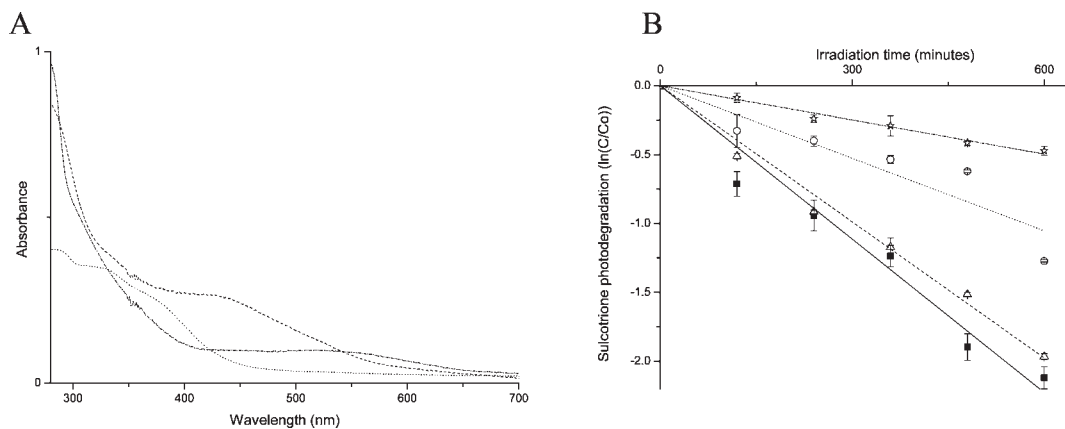
<sup>a</sup> The half-lives of photolysis were measured on wax film irradiated at 500 W m<sup>-2</sup> for the active ingredient alone and with photoprotection. The grape wine extract was added in a ratio of three toward the amount of active ingredient.

ingredients from photodegradation. Their characteristics are summarized in **Table 1**. The extracts came from various plant origins (trees, flowering plants, crop plants) and were mostly obtained by hydroalcoholic extraction. Some extracts present high polyphenol contents and are made of flavonoid-based molecules such as anthocyanins (grape and sorghum extracts) or morin (fustictree extracts). Other extracts contain aromatic organic compounds such as anthraquinones (madder extracts), hematoxylin (logwood extract), or indigo (woad extract) (**Figure 2**).

**Pesticide Photodegradation.** Because the photochemical evaluation of pesticides by direct measurements in the field is

time-consuming, expensive, and subject to weather conditions, laboratory tests were carried out on wax films that mimic the leaf surface. By setting the parameters close to outdoor conditions, we have already proved that the rates of photolysis measured in this model are on the same order as those measured in the field (22).

The photoreactivity of pesticides was assessed on a panel of molecules from separate chemical families (**Figure 1**). Pesticides were systematically used in their commercial formulation rather than pure. As only one commercial formulation is used for each active ingredient, the name of the formulation is not given in the text but under Materials and Methods.



**Figure 3.** (A) Absorption spectra of plant extracts in aqueous solution at 0.1% at pH 3.5: (---) madder extract; (···) coreopsis extract; (- · - ·) grape extract. (B) Sulcotrione rate of photolysis on carnauba wax at  $550 \text{ W m}^{-2}$  deposited at a rate of  $300 \text{ g ha}^{-1} \text{ L}$  (■) sulcotrione without photoprotection; ( $\Delta$ ) with madder extract; ( $\circ$ ) with coreopsis extract; ( $\star$ ) with grape extract. The pigments were added in 3-fold excess compared to sulcotrione.

**Table 3.** Photoprotecting Effect of Various Plant Extracts on Sulcotrione<sup>a</sup>

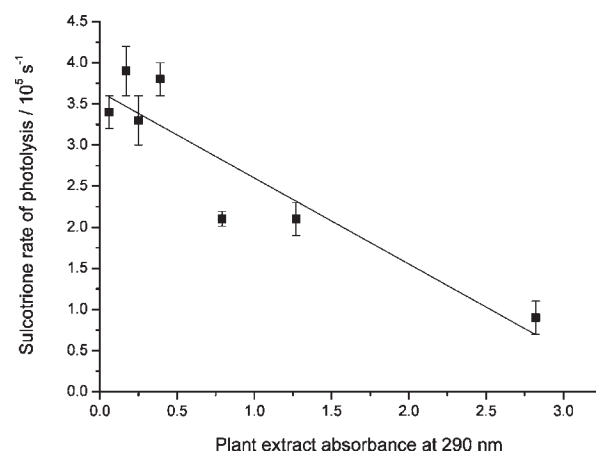
plant extract added	absorbance at 290 nm <sup>b</sup>	half-life of photolysis	gain in stability (%)
none		4 h $\pm$ 6 min	
grape wine	0.79	9 h 10 min $\pm$ 5 min	58
sorghum	0.25	6 h $\pm$ 30 min	34
logwood	1.27	9 h 10 min $\pm$ 5 min	58
madder	0.17	5 h $\pm$ 20 min	22
fustictree	2.82	21 h 20 min $\pm$ 1 h	82
woad	0.06	5 h 40 min $\pm$ 20 min	32
coreopsis	0.39	5 h $\pm$ 20 min	24

<sup>a</sup> Formulated sulcotrione is irradiated on carnauba wax films at  $500 \text{ W m}^{-2}$  at the rate  $720 \text{ g ha}^{-1}$ . Half-lives of photolysis were reported together with the gain in stability (in percentage). The plant extracts were added in a ratio of three toward the amount of sulcotrione; their absorbance at 290 nm is reported. <sup>b</sup> Absorbance of an aqueous solution of the plant extract at 0.1% in water at pH 3.5.

We observed that photoreactivity on wax films is rather important as the half-lives of photolysis were within the range from 30 min to 30 h (Table 2). These results highlight the sensitivity of some active ingredients toward sunlight and the importance of photolysis dissipation on leaf surfaces.

**Photoprotecting Effect of Plant Extracts.** The photoprotecting effect of seven colored plant extracts was evaluated on the wax film model (Figure 3A). The experiments were carried out on the herbicide sulcotrione, as its degradation reaction was well described (21, 22). Plant extracts were added in a 3-fold excess compared to the herbicide sulcotrione. The addition of the plant extract systematically reduced sulcotrione's photoreactivity (Table 3). The photolysis half-life of sulcotrione increased from 4 to 5 h when photoprotected with madder extract and up to 21 h with the fustictree extract, corresponding to gains in photostability of 22 and 82%, respectively (Figure 3B).

The photoprotection of seven pesticides was tested using grape wine extracts. Grape wine extract did not present the highest photoprotecting activity (which was fustictree), but provided a good photostabilization of sulcotrione. Grape wine extract was chosen for its potential industrial application as it is not very expensive. It is obtained from grape pomace, a byproduct of the wine industry. The addition of a 3-fold excess of grape wine extract reduced the photolysis rate of all tested pesticides except for triclopyr, which showed similar half-lives with or without photoprotection. For the other six pesticides, grape wine extract stabilized the active ingredients and increased the photolysis half-life by at least 38%. The best photostabilization was obtained for



**Figure 4.** Correlation between sulcotrione rate of photolysis and absorbance at 290 nm in aqueous solution at pH 3.5 (which is the pH of formulated sulcotrione). Formulated sulcotrione is irradiated on carnauba wax films at  $500 \text{ W m}^{-2}$  at the rate  $720 \text{ g ha}^{-1}$ ; plant extracts were added in a 3-fold excess compared to sulcotrione.

mesotrione, the half-life of photolysis of which was increased by a factor of 4.

To estimate the importance of the plant extract amount on the photoprotecting effect, photochemical tests were carried out with sulcotrione at  $900 \text{ g ha}^{-1}$  and the grape wine extract added in a 3-, 6-, and 9-fold excess. The results indicate that the photostabilization of sulcotrione increases with the amount of grape wine extract. The gain in stability increased from 37 to 53 and 62%.

We assumed that the photoprotecting effect of these plant extracts is mainly due to a light-shielding process. To establish a correlation, we have considered the UV part of the spectrum where photons have higher energy and a stronger impact on pesticide photodegradation. Indeed, sunlight irradiations start at the ground on earth at about 290 nm, where most of the pesticides still absorb. We found a good linear correlation ( $R^2 = 0.82$ ) between the absorption properties of plant extracts at 290 nm and their photoprotecting effects (Figure 4). This correlation demonstrates that the plant extracts certainly act as sunscreens. Some constituents of the plant extract, like the anthocyanins and the polyphenols, are well-known for their antioxidant properties (18); such properties could also contribute to slow the pesticides' degradation by scavenging the free radicals and the reactive oxygen species produced by plants.

**Conclusion.** Natural products have been a potential source of new plant protection applications that match the need to develop sustainable and innovative technology (23). We discovered that the use of natural plant extracts can be a very promising strategy to protect active ingredients from photodegradation. In the next step it is important to assess the plant pigment phytotoxicity and to verify that their use does not modify the bioavailability of the pesticides. Work is in progress to evaluate if the use of these photoprotecting agents can allow reduction of the pesticide applied dose with a maintained efficacy. These plant extracts presenting a photoprotecting activity could be used as adjuvants for pesticides, or they can also be added to the formulated pesticide prior to application (tank-mix adjuvant). The use of photoprotection can lower the recommended applied doses; it would be beneficial from both economic and ecological points of view.

#### LITERATURE CITED

- (1) Coelho, S. European pesticide rules promote resistance. *Science* **2009**, *323*, 450.
- (2) Beaudegnies, R.; Edmunds, A. J. F.; Fraser, T. E. M.; Hall, R. G.; Hawkes, T. R.; Mitchell, G.; Schaetzer, J.; Wendeborn, S.; Wibley, J. Herbicidal 4-hydroxyphenylpyruvate dioxygenase inhibitors – a review of the triketone chemistry story from a Syngenta perspective. *Bioorg. Med. Chem.* **2009**, *17*, 4134–4152.
- (3) Ruegg, W. T.; Quadranti, M.; Zoschke, A. Herbicide research and development: challenges and opportunities. *Weed Res.* **2007**, *47*, 271–275.
- (4) Green, J. Adjuvant outlook for pesticides. *Pestic. Outlook* **2000**, *11*, 196–199.
- (5) Knowles, A. Recent developments of safer formulations of agrochemicals. *Environmentalist* **2008**, *28*, 35–44.
- (6) Lebo, S. E. Method for producing improved biological pesticides. WO 1995033378, 1995.
- (7) Ryckaert, B.; Spanoghe, P.; Heremans, B.; Haesaert, G.; Steurbaut, W. Possibilities to use tank-mix adjuvants for better fungicide spreading on triticale ears. *J. Agric. Food Chem.* **2008**, *56*, 8041–8044.
- (8) Katagi, T. Surfactant effects on environmental behavior of pesticides. *Rev. Environ. Contam. Toxicol.* **2008**, *194*, 71–177.
- (9) Rabson, R.; Plimmer, J. R. Photoalteration of pesticides. *Science* **1973**, *180*, 1204–1205.
- (10) Caboni, P.; Sarais, G.; Angioni, A.; Garcia, A. J.; Lai, F.; Dedola, F.; Cabras, P. Residues and persistence of neem formulations on strawberry after field treatment. *J. Agric. Food Chem.* **2006**, *54*, 10026–10032.
- (11) Angioni, A.; Dedola, F.; Minelli, E., V.; Barra, A.; Cabras, P.; Caboni, P. Residues and half-life times of pyrethrins on peaches after field treatments. *J. Agric. Food Chem.* **2005**, *53*, 4059–4063.
- (12) Minello, E. V.; Lai, F.; Zonchello, M. T.; Melis, M.; Russo, M.; Cabras, P. Effect of sunscreen and antioxidant on the stability of pyrethrin formulations. *J. Agric. Food Chem.* **2005**, *53*, 8302–8305.
- (13) Wakefield, G.; Park, B. G.; Lipscomp, S.; Holland, E. Surface-doped particles of TiO<sub>2</sub> or ZnO and their use. WO 2005072680, 2005.
- (14) Si, Y.; Zhou, J.; Chen, H.; Zhou, D. Photostabilization of the herbicide bensulfuron-methyl by using organoclays. *Chemosphere* **2004**, *54*, 943–950.
- (15) ter Halle, A.; Richard, C.; Ledoigt, G. Phytosanitary formulations containing plant extracts as photoprotectants for pesticides. WO 2008090476, 2008.
- (16) Gould, K. S. Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. *J. Biomed. Biotechnol.* **2004**, 314–320.
- (17) Brandt, K.; Giannini, A.; Lercari, B. Photomorphogenic responses to UV radiation III: A comparative study of UVB effects on anthocyanin and flavonoid accumulation in wild-type and aurea mutant of tomato (*Lycopersicon esculentum* Mill.). *Photochem. Photobiol.* **1995**, *62*, 1081–1087.
- (18) Rice-Evans, C. A.; Miller, J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152–159.
- (19) Mazza, G.; Brouillard, R. The mechanism of copigmentation of anthocyanins in aqueous-solutions. *Phytochemistry* **1990**, *29*, 1097–1102.
- (20) Emmons, C. L.; Peterson, D. M.; Paul, G. L. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant activity and contents of phenolic and tocol antioxidants. *J. Agric. Food Chem.* **1999**, *47*, 4894–4898.
- (21) ter Halle, A.; Drncova, D.; Richard, C. Phototransformation of the herbicide sulcotrione on maize cuticular wax. *Environ. Sci. Technol.* **2006**, *40*, 2989–2995.
- (22) ter Halle, A.; Piquet, A.; Richard, C. An actual scenario that demonstrates sulcotrione photodegradation on maize leaves after spraying. *Environ. Chem.* **2007**, *4*, 256–259.
- (23) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorgan. Med. Chem.* **2009**, *17*, 4022–4034.

---

Received for review May 10, 2010. Revised manuscript received July 8, 2010. Accepted July 12, 2010.