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Potential of Combined Ethephon and Methyl Jasmonate Treatments for Improving Mechanical Harvesting of Wine Grapes

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Summary

Abscission agents could reduce the damage to grapes and the volume of juice released during mechanical harvesting, avoiding undesirable oxidation and fermentation processes before the winemaking begins. Plant growth regulators such as ethephon or methyl jasmonate have been shown to be effective separately to reduce fruit detachment force (FDF) in different grapevine cultivars, however, additive and interaction effects of both products in combined treatments have not been evaluated yet. The objective of this study was to evaluate the potential use of ethephon and methyl jasmonate, separately or in combination, as abscission agents to improve the quality of machine-harvested 'Verdejo' wine grapes. The experiments were conducted in 2009, 2010 and 2011, using a fully crossed factorial design where mature clusters of 'Verdejo' grapevines were sprayed with different solutions containing methyl jasmonate (0, 10, 20 or 40 mmol L^{-1}) and ethephon (0 or 1000 mg L^{-1}). FDF and stem end condition of the berries were monitored for a maximum of 8 days after the applications. Ethephon reduced FDF 4 days after treatment in all growing seasons, maintaining values below those of controls until harvest. Methyl jasmonate had less effect than ethephon and declined FDF from 6–8 days after the sprayings. An additive effect was detected in the analysis of variance, which makes the combination of ethephon and methyl jasmonate more effective than either agent alone. The lowest FDF values just before harvest corresponded to cotreated plants, with reductions up to 45 % relative to untreated controls. Moreover, combined treatments significantly increased the percentage of detached berries presenting dry stem scars up to 8 days after the application, reaching accumulated values up to 60 % in 2011. Both abscission agents little affected must composition parameters, and did not produce adverse effects as preharvest fruit drop or defoliation. These results demonstrate that combined treatments with ethephon and methyl jasmonate are potentially useful to improve quality of mechanically harvested 'Verdejo' grapes. Additional research is needed to provide the exact concentrations of abscission agents required for consistent fruit loosening in practical applications, and to establish the optimal harvester settings in treated vineyards at different growing sites.

Key words. Abscission – ethylene – fruit-loosening – must – plant growth regulators – *Vitis vinifera* L.

Introduction

Mechanical harvesting is an important technique in order to contain the operating costs of the vineyard, but may have adverse effects on wine characteristics. The must production during the process, mostly due to the energetic action of the shakers, which knock against the clusters to allow the detachment of the berries, is the major factor impacting on wine quality (Baldini and Intrieri 2004). The breaking of the grapes and the release of free must cause the trigger of some biochemical processes (oxidation and uncontrolled fermentation) before being delivered to the winery, that negatively affect the stability and organoleptic characteristics of the wine (Meyer 1969; Nagel and Graber 1988). Although harvesting equipment has been improved over the years to minimize berry damage, varieties with delicate skin are still problematic (Walg 2007).

The grape juice production depends on many factors, including physical-mechanical characteristics of the berry as its breaking strength and its detachment from the pedicel (Carrara et al. 2007), ambient factors (Catania et al. 2009), and speed and frequency of the shakers of the harvesting machine. A decrease in the fruit detachment force (FDF) could allow working at a lower varying shaker frequency and, therefore, must production and damages in berries and vines might be reduced (Carrara et al. 2007). In this sense, the preharvest application of fruit loosening agents (chemical products that stimulate the abscission) on wine grapes could be potentially useful to improve harvest yield and quality.

Abscission of leaves and fruits respond to developmental cues and biotic and abiotic stresses. The abscission zone is developed from the basal part of organ and further differentiated as separation and protection layer. The organ separation is produced by the degradation of the cell walls in the abscission zone, which is associated in grapes with an increase in the activity of cellulase and polygalacturonase (Deng et al. 2007). Abscission is a process highly regulated by phytohormones such as ethylene and jasmonates (Rohwer and Erwin 2008; Zhang and Zhang 2009).

Ethephon (2-chloroethylphosphonic acid) is a plant growth regulator that, once inside the tissues, breaks down to liberate ethylene (Maynard and Swam 1963). Several studies conducted over 30 years ago, have shown that ethephon treatments previous to harvest lead to easier detachment of the fruits in different *Vitis vinifera* L cultivars, increasing the presence of a dry stem scar in the abscission points (Szyjewicz et al. 1984). Both facts contribute to minimize physical damages and must losses in mechanical harvesting. However, the effects of ethephon are strongly influenced by concentration and time of treatment, cultivar differences and seasonal conditions (Peterson and Hedberg 1975; El-Zeftawi 1982; Szyjewicz et al. 1984).

Jasmonates are a group of phytohormones, including jasmonic acid, methyl jasmonate and closely-related analogues, which are involved in numerous physiological plant processes (Gross and Parthier 1994; Rohwer and Erwin 2008), specifically stress responses, senescence and abscission of leaves and fruits. Methyl jasmonate might be a useful abscission agent in citrus (Hartmond et al. 2000), but its efficacy seems to be lower than ethylene releasing compounds as ethephon in other plants (Burns et al. 2008). Fidelibus et al. (2007) and González-Herránz et al. (2009) have shown that methyl jasmonate selectively induces abscission of mature grape berries and promotes dry stem scars on the abscission zone, demonstrating that these effects are dose dependant.

As adverse effects, the use of fruit-loosening agents at relatively high concentrations can produce undesirable defoliation (El-Zeftawi 1982; Hartmond et al. 2000) or excessive natural detachment of fruits before harvest, which leads to significant yield losses (Fidelibus et al. 2007). Ethylene releasing compounds can delay budbreak and reduce vegetative growth the next spring (Eirs and Celik 1981).

Interactions between phytohormones could substantially affect abscission. Several ethylene-independent, ethylene-promoted or ethylene-antagonised jasmonate responses have been discovered at physiological and genetic levels (Rohwer and Erwin 2008). Methyl jasmonate application was shown to enhance ethylene production in apple (Saniewski et al. 1987) and citrus fruit (Hartmond et al. 2000), while in other crops, as olive, no increases or even decreases in ethylene production have been reported (Sanz et al. 1993). Abeles et al. (1989) suggested that the abscission processes initiated by methyl jasmonate are independent of ethylene production within the tissue. In any case, possible additive or synergistic effects between abscission agents could be exploited in combined treatments to facilitate mechanical harvesting.

The objective of this study was to evaluate the additive and interaction effects of ethephon and methyl jasmonate applications on fruit detachment force in different seasonal conditions, and their potential use for improving mechanical harvesting of 'Verdejo' wine grapes.

Material and Methods

A field trial was carried out in 2009, 2010 and 2011, in a cv. 'Verdejo'/110 Richter vineyard located in Rueda *Appellation d'Origine* area (North-Central Spain). 'Verdejo' is a white grape cultivar, native from Rueda, which produce famous aromatic wines. The vineyard was planted in 2003 at 3.0 × 1.5 m (2222 vines ha ^{1}). Vines are pruned to a double Guyot system, leaving twenty buds in each, and are grown in trellis under irrigation, according to the standard practice in the zone. The soil is sandy and deep, with basic pH and low contents of organic matter.

Temperature and precipitation data were provided by the weather station of Rueda (Valladolid), located near the field trial. Mean air temperature was 11.1 °C in 2009, 11.9 °C in 2010 and 12.8 °C in 2011. During these growing seasons, there were neither late frosts nor remarkable attacks of pests or diseases.

Different treatments were compared, resulting from combination in a fully crossed factorial experiment of two doses of ethephon (0 and 1000 mg L^{-1}) with two doses of methyl jasmonate (0 and 20 mmol L^{-1}) in 2009, and with three doses of methyl jasmonate (0, 20 and 40 mmol L^{-1}) in 2010 and 2011. 1 mmol L^{-1} methyl jasmonate approximately corresponds to 224 mg L^{-1}. Solutions consisted of Ethrel 48 (ethephon 48 % w/v, Nufarm España SA) and methyl jasmonate (95 % purify, Sigma-Aldrich). All solutions included PG Supermojante 1 %v/v (alquilphenol ethoxilated/propoxilated 99.6 % w/w; Dwo AgroSciences Iberica, SA) as wetting agent.

Experimental design was completely randomized with five replications and one plant per replication. Treatments were applied to the clusters until runoff with a hand sprayer (average around 0.25 L solution per vine) when grapes had amassed sufficient soluble solids: 8 Sept. 2009 (19.8º Brix), 14 Sept. 2010 (20.1º Brix) and 1 Sept. 2011 (23.1º Brix). In 2009 and 2011, there were no rainfalls from sprayings to harvest whereas, in 2010, 14.4 and 5.4 mm were registered 3 and 4 days after the applications, respectively.

One cluster per replication was taken carefully 2, 4, 6 and 8 days after treatments to measure the force required to detach each berry from the rachis. Sampled clusters were cut in three portions: top, middle and bottom part, and from each one, two berries were cut with pedicel to measure fruit detachment force (FDF). Each berry was placed in a jig attached for a force gauge (DS2-N5; Imada, Northbrook, IL) and force parallel to the fruit axis was applied to the rachis until it detached from the berry, at which time peak force was recorded.

The condition of the surface of the abscission zone (presence of a dry stem scar) in the berries detached in the course of making FDF measurements was checked 8 days after treatments, using a binocular magnifying glass. Further, in 2010 and 2011, a cluster from controls and vines cotreated with ethephon and 20 or 40 mmol L^{-1} methyl jasmonate was shaken by hand at harvest. Then, detached berries were classified depending on the presence or not of scar tissue (dry or wet) in the abscission zone.

To assess potential yield losses associated with treatments, in 2010 and 2011 one cluster per replication was randomly selected after the sprayings and was loosely enclosed in plastic mesh bags to catch any berries that might abscise. The weight percentage of dropped berries was determined. Possible leaf yellowing and/or leaf abscission in treated plants were observed until the end of growing season. Vines were monitored next spring for date of bud break.

At harvest (16 Sept. 2009, 22 Sept. 2010 and 11 Sept. 2011), 50 berries were randomly collected and weighed in each experimental treatment. Total soluble solids content, total acidity and pH were determined in the must obtained from each sample according to the official methods of analysis established by the European Commission (EEC 2676/90 Regulations; European Commission 1990). Absorbances at 420 nm (UV/VIS spectrophotometer Jasco V-530) also were measured.

Data were subjected to factorial analysis of variance (ANOVA) using SAS statistical software (SAS Inst., Cary, NC). The separation of means was accomplished using the Least Significant Difference (LSD) test.

Results and Discussion

The factorial analysis of variance (Table 1) shows that the fruit detachment force (FDF) and the condition of the surface of the abscission zone in berries detached were significantly modified by conditions during the growing season and experimental treatments. There were strong seasonal differences between 2009 versus 2010 and 2011 (Tables 1 and 2), probably due to differences in environmental conditions that prevailed at or after applications, particularly to differences in temperature and soil moisture levels (Peterson and Hedberg 1975). Within each year, FDF decreased throughout the course of the study whether the clusters were treated with abscission agents or not (Table 2).

Table 1. Mean squares of factorial analysis of variance of data ontained from ethephon (ET) and methyl jasmonate (MJ) sprayings at different days after treatments (DAT) in 2009, 2010 and 2011.

Parameter/Time		Source of variation					
		Model	Year	ET	MJ	ET*MJ	Error
Weight of dropped berries (%)							
From 0 to 8 DAT		44.15	22.25	46.54	59.06	44.18	26.60
Fruit Detachment Force							
4 DAT		2.560 **	7.829 **	1.322 **	0.105	0.056	0.100
6 DAT		2.867 **	9.092 **	1.383 **	0.349	0.046	0.120
8 DAT		3.838 **	12.955 **	1.006 **	1.667 **	0.026	0.137
Berries with dry stem scar (%)							
8 DAT		2036.1 **	1820.1 **	4584.1 **	2374.6 **	1272.8 **	168.8
Berry weight							
8 DAT		0.428 **	1.347 **	0.003	0.202	0.086	0.127
Must composition							
8 DAT	Brix	22.445 **	72.340 **	0.808	0.035	4.381	3.138
	pH	0.049 *	0.099 **	0.025	0.018	0.035	0.018
	Total Acidity	6.554 **	21.160 **	0.028	1.511 *	1.261	0.472
	Absorbance 420 nm	0.216	0.591 *	0.000	0.100	0.472	0.179

* Significant P < 0.05P < 0.05; ** significant P < 0.01

Table 2. Mean values of fruit detachment force and proportion of abscised berries exhibiting a dry stem scar, obtained with ethephon (ET) and methyl jasmonate (MJ) applications in different days after treatments (DAT).

Year	ET (mg L^{-1})	MJ (mmol L^{-1})	Fruit detachment force (N)				Dry scar berries (%)
			2 DAT	4 DAT	6 DAT	8 DAT	8 DAT
2009	0	0	1.250 a	1.358 a	1.718 a	1.017 a	0.0 a
		20	1.189 a	1.192 a	1.123 b	0.824 a	0.0 a
	1000	0	1.313 a	1.188 a	0.964 a	0.897 a	0.0 b
		20	1.200 a	0.951 a	0.774 a	0.555 b	33.3 a
2010	0	0	2.283 a	1.995 a	1.955 a	2.111 a	0.0 a
		20	1.979 a	2.131 a	2.225 a	2.060 a	0.0 a
		40	2.122 a	2.097 a	1.870 a	1.858 a	0.0 a
	1000	0	2.102 a	1.700 a	1.910 a	1.782 ab	0.0 a
		20	2.267 a	1.884 a	2.101 a	2.081 a	0.0 a
		40	2.127 a	1.689 a	1.834 a	1.745 b	3.3 a
2011	0	0	2.813 a	2.537 a	2.244 a	2.545 a	0.0 a
		20	2.957 a	2.349 a	2.439 a	2.554 a	0.0 a
		40	2.420 a	2.282 a	2.438 a	1.873 b	10.0 a
	1000	0	2.363 ab	2.166 a	2.154 a	2.272 a	0.0 c
		20	2.922 a	2.378 a	2.414 a	2.202 a	20.0 b
		40	2.311 b	1.853 a	1.776 b	1.380 b	60.0 a

Within years and ethephon treatments, means with different letters are significantly different ($P < 0.05$, LSD test).

Ethephon spraying reduced FDF 4 days after treatment ($P < 0.05$) in all studied seasons (Fig. 1), maintaining values below those of untreated controls until harvest (Table 2). In concordance, El-Zeftawi (1982) observed that dose of 1000 mg L^{-1} ethephon was effective to reduce the cluster detachment force 5–7 days after treatments in various wine grape cultivars.

Methyl jasmonate treatments presented a minor and later effect on FDF than those of ethephon. Differences between treated and untreated vines were not significant in 2010, whereas in 2009 (20 mmol L^{-1}) and 2011 (40 mmol L^{-1}) methyl jasmonate declined FDF from 6–8 days after the sprayings, with respect to controls. These FDF reductions did not reach the levels of 75–80 % observed by Fidelibus et al. (2007) in raisin grapes and Hartmond et al. (2000) in citrus, using similar doses of methyl jasmonate.

The lack of response to methyl jasmonate on FDF in 2010 can be associated with the rainfall registered 3 and 4 days after treatments (19.8 mm in total), which might have washed part of the product from the clusters. This precipitation may have eliminated the effects of methyl jasmonate but not those of ethephon, since they act faster (Fig. 1). Not only the rainfall after spraying (Mortensen 1980) but also an incomplete cover of product in the grapes or an insufficient maturity level of fruits when they receive the application may affect the efficacy of abscission agents (Fidelibus et al. 2007).

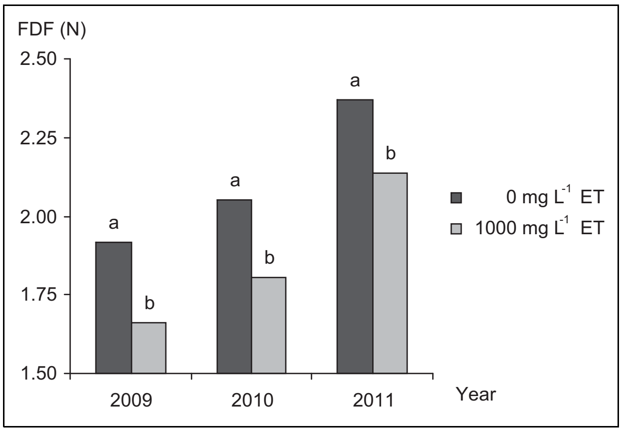

Fig. 1. Comparison of means of fruit detachment force (FDF) registered 4 days after applications in plants treated and untreated with ethephon (ET) in 2009, 2010 and 2011.

The additive effect reflected in ANOVA (Table 1) made the combination of ethephon (1000 mg L^{-1}) and methyl jasmonate more effective than either agent alone. Really, the lowest FDF values registered 8 days after treatments in the trial corresponded to cotreated plants (Table 2). The declines accounted for 47.2 % in 2009 (20 mmol L^{-1} methyl jasmonate) and 45.8 % in 2011 (40 mmol L^{-1} methyl jasmonate), relative to untreated controls. These reductions could minimize physical damage on mechanically harvested grapes.

Although methyl jasmonate treatments were shown to increase ethylene production in different crops (Saniewski et al. 1987; Hartmond et al. 2000), the abscission processes initiated by methyl jasmonate could be due, at least in part, to a direct effect of the hormone, independent of ethylene production within the tissue, on polysaccharide metabolism and mechanical weakening of cell walls in the abscission zone (Abeles et al. 1989; Sanz et al. 1993). The additive effect between methyl jasmonate and ethephon we have detected might be a result both of an enhancing of ethylene production and an ethylene-independent action.

Data of the condition of the surface of the abscission zone in the berries detached for FDF measurements showed that ethephon or methyl jasmonate had no effects on this parameter applied separately (Table 2). Despite this fact, it is interesting to note that combined treatments with both plant growth regulators significantly increased the presence of a dry stem scar in berries detached up to 8 days after the application with respect to the controls (Table 2), reaching accumulated values up to 60 % in 2011. When the clusters cotreated with methyl jasmonate (20 and 40 mmol L^{-1}) and ethephon (1000 mg L^{-1}) were shaken by hand at harvest, the percentages of detached berries presenting scar tissue in the abscission zone were greater than 50 % (Fig. 2). González-Herránz et al. (2009) in cv. 'Thompson Seedless', and Morris and Cawthon (1981) in cv. 'Concord', have reported increasea on the presence of dry stem scars after simple applications of methyl jasmonate (doses above 10 mmol L^{-1}) and ethephon (400 mg L^{-1}) respectively, There are no references in the literature about combined effects of both abscission agents on this variable.

Wet stem scars provide an entry point for pathogenic microorganisms (Ballinger and Nesbitt 1982; Kou et al. 2007) and enable juice to leak from the berries, promoting undesirable oxidation and fermentative processes before they are delivered to the winery (Meyer 1969). The dry scars prevent these problems and, on the other hand, allow the harvest of individual berries, having minimal mechanical damage. Therefore, the promotion of dry stem scars by combined application of methyl jasmonate and ethephon is highly interesting.

The average of dropped berries, collected in net bags from spraying to harvest, represented between 0.71 % (year 2010) and 1.35 % (2011) of total weight of the clusters, considering the full field test. The ANOVA of these values was not significant ($P < 0.05$) for experimental treatments (Table 1). Thus, the abscission agents did not produce representative yield losses if plants are harvested until 8 days after treatments. The appearance of vines treated with methyl jasmonate and ethephon and control vines were similar. No leaf yellowing, leaf abscission or delay in bud break date in the next spring were observed after the application of abscission agents (data not shown).

Berry weight and must composition parameters have shown significant interannual differences ($P < 0.05$), but have been little affected by the abscission agents applied, except for total acidity (Table 1). The juices from berries treated in 2011 with 40 mmol L^{-1} methyl jasmonate had significant higher titratable acidity than untreated berries (Fig. 3). The results obtained for this variable, in wine grapes, are consistent with those of studies on postharvest applications in stored fruits and vegetables where methyl jasmonate have contributed to maintain the acidity levels (Wang 1998; Jin et al. 2006; Ghasemnezhad and

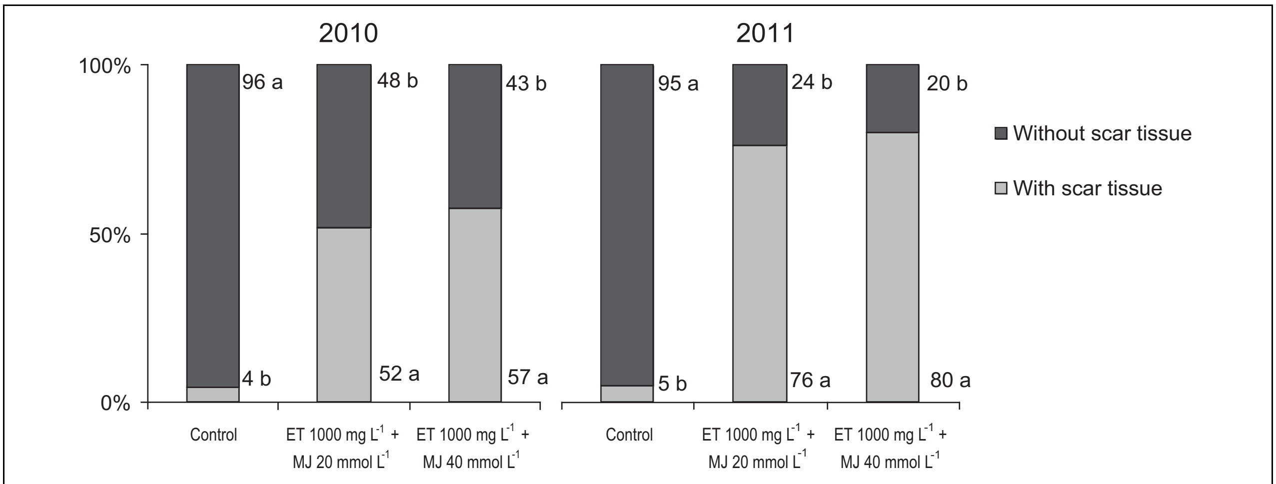

Fig. 2. Percentage of detached berries presenting scar tissue in the abscission zone, when the clusters were shaken by hand at harvest, in different experimental treatments combining ethephon (ET) and methyl jasmonate (MJ) applications. Within years and colours, means followed by a different letter are significantly different ($P < 0.05$, LSD test).

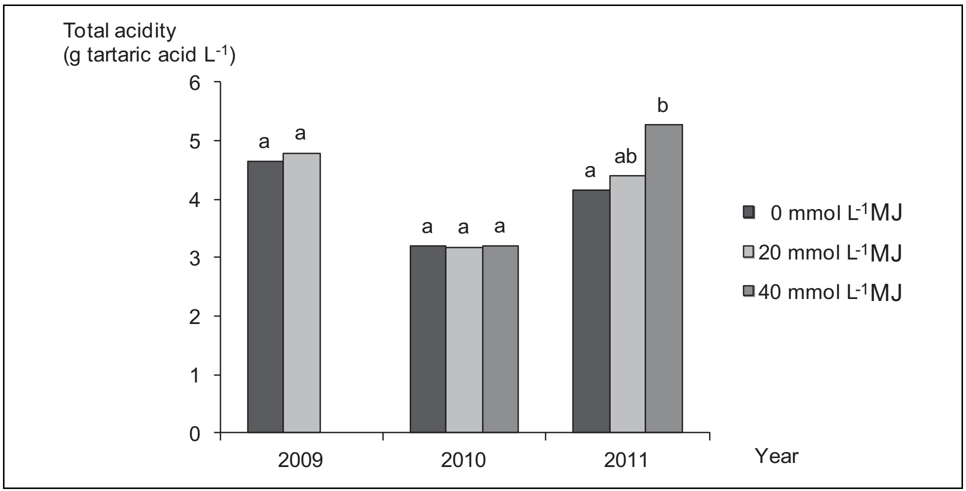

Fig. 3. Effect of different doses of methyl jasmonate on total acidity of must in the three years studied.

Javaherdashti 2008). On the other hand, Fidelibus et al. (2007) found that 'Cabernet Sauvignon' berries treated with 4500 mg L^{-1} methyl jasmonate had less total acidity than nontreated controls 14 days after the sprayings, while treatment had no significant effects in 'Merlot'.

Since ethephon and methyl jasmonate were applied late, probably they did not significantly affect ripening processes as when applied at veraison (Gallegos et al. 2006; González et al. 2011; Ruiz-García et al. 2012), and therefore they have not impact on must composition parameters at harvest. Responses observed to ethephon applications near the harvest have been little and variable, depending on cultivar and seasonal differences (Morris and Cawthon 1981; El-Zeftawi 1982).

In conclusion, fruit detachment force in 'Verdejo' grapevines has been significantly reduced by cotreatments with ethephon and methyl jasmonate applied 8 days before harvest, without causing yield losses, defoliation or delay in bud break the next spring. Moreover, the combined applications have promoted the formation of dry stem scars in the abscission zones of the berries. For these reasons, the preharvest treatment combining these two fruit-loosening agents is potentially useful as a tool for enhancing wine quality in mechanically harvested vineyards. They can enable less aggressive harvesting, leading to reduction of fruit damage, less material as rachis and leaves are sent to the winery, and less volume of juice is released during the process.

Practical treatments of abscission agents to the canopy (fruiting zone) might not be as effective as sprayings applied directly over the clusters have resulted in our study. Further research should be carried out to provide abscission agent concentrations necessary for consistent fruit loosening in practical conditions at multiple growing sites. Moreover, application of fruit loosening agents should be tested in larger scale mechanically harvested trials, to determine the optimal harvester settings necessary to achieve high fruit removal with low fruit damage, as function of the specific characteristics of treated vines.

Evaluation of musts and wines obtained from mechanically harvested grapes, treated and untreated with ethephon and methyl jasmonate, must be made to demonstrate these abscission agents can effectively improve wine quality. It will be especially important to assess oxidative stability and aroma characteristics.

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