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Original article

A preharvest treatment of ethephon and methyl jasmonate affects mechanical harvesting performance and composition of 'Verdejo' grapes and wines

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Summary

Previously we showed that the combined application of ethephon and methyl jasmonate to grapes (*Vitis vinifera* L.) may decrease fruit detachment force and promote the formation of an abscission layer between the pedicels and berries, without causing preharvest yield losses or defoliation (Uzquiza et al., 2013). Theoretically, such effects could help minimize the physical damage to berries and the volume of must released during mechanical harvesting which could, in turn, reduce the opportunity for undesirable oxidative processes to occur before the product comes into the winery. The aim of this work was to study the effects of this treatment on mechanical harvesting performance, and on must and wine quality. The experiments were conducted in 2011 and 2013, in a 'Verdejo' vineyard located in 'Rueda' *Appellation d'Origine* area (North-Central Spain). In a completely randomized factorial design different treatments were compared, combining the application of abscission agents (ethephon 1,000 mg L^{-1} plus methyl jasmonate 8,960 mg L^{-1} sprayed on the clusters 10 days before harvesting, *versus* untreated controls), and type of harvest (manual picking and mechanical at different shaking frequencies, 400 and 425 shakes min^{-1}). The preharvest treatment did not significantly affect product losses during mechanical harvesting at 400 shakes min^{-1} but, at 425 shakes min^{-1}, total losses in treated vines were 4.2% higher than in untreated. The percentage of free must in the harvested mass was lower in treated vines (20.6%) than in controls (23.2%) when shaking frequency was higher. The abscission agents tended to reduce potassium content in the must and increased hydroxycinnamic acid and total polyphenol contents, but they did not affect colour intensity or oxidative stability of wines.

Keywords
abscission, oxidation, plant growth regulators, polyphenol, *Vitis vinifera* L.

Significance of this study

What is already known on this subject?

- The combined application of ethephon and methyl jasmonate to grapes some days before harvest may decrease fruit detachment force and promote the formation of dry stem scars in the abscission zone, without causing significant preharvest yield losses or defoliation.

What are the new findings?

- For the first time, the effects of abscission agents on mechanical harvesting of grapes have been evaluated. Treatment of ethephon plus methyl jasmonate reduced the release of must, reduced potassium content in the product and increased total polyphenol content.

What is the expected impact on horticulture?

- Preharvest treatments of ethephon plus methyl jasmonate could help to minimize the physical damage to mechanically harvested wine grapes and contribute to enhance the quality of musts and wines.

Introduction

Compared to manual picking, mechanical harvesting reduces vineyard operating costs and may better enable harvesting at optimal levels of grape maturity since mechanical harvest is less dependent on labor availability and can proceed more quickly. However, mechanical harvest, unlike manual picking, generally detaches individual broken berries which release free must, and therefore promotes oxidative browning and uncontrolled fermentation processes that may begin before the fruit arrives at the winery (Meyer, 1969).

Polyphenols (anthocyanins and tannins) are responsible for the colour and astringency of wines (Ribéreau-Gayon and Glories, 1987). The oxidation of phenolic compounds in grapes and musts induces the formation of different polymers that may impart a brown colour to wines, reduce their stability, and modify their organoleptic characteristics (Nagel and Graber, 1988; Allen et al., 2011). Hydroxycinnamates are the major class of phenolics and therefore, the major oxidation substrates and browning precursors in white wines (Singleton et al., 1984). When grapes are crushed, polyphenol oxidase enzymes rapidly oxidize the hydroxycinnamates in the released must to quinones (Robards et al., 1999). By contrast, the wine oxidation is mainly a non-enzymatic process, in which oxygen does not directly react with phenolic compounds (Oliveira et al., 2011).

Numerous studies have shown that methyl jasmonate or ethylene releasing compounds such as ethephon (2-chloroethyl phosphonic acid), sprayed on clusters at the beginning of ripening, increase the phenolic content and the colour intensity of musts and wines (Gallegos et al., 2006; Ruiz-García et al., 2012). Applied a few days before the harvest, both plant growth regulators may stimulate grape berry abscission, thus reducing the force needed to detach the berries and also promoting the development of a dry stem scar at the abscission zone between the pedicels and berries (Fidelibus et al., 2007; González-Herranz et al., 2009; Uzquiza et al., 2013, 2014a, 2014b). These effects could allow harvester machines to work at lower shaker frequency and, therefore, reducing damage to the berries and minimize the release of free must. In this way, they would help to improve wine quality in mechanically harvested vineyards.

Additive or synergistic effects between abscission agents could be exploited in combined treatments to facilitate mechanical harvesting, reducing the amount of products needed and avoiding excessive residues in fruits. In previous studies (Uzquiza et al., 2013), we have demonstrated that combined applications of ethephon (1,000 mg L^{-1}) and methyl jasmonate (8,960 mg L^{-1}) 8 days before harvest have an additive effect on promoting fruit abscission-related processes in grapes 'Verdejo', without causing preharvest yield losses or defoliation. However, the effect of these treatments on must and wine quality has not yet been determined. Therefore, the aim of this work was to study the effects of the application of ethephon plus methyl jasmonate on mechanical harvesting performance of 'Verdejo' grapes, and on must and wine composition.

Materials and methods

The study was carried out in a 'Verdejo'/110 Richter vineyard located in "Rueda" *Appellation d'Origine* area (North-Central Spain). 'Verdejo' is a white grape cultivar, native from Rueda, which produces famous aromatic wines. The vineyard was planted in 2003 at 3.0 x 1.5 m (2,222 vines ha^{-1}). Vines were pruned to a double Guyot system, leaving twenty buds per plant, and were grown in trellis under irrigation, according to the standard practice in the zone. The soil is a deep sand, with basic pH (8.2) and medium organic matter content (1.8%).

In a trial carried out in 2011 and 2013, fully crossed factorial experiments were conducted. The factors included (i) application of abscission agents (ethephon 1,000 mg L^{-1} plus methyl jasmonate 8,960 mg L^{-1} *vs.* untreated controls), and (ii) type of harvest (manual picking and mechanical at different shaking frequencies as described below). Experimental design was completely randomized with three replications, including 10 adjacent vines per replication for mechanical harvest and 5 vines for the manual one. Each base plot was isolated from the rest by two vines to prevent overspray from contacting close plants. Two additional plants were included at the beginning of mechanically harvested treatments, to clean the machine after the adjacent treatment has been harvested.

Abscission agent solutions were prepared with Ethrel 48 (ethephon 48% w/v, Nufarm España, SA) and methyl jasmonate (95% purity, Sigma-Aldrich), and included PG Supermojante 0.1% v/v (alquiphenolethoxilated/propoxilated 99.6% w/w; Dow AgroSciences Iberica, SA) as wetting agent. Treatments were applied to the fruiting zone of the vines with a hand sprayer (average around 450 L ha^{-1}) when grapes had amassed sufficient soluble solids: 2 Sept. 2011 (23.0°Brix) and 23 Sept. 2013 (20.5°Brix).

Experimental treatments were harvested 10 days after spraying: 12 Sept. 2011 and 2 Oct. 2013. On these dates, 3 clusters per treatment and replication were taken carefully to measure the force required to detach each berry from the rachis. Sample 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 to a force gauge (DS2-N5; Imada, 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 weight percentage of berries with dry scar tissue in the abscission zone was checked at harvest in another two clusters per treatment and replication.

Mechanical harvesting was carried out using a Gregoire G8.260 (Gregoire Group, France) grape harvester. This 125 kW powered machine is characterized by fourteen horizontal bow beaters with collecting buckets as a transport system. It presents a pendulum suspension which could follow perfectly the vines limiting damage to the wood. The destemming system was not used. Mechanical harvester speed and shaker frequency were set at 4 km h^{-1} and 400 shakes min^{-1} respectively in 2011. In 2013, an additional experimental treatment with the same speed and 425 shakes min^{-1} were included in the trial. For this, all treatments and repetitions were randomly distributed again to maintain the experimental design of the previous year. After mechanical harvesting, 120 mg kg^{-1} of potassium metabisulfite were added to the product.

The total amount of picked grapes and the product losses for mechanical harvesting were monitored. Product losses were represented by the grapes left on the vines (unpicked bunches) and the grapes falling ahead of the harvester (on ground losses), and were expressed as a percentage of overall production (harvested fruit + losses). Ground losses were collected in plastic sheets placed on both sides of the vines, while unpicked bunches were collected by hand after the machine had passed. In 2011, abscised berries between the application of the treatments and harvest day also were collected (preharvest losses).

In the mechanically harvested product, the amount of released must was determined by draining the mass harvested until runoff ceased, using a 2 mm mesh. Then, liquid and solid parts were weighted to calculate the percentage of must released.

A standard wine-making process was used to produce the wine from 10 kg of the harvest of each experimental treatment. Hand-picked clusters were destemmed, and then all treatments were pressed using a pneumatic press (maximum pressure = 2 bar). For each mechanical harvesting treatment, the quantity of liquid (must) and solid part in the harvested mass were calculated, so proportional amounts were taken to press 10 kg. In all musts obtained, both manual and mechanical treatments, potassium metabisulfite was added to set free sulfur at 30 mg L^{-1}.

Approximately, 4 L of must for each experimental treatment were held for 24 hours at 5°C, and then racked for vinification. Alcoholic fermentation was induced by yeast; *Saccharomyces cereviseae* Zymaflore X16 in 2011, and *Saccharomyces cereviseae* Zymaflore Spark (Laffort, Laguardia, Spain) in 2013. Then, must in glass jars were kept in a refrigerated cell at 17°C to improve yeast growth. Alcoholic fermentation showed a regular trend, and was considered finished with a value of reduced sugar lower than 4 g L^{-1}. Free sulfur was set at 30 mg L^{-1} with potassium metabisulfite, and wines were clarified and decanted at 4°C during 48 hours. Then wines were 30 days at 1°C for tartaric stabilization in fully filled jars of 2 L volume, without "air space" to avoid oxidations. Before bottling, free sulfur was set at 30 mg L^{-1}.

Total soluble solids content in the musts, and total acidity, pH, and potassium content in the musts and wines, were determined according to the official methods of analysis established by the European Union (Commission regulation (EEC) No 2676/90, 1990). Absorbances at 280 (total polyphenol index), 320 (estimation of hydroxycinnamic acids content) and 420 nm (colour intensity) also were measured using a UV/VIS spectrophotometer Jasco V-530. The oxidative stability of the wines was evaluated registering the absorbances at 420 nm before and after a forced oxidation in a stove, at 50°C for 48 h (Arfelli et al., 2010).

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 Student's test (comparison between two means) and the Least Significant Difference test (LSD, for multiple comparison).

Results and discussion

Berry abscission

Confirming earlier observations with 'Verdejo' grapes (Uzquiza et al., 2013), the preharvest application of ethephon and methyl jasmonate significantly reduced fruit detachment force (FDF) with respect to controls (2.02 *vs.* 3.00 N in 2011; 2.59 *vs.* 3.22 N in 2013, $p<0.05$). Others (Fidelibus et al., 2007; Gonzalez-Herranz et al., 2009; Uzquiza et al., 2014b) observed a similar, but more striking effect of methyl jasmonate treatments on FDF of 'Thompson seedless', with lower doses than used here. Thus, there seems to be striking differences among grape cultivars in responsiveness to abscission agents, as Malladi et al. (2012) also observed for blueberry (*Vaccinium* sp.).

The lower FDF of treated grapes did not significantly affect preharvest yield losses measured in 2011. The mean value of preharvest fruit drop in 'Verdejo' was 1.4%, lower than the 3% observed in 'Sauvignon blanc' in a similar experiment (Uzquiza et al., 2014), and much lower than the 20% observed on 'Thompson Seedless' vines treated with methyl jasmonate alone (Fidelibus and Cathline, 2010).

Besides the decrease in FDF, promotion of dry stem scars is another potentially desirable effect of abscission agents. In 2011, approximately 63% (w/w) more of berries from treated vines developed a dry stem scar in the abscission zone compared to untreated controls ($p<0.01$). In 2013, about 24% more berries from treated vines had dry stem scars than berries from control vines. Dry scars could help minimize entry points for microorganisms (Ballinger and Nesbitt, 1982; Kou et al., 2007) and reduce juice leakage from berries during mechanical harvesting and transporting to the winery.

Mechanical harvesting

Yield was not significantly affected by the abscission agent treatment (averaged values were 2.54 kg $vine^{-1}$ in 2011 and 4.64 kg $vine^{-1}$ in 2013). Moreover, the treatments did not affect unpicked bunch losses nor ground losses (detached fruits that were not harvested) during mechanical harvesting at 400 shakes min^{-1} in 2011 and 2013 (Figure 1). However, at 425 shakes min^{-1}, treated vines had approximately 4% higher ground and total losses than untreated vines ($p<0.05$). Both unpicked bunches and total losses were greater at 400 than at 425 shakes min^{-1} regardless of preharvest treatment (Figure 1), but ground losses were similar at different shaking frequencies in 2013, agreeing with Pezzi and Caprara (2009) and Arfelli et al. (2010). Other possible losses of product during harvesting process, such as dispersed must on the leaves or in the harvester system, were not measured, but other studies suggest that they represent a low percentage in the total product losses and did not vary at different shaking frequencies (Arfelli et al., 2010).

There was less free must ($p<0.05$) in the harvested mass from vines treated with abscission agents (20.6%) than there was from control vines (23.2%), when the harvester frequency was 425 shakes min^{-1}, possibly due to the fact that lower FDF and the increased presence of scar tissue in the abscission zone reduced mechanical damage during harvest. Working at 400 shakes min^{-1} the same tendency was observed, both in 2011 and 2013, but differences were not statistically significant (data not shown). Shaking frequencies alone did not affect the quantity of must released in 2013 ($F=0.35$, $p>0.05$), in agreement with Arfelli et al. (2010) and Caprara and Pezzi (2011). In contrast, Pezzi and Caprara (2009) and Catania et al. (2009) observed that must percentages decreased when shaking frequencies were lower.

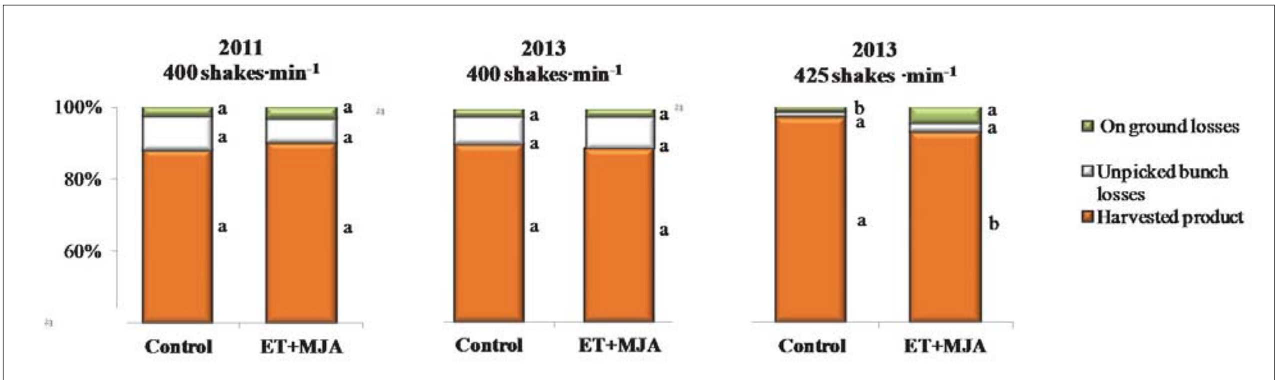

Figure 1. Influence of the preharvest treatment with ethephon plus methyl jasmonate (ET+MJA) and shaking frequencies on weight percentage of product losses registered during mechanical harvesting in 2011 and 2013. Within a year and shaking frequency, values followed by a different letter are significantly different ($p<0.05$).

Must composition

Must and wine composition were strongly affected by the year, probably due to interannual meteorological differences during grape ripening (Table 1). Except for total soluble solids content, must composition variables were little affected by harvest method. As Table 2 shows, the abscission agents applied did not affect total soluble solids content or pH, whereas their impact on total acidity and potassium content depended on the year.

The interaction between sources of variation in ANOVA of total soluble solids of must (Table 1) revealed that hand-picked grapes had higher soluble solids than mechanically harvested grapes in the absence of abscission agents (Figure 2). However, hand-picked and machine harvested grapes had similar soluble solids when abscission agents were applied (Figure 2). Treated grapes tended to have higher levels of total acidity and lower levels of potassium than non-treated grapes, though differences were only significant in 2011 (Table 2). Reduced potassium content could be considered a positive effect in mechanical harvesting, where skin maceration occurring during the harvest process and transport can increase the inorganic cation content (Ough, 1969; Guerzoni et al., 1981; Arfelli et al., 2010).

Regardless of the type of harvest (Table 2), musts from grapes treated with abscission agents had a higher total polyphenol index (280 nm absorbance) than controls. In treatments 10 days before harvesting, grapes might rap-

Table 1. F values of the factorial analysis of variance of grape and wine composition data, obtained with different preharvest treatment (TR: application of ethephon plus methyl jasmonate *vs.* control) and harvest method (HM: hand picking *vs.* mechanical) in 2011 and 2013. A_x: absorbance at x nm.

Parameters	Source of variation									
	Model		Year		TR		HM		TR*HM	
Must										
Brix	11.70	**	22.35	**	3.66		11.09	**	5.42	*
pH	63.43	**	323.22	**	2.54		2.52		3.26	
Total acidity	0.99		0.01		4.11	*	0.35		0.31	
Potassium content	28.51	**	119.61	**	5.91	*	1.39		0.01	
A_{280} (1)	2.03		.		8.98	*	0.55		0.03	
A_{320}	12.85	**	32.46	**	15.40	**	3.23	*	0.47	
A_{420}	17.19	**	70.54	**	0.00		1.86		0.90	
A_{420}/A_{320}	9.41	**	37.25	**	0.97		0.72		0.80	
Wine										
pH	17.51	**	94.69	**	0.21		1.03		0.69	
Total acidity	9.03	**	46.08	**	0.00		2.60		0.80	
Potassium content	27.51	**	142.82	**	0.49		0.72		1.83	
A_{280}	11.05	**	44.54	**	13.21	**	0.77		0.06	
A_{320}(1)	3.40	*	.		10.18	**	1.92		1.49	
A_{420}	105.21	**	543.11	**	2.01		2.11		3.13	
$A_{420\,ox}$(2)	1.32		0.65		3.20		0.06		0.88	

1 Determined only in 2013.
2 Value registered after browning test (Arfelli et al., 2010).
* Significant p<0.05; ** Significant p<0.01.

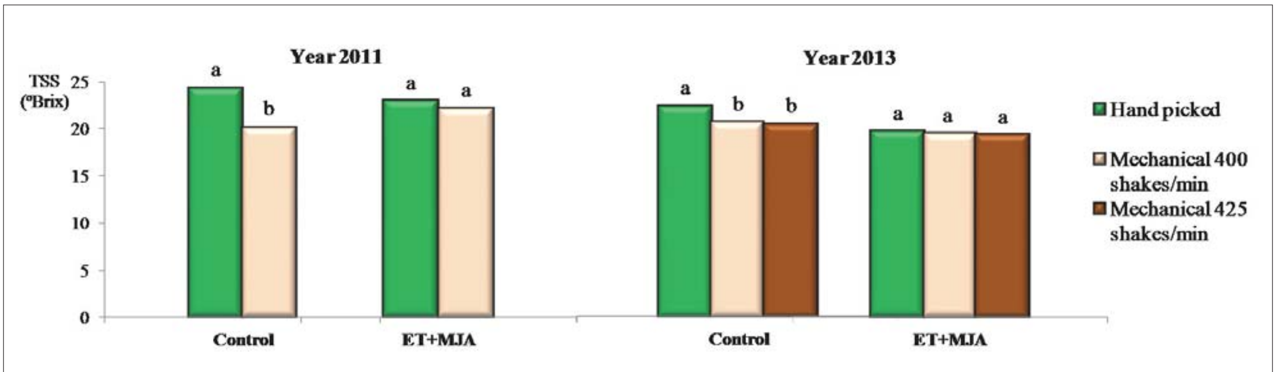

Figure 2. Effects of the preharvest application with ethephon plus methyl jasmonate and type of harvest on total soluble solid content of the must (TSS). Within treatments, harvest methods with different letter are significantly different (p<0.05).

idly increase ethylene production (Uzquiza et al., 2014b), and accordingly phenolic content, just as they do following applications of ethephon (Gallegos et al., 2006) or MeJA (Ruiz-García et al., 2012) around veraison. Despite having different polyphenol indexes, there were no significant treatment effects on colour intensity of must (Table 1).

Compared to controls, the application of abscission agents increased the 320 nm absorbance of must (Table 2), an estimate of the content of hydroxycinnamic acids such as caftaric acid and S-glutathionyl-caftaric, which are quickly transformed in benzoquinones by polyphenol oxidase enzymes released when the berries are broken (Oliveira et al., 2011; Allen et al., 2011). Musts from hand-picked grapes registered similar values as musts from mechanically harvested fruit in 2011 (around 10.50) but higher values in 2013 (9.42 *vs.* 8.48, $p<0.05$). The ratio of absorbances at 420 nm (browning) and at 320 nm (non oxidized substrate) reflected less enzymatic oxidation of musts from treated grapes than controls in 2011, without significant differences in 2013 (Table 2).

Table 2. Effect of preharvest treatment with ethephon plus methyl jasmonate on must and wine composition. A_x: absorbance at x nm.

Parameters	Year 2011		Year 2013	
	Control	Treatment	Control	Treatment
Must				
Brix	22.28±2.50 a	22.77±0.59 a	21.22±0.95 a	19.82±0.48 b
pH	3.25±0.05 a	3.26±0.04 a	3.04±0.03 a	3.01±0.02 b
Total acidity (g L^{-1})	4.10±0.25 b	4.61±0.33 a	4.29±0.33 a	4.36±0.22 a
Potassium (mg L^{-1})	638±78.0 a	512±77.0 b	235±64.0 a	200 ±41.0 a
A_{280}	–	–	13.11±0.84 b	14.89±1.38 a
A_{320}	10.09±0.81 b	10.93±0.51 a	8.22±0.43 b	9.37±0.90 a
A_{420}	2.24±0.31 a	1.86±0.19 b	0.93±0.18 b	1.15±0.27 a
A_{420}/A_{320}	0.22±0.02 a	0.17±0.02 b	0.11±0.02 a	0.12±0.03 a
Wine				
pH	3.12±0.05 a	3.11±0.03 a	2.96±0.04 a	2.95±0.04 a
Total acidity (g L^{-1})	5.89±0.34 a	5.79±0.27 a	6.57±0.27 a	6.58±0.34 a
A_{280}	7.23±0.62 b	8.22±0.82 a	9.28±0.75 b	10.44±0.87 a
A_{420}	0.05±0.01 a	0.05±0.01 a	0.27±0.02 a	0.29±0.04 a
$A_{420\ ox}$ (1)	0.26±0.02 a	0.33±0.08 a	0.30±0.02 a	0.32±0.03 a

[1] Value registered after browning test (Arfelli et al., 2010). Within a year, means followed by different letters are significantly different ($p<0.05$, Student's test).

Wine composition

Neither field treatments nor harvest method affected total acidity, pH or potassium content of wines (Table 1). Accordingly with the results obtained for must composition analysis, the values of absorbance at 280 nm were higher in wines from treated grapes than controls, regardless of harvest type (Table 2), while the values at 420 nm were similar in all experimental treatments (Table 1). These results disagree with those of Noble et al. (1975) and Arfelli et al. (2010) who have found higher total polyphenol contents and more colour intensity in mechanical than manual harvested grapes. Darías-Martín et al. (2000) also found differences in 280 and 320 nm absorbances of wines as function of harvest type, demonstrating a greater extraction of flavonoid and non-flavonoid compounds in maceration that occurs during harvest. In this line, Catania et al. (2009) found significant effects of different shaking frequencies on the yellow hue in wines from mechanically harvested fruit.

The final colour intensity of wines after forced oxidation in stove were independent from preharvest treatment or harvest methods, with mean values of 420 nm absorbances around 0.3 (Tables 1 and 2). These results disagree with those of Arfelli et al. (2010), who observed that wines from manually harvested fruit were less sensitive to oxidation than wines from mechanically harvested grapes.

In this work we have shown, for the first time, the effects of preharvest treatments with abscission agents on mechanical grape harvesting and wine composition. Our results show that treatment with ethephon plus methyl jasmonate might slightly reduce yield due to greater on ground losses, especially at high shaking frequencies. However, the abscission agents may improve must quality, by raising hydroxycinnamic acids or decreasing potassium content, though they did not affect colour intensity and oxidative stability of wines.

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