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Improving Postharvest Resistance in Fruits by External Application of trans-Resveratrol

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As it is well-known, one of the main problems of modern agriculture is the postharvest fruit losses due to pathogen's attack and natural senescence during storage. Well established solutions to improve this situation, such as, for example, storage under controlled conditions and the use of synthetic pesticides, are not free of problems due to human health risks and environmental effects caused by chemical pesticides. A new strategy to solve these problems consists of developing methods to improve the natural plant resistance by using, upon their identification, the plant's own defense molecules, in other words, applying methods based on the plant's own natural processes of pest suppression to control spoilage. This requires the identification of components of the natural defense response in plants, which, in turn, demands highly sensitive, fast, and versatile analytical methods especially for trace, nonvolatile, compounds. In this work a laser-based technique has been applied for screening the postharvest elicitation of resveratrol by *Botrytis cinerea* in grapes. Besides antifungal character, resveratrol is known to present important antioxidant properties, which could also have positive effects on fruit conservation during storage. Consequently, several experiments were carried out in which exogenous application of resveratrol to several fruits maintained their postharvest quality. The quality of both resveratrol-treated and untreated fruits has been studied by the assessment of the biochemical composition and sensory analysis. Indeed, the present work demonstrates that the external application of resveratrol does not alter the sensorial and biochemical properties of the fruit.

KEYWORDS: ***Botrytis cinerea;*** **fruit quality; laser spectrometry; plant natural pesticides; resveratrol**

INTRODUCTION

As it is well-known, large amounts of fruits have to be stored for more or less extended periods of time, before they are sold to consumers, causing considerable losses due to pathogens' attack and natural senescence. Well-established solutions to improve this situation, such as the use of synthetic pesticides, are not free of problems due to human health risks and environmental effects caused by chemical pesticides. A new strategy to solve these problems consists of developing methods to improve the (natural) plant resistance by using, upon their identification, the plant's own defense molecules, in other words, by applying methods based on the plant's own natural processes of pest suppression to control spoilage. In this paper several examples of such a "natural pesticide" approach are reported.

A considerable number of investigations have been conducted on the identification of these secondary plant metabolites and on the understanding of host−parasite interactions (*1*−3). For example, during the past decade many studies have been published on the development of disease-resistant transgenic plants (*4*−6). However, a comprehensive genetic analysis of host−pathogen interactions is in many cases still impractical, such that a more classical phytopathological approach to the activation of plant defense responses is still in use (*7*, *8*).

Regarding the identification of plant natural defense compounds, it has been described that one of the most important mechanisms for resistance of grapevines to fungal diseases involves the synthesis of *trans*-resveratrol (3,5,4′-trihydroxystilbene), as response to the infection and other stresses such as UV irradiation, chemicals, and so forth (*9*−11). Thus, in this direction of identifying natural defense molecules, we have recently developed a direct and fast analytical laser-based technique optimized for nonvolatile compounds in fruits (*12*, *13*). Its application to analyze the resveratrol content in grapes revealed a significant concentration of this polyphenol on grape skin and vine leaves (*13*, *14*).

This selective accumulation of *trans*-resveratrol in grape skin and both its broad antifungal character and antioxidant properties

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make it a good candidate as a "natural pesticide" to improve the natural fruit resistance to fungal infection. Although several in vitro investigations have been carried out on the antifungal activity of *trans*-resveratrol (*15*, *16*), to the best of our knowledge, there is no bibliographic record on the direct exogenous application of resveratrol on fruits and vegetables to maintain their postharvest quality.

We anticipate that one of the main findings of this work will be the demonstration of the activity of resveratrol as a natural pesticide by its exogenous application to several fruits. Indeed, grapes and apples maintained their postharvest quality for weeks or months with clear differences from the untreated samples regarding the health and quality of the fruit. This result opens a new way to maintain the postharvest quality of the fruit.

MATERIALS AND METHODS

Laser Desorption and Resonant Ionization Mass Spectrometry. A new laser technique for fast and direct analysis of nonvolatile compounds in fruits, particularly resveratrol in grapes, has been developed in our lab (*13*, *14*). This has been accomplished by the combination of laser desorption (LD) with laser resonance-enhanced multiphoton ionization (REMPI) coupled to time-of-flight mass spectrometry (TOFMS) detection. One of the main advantages of the technique is that both desorption and ionization processes are separated and can be independently optimized in order to obtain a higher sensitivity. This has included (a) a 20-fold enhancement in the desorption yield by mixing the analyte with Zn powder (metal powder enhanced desorption), (b) the determination that *trans*-resveratrol is ionized through a one-color two-photon process, and (c) the resonant ionization region between 301.8 and 307.5 nm with the maximum at 302.1 nm, which is the optimal wavelength for *trans*-resveratrol analysis in complex samples.

The experimental setup has already been described elsewhere (*17*), so only a brief report is given here. Essentially, it consists of two independent high vacuum chambers; the first chamber is used for both laser desorption and laser postionization of the sample followed by the ions' acceleration toward the second chamber, basically a time-of-flight unit with a two-microchannel plate detector. A few nanosecond laser pulses from the fundamental emission of a Nd:YAG laser are used for sample desorption. A frequency-doubled dye laser is then used to selectively ionize the desorbed neutral compound by resonant-enhanced multiphoton ionization (REMPI). To this end, active wavelength laser scanning is achieved with tunability from 230 up to 730 nm. In addition to the selective ionization due to REMPI, additional selectivity is provided by the use of mass spectrometry, that is, providing mass identification and making the technique more sensitive and universal.

A basic feature of the technique is the absence of any separation method for sample preparation. The samples were prepared by cold-pressing the grape skin by means of a hydraulic press, after verification that with this easy procedure all the resveratrol is extracted from the skin. Thus, the combination of laser desorption followed by REMPI-TOFMS detection can overcome the main error sources, present in the chromatographic methods generally employed for resveratrol analysis (*13*).

After the optimization of the experimental conditions and the location of the resonant wavelength of the analyte, the validation of the method has been carried out with excellent results, including a variation of the signal with the concentration, giving a linear fit with a regression coefficient of 0.9997 in the range of interest, a precision better than 5% in both repeatability and reproducibility studies, and an accuracy of 96%. The combination of laser resonant ionization and mass spectrometry detection allows us to reach a detection limit of 2 ppb and a sensitivity on the order of 20 ng per single laser shot.

Preparation of *Botrytis Cinerea* Conidial Suspension and Inoculation of Grapes. The strain (*B. cinerea* 2100 from a Spanish Type Culture Collection) was grown on potato dextrose agar at 24 °C and high humidity with a 14 h light photoperiod. Conidial suspensions were obtained by the addition of 2 mL of sterile water to a sporulating culture and filtered through three layers of gauze in order to obtain a single spore suspension. The spore counts were obtained with a Neubauer counter to obtain a final concentration of 1×10^3 conidia·mL^{-1}.

Three groups of grapes were monitored: noninfected (natural evolution of resveratrol during the experiment), mock-infected, and *Botrytis*-infected. To have enough material for the spectrometric analysis, each group was prepared with 40 grapes of a similar size; day by day, seven grapes of each group were peeled-off and their skin was analyzed (3 replicates) by the LD+REMPI−TOFMS technique to monitor the resveratrol content evolution. The full experiment was repeated three times.

For mock-infected and *Botrytis*-infected groups, each grape was wounded at the equator with a small nail head having a stop in order to produce always the same depth (about 4 mm); the mock-infected group was inoculated with 5 μL of buffer (0.11 M glucose, 67 mM KH_2PO_4), and the *Botrytis*-infected ones were inoculated with 5 μL of the conidial suspension in the same buffer.

All the grapes were individually placed on a grid with wet paper below the grid and covered by a plastic film in order to maintain a high humidity (80−85% relative humidity) during the experiment, and they were incubated at room temperature.

Exogenous Application of Resveratrol in Fruits. The essential procedure for the treatment of the fruits with *trans*-resveratrol (*18*, *19*) is as follows. Commercial *trans*-resveratrol was purchased from Sigma Chemical Co. Several concentrations were tested so as to get optimal results with the minimum concentration possible of the compound. The best results (here showed) were obtained with a 1.6×10^{-4} M solution of resveratrol in water. Due to the slight solubility of *trans*-resveratrol in water and to ensure an homogeneous application, the solution was stirred during the treatment. The first experiment was conducted on grapes: the treatment was done by dipping them in this solution during a few seconds (ca. 5 s). A second group was immersed in water for the same time period, as control. The (mature) grapes (Aledo variety) were directly purchased in the market and directly treated during the same day, without any previous cleaning. To avoid effects of different maturity stage between bunches, they were cut in two similar moieties, and each one was incorporated into one of the groups. After this short treatment, the fruits were kept in open air at (controlled) room temperature. Each experiment contained three half-bunches per group.

The same procedure was employed with apples (*Golden*); in these cases, six pieces per experiment were used (three treated and three nontreated). To have some statistics, the experiment was repeated up to five times (in each case) with similar results in each round.

Biochemical Analysis. Both treated and nontreated fruits were stored in market storage condition. After 8 days of storage, a part of the samples was taken for analysis, and after 18 days, the rest of the fruits was also analyzed. The analysis consisted of determining the biochemical parameters which give information about the nutritional quality of the fruit, namely energy (*20*); protein by the Kjeldhal method (*21*); fat by the Soxhlet method (*22*); fiber by the AOAC method (*23*); special carbohydrates (glucose and fructose) by the HPLC−refraction detector method (*24*); minerals by flame spectroscopy, namely, magnesium (*25*) and potassium (*26*); vitamins (pyridoxine and niacin) by the HPLC−diode array method (*27*); ascorbic acid by HPLC (*28*); amino acids (proline) by the colorimetric method (*29*); organic acids (tartaric acid and malic acid) by the HPLC−UV−vis method (*30*).

Sensorial Analysis. The sensory parameters give information about the quality of the product as well as the consumer's degree of satisfaction. These are (1) a triangle test (discriminative test) (*31*) [a method of difference testing involving the simultaneous presentation of coded samples, two of which are identical, to the sensory assessor, who is asked to select the sample perceived as different]; (2) a hedonic scale (*32*) [a method to obtain the assessor's degree of satisfaction, in a number of parameters related to the life span of the fruit (namely, appearance, taste, and texture)]; and (3) an ordinal scale (*32*) [a method to obtain the organoleptic quality considering only the fruit appearance as a parameter for the items (perfect, typical, with some minor imperfections, with some noticeable imperfections, and totally imperfect fruits)].

It is also important to note that all these sensory analyses have been conducted in a chamber for this purpose, as described in UNE 87-004-

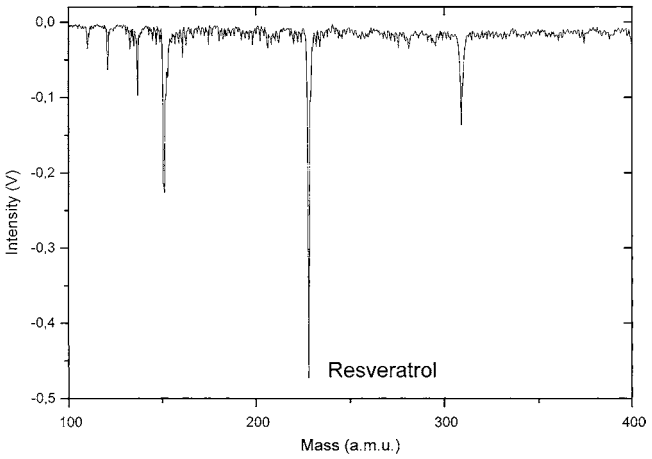

Figure 1. TOFMS spectrum of a grape skin sample obtained at normal experimental conditions. See text for comments.

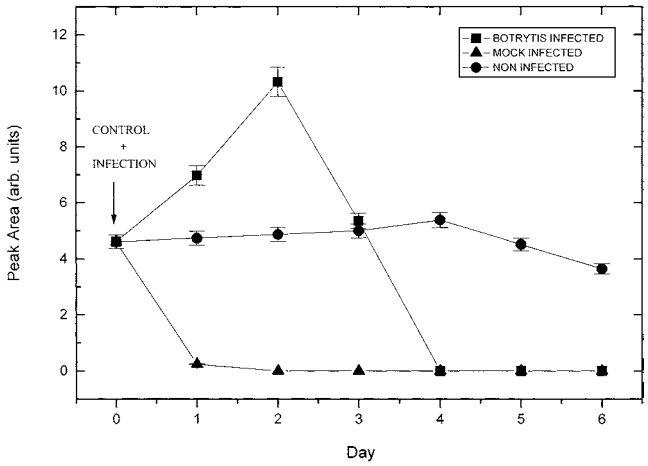

Figure 2. Evolution of the resveratrol content in grape skin in noninfected, mock-infected, and *Botrytis*-infected grapes. The clear elicitation of resveratrol by the *B. cinerea* can be noticed. (See text for details.)

79 (*33*), and that all these analyses have been done following the criteria of the European Cooperation for Acreditation of Laboratories (*34*).

In general, the statistical analysis done in this study has applied several procedures, such as homogeneity of two samples, a table of significant levels in the triangular test, and a frequency distribution (*35*, *36*).

RESULTS AND DISCUSSION

Resveratrol Elicitation by *B. Cinerea* in Grapes. Since the publication of the action of *B. cinerea* as elicitor toward the production of resveratrol in grapevines (*37*), many investigations (mostly in vitro) have been carried out on this host−plant interaction (*10*, *38*−40), mainly by monitoring the resveratrol production in leaves, aiming to enhance the resistance of vine plants to this fungus.

Figure 1 shows a time-of-flight spectrum obtained from a sample of grape skin, corresponding to a desorption area with 48 μg of grape skin and 79 μg of Zn. The resveratrol peak as indicated is clearly noticeable. For this sample the resveratrol content has been determined using the standard additions method, that is, adding known quantities of resveratrol to several identical samples of grape skin; the value obtained for the intercept with the concentration axis gives the quantity of analyte in the blank. A value of 16.0 ± 0.5 μg of resveratrol/g of grape skin was obtained, that is, 16 ppm of resveratrol. The resveratrol content in grape flesh was also investigated; however, no significant signal of it was found (i.e. content below 2 ppb). This finding proves that the main content of resveratrol selectively accumulates in grape skin, which is consistent with previous investigations (*41*).

This figure shows the capabilities of our technique for the detection of resveratrol in grapes. Thus, in order to investigate the postharvest elicitation of this compound in grapes upon *B. cinerea* infection, three batches of grape samples were monitored for their resveratrol content: not infected, mock-infected, and *Botrytis*-infected ones. **Figure 2** displays the evolution of the resveratrol content in each case: while the noninfected grapes show a constant resveratrol content through the experiment, in the mock-infected ones a sudden decrease is observed the first day after the buffer inoculation with a smooth diminution during the subsequent days. For the *Botrytis*-infected group a significant increase in the resveratrol content is observed subsequent to the infection, with an approximately 50-fold enhancement of resveratrol with respect to the case of the mock-infected grapes (i.e.: twice the initial content) by the second day after the infection; afterward the resveratrol shows a rapid decrease leading to the disappearance of the compound by the fifth day after infection. This is probably due to the oxidative dimerization of the compound by the *B. cinerea* (*39*, *40*, *42*).

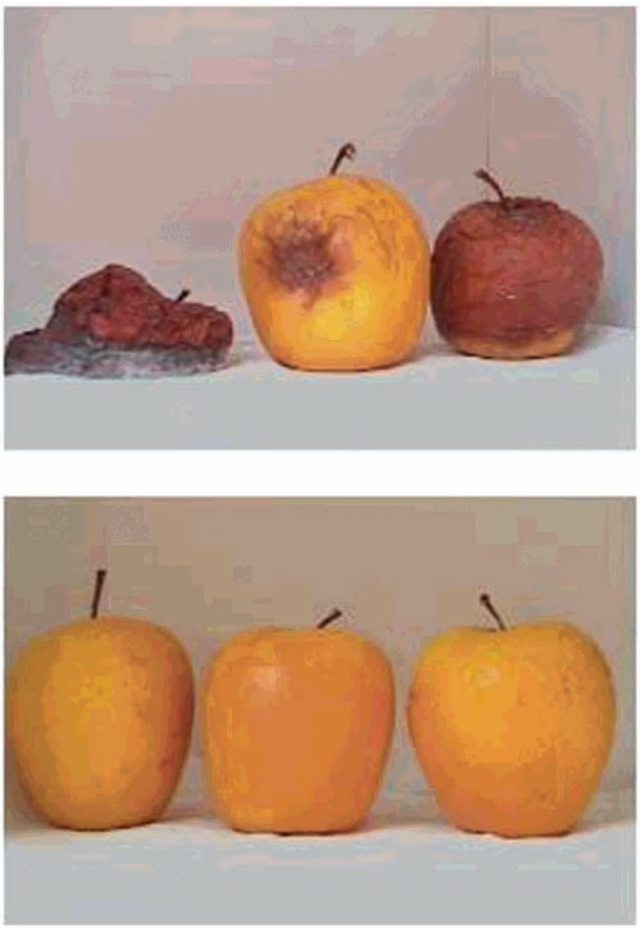

Figure 3. (top) Apple samples immersed 5 s in bidistilled water after 75 days of storage under room temperature. (bottom) Apple samples immersed 5 s in a 1.6×10^{-4} M resveratrol solution and stored at the same conditions.

Previous investigations on the production of resveratrol by grapes in response to *Botrytis* infection (*10*, *43*) found that its

Table 1. Nutritional Values Obtained in Grapes (*Ideal*, White) Untreated and Treated with Resveratrol

	untreated grapes[a]				treated grapes[a]				
	t_0+8 $n=5$	t_0+18 $n=5$	X	SD	t_0+8 $n=5$	t_0+18 $n=5$	X	SD	txsd[b]
energy (kcal/100 g)	72	76	74.3	4.85	75	78	76.9	6.32	5.3^{ns}
protein (g/100 g)	0.8	0.7	0.78	0.08	0.7	0.9	0.83	0.05	0.06^{ns}
fat (g/100 g)	0.3	0.4	0.36	0.15	0.3	0.4	0.37	0.09	0.12^{ns}
fiber (g/100 g)	1.3	1.3	1.28	0.30	1.0	1.0	1.02	0.43	0.35^{ns}
special carbohydrates:									
glucose (g/100 g)	6.9	7.6	7.33	0.58	8.1	7.8	7.98	0.86	0.69^{ns}
fructose (g/100 g)	8.4	8.7	8.56	0.38	8.5	9.1	8.80	0.96	0.69^{ns}
minerals:									
magnesium (mg/kg)	74	78	76.0	14.8	75	80	77.5	12.8	13^{ns}
potassium (g/100 g)	0.10	0.11	0.13	0.05	0.11	0.13	0.14	0.01	0.03^{ns}
vitamins:									
pyridoxine (mg/100 g)	<0.01	<0.01			<0.01	<0.01	*ns*		
niacin (mg/100 g)	0.13	0.14	0.14	0.02	0.20	0.15	0.18	0.06	0.04^{ns}
ascorbic acid (mg/kg)	185	210	197.6	15.3	196	185	190	12.3	s
amino acids:									
proline (mg/100 g)	14	14	14	3.4	15	15	14.7	3.2	3.1^{ns}
organic acids:									
tartaric acid (mg/kg)	0.46	0.48	0.46	0.06	0.48	0.45	0.48	0.03	0.04^{ns}
malic acid (g/100 g)	0.51	0.54	0.57	0.14	0.45	0.46	0.45	0.12	0.12^{ns}

[a] n is the number of times the analysis has been done. [b] ns = no differences < 0.05.

elicitation occurred predominantly in the noninfected grapes surrounding the infected ones, while in the latter the resveratrol content was always lower than that in the noninfected grapes. Although the authors gave no information on the infection method (spray, punching, etc.), conidial concentration, time of analysis after the infection, and so forth, this apparent contradictory result is not so when the different time scale of both experiments is considered: it seems that in their case the resveratrol analysis was done several days after the *Botrytis* infection and (as the authors claim) the low resveratrol content then found was due to the degradation of the compound by the fungus (as happened in the present case after the second day). Moreover, the resveratrol evolution found in the present work is consistent with previous in vitro investigations on the induction of resveratrol by *B. cinerea* in leaves (*16*): in this case, the maximum yield of resveratrol was found at the third day after infection, followed by a rapid reduction of the resveratrol content by the fifth day.

Exogenous Application of *trans*-Resveratrol on Fruit. Most of the investigations of the fungitoxic character of resveratrol have been carried out on its role against *B. cinerea*, which is the most destructive of the postharvest diseases of table grapes. However, resveratrol has also been shown to enhance the resistance of vine plants to other pathogens, such as Phomopsis viticola (*15*), Plasmopara viticola (*44*), or Rhizopus stonifer (*45*). This rather unspecific antifungal character and the selective accumulation of *trans*-resveratrol in grape skin makes it a good candidate as a "natural pesticide" against pathogen attack and therefore to improve the natural resistance of grapes to fungal infection. In addition, resveratrol is known to present important antioxidant properties that could also have positive effects on fruit conservation during storage. Consequently, both exogenous application and endogenous enhancement could be exploited to reduce grape spoilage.

To demonstrate this possibility, several tests were carried out with different fruits by immersing them in a 1.6×10^{-4} M resveratrol solution in water, for a few seconds, as indicated in the Materials and Methods section. Ten days after treatment, significant differences were observed in the two sets of bunches; while the resveratrol-treated bunches still maintained a physical aspect with no signal of losses or deterioration, the untreated ones were not only dehydrated but also clearly infected and deteriorated with local development of fungi, as can be expected after that period of time. The experiment has been repeated up to five times following the same procedure with similar results. In all cases, the untreated grapes were already infected and deteriorated after 5−7 days of storage at room temperature while the grapes treated with resveratrol maintained during twice this time (i.e.: 10−15 days) a physical aspect with no external signs of deterioration.

Table 2. Results of Triangular Test over Treated with Resveratrol and Untreated Grapes 8 days after the Treatment

variety of grape	no. of assessors	assessors that found differences	assessors that did not find differences
Cape (red)	25	8	17
Ideal (white)	25	6	19

An important question arises as to how much resveratrol is added to the grape skin during the 5 s dipping into the 0.16 mM solution of resveratrol and why it is so efficient in the improvement of the fruit resistance. The total volume loss originated by dipping a bunch of 147 grapes was measured to be 370 mL, which corresponds to 3.82 μg of resveratrol added per grape or, equivalently, 7.64 μg of resveratrol per g of grape skin. This is nearly 50% of the reported natural concentration in grape berry skins.

The reason this added resveratrol is so efficient to prevent postharvest rot is not totally clear to us at present. To fully understand this process, further experiments will be required. However, we think it is worthy to mention the possibility that selective accumulation of this compound on the external face of the grape skin, as one would expect from the short submergence of the fruit, may play a crucial role in protecting it from pathogen attack. In this view, the fruit resistance would not only depend on the skin resveratrol content but also depend on how it would be distributed. To be more specific, the key factor could be the actual concentration of this natural pesticide in the external layer of the fruit skin. Thus, for a given overall concentration of the resveratrol in the skin, the more it accumulates on the external layer, the higher the postharvest

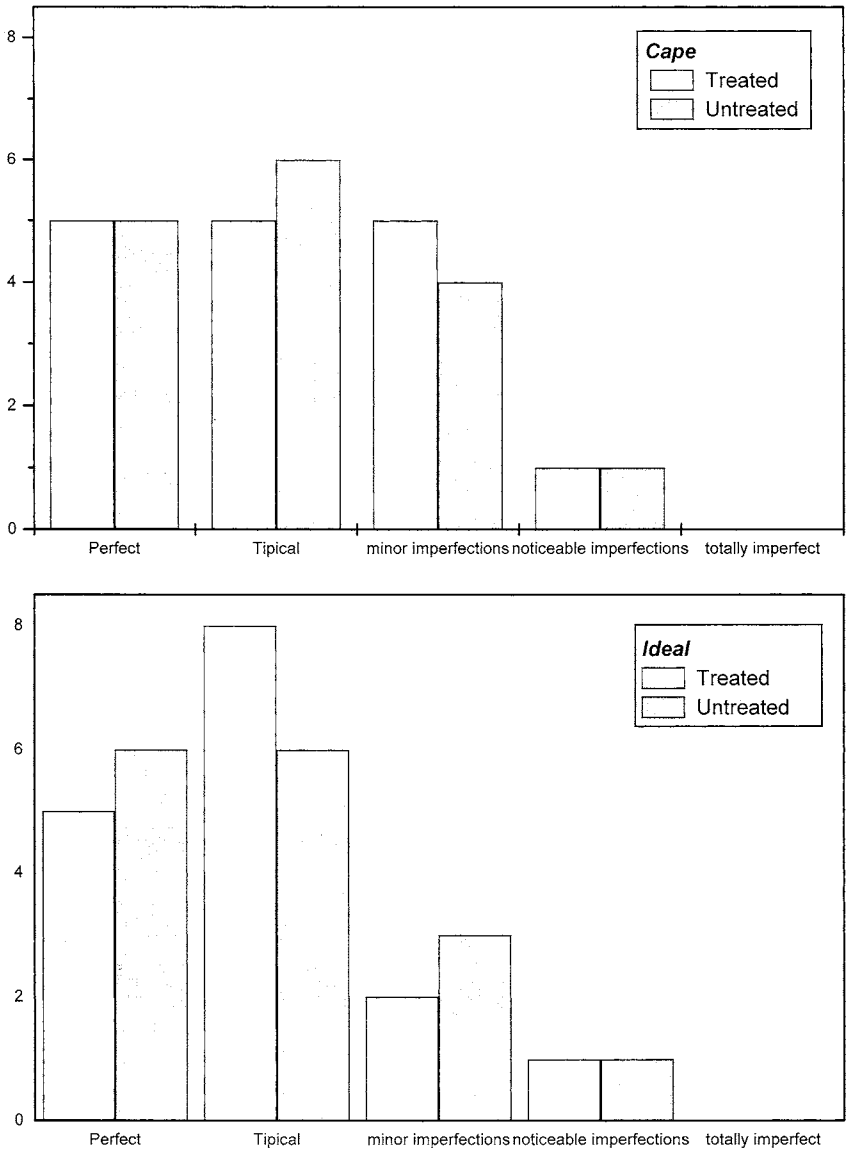

Figure 4. Frequency distribution of the assesor's replies in the sensorial analysis of treated and untreated grapes. Results are displayed using an ordinal scale (Appearance).

resistance of the fruit. This working hypothesis will be tested in future experiments in our lab.

This interesting result opened the way to subsequent investigations with other fruits. In fact, the phytopathogenic fungus *B. cinerea* can infect a huge range of host plants with no apparent specialization (berry fruits, horticultural vegetables, monocotyledons, bulbs, ornamentals, ...). In addition, the grapevine genes encoding for the resveratrol synthase have been transferred to plants which usually do not produce this compound, such as tobacco (*46*), rice (*47*), and tomato (*48*), with satisfactory results: the antifungal activity of *trans*-resveratrol was transferred to the transgenic lines, obtaining more resistant plants.

To demonstrate the capabilities of resveratrol as a natural pesticide, work has been conducted in our lab on the application of resveratrol to fruits other than grapes with similar results (except for the decay time). In each round, six pieces of fruit were monitored (three treated and three nontreated) and, as in the precedent case, all the experiments were repeated up to five times in order to have some statistics.

Figure 3 shows the results obtained with apples (*Golden* variety) 75 days after resveratrol application: as before, the treated samples maintain a clear nondeteriorated aspect with no external signs of decay, while the nontreated ones are already completely rotten and useless. The picture only shows one of the experiments to illustrate the effect of the treatment with *trans*-resveratrol. Similar results were obtained from the five experiments carried out. Specifically, 86.6% of the treated samples maintained a healthy aspect during at least 60 days while the nontreated ones started to decay, showing clear signals of shrivelling and dehydration, already during the second week of the experiment.

Obviously, *B. cinerea* is not the only fungus causing the symptoms of deterioration found in grapes and apples; a recent work (*49*) on the development influence of *B. Cinerea* on infected grapes (from the same variety and experimental conditions as in the present work) demonstrated that the deterioration of the grapes is not only due to the development of *B. Cinerea* but also due to other organisms present on the fruit, mainly yeast and imperfect fungi such as *Penicillium*, *Aspergillus*, and *Alternaria* spp. In any case, as indicated above, *trans*-resveratrol has been shown to have fungitoxic effects against many other fungi, and the results here shown demonstrate a clear preservation of the fruits treated with *trans*-

Table 3. Results in the Method Using a Hedonic Scale with 30 Assessors 8 days after the Treatment (Homogeneity of Two Samples)

	Cape, red			*Ideal*, white		
	appearance	taste	texture	appearance	taste	texture
SD (standard error)	0.32	0.30	0.33	0.35	0.34	0.32
txsd	0.63	0.59	0.64	0.65	0.58	0.65
diff between means	0.06	0.31	0.31	0.08	0.38	0.42
results	no differences	no differences	no differences	no differences	no differences	no differences

resveratrol as compared with the nontreated ones. Despite the fact that the antifungal properties of *trans*-resveratrol have been mainly studied by in vitro investigations, to the best of our knowledge, this is the first time that the improvement of the postharvest resistance in fruits, especially in fruits other than grapes, by the direct external application of *trans*-resveratrol is reported.

Finally, it is interesting to notice that although some authors have claimed that the risks for human health related to the consumption of natural chemicals in foods are even greater than the risks from pesticide residues (*50*, *51*), the lack of toxicity of resveratrol has already been demonstrated. One of the main stages in the development of new natural pesticides is the study of the toxicological and environmental properties of the compound to be used (*52*). Biological control agents are one of the more interesting alternatives to the use of harmful chemical pesticides, but it has to be demonstrated that they are safe for human consumption. As stated above, in the case of resveratrol, a considerable number of investigations are currently focused on the health benefits of resveratrol consumption (see refs 53−55 for recent reviews on this subject), giving it an added value as a candidate for biocontrol experiments against *B. cinerea*.

Biochemical Analysis. Table 1 shows the values of the physicochemical parameters evaluated, in grapes and grapes treated with resveratrol (*Ideal*, white). For each group (untreated and resveratrol-treated grapes) similar results were obtained 8 and 18 days after treatment. After applying a statistical analysis (homogeneity of two samples) to both groups, we concluded there were no statistical differences between the two types of fruits (significance level <0.05) under the present analysis conditions.

Similar results to those shown in **Table 1** were obtained with a different variety of grape (*Cape*, red) as well as with other fruits, namely apples, tomatoes, peaches, and cherries. In all cases, a similar conclusion was reached (that is, there were no differences in their biochemical properties between treated and not treated samples), so the details are omitted for brevity.

Sensory Analysis. This analysis includes three different ones: the triangle test, the ordinal scale, and the hedonic scale. **Table 2** shows the results obtained by applying the triangular test to grapes (varieties *Cape* and *Ideal*) and the information about the number of assesors participating in the test: those being able to differentiate the samples and those not being able to do so. Therefore, after applying the abovementioned statistical analysis, we concluded there were no differences between treated and nontreated fruits (significance level <0.05).

By means of an ordinal scale, the organoleptic quality of the fruit has been evaluated. The assessors have classified the fruits following the criteria included in the Materials and Methods section in this paper. **Figure 4** reflects the frequency distribution of the assesor's replies. What is relevant in all these figures is that the frequencies for the two types of fruits—treated and nontreated—are similar.

Finally, the appearance, taste, and texture were evaluated by applying a hedonic scale in order to obtain the assessor's degree of satisfaction. **Table 3** shows the statistical results obtained from each sample fruit, and after the statistical analysis (homogeneity of two samples), it can be concluded that there were no differences between treated and untreated fruits for all the evaluated organoleptic features.

CONCLUDING REMARKS

Our novel technique based on laser desorption coupled with laser REMPI−TOFMS was applied to monitor natural pesticides: in this case, resveratrol content in grapes. The technique that allows fast, direct, and highly sensitive analysis of *trans*-resveratrol in grapes with great sensitivity and resolution was used to demonstrate the postharvest elicitation of resveratrol by *B. cinerea* in grape skin. According to previous in vitro investigations, the resveratrol yield shows a maximum on the second day after infection, followed by a rapid decrease probably due to the metabolism of the compound by the fungus. Despite the fact that several investigations have been carried out on the elicitation of resveratrol by *B. cinerea* both in the field and in vitro, to the best of our knowledge, this is the first time that the *Botrytis*-induced production of resveratrol in infected grapes after harvest is reported.

One of the main findings of this work is the demonstration of the activity of resveratrol as a natural pesticide by its exogenous application to grapes and apples, which maintained their postharvest quality for weeks or months with clear differences from the untreated ones regarding the health and quality of the fruit. This result opens a new way to maintain the postharvest quality of the fruit.

In addition, the influence of the external application of resveratrol on the fruit was assessed by proper biochemical and sensorial analysis. It was demonstrated that the resveratrol application does not alter the fruit organoleptic and biochemical properties.

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