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Effects of 'hoja de malvón' disease on the composition, sensory properties and preference of Malbec wines from Mendoza, Argentina

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Abstract: 'Hoja de malvón' is a grapevine wood disease widespread in Argentina that decreases vineyard productivity and longevity. This study was conducted during two consecutive vintages to assess its influence on the general composition, sensory attributes and preference of *Vitis vinifera* L. cv Malbec wines. Batches of 120 kg of grapes harvested from vines with different degrees of (visual) symptoms of the disease were separately vinified. Grapes were grouped in three treatments: **T1**, grapes from vines with no symptoms; **T2**, grapes from vines with 10%–50% symptoms; and **T3**, grapes from vines with 50%–100% symptoms. Basic analyses of the grapes prior to crushing, and basic wine analysis were undertaken, together with spectrophotometric analyses of the wines after three months of bottle aging. The kinetics of alcoholic fermentation (AF) was followed daily and additionally the wines were assessed by a sensory panel. T3 grapes were characterized by comparatively lower initial sugar contents and higher titratable acidity. The AF kinetics were unaffected in the wines arising from the diseased grapes. There was no clear-cut effect of the disease on the wines' pH, volatile acidity, and total acidity; however, the later spectrophotometric analyses showed that the total phenolic index was the highest in T2 wines for both vintages. In 2005, the color index (CI) was higher in T3 and showed the lowest value in 2006. The sensory properties of the wines were different in the two vintages. In 2005, T2 and T3 wines were perceived as having higher color intensity, violet hue and spicy notes, T2 being the most preferred wine. In 2006, T1 and T2 showed a much better sensory profile than T3, but no preference for any wine was detected. This study demonstrates that only in 2005 wines made from grapes with 10%–50% symptoms showed an improved and recognizable compositional and sensory profile. Our results suggest that the effects of the 'hoja de malvón' disease on wine quality are not always positive. Therefore, the reduction in productivity, consistency, uniformity, and vineyard longevity that this disease causes may be more important than the slight improvement, if any, in wine quality.

Keywords: 'hoja de malvón' disease, wine, chemical composition, sensory properties, wine preference

Introduction

The grapevine disease known as 'hoja de malvón' (which literally means 'geranium-like vine leaf') is a wood disease widespread in the different Argentinean viticulture regions.[1–3] The disease is associated with an array of fungal species including *Inocutis jamaicencis*, *Phaeomoniella chlamydospora, Phaeoacremonium aleophilum, Phaeoacremonium parasiticum* and *Botryosphaeria* species[1,3–5] that affect the longevity, productivity and homogeneity of the vineyard, causing important economic losses in wine, table and raisin grapes.[6] The disease causes wood necrosis, decline and death of vines. Leaves are smaller than normal and chlorotic, with margins curling downwards resembling a geranium leaf.[1]

From both a viticulture and winemaking perspective, low vigor in the vineyard has traditionally been linked to an improved phenolic composition in the resulting wines.[7–9] Provided that the berry native phenolic composition imparts a huge impact on wine composition and overall quality[10,11] winemakers often seek grapes arising from moderate-to-low vigor vineyards. In vines, low vigor has been classically associated with deficit irrigation,[12,13] insect attack,[14] and viral[15] and fungal diseases.[3,16] In fact, in the Argentinean viticulture regions, it is a common belief among certain winemakers that the smaller berry size and the greater sun exposure of the bunches in 'hoja de malvón' infected vines (presumably resulting from a reduction in vine vigor) would improve the sensory profile of the wines (Carlos Catania, personal communication). To date, however, the effect that the 'hoja de malvón' disease exerts on wine chemistry and its sensory composition has not been assessed.

The effects of powdery mildew (*Erysiphe necator* Burr) in diminishing must and wine quality have been reported.[17–18] For instance, Stummer and colleagues detected changes in the composition and sensory attributes of both musts and wines coming from as low as 1% to 5% of affected bunches[18] and Calionec and colleagues found that this disease diminished the concentration of 3-mercaptohexanol, a major varietal aroma of Sauvignon Blanc wines.[19] Calzarano and colleagues reported a decrease in the enological quality of grapes and wines affected by 'esca' disease, due to the negative effects this fungal wood disease imparts on vine photosynthesis.[20]

Specifically, the aim of this work was to evaluate the basic chemical composition, sensory attributes and preference of Malbec wines produced with grapes affected by two different degrees of the 'hoja de malvón' disease (10%–50% and 50%–100% of visual symptoms in the vines that provided the grapes) and to compare them with the same parameters in a wine produced with grapes from vines with no symptoms. In order to accomplish this objective, we carried out a small-scale winemaking process of three different batches of grapes (ie, no visual symptoms, 10%–50% and 50%–100% of visual symptoms) during two consecutive vintages.

Materials and methods

Plant material and experimental setting

Grapes of *Vitis vinifera* L. cv Malbec from the 2005 and 2006 vintages were sourced from a 20-year-old vineyard (row orientation North-South, plant spacing 2.50 × 1.5 m) located in Mayor Drummond, Luján de Cuyo, in the province of Mendoza (middle-West of Argentina) at 33° 00′ South latitude, 68° 51′ West longitude and 912 meters above the sea level. Studies previously conducted to assess the spatial pattern of the disease in the vineyards showed a random spatial distribution for vines within each row, between adjacent rows and for the whole plot analyzed.[21] In the current experiment, the plot consisted of 10 rows of 75 vines each and on being analyzed by Chi-square analysis, results again showed a random spatial distribution of the disease vines inside the plot ($\alpha = 0.01$ and $P = 0.091$, data not shown).

The treatments were defined by direct visual inspection of foliar symptoms that the vines showed on the field during the corresponding growing season.[20] This methodology yielded three different treatments according to the extent and severity of visual foliar symptoms:

- **T1:** grapes from vines with no foliar symptoms of the disease; also referred to as control wine.
- **T2:** grapes from vines with a percentage of foliar symptoms between 10% and 50%.
- **T3:** grapes from vines with a percentage of foliar symptoms between 50% and 100%.

Harvest was performed when T1 grapes reached the pH, total acidity and sugar content commonly recognized as typical for production of Malbec wines in the region.[22] Batches of 120 kg of grapes for each treatment were hand-harvested on March 23rd (2005) and March 30th (2006) in 20 kg plastic boxes and transported to the winery facility of the 'Centro de Estudios de Enología' (CEE) at INTA EEA Mendoza.

Grape basic analyses at harvest

Prior to crushing, a set of 900 berries was randomly taken from each batch of clusters according to the methodology proposed by the Institut Coopératif du Vin.[23] The berries from each treatment were manually crushed, the must roughly filtered, and then analyzed in triplicate as follows. Sugar content was measured by a hand-held refractometer (PR-101, Atago Co, Japan) and expressed as °Brix (percent of soluble sugars), titratable acidity was determined by direct titration with NaOH 0.1M using bromothymol blue as an indicator and expressed as g/L of tartaric acid, and pH was measured with a pH-meter (Orion, Thermo Scientific, Waltham, MA,USA).

The basic initial analyses of the grapes from the three different treatments during both vintages are summarized in Table 1.

Table 1 Vintage year, treatment, and the basic composition of Malbec grapes harvested from vines with no symptoms and with two different severity levels of visual foliar symptoms of 'hoja de malvón'

Vintage year	Treatment*	°Brix	Titratable acidity (g/L tartaric acid)	pH
2005	T1	24.55 ± 0.07	4.27 ± 0.04	3.30 ± 0.01
	T2	25.50 ± 0.71	4.02 ± 0.05	3.31 ± 0.01
	T3	24.35 ± 0.07	4.62 ± 0.11	3.26 ± 0.01
2006	T1	26.15 ± 0.49	4.08 ± 0.04	3.67 ± 0.02
	T2	25.45 ± 0.07	4.11 ± 0.01	3.63 ± 0.01
	T3	25.25 ± 0.35	4.61 ± 0.06	3.62 ± 0.01

*T1, no symptoms; T2, 10%–50% symptoms; T3, 50%–100% symptoms.
Notes: The results are presented as the average (arithmetic mean) of three measures ± standard error.

Winemaking procedure

For each vintage the grapes were: de-stemmed, crushed with 50 mg/L of sulfur dioxide (SO_2) added and transferred to 3 sealable 100 L stainless steel tanks. At the onset of alcoholic fermentation (AF), the titratable acidity of the musts was raised to 7 g/L (2005 vintage) and to 7.5 g/L (2006 vintage) with food grade tartaric acid (Duperial, Argentina). The musts were inoculated using 30 g/hL of a 50/50 mixture of two active dry yeasts (ADY) inoculums (*Saccharomyces cerivisiae,* strains EC-1118 and ICV D-254, Lallemand Inc, Denmark), and added with 30 g/hL of an AF booster (Go-Ferm, Lallemand Inc, Denmark).

The musts were fermented to dryness (ie, below 1.8 g/L of reducing sugars) at a temperature between 26 and 30°C for 20 days, which was the maceration length for all the treatments. In order to monitor temperature evolution and the kinetics of sugar consumption (SC) during AF, daily measures of temperature and density were taken throughout the maceration process, and the density values were then converted into reducing sugars by equivalence table. Temperature data were very similar among tanks of the same vintage, and thus the arithmetic mean value from three tanks was obtained. During the maceration length, the musts/wines received two daily pumping-over's followed by a gentle punching-down. Drain-off was performed once the maceration time was completed. Press-wines were discarded and run-off wines were placed into 25 L glass-carboys at a temperature of 21°C ± 2°C, where malolactic fermentation (MLF) occurred spontaneously. MLF was followed weekly by paper chromatography[24] until depletion of the malic acid, which was confirmed by enzymatic determination (Vintessential Lab, Dromana, VIC, Australia). MLF was considered completed once the malic acid content of the wines fell below 0.1 g/L.[25]

After MLF was completed, the wines were racked, 30 mg/L of SO_2, added stored at 1°C for 30 days to allow tartaric acid stabilization, racked again, and allowed to settle overnight at 18 °C. Afterwards, free SO_2 was adjusted to 35 mg/L, then the wines were bottled and stored horizontally in the CEE cellar at INTA EEA Mendoza under controlled conditions of temperature and humidity until needed.

Basic wine analysis

The wines were analyzed after three months of bottle aging as follows. Alcohol content (ethanol [Eto], expressed as % v/v), volatile acidity (VA, expressed as g/L of acetic acid), titratable acidity (TA, expressed as g/L of tartaric acid) and reducing sugars (RS, expressed as g/L of reducing sugars) were determined according to Argentinean official methods.[26] pH was measured with a pH-meter (Orion, Thermo Scientific, Waltham, MA, USA) and malic acid depletion was confirmed by enzymatic determination (Vintessential Lab, Dromana, VIC, Australia). Free and total SO_2 levels were determined using the aspiration method.[27]

Wine spectrophotometric analysis

Several spectrophotometric measures were performed. The chromatic parameters were determined as follow. The color index (CI), was determined as the sum of the sample absorbance's at 420, 520 and 620 nm,[28] and the analytical hue (H), as the ratio between the absorbance at 420 and 520 nm.[29] The total polyphenolic index (TPI) was determined as the absorbance of the sample diluted 1/100 under UV light in a 1 cm-path quartz cell.[29] The color fractions due to free anthocyanins (FA), copigmented anthocyanins (CA) and polymeric anthocyanins (PA) were assessed following the methodology proposed by Levengood and Boulton.[30] The total anthocyanin content (TAC) was determined according to the method proposed by Amerine and Ough,[31] and modified by Jofré and colleagues.[32] TAC was expressed as mg/L of malvidin chloride according to the following calculation:

Malvidin chloride (mg/L) = [(A2–A1) – p]/q, where: A1 = reading in water; A2 = reading with bisulphite to 5%; $p = 0.0042$; q = 0.0002.

Prior to each spectrophotometric measurement, the samples were centrifuged for 30 minutes at 3500 rpm and then filtered through a 0.45 µm membrane (Sartorius, Goettingen, Germany). All the measurements were performed in a Perkin-Elmer Lambda 3B spectrophotometer (Norwalk, CT, USA).

Wine sensory analysis and preference test

The sensory analysis of the wines was carried out by a sensory panel composed of 9 (2005 vintage) and 10 (2006 vintage) experienced panelists belonging to the permanent staff of the CEE at INTA. Panelists' ages ranged from 25 to 63 years old. The wines were analyzed after 3 months of bottle aging, so the first session took place in August 2005 and the second in September 2006. Prior to each session, the wine attributes were defined by consensus among all the panelists, engaging in discussions on the intensity and worthiness of including a particular attribute. During each sensory analysis session, the panelists measured the intensity of each selected attribute by means of a structured scale ranging from 0 (total absence of sensation) to 5 (maximum presence of sensation); the arithmetic mean of each attribute for each wine was then obtained.

In addition to wine attributes, the panel preference for the different wines was determined by means of the Kramer test, also known as 'rank test'.[33] To carry out this test the panelists are asked to rank the wines according to their preference, from the most preferred to the least, beginning with 1. The sum of the preferences for each wine constitutes a rank or preference order (SP). The Kramer test establishes, in accord with the number of panelists, an interval ('preference interval'). The wines whose SP values are below the lower value of the interval are considered the most preferred, the ones that fall inside the interval are considered moderately preferred, and, finally, the ones are above the higher value of the interval are considered the least preferred.

The wines of each treatment were randomly served in tulip-shaped transparent (INAO) glasses and covered with plastic lids over the rim just after pouring. In order to minimize sample taste carryover, judges were asked to rinse their mouth with mineral water and to eat an unsalted cracker between samples. Both sessions were carried out at room temperature (20°C ± 2°C) under natural day-light.

Data treatment and statistical analysis

Even if all the analyses were done in triplicate, the treatments at grape and wine level had no replicas, so the basic grape and wine composition as well as the spectrophotometric analyses for each wine were not submitted to any statistical analysis and the raw data are presented as the arithmetic mean of three measures followed by the standard error of three measures. Conversely, during the sensory analyses each panelist was considered as a replica; thus, the sensory data were suitable to be submitted to one-way analysis of variance (ANOVA) in order to assess the influence of each treatment on the sensory composition of the wines. The Tukey's test at $\alpha = 0.05$ level was applied, and differences were expressed as the arithmetic mean of each variable followed by the standard error. In order to confirm the fulfillment of the ANOVA's assumptions in the data collection, error independence and normality assumption were tested by the Shapiro-Wilks modified normality test, confirming that there was no violation at the principles of normality. The statistical software Statgraphic Plus version 5.1 (Statistical Graphics Corp, Warrenton, VA, USA) was employed to analyze the data.

Results

Grape basic analyses at harvest

Table 1 shows the basic analyses performed in the grapes of each treatment prior to crushing. In 2005, T2 showed an initial sugar content (expressed as °Brix) slightly higher and a lower TA than the other two treatments. Conversely, T3 showed both the lowest sugar content and pH and the highest TA. In 2006, the initial sugar content of the grapes was higher in T1 whereas TA was slightly lower in this treatment (compared with T2) or much lower (compared with T3). The sugar content and pH were closer between T2 and T3, but TA was slightly higher in T3.

Alcoholic fermentation development

Figure 1 shows the time course of SC and temperature evolution during AF in the wines of each treatment during both vintages. General musts' temperatures during AF were slightly lower in the 2005 vintage than in the 2006 vintage. As for SC, in the 2005 vintage, the general trend in all must fermentations was that they occurred in a somewhat slower fashion than in 2006, especially for T1 wine. The later wine did not reach the value threshold that indicates dryness (which means that only traces of residual sugars are left in the wines, ie, <1.8 g/L of reducing sugars) until the 15th day, whereas T2 and T3 reached this value at the 13th and 11th days from the onset of AF, respectively (data not shown).

In the 2006 vintage, even if the grapes had a higher sugar content, the kinetic of the AF, especially for T2 and T3 wines, progressed faster than in the 2005 vintage. No remarkable differences among treatments were found on the AF process from the onset of this process until the 6th day. Hereafter, the AF of T1 wine turned somewhat sluggish for the following 4 days, which ended with this wine having a higher content of remaining reducing sugars at the end of the AF period and thus after bottle aging (Table 2). Conversely, T2 and T3 wines reached dryness at the 11th and 9th days from the onset of AF, respectively (data not shown).

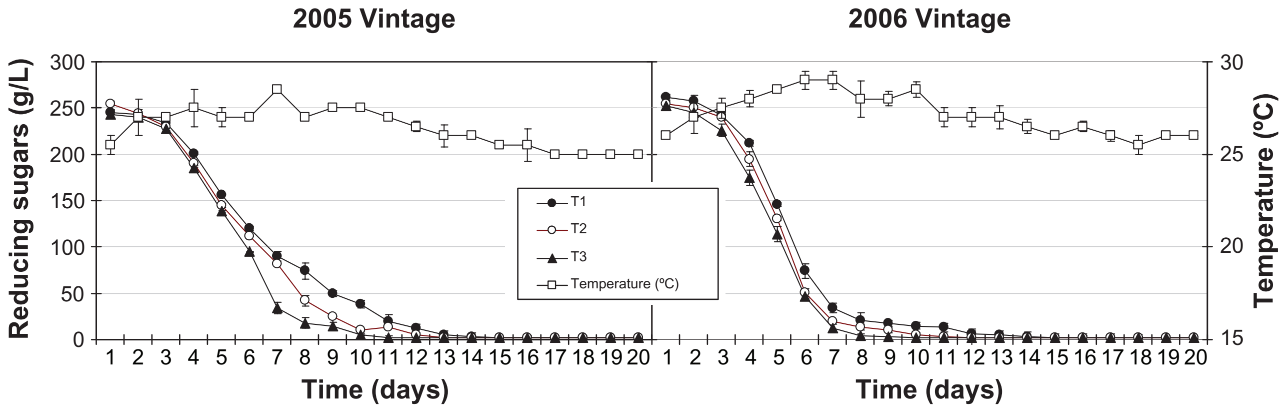

Figure 1 Time course of sugar consumption (SC) and the average temperature (T °C) during alcoholic fermentation of Malbec musts/wines made with grapes harvested from vines with no symptoms and with two different severity levels of visual foliar symptoms of 'hoja de malvón'.
Notes: Vertical bars denote the standard error of three measures. T1, no symptoms; T2, 10%–50% symptoms; T3, 50%–100% symptoms.

Wine basic analysis

Table 2 shows the vintage year, treatment and the basic analyses of the wines after three months of bottle aging.

In the 2005 vintage, no remarkable differences between the wines were found in Eto, TA and pH. VA (a parameter that indicates the wine's microbiological and sanitary conditions), was higher in T3 wine, although in all the three wines the VA values were well below the legal limit (locally established: 1.20 g/L of acetic acid).[26] In this same vintage, as already stated, all the wines reached dryness, so it was expected that the wines after three months of bottle aging showed the same value of RS ($<$1.80 g/L).

In the 2006 vintage, all the three wines showed a higher Eto and pH values than in the 2005 vintage, with T1 and T2 showing higher Eto values than T3. These results are consistent with higher sugar levels and lower TA values for the grapes of the 2006 vintage (Table 1). In spite of having performed the same TA correction for all three treatments at the onset of AF, TA was slightly lower in T2. VA was somewhat higher in both disease-affected treatments (T2 and T3), nevertheless such values still remained well below the legal limit of 1.20 g/L of acetic acid.[26] As for the RS content of the wines, this value did not change from that obtained at the end of the AF, ie, T2 and T3 showing dryness values, whereas T1 showed a few grams of residual sugar above the dryness threshold.

Wine spectrophotometric analysis

Table 3 shows the spectrophotometric analyses of the wines after three months of bottle aging.

In the 2005 vintage, T2 showed the highest TPI value whereas T3 showed the highest CI value. H, a parameter that relates the absorbance at 420 nm (brownish wine pigments) and 520 nm (reddish wine pigments) was higher in the control wine, suggesting that this wine was more brownish in color than both wines arising from disease-affected vines. TAC was higher in T3 wine as well as the FA and PA values, whereas CA was the lowest.

Table 2 Vintage year, treatment, and the basic analyses of Malbec wines made with grapes harvested from vines with no symptoms and with two different severity levels of visual foliar symptoms of 'hoja de malvón'

Vintage year	Treatment*	Alcohol content (% v/v)	Total acidity (g/L tartaric acid)	Volatile acidity (g/L acetic acid)	Reducing sugars (g/L)	pH
2005	T1	14.87 ± 0.04	5.09 ± 0.08	0.62 ± 0.04	$<$1.80	3.59 ± 0.01
	T2	14.85 ± 0.01	4.98 ± 0.04	0.63 ± 0.04	$<$1.80	3.58 ± 0.02
	T3	14.85 ± 0.07	5.21 ± 0.08	0.68 ± 0.03	$<$1.80	3.60 ± 0.01
2006	T1	15.85 ± 0.07	5.13 ± 0.11	0.49 ± 0.02	2.55 ± 0.30	3.91 ± 0.01
	T2	15.88 ± 0.04	4.92 ± 0.05	0.54 ± 0.01	$<$1.80	3.94 ± 0.01
	T3	15.65 ± 0.07	5.22 ± 0.07	0.53 ± 0.04	$<$1.80	3.84 ± 0.02

*T1, no symptoms; T2, 10%–50% symptoms; T3, 50%–100% symptoms.
Notes: The results featured were obtained after three months of bottle aging, and are presented as the average (arithmetic mean) of three measures ± standard error.

Table 3 Vintage year, treatment, and the spectrophotometric analyses of Malbec wines made with grapes harvested from vines with no symptoms and with two different severity levels of visual foliar symptoms of 'hoja de malvón'

Vintage year	Treatment*	Total polyphenol index	Color index	Hue	Total anthocyanins (mg/L)	Copigmented color (%)	Free anthocyanins color (%)	Polymeric color (%)
2005	T1	49.85 ± 0.21	0.96 ± 0.02	0.58 ± 0.01	406.25 ± 55.30	49.90 ± 5	21.40 ± 4	28.70 ± 8
	T2	54.00 ± 0.71	1.08 ± 0.03	0.56 ± 0.01	464.45 ± 77.86	51.30 ± 4	18.60 ± 4	30.10 ± 5
	T3	46.75 ± 0.35	1.18 ± 0.03	0.55 ± 0.02	480.23 ± 83.82	41.25 ± 4	27.25 ± 4	31.50 ± 6
2006	T1	44.10 ± 0.57	0.97 ± 0.01	0.70 ± 0.03	312.82 ± 33.80	43.60 ± 4	21.20 ± 7	35.20 ± 7
	T2	45.35 ± 0.92	0.93 ± 0.01	0.73 ± 0.04	277.89 ± 31.28	44.50 ± 5	19.60 ± 7	35.90 ± 5
	T3	43.00 ± 0.71	0.91 ± 0.01	0.70 ± 0.01	393.01 ± 40.10	38.75 ± 3	24.10 ± 4	37.15 ± 5

*T1, no symptoms; T2, 10%–50% symptoms; T3, 50%–100% symptoms.
Notes: The results featured were obtained after three months of bottle aging, and are presented as the average (arithmetic mean) of three measures ± standard error.

Generally speaking, the 2006 wines showed lower TPI and CI values and higher H values than their counterparts from the 2005 vintage. TPI showed the highest value in T2 wines whereas CI showed the highest value in T1. Also, T2 wines showed higher H values. As in the 2005 vintage, TAC was higher in T3 wine, intermediate in T1, and the lowest in T2. Regarding the color fractions in the 2006's wines, there was no clear trend for a particular treatment, although, as in the 2005 vintage, T3 showed higher FA and PA values and a lower CA value than the two remaining wines.

Wine sensory analysis and preference test

Figure 2 presents the sensory profile plots of the wines made from the three treatments in both vintages under analysis.

In the 2005 vintage, both T2 and T3 wines were perceived by the panelists as having more visual color and violet hue than T1. The control wine was perceived as being significantly fruitier though less spicy than the wines arising from grapes of the disease-affected vines. In the in-mouth appraisal of the wines, only bitterness was found to be emphasized in T3, whereas no differences among the wines were detected in concentration, astringency or acidity. T2 was preferred over T1 and T3, and between these last two wines, T3 was ranked as the least preferred (Table 4).

In the 2006 vintage, T1 and T2 wines showed more violet hue than T3. In addition, T1 showed a significantly higher global aromatic intensity, and T2 was perceived as significantly more floral. In the 2006 vintage, there were

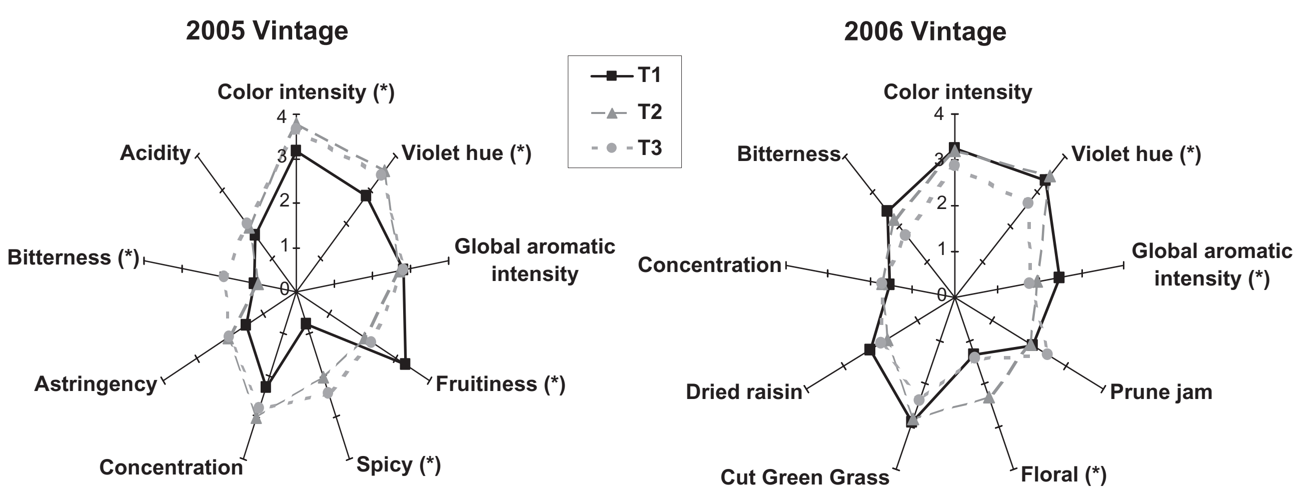

Figure 2 Sensory profile plots of wines made with grapes harvested from vines with no symptoms and with two different severity levels of visual foliar symptoms of 'hoja de malvón'.
Notes: The results featured were obtained after three months of bottle aging, and are presented as the average (arithmetic mean) of 9 (2005) and 10 (2006) non-trained judges. Sensory attributes followed by (*) denote significant differences for Tukey's test and $P < 0.05$. T1, no symptoms; T2, 10%–50% symptoms; T3, 50%–100% symptoms.

Table 4 Vintage year, treatment, and the panel's preference of Malbec wines made with grapes harvested from vines with no symptoms and with two different severity levels of visual foliar symptoms of 'hoja de malvón'

Vintage year	Treatment*	Preference interval	SP	Significance ($P < 0.05$)**
2005	T1	13–23	17	b
	T2		10	a
	T3		27	c
2006	T1	14–26	17	b
	T2		26	b
	T3		17	b

*T1, no symptoms; T2, 10%–50% symptoms; T3, 50%–100% symptoms.
**Different letters within the column denote a statistically different preference according to the Kramer test.[27]
Notes: The results featured were obtained after three months of bottle aging.

no differences found in the in-mouth appraisal of the wines (Figure 2) or in the preference for any wine (Table 4).

Discussion

Grape basic analyses at harvest

Even without statistical support, there were two trends on the basic grape compositions that are worth noting. T2 (2005) and T1 (2006) showed higher °Brix values than the two remaining treatments. Likewise, TA values were slightly lower in these same treatments, probably due to the general higher maturity level of the corresponding grapes at harvest.[34] It is not clear why the sugar levels were higher in the grapes arising from vines with 10%–50% symptoms (T2) in the 2005 vintage. The grapes arising from more affected vines (T3) showed slightly lower °Brix values in the 2005 vintage and almost 1 °Brix less than T1 in the 2006 vintage. The latter suggests that in both vintages, the more affected vines gave rise to grapes with comparatively lower initial sugar contents. This result is in line with the reported fact that some grape fungus diseases such as 'esca'[20,35] or powdery mildew[17] decrease the initial sugar contents of juices obtained from diseased grapes compared with juices obtained from healthy grapes. One of the main foliar symptoms triggered by the 'hoja de malvón' disease is that leaves become smaller than normal, chlorotic and with the edges rolled downwards.[1] It may be possible that the lower photosynthetic surface fostered by a severe attack of this disease could have affected photosynthesis, thus reducing carbon fixation and the sugar content in T3 grapes accordingly. Unfortunately, since no real replicas were taken in the berries at harvest, we cannot draw a definitive conclusion on this point.

Also, T3 showed comparatively higher TA values at harvest during both vintages. This trend may be explained as a result of a comparatively higher malic acid concentration in the berries from T3 vines. Indeed, Calzarano and colleagues postulate that in musts from vines affected by 'esca' disease, the pathological state would lead to an increase in the respiration rate of sugars, thus yielding malic acid via glycolysis,[35] which may explain the higher TA (and also the lower initial sugar content) in the T3 musts. TA has also been shown to increase in grapes affected by powdery mildew[17,19,36] or *Botrytis*.[37]

Alcoholic fermentation development

On the whole, the AF of the musts arising from affected vines (T2 and T3) progressed in a typical fashion during both vintages, showing no effect of the 'hoja de malvón' disease on the kinetic of this process. In fact, T3 wines showed a faster AF kinetic change (especially compared with T1) during both vintages. Interestingly, even if the general sugar contents were higher in the 2006 vintage, the wines from this vintage showed a faster AF (particularly T2 and T3 wines) than the ones of the 2005 vintage. Since both the yeast inoculum and winemaking procedure was exactly the same in both vintages, the slower AF rate in 2005 was probably related to lower AF temperatures in 2005 (ranging from 26–28 °C, Figure 2), for even slight differences in FA temperatures lead to different FA kinetics.[38,39] However, T1 wine from the 2006 vintage was the only one that did not achieve full dryness once the maceration time was completed (Table 2), which could be linked to its grapes having the highest initial sugar content (Table 1).

On the other hand, wines from affected grapes showed a normal AF kinetics during both vintages. A possible explanation is a higher content of yeast assimilable nitrogen (YAN) in both T2 and T3 grapes as a result of the disease. An increase in YAN values has been reported in grapes affected by 'esca'[20] and powdery mildew,[36] as a result of the degradation of the protein component of the wood effected by these fungi that results in the release of free amino acids into the xylem[40] or through direct synthesis of these compounds by fungi metabolism.[35] In any case, the higher YAN levels in the affected grapes have helped overcome the overall high sugar levels of the grapes from T2 and T3 during both vintages, thus allowing these wine to reach dryness. Conversely, T1, whose grapes had the highest sugar content in 2006, may have had very low YAN levels, thus explaining the sluggishness of its AF kinetics compared with the two other wines.

Wine basic analysis

There was no clear-cut effect of the disease on the wines for pH, VA, and TA, which is in agreement with previous data recorded in wines obtained from healthy and 'esca' affected vines.[20] From a technological standpoint, neither Eto nor VA values were affected by the disease during both vintages. As for the Eto content, Calzarano and colleagues have shown that the alcohol content of Trebbiano d'Abruzzo wines from symptomatic vines affected by 'esca' was about 1% v/v lower than that of wines from healthy and asymptomatic vines,[20] which did not match our results.

General values of Eto and pH were higher in the 2006 wines, and VA was higher in the 2005 wines. It is known that Eto, pH, TA and VA values are strongly affected by a particular growing season.[41] Thus it may be that this variable, rather than the disease, is the factor responsible for these small differences seen between the vintages.

Wine spectrophotometric analysis

Unlike the basic wine analyses, the spectrophotometric analyses showed a more pronounced effect of the disease on the chemical composition of the wines. This may be due to the fact that the spectrophotometric measures carried out in this study involve either directly (eg, TPI, TAC, CA, FA, PA) or indirectly (eg, CI, H) the phenolic composition of the wines. It has been shown that wine phenolics can be greatly affected by several vine fungal diseases such as 'esca',[20,35] powdery mildew[17] or *Botrytis*,[37] even more than the basic composition of the wine.[35] Thus, higher differences among treatments in the spectrophotometric measures would be expected.

In the 2005 and 2006 vintages, although especially in the former, T2 showed the highest TPI value, whereas T3 showed the lowest value. These results are in partial agreement with previous data in Trebbiano d'Abruzzo wines, where the authors observed an increase in the total polyphenol content of the wines from symptomatic vines affected by 'esca'.[20]

As a trend, in T3 wines from both vintages, TAC and FA achieved the highest values but also showed the lowest CA values. Lower anthocyanin content in the grapes affected by some biotic stress such as powdery mildew[42] has been previously reported, which contradicts our results. In our case, however, the lower vigor induced by the disease (without seriously affecting photosynthesis, as it can be deduced by analyzing the initial sugar contents) could have led to a smaller berry size and a concomitant rise of the skin to juice ratio,[43] thus explaining higher TAC values in T3 wines. As for FA values in T3 wines, they could be regarded as a negative outcome since, from a color evolution point of view, a higher content of FA together with lower CA values may result in an early color loss.[44]

In general, the 2005 wines showed higher TAC and CA with lower PA values. The 2005 vintage was a year of higher polyphenol extractability compared with the 2006 vintage (Carlos Catania, personal communication), thus explaining higher TAC levels in the 2005 wines. The higher CA values in the wines of the 2005 vintage could be explained by the comparatively lower levels of Eto in these wines. Indeed, Eto levels were as much as 1% lower in the 2005 vintage compared with the 2006 vintage. It has been established that the copigmentation phenomenom is readily disrupted by higher levels of Eto,[44] a fact that can account for the aforementioned differences.

Wine sensory analysis and preference test

Different disease treatments gave rise to different wine profiles, which we assume were correlated with the disease. Moreover, the sensory properties of the wines obtained from healthy and affected grapes were different in the two vintages.

In the 2005 vintage, T2 showed a better sensory profile than T1 and T3, which was readily confirmed by the preference test (Table 4); T3 showed a greater color intensity and violet hue, which is in agreement with the spectrophotometric results (notably IC) presented above (Table 2). An improved color and violet hue in T3 wine from 2005 confirms the hypothesis held by some winemakers that wine from 'hoja de malvón' affected grapes are deeper in color and their overall sensory properties are preferable. However, in this vintage T3 was found to be the least preferred by the panelists (Table 4), which is in agreement with previously reported data on the lack of preference for Cabernet Sauvignon, Thompson Seedless, Carignan and Ribier wines arising from powdery mildew affected grapes.[19,45] For the in-mouth appraisal, T3 was perceived as being significantly more bitter than its two other counterparts. It is widely known that flavonols, a class of phenolic compound located in berry skins, the largest proportion of which is quercetin-3-glucosyde,[46] have the ability to elicit bitter sensations transferred in wine.[47,48] As in other grape varieties, in Malbec, flavonols are known to increase under increased sun exposure, as a defense mechanism of the vine to absorb high amounts of UV light[49], these compounds acting as a natural sunscreen.[10,50] It may be possible that, as a result of a higher sun exposure in T3 grapes (presumably achieved by the effect of 'hoja de malvón' disease), the concentration of flavonols at berry level was higher than in T1 and T2, where these compounds are extracted during the winemaking process to finally give the resulting wines a discernibly bitter palate. Additionally, it has been demonstrated that some fungal diseases, such as powdery mildew, decrease the wine varietal aroma.[19] These assumptions may also help explain why this wine was ranked as the least preferred.

In the 2006 vintage, T1 and T2 showed a much better sensory profile than T3. This improvement was noticeable by the tasting panel and by statistical analysis (notably for violet hue, Figure 2). Interestingly, T3, which showed a significantly higher violet hue in 2005, showed a significantly lower intensity of this attribute in 2006, thus emphasizing the lack of consistency of the 'positive' sensory effects triggered by the 'hoja en malvón' disease. As for the control treatment, this wine displayed a higher global aromatic intensity, whereas T2 was particularly high in the floral attribute. However, no preference for any wine was found, suggesting that these differences among wines in some particular attributes were not strong enough to prompt the panel preference towards a particular wine. Globally, the sensory appraisal of wines from the 2006 vintage showed that there was no sensory positive effect induced by this disease; indeed, in the two vintages none of the affected wines showed better in-mouth properties than its control counterparts. The better effects were restricted to color in the 2005 vintage.

Conclusions

To the best of our knowledge, this is the first time that the effect on the chemistry and sensory properties of wines made from vines affected by 'hoja de malvón' has been reported.

The kinetics of the alcoholic fermentation was unaffected in the wines arising from diseased grapes, thus suggesting that the disease neither releases any harmful compounds for yeast development nor deprives the must of the main growing factors for yeast. The TPI was the highest in T2 wines for both years, suggesting that the moderate stress the vines are supposed to be under from a mild attack would enhance phenol accumulation, perhaps as a protection mechanism. However, it may be possible as well that the lower berry size, as a result of the decreased vigor induced by this disease, had increased the skin to pulp ratio, thus enhancing polyphenol concentration. The color index was higher in T3 (2005) but showed the lowest value in 2006, an indicator that the effect of this disease on the enhancement of the color index did not endure in subsequent harvest years. It is possible that the long term effects of this disease may have affected some target berry compounds in color development, such as tannins, thus jeopardizing the stability of wine color during winemaking and aging. In fact, in 2006, the control treatment showed the highest color index and the lowest H value, which supports the latter assumption. During sensory analysis in 2005, T2 and T3 wines were perceived to have greater color intensity, violet hue and spicy notes whereas T1 was perceived as the fruitiest in aroma. In 2006, T1 and T2 were perceived with greater violet hue than T3, and T2 wine was perceived with highest floral intensity of aroma. While in 2005 T2 was preferred, no preference among the wines was found in 2006.

It is a folk belief among some Argentinean winemakers that wine made from vines infected with the 'hoja de malvón' disease results in an improved wine quality because of the low vine vigor. The better quality may be associated with an increase in total phenolic concentration by synthesis or by increasing the skin to pulp ratio, as suggested above. This study shows, however, that only in the 2005 vintage did wines made from the grapes with 10%–50% symptoms show an improved sensory profile (but not in 2006). Moreover, from a sensory point of view, the positive effects of this disease on wine quality showed a lack of repeatability among the studied vintages. This may indicate that the effects of the disease on wine quality are not always positive and that the reduction in productivity, consistency, uniformity, and vineyard longevity, which this disease causes may be more important than the slight improvement, if any, in wine quality. Other viticulture practices (eg, canopy and crop level management, deficit irrigation, intercrop management) would be better options for the improvement of grape and wine quality (eg, TPI, color intensity) without compromising vineyard sustainability.

Disclosure

The authors declare no conflicts of interest.

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