

Post-pruning storage and toasting of vine-shoots decrease fungicide residues

Sourced from the research article "Evolution of fungicide residues in pruned vine-shoots" (OENO One, 2021)¹.

>>> The potential use of vine-shoots as enological additives has been recently demonstrated. They can make a positive contribution to the sensorial profile of wines when they have been stored for 6 months after pruning, granulated, toasted under specific conditions and finally added to wines during winemaking. Since fungicide application is a common practice in vineyards, it is necessary to know the possible presence of such residues. In this study, a liquid chromatography-mass spectrometry analytical technique method for determination in vine-shoots of the main vineyard fungicides was developed, and their dissipation after post-pruning storage and toasting process was demonstrated. It was confirmed that the use of vine-shoots as enological additives will not pose a risk to the consumer, if good agricultural practices are used. <<<

■ Study context

Pruned vine-shoots are the most common waste produced in viticulture, with an estimated average world production of 1.95×10^7 tons per year. Nevertheless, due to their lower value, they are generally left or burnt in the vineyard, which generates environmental problems.

However, the chemical composition of vine-shoots is characterized by some phenolic and aromatic compounds which have opened up the possibility of using them in viticultural and enological practices. Therefore, some authors² have proposed the use of vine-shoots as enological additives, due to their contribution to the aroma profile of wines when they are elaborated in contact with vine-shoot fragments. Due to this innovative use of vine-shoots, it is necessary to evaluate the presence of pesticide residues, especially fungicides since their use in vineyards is very widespread. To ensure that such wines do not compromise consumer safety, the presence of fungicides residues must be below the maximum residue limits (MRLs), or even better, totally exempted. The control of pesticide residues, their evolution and transfer to plant material to grapes and wine has been widely studied. Therefore, the possible effects of using vine-shoots during winemaking still needs to be studied.

■ Trials

The aim of this work was to determine the content of fungicide residues in vine-shoots when used as enological additives. It was therefore necessary to develop a method for the extraction and analysis of several fungicide active substances; these were chosen from among the most used in Spanish vineyards.

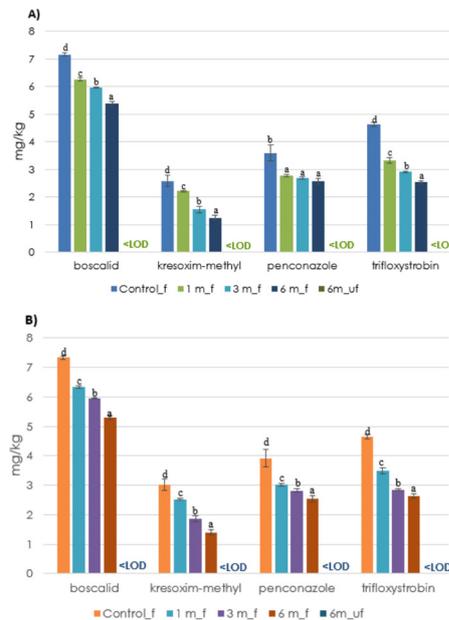


Figure 1. Evolution of fungicide residues in Airén (A) and Cencibel (B) vine-shoots during 6 months of storage. f = fortified vine-shoots treated under critical agricultural practices; uf = unfortified vine-shoots treated in vineyard under good agricultural practices. LOD: limit of detection, 0.01 mg/kg. For each substance, different letters indicate significant differences between sample times according to the Tukey test ($p < 0.05$). The mean values ($n = 4$) are shown with their standard deviation.

Vine-shoots from two *Vitis Vinifera* cultivars, Airén and Cencibel, were pruned from 50 vines and grouped into 2 kg batches, which were subsequently separated into two groups: the first was stored intact (unfortified vine-shoots) and the second was sprayed with 200 mL of the different fungicides under *critical agricultural practices* at a dosage ten times higher than the legal limit (fortified vine-shoots). The active substances used were: trifloxystrobin, boscalid, kresoxim-methyl and penconazole.

After that, vine-shoots were stored intact in the dark at room temperature (18 ± 3 °C) for 1, 3 and 6 months. Afterwards, the fortified vine-shoots stored for 6 months were also toasted at 180 °C for 45 min.

■ Validity of the HPLC-MS/MS method for analysing vine-shoot fungicides

Vine-shoots were extracted and analysed according to Martínez *et al.* (2015)³. The extraction was made according to the modified version of QuEChers method for the multiresidue analysis of grapes. Finally, the separated extract was injected directly into the chromatograph after acidification with formic acid. MS/MS detection was carried out in positive mode under the conditions established by Oliva *et al.* (2018)⁴. Good calibration curves and analytical linearity of the vine-shoot fungicides were obtained, with correlation coefficients (R^2) higher than or equal to 0.999 and a relative standard deviation (RSD) lower than 10.5 %. Therecovery results were acceptable, with recoveries ranging from 96 % to 100 % and $RSD \leq 4$ %, which confirmed that the method was sufficiently reliable for these four fungicides.

■ What was the evolution of fungicide residues during storage?

The presence of fungicide residues in vine-shoots after pruning and 1, 3 and 6 months of storage, along with the unfortified vine-shoots at this last sample point, are shown in Figure 1. In general, the results showed a tendency for the residues to decrease over time in both varieties, reaching their lowest levels after 6 months, trifloxystrobin being the most reduced active substance. This time point agrees with the one set by Cebrián *et al.* (2017)⁵, because it is when the highest content of high-value enological compounds was reached. Therefore, if under these unfavourable conditions vine-shoots did not show any residues from these fungicides, then they will likely show similar behavior when treatment is applied in accordance with good agricultural practices. In both varieties, trifloxystrobin and kresoxim-methyl levels decreased after storage by about 40 % and 50 % respectively to below the maximum residue limit (MRL) established in grapes. On the other hand, penconazole decreased by approximately 30 % in both varieties, and boscalid decreased by nearly 25 %.

■ What is the evolution of fungicide residues with toasting procedure?

The toasting process of vine-shoots is necessary for enhancing certain compounds which are important for wines, especially in relation to their aroma compounds⁶. It is therefore necessary to study the presence of fungicide residues in the fortified vine-shoots after this heat treatment. Figure 2 shows that when fortified Airén (A) and Cencibel (B) vine-shoots are toasted the concentration of fungicides significantly decreases for some active substances, in contrast to the *critical agricultural practices treatment* (Control). The active substance boscalid was found to have the highest decrease in concentration: by 84 % and 90 % in Airén and Cencibel respectively, with concentrations of 1 and 0.8 mg/kg, which is almost five times lower than its MRL in wine grapes.

Kresoxim-methyl levels decreased by 54 %, which was its initial concentration during storage, thus remaining constant with toasting, but with levels below than the MRL. By contrast, kresoxim-methyl and trifloxystrobin in Cencibel decreased by 77 % and 74 % respectively in toasted vine-shoots with respect to the Control, and by around 50 % in both cases with respect to storage, with concentrations close to 1 mg/kg.

Compared to their initial concentrations, which were measured when vine-shoots were analysed after storage, trifloxystrobin decreased by 62 % and 77 % in Airén and Cencibel vine-shoots, thus also showing levels below the MRL in wine grape matrices.

As regards penconazole, there was approximately a 30 % decrease in its initial concentration with storage in both varieties, and a further 10 % with toasting in Airén and 20 % in Cencibel vine-shoots. It should be noted that the vine-shoots had been fortified under *critical agricultural practices* (10 times over the legal limit).

■ Significance and impact of the study

The results of this work confirm a decrease in the levels of boscalid, kresoxim-methyl, penconazole and trifloxystrobin

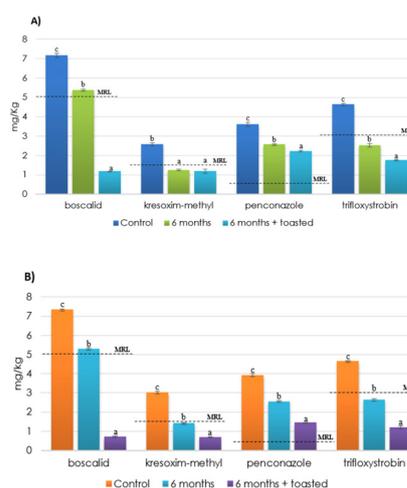


Figure 2. Effect of toasting on Airén (A) and Cencibel (B) vine-shoot fungicide residues. Control = analysed immediately after treatment; 6 months = analysed after 6 months of storage after treatment; 6 months + toasted = analysed after 6 months of storage and toasting; MRL = maximum residue limit in wine grapes. For each substance, different letters indicate significant differences between sample times according to the Tukey test ($p < 0.05$). The mean values ($n = 4$) are shown with their standard deviation.

residues in Airén and Cencibel vine-shoots when treated under *critical agricultural practices* and stored for 6 months and toasted. Therefore, these data indicate that the use of vine-shoots as an enological additive for enhancing and differentiating wines is not likely to expose consumers to the four studied fungicides. ■

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