Research Note:
Influence of Blackberry Plants on the Aroma Profile of *Vitis vinifera* L. cv. Pinot Noir

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***V. vinifera* cv. Pinot Noir vines were grown in pots together with blackberry plants and the effect of this association on the grape aroma was assessed. Preliminary data showed that vines that cohabited with blackberry had a different aroma profile compared to vines grown alone. The association with blackberry increased the concentration of 30 out of 74 free aroma compounds and 24 out of 95 bound ones. No aroma compound was identified exclusively in the treated grapes.**

INTRODUCTION

The aroma profiles of grapes and wine are greatly influenced by genotype, viticultural practices, oenological techniques and environmental factors, such as soil type, climate, water availability, sunlight, temperature and vineyard topography (González-Barreiro *et al*., 2015). To date, only a few studies have investigated the effect of native flora on the grape aroma profile.

In a study performed in Australia, Capone and co-workers (2012) reported that the proximity of *Eucalyptus* trees to Shiraz vines significantly influenced the 1,8-cineole concentration in the resultant red wines. Increased concentrations of this aromatic compound were found in the leaves, stems and grapes of vines grown close to *Eucalyptus* trees, and this was due to airborne transmission of 1,8-cineole, as first suggested by Herve *et al*. (2003). The influence of native vegetation surrounding the vineyard on the Argentine white variety Torrontes Riojano (Romano *et al*., 2010; 2014) was also investigated. From analysing the volatile components of the wine, the authors found that five out of the 71 aromatic compounds detected in the wine might have come from *Larrea* spp., a native shrub commonly surrounding the local vineyards, and these five "exogenous" volatiles were present in high concentrations. The authors hypothesised that the transmission of these compounds from native plants to the grapevines might have occurred through root absorption or by air transmission.

The aim of this paper is to assess if native species, such as the blackberry, have an influence on grape aroma profiles. In order to better assess this effect, we used plants grown in pots under semi-controlled conditions.

MATERIALS AND METHODS

The experiment was carried out in Verona, Northern Italy, in 2013. The plant material was *Vitis vinifera* L. cv. Pinot noir clone 777 grafted onto 420A rootstock. The vines were planted in 2011 in plastic pots of 45 L containing a mixture of soil and peat (2:1). Two treatments were set up at planting time: 1 – vines planted in association with blackberry plants (*Rubus laciniata* L.), denominated as "treated vines"; and 2 – vines grown alone as "control vines". Each treatment had three replicates of two vines. Vines were trained to the Guyot system, and the samples were irrigated and fertilised in the same fashion.

The influence on grape aroma of blackberry plants grown in association with vines was assessed in 2013, when the vines were three years old. During the vegetative period (from 1 April to 30 September), air temperature data were collected from a weather station located near the study site (http://www.arpa.veneto.it/). The mean minimum, medium and maximum daily temperatures recorded during that period were 12.5°C, 19.5°C and 26.1°C respectively.

One month after berry set, the vines were adjusted to similar crop loads through bunch thinning. At harvest, 100-berry samples were collected from each replicate and stored at -20°C for aroma analysis. Free and bound aroma compounds were identified by gas chromatography mass spectrometry analysis (GC-MS), using the protocol described by Flamini *et al*. (2001). The quantification was carried out by comparing the peak area of each compound to that of the internal standard, using 1-heptanol for the free form and 1-decanol for the bound form.

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The effect on grape aroma of the association between blackberry and Pinot noir vines was initially assessed by principal composition and classification analysis (PCCA), using STATISTICA version 8 (StatSoft Inc., Tulsa, OK). The identified aromatic compounds (variables) were screened according to the resulting correlation matrix. Volatiles showing a low discrimination effect between the two treatments were removed, and the remaining aromatic compounds were analysed by a multiple t-test using GraphPad Prism, version 6 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

The aroma analysis identified 74 free and 95 bound compounds in the grapes, displaying the peculiar volatile profile of the Pinot noir variety (Yuan & Qian, 2016). Based on the results from the PCCA analysis (data not shown), 64 free and 65 bound compounds were selected according to the higher discriminatory effect between the treatment and control.

Statistical analysis identified 30 free and 24 bound compounds showing statistically different concentrations between the blackberry sample and the control (Table 1). The concentration of all these compounds was higher in the treated grapes than in the control vines. For several aromatic compounds, the concentration showed values of two- to threefold higher in the treated grapes than in the control ones.

Among terpenes and norisoprenoids, the most important compounds responsible for the varietal aroma in grapes – geranic acid, epoxylinalol and vomifoliol – were the most abundant compounds for both the free and glycosylated forms of these classes.

Among the free benzene derivatives that were significantly higher in the blackberry sample, benzyl alcohol and phenylethyl alcohol were the most representative, whereas phenylethyl alcohol, homovanillyl alcohol, vanillyl alcohol and acetovanillone predominated in the bound benzene fraction.

With respect to alcohols and acids, the Pinot noir grapes were characterised by high concentrations of hexanoic acid, 1-hexanol and 2-hexen-1-ol, (*E*)- in the free aroma class, and high values of 1-butanol, 3-methyl- and 1-butanol, 2-methyl in the bound class. No aroma compound was identified exclusively in the treated grapes.

DISCUSSION AND CONCLUSIONS

The preliminary results obtained from this study suggest that native flora grown in close proximity to grapevines can exert an effect on the aromatic profile of the grapes. In this experiment, blackberry plants grown in association with Pinot noir vines significantly influenced the concentration of several grape-aroma compounds, increasing their content compared to that of the control vines grown alone.

We can hypothesise two different mechanisms to explain

TABLE 1

Concentration of free and bound aromatic compounds from Pinot noir grapes of vines grown alone ("control") and in association with blackberry plants ("treated"). Only the compounds showing statistically different contents between the two treatments are shown.

Free aroma compounds (µg/L)				Bound aroma compounds (µg/L)			
Compound	**Control**	**Treated**			**Control**	**Treated**	
Terpenes				**Terpenes**			
geranic acid	5.45	19.17	***	geranic acid	69.82	138.57	*
epoxylinalol	1.76	6.61	*	epoxylinalol	220.88	446.64	**
Norisoprenoides				linalool oxide	23.60	44.97	***
vomifoliol	40.35	176.19	***	exo-2-hydroxycineole	6.78	15.21	***
β-ionone epoxide	22.41	83.95	**	*α*-terpineol	15.60	37.56	**
3-oxo-*α*-ionol	2.68	32.83	*	epoxylinalol 2	23.13	42.56	**
3-hydroxy-7,8-dihydro-*β*-ionol	0.54	3.08	*	**Norisoprenoides**			
Benzene derivatives				vomifoliol	3523.73	7674.15	*
o-hydroxyphenethyl alcohol	1.24	4.89	**	*β*-ionone epoxide	48.88	82.18	*
acetovanillone	10.33	51.57	**	3-oxo-7,8-dihydro-*α*-ionol	70.84	133.40	**
benzoic acid	6.86	20.74	**	**Benzene derivatives**			
2,4'-dihydroxy-3'-methoxyacetophenone	3.10	12.38	**	*o*-hydroxyphenethyl alcohol	87.86	142.96	*
methyl vanillate	4.66	24.37	**	acetovanillone	274.57	473.02	*
phenol	1.67	5.53	**	methyl vanillate	143.30	259.55	*
p-hydroxyacetophenone	2.36	11.17	**	phenylethyl alcohol	1264.82	2028.00	*
ferulic acid	13.33	89.05	*	vanillyl alcohol	269.44	479.41	*
benzyl alcohol	538.51	1597.95	**	homovanillyl alcohol	620.65	1945.20	**
phenylethyl alcohol	296.87	766.56	*	acetosyringone	71.19	144.59	*
vanillyl alcohol	4.35	14.97	*	pyrogallol 1-methyl ether	7.67	67.65	*

TABLE 1 (CONTINUED)

Free aroma compounds (µg/L)				Bound aroma compounds (µg/L)			
Compound	**Control**	**Treated**			**Control**	**Treated**	
3-allylguaiacol	0.34	2.46	*	**Alcohols and acids**			
β-hydroxypropiovanillone	0.43	4.23	*	hexanoic acid	31.91	64.73	*
acetosyringone	0.83	6.21	*	3-hexen-1-ol	19.03	39.36	**
o-pyrocatechuic acid, methyl ester	2.23	19.67	*	1-butanol, 2-methyl	71.80	140.09	**
Alcohols and acids				1-octanol	2.45	6.72	*
hexanoic acid	515.10	1159.34	**	3-penten-1-ol, 2-methyl-	10.20	14.53	*
2-hexen-1-ol, (*E*)-	302.26	800.63	**	1-butanol, 3-methyl-	240.62	381.95	*
2-hexanol	33.72	51.90	**	**Ketones**			
3-hexen-1-ol, (*E*)-	93.21	245.02	**	4-oxoisophorone	5.99	8.31	*
1-hexanol	472.39	904.26	*				
3-hexen-1-ol	12.56	33.05	*				
dihydrophytol	0.90	5.24	*				
furfuryl alcohol	16.28	66.36	*				
Ketones							
3-pentanone, 2-methyl	1.58	2.42	**				

Statistical analysis was carried out using a multiple t-test. The data are mean values of triplicates. *, ** and *** denote significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

the increase of aroma concentration observed in the treated grapes compared to the control. For some aroma compounds, such as 3-hydroxy-7,8-dihydro-*β*-ionol, benzoic acid, benzyl alcohol, 1-octanol and 1-hexanol, airborne transfer from the blackberry fruit and leaves to the surface of the berries might have occurred, as reported previously for 1,8-cineole (Capone *et al*., 2012). These compounds, in fact, are the main volatile components of blackberry aroma (Georgilopoulos & Gallois 1987; Humpf & Schreier 1991), and display high concentration in blackberry plants.

Beside for aerial transfer, a root-mediated transfer can also be hypothesised. It has been well documented that plant roots can secrete many compounds that are able to influence the rhizosphere, the community of soil microorganisms and neighbouring plants (Bertin *et al*., 2003). Blackberry roots might have secreted exudates that are able to modify the rhizosphere, enhancing the mobilisation, availability or assimilation of mineral nutrients by vines grown in association with blackberries. This might well have promoted the aromatic biosynthesis, leading to increased volatile contents in grapes.

In conclusion, our preliminary data suggest that the association between blackberry plants and 'Pinot noir' grapevines has a quantitative effect on the grape aroma profile by promoting a higher aromatic concentration. This association seems not to exert any qualitative effects, since no aroma compound was identified exclusively in the treated grapes. The mechanisms of this interaction are still unknown and merit further investigation.

LITERATURE CITED

Bertin, C., Yang, X. & Weston, L.A., 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256, 67-83.

Capone, D.L., Jeffery, D.W. & Sefton, M.A., 2012. Vineyard and fermentation studies to elucidate the origin of 1,8-cineole in Australian red wine. J. Agric. Food Chem. 60, 2281-2287.

Flamini, R., Dalla Vedova, A. & Calò, A., 2001. Study on the monoterpene contents of 23 accessions of Muscat grape: Correlation between aroma profile and variety. Riv. Vitic. Enol. 2(3), 35-49.

Georgilopoulos, D.N. & Gallois, A.N., 1987. Aroma compounds from fresh blackberries (*Rubus laciniata*, L.). Z. Lebensm. Unters. Forsch. 184, 374-380.

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B. & Simal-Gándara, J., 2015. Wine aroma compounds in grapes: A critical review. Crit. Rev. Food. Sci. Nutr. 55(2), 202-218.

Herve, E., Price, S. & Burns, G., 2003. Eucalyptol in wines showing a "eucalyptus" aroma. In Proc. VIIeme Symp. Internat. d'Oenologie, Actualites Oenologiques, 19-21 June 2003, Bordeaux, France.

Humpf, H.U. & Schreier, P., 1991. Bound aroma compounds from the fruit and the leaves of blackberry (*Rubus laciniata* L.). J. Agric. Food Chem. 39, 1830-1832.

Romano, R., Trebes, V. & Barbeito, M.E., 2010. The influence of native flora on Argentine white wine terroir cv. Torrontes Riojano. VIII International Terroir Congress, 1(2), 62-68, 14-18 June 2010, Soave, Italy.

Romano, R., Trebes, V. & Gargantini, R., 2014. Influence of landscape on the Argentinean white wines "terroir": cv. Torrontes Riojano. BIO Web of Conferences 3, 02005. 37th World Congress of Vine and Wine and 12th General Assembly of the OIV, 9-14 November 2014, Mendoza, Argentina.

Yuan, F. & Qian, M.C., 2016. Aroma potential in early- and late-maturity Pinot noir grapes evaluated by aroma extract dilution analysis. J. Agric. Food Chem. 64, 443-450.