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Is It Possible To Increase the Aloin Content of *Aloe vera* by the Use of Ultraviolet Light?

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ABSTRACT: In this paper, the effects of ultraviolet (UV) treatments on the aloin content of *Aloe vera* L. gel have been analyzed. UV-A treatment to *A. vera* plants for 36 days led to an increase in the aloin concentration in gel, rind tissue, and latex, while a decrease in chlorophylls *a* and *b* occurred in the photosynthetic tissue as a consequence of UV treatment. The growth of *Penicillium digitatum* and *Botrytis cinerea* (artificially inoculated on the leaf surface) was drastically decreased in UV-A-treated leaves, which could be attributed to the increase in the aloin concentration by the UV-A treatment. In addition, UV-C treatment to detached leaves also led to an increase in the gel aloin concentration, at higher levels than occurred with UV-A treatment, although leaves showed severe lesions after 48 h of treatment.

KEYWORDS: *Aloe vera, aloin, UV treatment, antifungal activity*

INTRODUCTION

Aloe vera (*Aloe barbadensis* Miller) is a traditional medicinal plant and actually being used in food, pharmaceutical, and cosmetic industries.[1] *A. vera* leaves are formed by two distinct layers: the green outer leaf rind (epidermis covered with cuticle and chlorenchyma cells) and the soft colorless gel inner parenchyma and the yellow latex, which drips from canals (the so-called aloin cells) located just below the rind.[2,3] Thus, the *A. vera* industry produces different commercial raw materials, such as yellow latex, gel, and whole leaf extract. The chemical composition of the gel is very complex, composed mainly by polysaccharides (acemannan and a wide variety of polysaccharides forming the cell wall matrix) and soluble sugars, followed by proteins, many of which are enzymes, amino acids, vitamins, and anthraquinones,[4−6] with concentrations being different depending upon the leave size and growth stage.[7]

There is an increasing interest for the use of *A. vera* gel in the food industry, being used as a resource of functional foods in drinks, beverages, and ice creams,[6,8] because evidence exist on the beneficial health properties after ingestion of *A. vera* gel, such as antitumoral, anti-infection, anti-inflammatory, and antidiabetes, among others.[6,9] On the other hand, *A. vera* gel has been proposed as a new and valuable edible coating,[10] with benefits for preserving postharvest quality and safety of fruits, such as sweet cherry and table grape.[11−13] In addition, it has been shown that *A. vera* gel has antimicrobial properties, because nectarine coated with *A. vera* gel and artificially inoculated with several fungi (*Penicillium digitatum*, *Botrytis cinerea*, and *Rhizopus stolonifer*) developed lower decay and reduced the postharvest ripening process.[14] This antifungal activity of *A. vera* gel has also been shown in *in vitro* tests by reducing the mycelium growth, with the efficacy being higher for *P. digitatum* than for *B. cinerea*.[15] In this report, preharvest application of *A. vera* gel was also effective in reducing table grape microbial spoilage at harvest and during prolonged storage and led to a reduction of the parameters related to ripening.

Anthraquinones, among other moieties, are generally used as key components for the quality control of this plant and its derivatives. The most important anthraquinone is aloin (10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10*H*)-anthracenone), which is found in nature as a mixture of two diastereosiomers, aloin A (10*S*) and aloin B (10*R*). Aloin is synthesized in the plastids of the assimilating tissue, released to the apoplast through exocytosis, and finally stored in the aloin cell, adjacent to the vascular bundle sheath cell.[16] Thus, aloin is the major phenolic compound in *A. vera* leaves, and it is mainly contained in the bitter, smelly exudates seeping out from freshly cut leaves, while very low amounts of aloin exist in the gel.[17] However, variations in gel composition and especially in the aloin concentration have been found among several *Aloe* species, with the concentration increasing from winter to summer, which was attributed to the increase in the temperature and light photoperiod.[18] In the above comparative study, the aloin level in *A. vera* gel was very low compared to other *Aloe* species, such as *Aloe ferox*, *Aloe mitriformis*, and *Aloe saponaria*, with these species being the most effective at inhibiting the growth rate of spores from *B. cinerea*, *P. digitatum*, *Penicillium expansum*, and *Penicillium italicum*, previously inoculated on the leaf.

However, *A. vera* is the *Aloe* species most cultivated worldwide, and its gel is the most produced and commercialized, with potential application as pre- or postharvest treatment to preserve fruit quality and reduce decay,[14,15] with these antifungal properties being attributed to its aloin concentration.[18] During the past decade, it has been shown that light

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irradiation could be a good strategy for increasing the commercial and nutritional values of vegetable products.[19] In fact, ultraviolet (UV)-B radiation is an important regulator of plant secondary metabolism, leading to increases in phenolic compounds, carotenoids, and glucosinolates, among others.[20] Specifically, UV-B radiation (6 h per day for 20 days) increased the total anthraquinone content, barbaloin content, and aloe-emondin content in *A. vera* leaves by 31.8, 11.3, and 22.0%, respectively.[21] Thus, the overall objective of this work was to increase the aloin concentration in *A. vera* leaves through UV treatments as an elicitor, to obtain *A. vera* gel with higher antifungal activity to be applied as pre- or postharvest treatment to preserve fruit quality.

MATERIALS AND METHODS

Experimental Design. The experiments were carried out using *A. vera* plants grown under organic farming at the High Polytechnic School of Orihuela, Alicante, Spain. In the first experiment, 10 plants from this farm (3 years old) were transplanted to 50 L pots containing soil from the organic farm compost and left at field conditions 30 days previous to start of the experiments. Half of the *Aloe* plants were kept in a chamber room (2 × 2.5 × 4 m) with natural light coming through three windows (1 × 1.5 m). The other half was kept in a similar chamber but with 2 UV-A lights (wavelength of 365 nm, Phillips TL-D, 18 W) at 1.2 m above the plants. The UV-A light regime was set up at 15 min per hour during the whole experiment. The treatments started on Jan 10, 2011 (winter season). At 4 day intervals, one external leaf from each pot was picked at the early morning (9:00 a.m.), by cutting their point of attachment with a sharp knife. Leaves were washed with tap water to remove the adhering soil particles and deposited inside a container for 1 h to permit the efflux of the yellow latex. Then, color (in both lower and upper epidermis) was measured. For each leaf, the spikes placed along their margins were removed before longitudinally slicing to separate the rind from the inner leaf gel. The gel filets were crushed to yield a mucilaginous gel, which was filtered to discard the fibrous fraction. The gel yield was expressed as a percentage of the obtained gel with respect to the whole leaf weight. Similarly, the rind (epidermis and sub-epidermis tissues) was milled and homogenized. In both, fresh gel and rind, pH, total soluble solids (TSS, °Brix), and total acidity (TA) were analyzed, while chlorophylls were quantified in the rind tissue. Finally, aloin was quantified in latex, rind, and gel.

In addition, after 14 and 28 days of UV-A treatments, 5 freshly harvested leaves were picked (1 leaf from each plant), in which *P. digitatum* and *B. cinerea* spores were inoculated. For each leaf, 8 injuries (2 × 2 mm in length and width and 2 mm in depth) were performed along the leaf surface with a sterile lancet for fungi inoculation. Four of these injuries were inoculated with *P. digitatum*, and the other four were inoculated with *B. cinerea*, by depositing 20 μL containing 100 spores of the corresponding fungi stock. Leaves were stored in a controlled chamber at 20 °C with normal light, and after 5 days, the injury dimension was evaluated by measuring the external and depth diameters of the injury, the infection volume was calculated, and then the respiration rate and ethylene production were determined in each leaf.

For the second experiment, 45 external leaves were cut from 15 plants grown on the farm. A total of 20 leaves were treated continuously with UV-C lamp (Osram Sylvania G15T8, 15 W) at 254 nm; another 20 leaves served as the control (treated with white lamp, Lumilux 15 W, Osram); and 5 leaves were used as day 0. For both treatments, samples of 5 leaves were taken after 24, 48, 96, and 168 h, in which the external color (hue angle), gel yield, and gel aloin concentration were determined.

Fungus Strains. The fungi used in this study were *P. digitatum* CECT 2954 and *B. cinerea* CECT 2100, purchased from the Spanish Type Culture Collection (CECT) and routinely cultured on potato dextrose agar (PDA). The fungi spores were collected and diluted with sterile water until 2500 colony forming units (CFU) mL^{-1} and used as stock.

Color, pH, TSS, and TA. Color was determined at three points along the leaf in both lower and upper epidermis using a Minolta colorimeter (Minolta, Oxaca, Japan). After recording L^*, a^*, and b^* parameters, color was expressed as the hue angle (arctangent b^*/a^*) and results were the mean ± standard error (SE). TSS was determined for each leaf (rind and gel) in duplicate with a digital refractometer Atago PR-101 (Atago Co., Ltd., Japan) at 20 °C, and results were the mean ± SE and expressed as g 100 g^{-1}. The pH of the rind or gel was recorded. Then, TA was determined in duplicate by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL of distilled H_2O, and results were the means ± SE and expressed as g of malic acid equivalent 100 g^{-1}.

Chlorophyll Determination. Chlorophyll *a* and *b* measurements were made in duplicate on the rind tissue from each leaf by extraction in 85% acetone.[22] Absorbance was read at 664 and 647 nm, using an Uvikon XS spectrophotometer (Bio-Tek Instruments, Winooski, VT). Results were expressed as mg g^{-1} on a fresh weight basis.

Aloin Concentration. The protocol described by Zapata et al.[18] was used for aloin quantification. Yellow latex (0.1 g) and gel or rind (5 g) were homogenized in 25 mL of methanol (50%, v/v) containing 2 mL of 0.1 N HCl and 5 mM NaF using a Polytron at 9500 rpm for 2 min. After homogenization, samples were sonicated at 10 °C for 60 min and then centrifuged at 20000*g* for 15 min. The supernatant was filtered through a 0.45 μm Millipore filter, and then 10 μL was injected into a high-performance liquid chromatography with diode-array detection (HPLC−DAD, Hewlett-Packard HPLC-1100 Series) system equipped with a C_{18} column (Supelcogel C-610H, 30 cm × 7.8 mm, Supelco, Inc., Bellefonte, PA). Aloin A was eluted isocratically by methanol/water as the mobile phase (64:36, v/v, containing 0.5% formic acid) at a flow rate of 1 mL min^{-1} and detected at 254 nm wavelength. A calibration curve was performed using aloin A (barbaloin) standard (Sigma, Madrid, Spain) at concentrations ranging from 0 to 100 mg L^{-1} ($y = 4.71x + 4.25$; $R^2 = 0.9979$). Results were expressed as mg 100 g^{-1} of fresh weight.

Respiration and Ethylene Production Rates. CO_2 and ethylene productions were measured by placing each leaf in 5 L glass jars hermetically sealed with a rubber stopper for 1 h. A total of 1 mL of the holder atmosphere was withdrawn with a gas syringe, and ethylene was quantified using a Hewlett-Packard model 5890A gas chromatograph (Wilmington, DE) equipped with a flame ionization detector and a 3 m stainless-steel column with an inner diameter of 3.5 mm containing activated alumina of 80−100 mesh. The column temperature was 90 °C, and injector and detector temperatures were 150 °C. Results were the mean ± SE and expressed as nL g^{-1} h^{-1}. For respiration rate determination, another sample of 1 mL of the same atmosphere was withdrawn and CO_2-quantified using a Shimadzu 14A gas chromatograph (Kyoto, Japan) with a thermal conductivity detector and a molecular sieve 5A column, 80−100 mesh (Carbosieve SII, Supelco, Inc., Bellefonte, PA), of 2 m length and 3 mm inner diameter. Oven and injector temperatures were 50 and 110 °C, respectively. Helium was used as the carrier gas at a flow rate of 50 mL min^{-1}. Results were the mean ± SE and expressed as mg of CO_2 kg^{-1} h^{-1}.

Statistical Analysis. Data from analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were treatment and time. Mean comparisons were performed using the high significant difference (HSD) Tukey's test to examine if differences were significant at $p < 0.05$ and are shown in tables and figures with significant letters. All analyses were performed with Statistical Products and Service Solutions (SPSS) software package version 11.0 for Windows.

RESULTS AND DISCUSSION

Experiment 1. The measurement of color at both upper and lower epidermis showed no significant differences between treated and control leaves along the experiment, with color coordinates at day 0 being $L^* = 42.36 \pm 0.41$, $a^* = -12.59 \pm$

0.29, and b^* = 20.49 ± 0.52 for the upper epidermis and L^* = 45.40 ± 3.45, a^* = −12.79 ± 0.84, and b^* = 19.68 ± 0.82 for the lower epidermis. However, when chlorophylls (*a* and *b*) were analyzed in the rind tissue, a significant reduction was observed in UV-A-treated leaves. Thus, at day 0, chlorophyll *a* and *b* concentrations were 11.66 ± 0.52 and 3.42 ± 0.41 mg g^{-1}, respectively, and decreased until 9.48 ± 0.31 and 2.26 ± 0.17 mg g^{-1} in treated leaves, while no significant changes were observed in control leaves (Figure 1). This is in agreement with

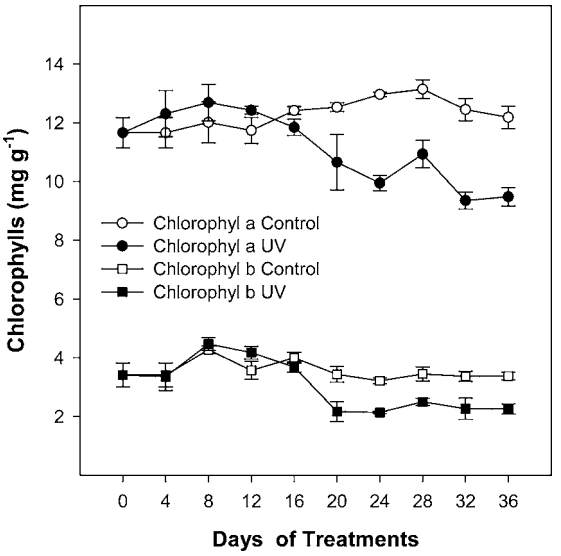

Figure 1. Chlorophyll *a* and *b* concentrations in *A. vera* rind tissue from leaves in the control or treated with UV-A. Data are the mean ± SE (n = 5).

data reported for grapes treated with UV-C during postharvest storage at 22 °C, in which decreases in chlorophyll *b* were observed.[23] The effect of UV treatments on decreasing chlorophyll *a* and *b* concentrations has also been reported for a wide range of plant species, and it is due to a breakdown of the structural integrity of chloroplasts.[24,25]

Initial yield gel (day 0) was 68.1 ± 0.4% and slightly increased in control leaves, reaching final levels of 71.6 ± 0.7%, while in those treated with UV-A, a slight decrease in the gel yield was obtained (66.5 ± 0.4%). Taking into account the overall data collected of the gel yield for all sampling dates, UV-A treatment led to a significant decrease in gel production (66.6 ± 0.4%) compared to the control (69.1 ± 0.5%). In any case, the reported gel yields could be considered appropriated for commercial purposes, because values were in the same range of previous reports, in which yields of ≅65% were obtained from *A. vera* leaves harvested along different growing seasons.[18]

TSS, expressed as °Brix, was 2.86 ± 0.04 and 1.25 ± 0.08 for rind and gel at day 0, respectively, and did not significantly change along the experiment in either control or treated leaves (data not shown). On the other hand, TA in the rind decreased along the experiment, in both control and UV-A-treated leaves, although levels were significantly higher for control samples. Contrarily, the treatment with UV-A did not significantly change the TA levels in the gel (Figure 2). Because *A. vera* has the crassulacean acid metabolism (CAM) photosynthesis pathway, malic acid accumulation in the vacuole occurs at night, leading to an increase in TA. At sunrise, acids are decarboxylated, with the released CO_2 being fixed in the Calvin cycle, and TA decreases as the day proceeds. The decrease

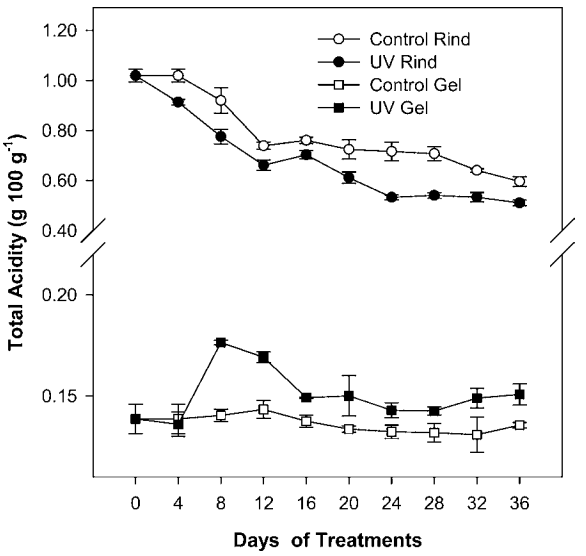

Figure 2. TA in *A. vera* gel and rind tissue from leaves in the control or treated with UV-A. Data are the mean ± SE (n = 5).

could be attributed to the fact that sunrise occurred earlier during the experimental period, from January to February.

Moreover, the lower TA on the gel from UV-A-treated leaves compared to those of control leaves could be due to a lower capacity to accumulate acid in the vacuole during the night period and, in turn, to a lower net photosynthesis. In fact, a decrease in photosynthesis (3−90%), particularly at higher UV-B doses, has been reported in a wide range of plant species, because of both direct (effect on photosystem and CO_2 fixation) and indirect (decrease in pigments and leaf area) effects.[24]

Aloin was determined in the yellow latex, rind, and gel, and its concentration was 200-fold higher in the latex than in the rind or gel. However, aloin increased in all samples as a consequence of the UV-A treatment to *A. vera* plants (Figure 3). Thus, initial aloin was 220 ± 22, 1.39 ± 0.10, and 0.58 ± 0.29 mg 100 g^{-1} for latex, rind, and gel, respectively, and increased up to ≅800, 5.8, and 2.5 mg 100 g^{-1} at the end of the experiment. This increase in the aloin concentration in *A. vera* leaves of treated plants could be attributed to a defense mechanism of plants to tolerate the UV radiation, as reported for other secondary metabolites, including phenolics and flavonoids in a wide range of plant species.[24,25] However, up to date, most studies have been performed using UV-B treatment, at either pre- or postharvest treatments, which showed that the increased phenolic accumulation was due to the enhancement of the activity of the enzymes involved in the phenylpropanoid and flavonoid pathways.[20] In fresh medicinal plant tissues, short-term UV-A and UV-B radiations stimulated the production of secondary metabolites, such as iridoids, and in turn improved their pharmacological properties.[26] When both UV lights are compared, UV-B showed higher activity upon increasing secondary metabolites and the corresponding antioxidant power.

After 14 and 28 days of UV-A light treatment to *A. vera* plants, leaves were picked, transferred to normal light, and inoculated with *P. digitatum* and *B. cinerea*. Results showed that the growth of both fungi species on the leaf surface was drastically decreased in those leaves previously treated with UV-A, with the infection volume being 5−6-fold lower in UV-A-treated leaves than control leaves after 5 days of spore

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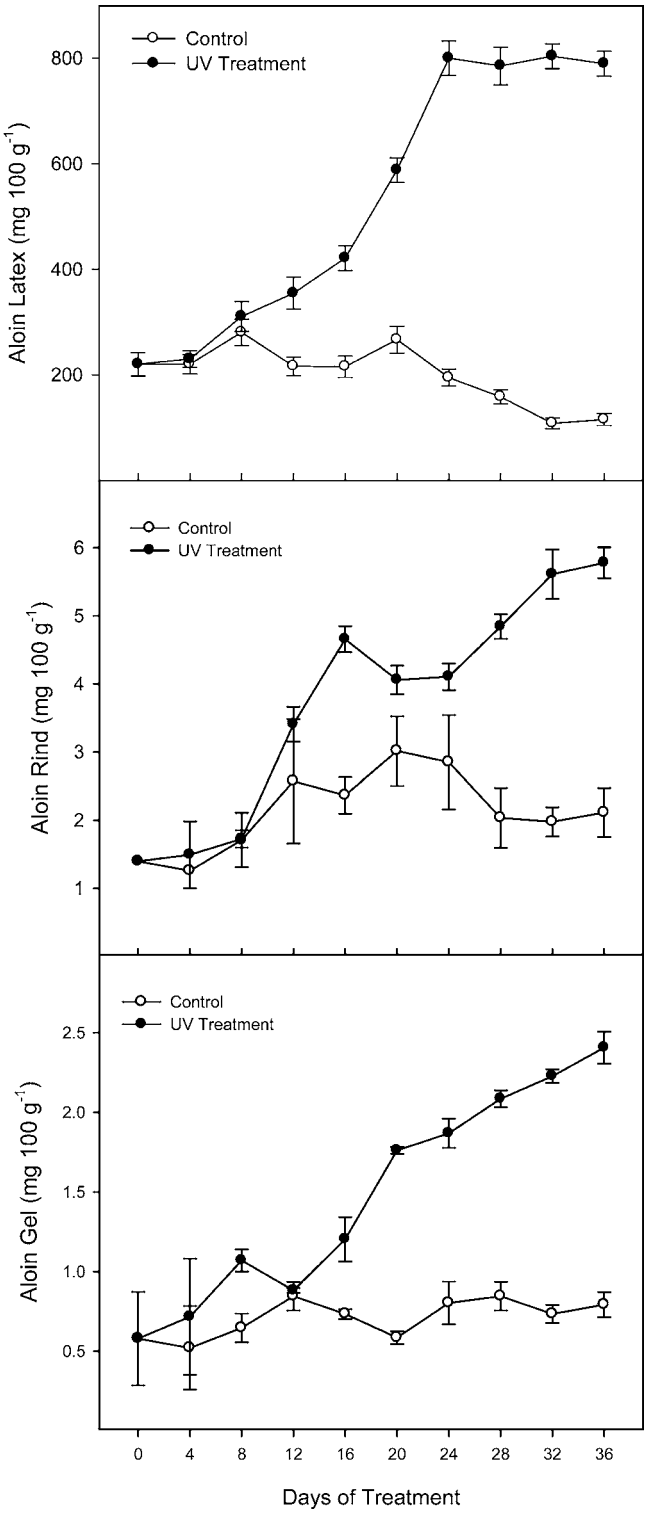

Figure 3. Aloin concentration in latex, gel, and rind tissues from *A. vera* leaves in the control or treated with UV-A. Data are the mean ± SE (n = 5).

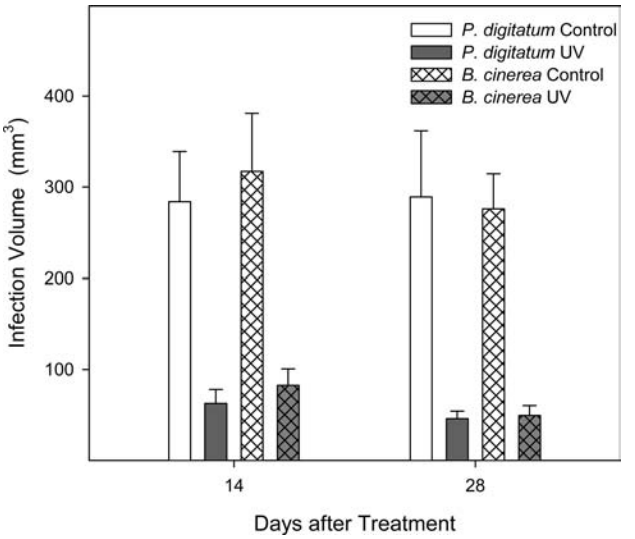

Figure 4. Infection volume on *A. vera* leaves after 5 days of inoculation with *P. digitatum* or *B. cinerea* in control and UV-A-treated leaves for 14 and 28 days. Data are the mean ± SE (n = 5).

inoculation (Figure 4), which is a reduction of 85 and 82% for *P. digitatum* and *B. cinerea*, respectively. These reductions could be attributed to the increase in the aloin concentration by the UV-A treatment. In fact, in a previous study with eight *Aloe* spp., it has been reported that the growth potential of fruit pathogenic fungi (*B. cinerea*, *P. digitatum*, *P. italicum*, and *P. expansum*), artificially inoculated on the whole leaves, was inversely correlated with the gel aloin concentration of each *Aloe* species.[18] It has been proposed that the antifungal and anti-Gram-negative and anti-Gram-positive bacteria activities of aloin are due to the induction of changes in the lipid/water interface in negatively charged membrane phospholipids and then alteration in the core of the bilayer.[27,28]

When the leaf respiration rate and ethylene production were measured, it could be observed that both parameters were significantly lower in UV-A-treated leaves than control leaves, with the same effect being observed after 14 or 28 days of treatment (Figure 5). The increased ethylene production in inoculated leaves could be a consequence of the fungal infection, which stimulates the ethylene biosynthesis pathway in plant tissues that usually shows a low ethylene production rate, such as non-climacteric fruits[29,30] or climacteric fruits.[14] On the contrary, UV-A-treated leaves developed lower infection, which was accompanied by a reduced ethylene production and respiration rate.

Experiment 2. From the previous experiment, results showed that UV-A applied during prolonged periods to *A. vera* plants increased the aloin concentration in the latex, rind, and gel. *A. vera* gel has been reported as a good tool for practical application as pre- or postharvest treatment for controlling fruit and vegetable decay, ripening, and senescence during storage,[5,12,14,15] with the efficacy being attributed to the aloin concentration.[18] Thus, *A. vera* gel with an increased aloin concentration could be expected to be more effective for practical purposes. Then, in this experiment, *A. vera* detached leaves were treated continuously, for a short time, with UV-C light, which is known to be more detrimental than UV-A to plants, with the objective of further increasing the aloin content. Results showed that the gel yield from the leaves was not affected by UV-C treatments, because gel yields were ≅69% for all samples (data now shown). However, UV-C treatment had a significant effect on increasing the aloin concentration in the gels compared to control ones. The highest increase was obtained from 24 to 48 h of UV-C treatment, from initial levels of 4.49 ± 0.23 to 25.31 ± 1.77 mg 100 g^{-1}, with the increase being much lower from 48 to 96 h, while the aloin content

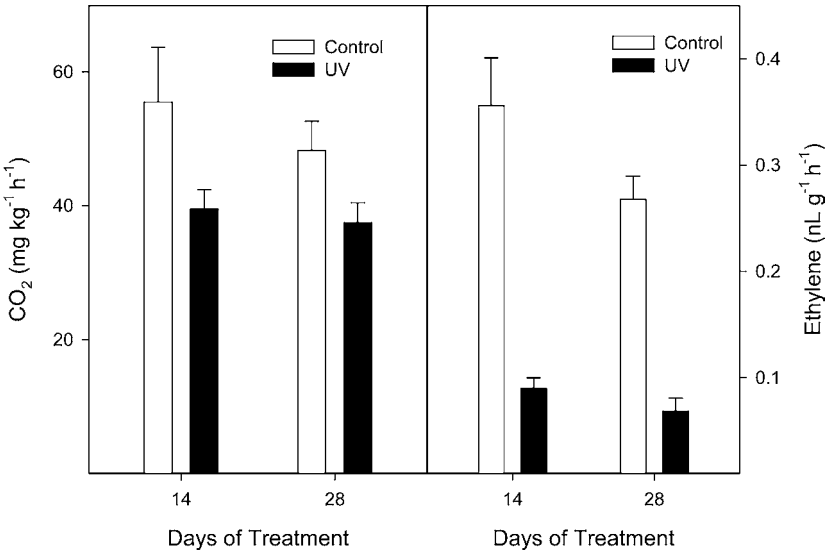

Figure 5. Respiration rate and ethylene production of *A. vera* leaves after 5 days of inoculation with *P. digitatum* or *B. cinerea* in control and UV-A-treated leaves for 14 and 28 days. Data are the mean ± SE (n = 20).

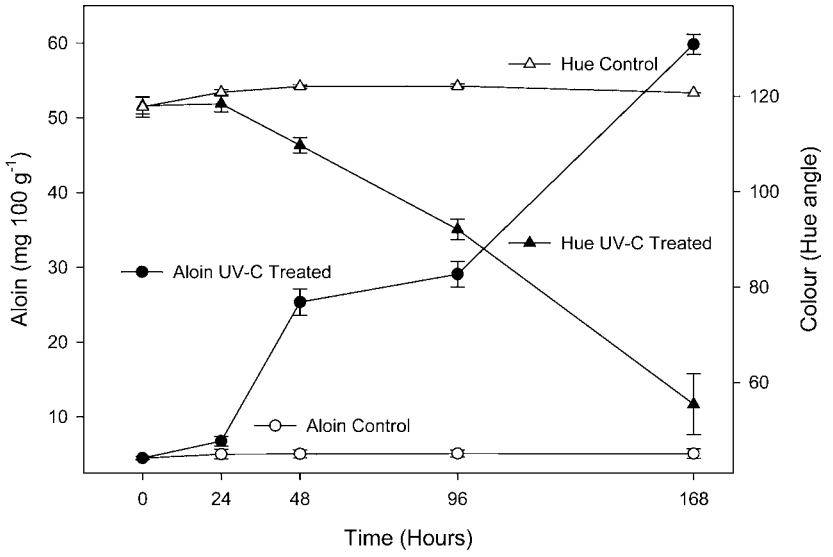

Figure 6. Color hue angle of adaxial epidermis of control and UV-C-treated leaves and the aloin concentration in their gels. Data are the mean ± SE (n = 5).

continuously increased until 168 h, reaching concentrations of 59.82 ± 1.36 mg 100 g^{-1} (Figure 6).

On the other hand, the continuous treatment of detached leaves with UV-C led to a browning process on the adaxial epidermis (because this was the leaf surface exposed directly to UV-C light), which started at the base and progressed to the apex. This browning coloration was manifested by a reduction in the color hue angle from initial values of ≅120 to ≅55 after 168 h of UV-C treatment, showing the harmful effect of UV-C, although this decrease started after 48 h of treatment (Figure 6). However, no significant changes were detected on abaxial epidermis color, as a consequence of UV-C treatment (data not shown).

Overall results suggest that UV-A induced an accumulation of aloin in both rind, latex, and gel tissues of *A. vera* leaves, which was correlated with an inhibition on the growth of *P. digitatum* and *B. cinerea*. Moreover, the treatment with UV-C light for a period of 48 h on detached *A. vera* leaves led to an increase in the aloin concentration in the gel. Then, for commercial purposes, the treatment with UV-C could be a useful tool to increase the aloin content in the gel and, therefore, its antifungal properties, which could be applied as pre- or postharvest treatment to preserve fruit and vegetable quality.

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Notes

The authors declare no competing financial interest.

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