

Under-vine cover crops: impact on weed development, yield and grape composition

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ABSTRACT

This study aims to evaluate the interest of using an under-vine cover crop as a sustainable management tool replacing herbicides or tillage to control weeds, evaluating its effects on yield and berry parameters in a semi-arid climate. The performance of *Trifolium fragiferum* as an under-vine cover crop was evaluated in 2018 and 2019 in a Merlot vineyard in Traibuenas (Navarra, Spain). This trial showed that the soil under the vines was covered by 80 % of the cover crop in August 2018 and 100 % in Aug 2019, with clover (*T. fragiferum*) comprising around 26 % and 70 % of the cover crop surface, respectively. The presence of the cover crop only reduced the number of shoots in the second year, although both years there was an increment in water stress. Neither yield, cluster weight nor berry weight were affected by the presence of the under-vine cover crop. Similarly, no changes in grape composition were observed. The use of *T. fragiferum*-like cover crops under the vine allows for better control of weeds, provided a good installation is achieved. In the first two years, this cover crop reduced vegetative growth and increased water deficit slightly. However, no changes in yield and grape composition were observed. In a context of herbicide suppression and search for sustainable management, under-vine clover cover crops constitute a viable alternative in semi-arid regions provided drip irrigation can be applied.

KEYWORDS

Trifolium fragiferum L., vine, water potential, Carbon isotope ratio

INTRODUCTION

Many vineyards worldwide use cover crops in the inter-row as a soil management strategy as, under many circumstances, their benefits outnumber the potential drawbacks they may have (Steenwerth and Guerra, 2012). However, the space under the vines (i.e., the rows) are frequently kept free of vegetation, at least in Mediterranean climate conditions, to avoid high competition with the crop, both for nutritional and water resources. This bare area is commonly maintained through mechanical tillage or using herbicides. As herbicides are currently being questioned for their environmental impact, and legal constraints to their use increase (AFP, 2019), there is a great

interest in reducing or eliminating their use. Additionally, focusing on the wine sector, some studies have demonstrated that herbicides can reduce grapevine root mycorrhization and soil microorganism populations or alter nutrient composition in grapevine roots, leaves, grape juice and xylem sap (Chou *et al.*, 2018; Donnini *et al.*, 2016; Zaller *et al.*, 2018), and that these changes may affect wine fermentation (Morozova *et al.*, 2017). Although less questioned, mechanical tillage of the row area to keep it free of vegetation also has some drawbacks as it generally results in an increased cost associated with the higher frequency of tilling operations compared to herbicides, while it also requires

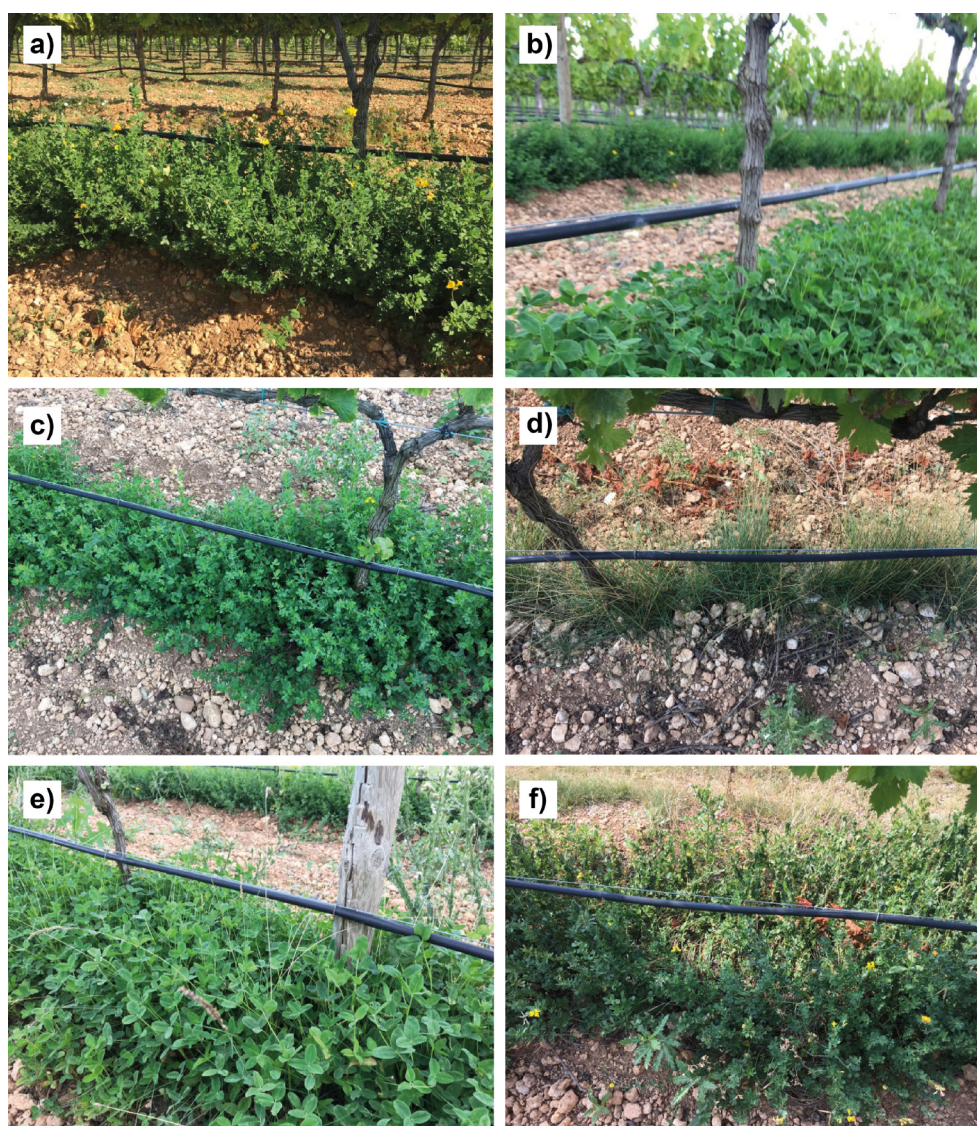


FIGURE 1. The appearance of the different under-vine covers in their second evaluation season in a preliminary test.

a) *Lotus corniculatus*, b) *Trifolium fragiferum*, c) *L. Corniculatus* + *T. fragiferum*, d) *Festuca ovina*, e) *F. ovina* + *T. fragiferum*, f) *Lolium rigidum* + *L. corniculatus*

specific equipment. Additionally, soil disturbance associated with mechanical tilling enhances organic matter degradation and, in turn, alters the population of soil microorganisms, decreases water infiltration and increases the soil susceptibility to erosion (Ben-Salem *et al.*, 2018; Ruiz-Colmenero *et al.*, 2011; Virto *et al.*, 2012).

Given the drawbacks of using herbicides or mechanical tillage for soil management under the vines, the establishment of cover crops, with low competition potential, appears as an appealing alternative (Jordan *et al.*, 2016; Karl *et al.*, 2016). This option has been evaluated in a very reduced number of research works (Chou and Vanden Heuvel 2018; Coniberti *et al.*, 2018; Hickey *et al.*, 2016; Jordan *et al.*, 2016; Karl *et al.*, 2016; Penfold *et al.*, 2018), out of which nearly none has been conducted in Mediterranean conditions. In this context, this work aims to evaluate the interest of using under-vine cover crops as a feasible and sustainable management option for vineyards in Mediterranean areas. As a preliminary test, six different cover crop mixes (*Lotus corniculatus*, *Trifolium fragiferum*, *L. Corniculatus* + *T. fragiferum*, *Festuca ovina*, *F. ovina* + *T. fragiferum* and *Lolium rigidum* + *L. corniculatus*) were evaluated during 2016–2018 to check their adaptation to the vineyard and competition with other adventitious species (Figure 1). Among them, *T. fragiferum* was selected as the most suitable species due to its ability to compete with other species, its reduced cost of the establishment (it is perennial) and to its ability to supply nitrogen to the crop through nitrogen fixation (Abad *et al.*, 2019).

METHODS

The trial was carried out in a vineyard belonging to Bodegas Ochoa winery, located in the village of Traibuenas (Navarra-Spain) during 2018 and 2019. The cultivated variety is Merlot (clone 343) on rootstock 420A, planted in 2001, with a distance between vines of 1 m and between rows of 3 m, and trained as a vertical shoot positioned double Cordon Royat. The plot has a drip irrigation system, with 3.5 L h⁻¹ drippers spaced 0.75 m. The irrigation started the 6th of June in 2018 and the 16th of June in 2019, applying 6-hour watering once a week until August, when it was changed to two 3-hour irrigation days per week until the 10th September in 2018 and until the 20th of September in 2019.

The soil can be classified as Typic Calcixerepts, with a loam texture up to the first 90 cm and a sandy loam in depth. The level of organic matter is 1.26 %, the total carbonates are 33 % and the active limestone is 8.94 %. The climate, according to Papadakis classification, can be defined as a humid temperate Mediterranean climate. Climate data for the 2018 and 2019 seasons were obtained from an automatic weather station belonging to the regional meteorological network, located close to the vineyard (1500 m in a straight line), and are summarized in Table 1 and Figure 2.

TABLE 1. Mean temperature, rainfall, Heliothermal Index (HI), Cool Night Index (CI) and Dryness Index (DI) calculated for both seasons, according to (Tonietto and Carbonneau, 2004).

	2018	2019
Mean temperature (Apr–Oct, °C)	19	19
Rainfall (Apr–Oct, mm)	52	7
Heliothermal Index, HI	2378	2391
Cool night Index, CI (°C)	13.5	11.9
Dryness Index, DI (mm)	45	-113

Data obtained from Traibuenas meteorological station belonging to the regional network. Sensor models are Vaisala HMP45C for temperature and Campbell ARG100 for rainfall.

The experimental design included two treatments: cover-crop under the vines (UV) and mechanically tilled (T) control, with five replicates per treatment in alternate rows. The first six vines in each row were not considered for sampling, and out of the 60 vines available per replicate, 20 vines were selected for homogeneity using measurements of the trunk cross-sectional area, estimated measuring two orthogonal diameters 30 cm above the ground. These 20 vines were marked and all the measurements performed in them.

For cover cropped vines (UV), seeds of *Trifolium fragiferum*, at a 15 g m⁻² dose were sown on a 40 cm-wide strip. Sowing was done manually on late February in 2018 after an inter-vine cultivator operation. Two days later, there was a light snowfall which helped to settle the seed on the ground. In the bare rows (T), inter-vine tilling work was carried out on the same sowing preparation dates. The area was kept relatively free of vegetation tilling four dates: at the beginning of November, March, May and July in 2018, and beginning of November, March, May and end of July in 2019 (Figure 3).

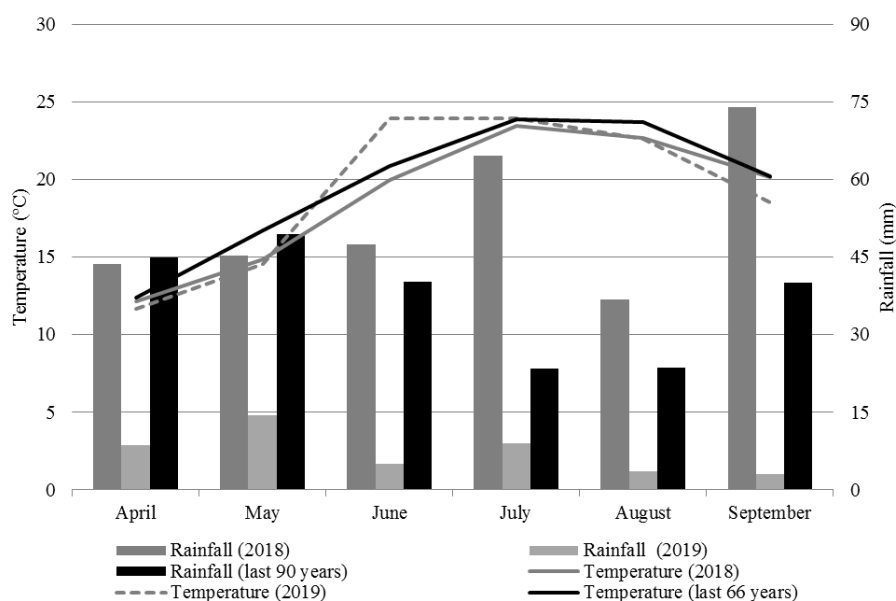


FIGURE 2. Climate conditions of the two seasons, 2018 and 2019 different seasons (April–September): mean monthly temperature (°C) and accumulate monthly rainfall (mm).

At the beginning of August, approximately coinciding with the mid-veraison, a detailed visual assessment of the presence of adventitious vegetation was carried out. All the species present were identified, and a percentage of soil cover for each was visually quantified. To perform this quantification, a modified version of the Horsfall and Barratt (1945) scale was used, where two additional intervals were added to allow detailing when certain species appeared only once (0–0.1%), twice (0.1–0.5), etc. Although this scale was initially conceived to evaluate the incidence of plant diseases, it is also very useful for cover crop diversity evaluation, as it allows reporting with greater detail at both ends in the scale, i.e., scarce and very frequent species.

The effect of cover crop on plant water status was estimated through the measurement of midday (11 am to 1 pm) stem water potential (Ψ_m) between early July and harvest. Determinations were carried out on four healthy leaves per replicate, each one in different vines, which had been bagged 1.5 hours prior to measurement using ziplock-bags covered with a metallic high-density polyethylene reflective film (SonocoRF, Sonoco Products Co., Hartsville, South Carolina, USA). Measurements were carried out with a Scholander pressure chamber (P3000, Soil Moisture Corp., Santa Barbara, CA, USA). Sampling and measurements were performed according to Turner and Long (1980). Additionally, a 100-berry sample at harvest from each replicate was

collected. The harvest was September 24, 2018, and September 5, 2019, according to the criteria of the winery. To determine the carbon isotope ratio ($\delta^{13}\text{C}$) using an Elemental analyzer (NC2500, Carlo Erba Reagents, Rodano, Italy) coupled to an Isotopic Mass Spectrometer (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany). Carbon isotope ratio allows for an integration of the water deficit experienced by grapevines along the ripening into a single value (Santesteban *et al.*, 2015).

The agronomical implications of the under-vine cover crop were evaluated by determining yield components and grape composition at harvest, which was performed on the same date both in UV and T vines. The yield was determined by counting and weighing all the clusters produced in the 20 vines at each replicate, whereas grape composition was determined in one 100-berry sample per replicate. The berry samples were formed by 5 berries per vine, picked from 1 cluster per vine and taken from each part in the cluster (shoulder, middle, and tip; outside and inside). Samples were carried to the lab at low temperature (4–6 °C) for analysis, weighed to determine mean berry weight (BW), and immediately homogenized with an LMU 9018 American blender (Man, México) for 10 s at full speed part of this homogenate (100 g approx.) was filtered with a gauze tissue and used to measure total soluble solids (TSS), pH, titratable acidity (TA), malic (MalA) concentrations and yeast assimilable nitrogen



FIGURE 3. Soil conditions with cover crop under-vine (left) and tillage (right) on June 18, 2019.

(YAN). All measures were made with a Miura 200 (TDI analysers, Gavá-Barcelona). The phenolic parameters were measured according to Cromoenos® method. This method consists of a fast extraction of phenolics following a procedure and reagents provided by Bioenos company (www.bioenos.com) and has been proved to predict wine colour and phenolic composition similarly or even better than other classical procedures (Kontoudakis *et al.*, 2010). In December, the 20 selected vines were pruned, counting the number of shoots and the total weight of shoots per vine.

Data were compared through t-tests, all analyses being performed using the R computing environment (R Development Core Team, 2016).

RESULTS

In 2018, 21 plant species were identified on UV treatment, covering 82 % of the under-vine surface, though just nine reached representativeness of more than 2 % of the total surface. The clover that had been sown occupied only 26 % of the surface in this first year. In 2019, 25 plant species were identified, and in this second year, clover covered around 70 % of the surface, with eight species with more than 2 % of the surface. In T vines, in 2018, 19 plant species could be identified, occupying 66.2 % of the surface, and only four represented more than 2 %. The presence of *Convolvulus arvensis* covered the majority of the surface at 40 %. In 2019, 23 weeds were identified, with only three occupying more

than 2 %, and once again, *Convolvulus arvensis* covered the greatest surface area, reaching 27.5 % (Table 2).

The presence of the cover crop under the vines resulted in differences in stem water potential for some of the dates of measurement. The greatest impact was observed in August both seasons. UV plants showed lower water potential (Figure 4). Nevertheless, the water deficit was not severe at any moment due to the contribution of irrigation. The carbon isotope ratio did not show differences between treatments (Table 3).

The use of *T. fragiferum* as under-vine cover did not impact the yield components and the vegetative development was also unaffected by soil management strategy, except for a slight difference in shoot number in the second season (Table 4). No differences were found between treatments for grape composition, although a trend towards higher levels of phenolic maturity parameters was observed for UV vines (Table 5).

DISCUSSION

Sowing *T. fragiferum* clover under the vines (UV) resulted in a progressive increase in its presence, with a larger covered area in the second year of growth. McGourty *et al.* (2008) reported a similar increase over time with subterranean clover. Regarding the presence of adventitious vegetation, this was similar both seasons for UV. In T treatment, the conventional management of the soil through mechanical tillage under the vines did not provide the complete elimination of adventitious vegetation, as growers tolerate certain presence before repeating tillage.

TABLE 2. Surface covered with adventitious vegetation species under the vines for each treatment and season.

Species	2018		2019	
	UV	T	UV	T
<i>Trifolium fragiferum</i>	26		67.5	
<i>Convolvulus arvensis</i>	27.5	40	10.5	27.5
<i>Aster squamatus</i>	5		3.25	
<i>Chenopodium album</i>	5	5		
<i>Sonchus oleraceus</i>	5	7.5		
<i>Amaranthus retroflexus</i>	3	5		
<i>Coniza</i> sp.	3		5	
<i>Salsola kali</i>	3			
<i>Stellaria media</i>	2			
<i>Picris echioides</i>			3.25	
<i>Lactuca serriola</i>			3.25	
<i>Picnomon acarna</i>			2.5	
<i>Rubia peregrina</i>				5
<i>Setaria viridis</i>				2.5
<i>Crepis foetida</i>			3.25	

Only species with more than 2 % presence are reported.

TABLE 3. Effect of the cover crop under-vine on the carbon isotope ratio ($\delta^{13}\text{C}$).

Year	Treatment	$\delta^{13}\text{C}$ (‰)
2018	UV	-26.873
	T	-26.849
	P	0.838
2019	UV	-27.110
	T	-27.378
	P	0.058

Mechanical tillage proved to favour the presence of some summer species such as *Convolvulus arvensis*, a species that becomes dominant in summer in many vineyards, as reported in Steinmaus *et al.* (2008).

The water potential was somewhat lower when the cover crop was used, especially after veraison, both treatments being in general under light to

moderate water deficit conditions (Carbonneau and Ojeda, 2013). In one study using a legume cover (*Lotus corniculatus*) - in British Columbia - lower leaf water potential was also observed in the vineyard where crops under the vines were used (Vukicevich *et al.*, 2019). However, in other studies where coverage under the vineyard was used, no differences appeared, maybe due to the fact the climate was more humid (Coniberti *et al.*, 2018; Karl *et al.*, 2016) or, if there were variations, they did not occur for all the cover-crop species used (Chou and Vanden Heuvel, 2018). The fact our experiment was performed in an irrigated vineyard has undoubtedly favoured clover survival and decreased differences between treatments. McGourty *et al.* (2008) detected greater water deficit when a legume cover crop was used when water potential was measured just before irrigation, but differences attenuated after irrigation had been applied. The carbon isotope ratio that indicates the water stress accumulated in the plant throughout the season did not show the specific differences observed for water potential in August, and confirms as weak the water stress suffered by the vineyard (Brillante *et al.*, 2020; Santesteban *et al.*, 2015).

The yield was not affected in the two years of the study. This effect does not agree with what was observed by Hickey *et al.* (2016) with a *Festuca rubra* cover in Virginia, or by Karl *et al.* (2016) with *Trifolium repens* and native vegetation in Finger Lake. Conversely, Chou and Vanden Heuvel (2018) in the Finger Lake district, did not observe yield variations, and even in one of the seasons, they obtained higher yield with a spontaneous cover crop compared to glyphosate-maintained bare soil. Penfold *et al.* (2018), in Australia, obtained increased yield in wetter years with a cover crop that combined *Trifolium fragiferum* and *Festuca ovina*. In our case, with warmer climatic conditions, the lack of variations in yield may be due to the use of irrigation, which diminishes the competition between the cover crop species and the vines, as indicated by Steenwerth *et al.* (2013) with the using of cover crops in an alleyway in California.

Berry composition was nearly unaffected, which agrees with the overall effects observed for water status, and yield, and in accordance to the results observed for TSS and phenolics in Hickey *et al.* (2016). There is only a certain trend to observe higher phenolics content in UV treatment, which needs to be confirmed. Concerning

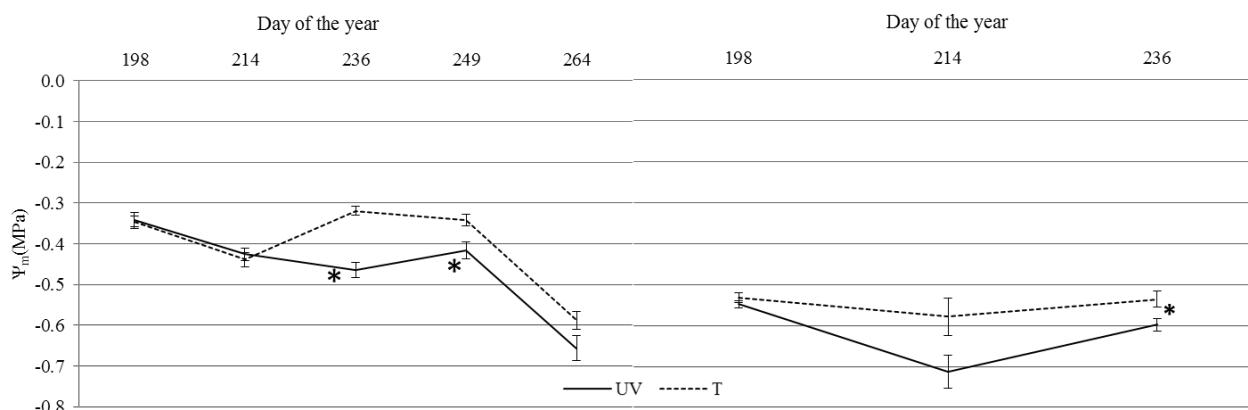


FIGURE 4. Effect of under-vine cover crop on the evolution of midday stem water potential (Ψ_m) during the growing season in 2018 (left) and 2019 (right). Vertical bars for each date and treatment correspond to the standard error, $n = 5$, and significant differences at $p < 0.05$ Have been marked with *.

TABLE 4. Effect of under-vine cover crop on yield components, pruning wood components and Ravaz Index for each season. P-values < 0.05 have been highlighted in bold.

Year	Treatment	Cluster no.	Yield (kg vine ⁻¹)	Cluster weight (g)	Berry weight (g)	Shoot no.	Pruning wood weight (kg vine ⁻¹)	Ravaz Index
2018	UV	20.2	2.8	139	1.35	11.6	0.446	6.96
	T	20.1	2.9	145	1.49	11.9	0.431	6.87
	P	<i>0.838</i>	<i>0.554</i>	<i>0.41</i>	<i>0.12</i>	<i>0.64</i>	<i>0.646</i>	<i>0.916</i>
2019	UV	19.7	2.01	102.1	1.04	12.04	0.27	8
	T	20.7	2.2	105.8	1.04	13.18	0.295	7.8
	P	<i>0.349</i>	<i>0.415</i>	<i>0.499</i>	<i>0.898</i>	0.017	<i>0.213</i>	<i>0.813</i>

nitrogen, some additional effect could be expected due to the fact an N-fixing legume was being used. Nevertheless, YAN was not increased due to the presence of the cover crop, agreeing with the results by Chou and Vanden Heuvel (2018), who did not find variations with respect to this parameter, nor with the use of legumes or grasses with respect to management with herbicide or tillage. Sulas *et al.* (2017) reported that a cover of *Medicago polymorpha* in an alleyway, despite being able to assimilate 125 kgN.ha⁻¹.year⁻¹, it just contributed 10 % to the vineyard N use. Under our experimental conditions, a longer-term cover crop establishment is probably required to generate noticeable effects of legume N-fixation on the crop.

Last, concerning vegetative development, the pruning weight, although not significantly reduced, showed a trend to decrease in the second year due to a reduction in the number of shoots per vine. This decrease in the pruning wood weight is usually the most remarkable

effect of the use of cover crops. Vukicevich *et al.* (2019), with a cover of *Lotus corniculatus* in British Columbia, experienced decreased vine growth. There was also a decrease of 26 % with the use of a *Fescue rubra* cover in Virginia (Hickey *et al.*, 2016) or 49 % with *Trifolium repens* in Finger Lake (Karl *et al.*, 2016). These vigour reductions could help to reduce thinning (Hickey *et al.*, 2016), or to decrease the incidence of fungal diseases (Valdés-Gómez *et al.*, 2008).

CONCLUSIONS

The use of *T. fragiferum* as an under-vine cover crop has proved to be a potentially useful tool for soil management in Mediterranean conditions, causing nearly no changes in vine performance, and competing well against the installation of undesired adventitious species. This first experiment under such conditions shows that management free of herbicides and mechanical tilling is feasible, and further research is required to fully explore the potentiality and limitations of under the vine cover-cropping.

TABLE 5. Effect of under-vine cover crop on berry composition: Total Solid Soluble (TSS), Total Acidity (TA), Malic Acidity (MaA), Yeast Assimilable Nitrogen (YAN) and on phenolic parameters of berry for each season.

Year	Treatment	TSS (°Brix)	TA (g TarA L ⁻¹)	pH	MaA (g L ⁻¹)	YAN (mg L ⁻¹)	Phenolic maturity index	Total polyphenol index	Anthocyanin berries (mg L ⁻¹)	Tannins in berries (mg L ⁻¹)
2018		15.3	5.5	3.27	0.58	128	1.31	42.67	2209	1151
	T	15.2	5.54	3.26	0.46	102.6	1.38	41.41	2031	1120
	P	0.443	0.731	0.61	0.372	0.323	0.382	0.086	0.237	0.085
	UV	14.4	6.62	3.208	0.66	100	1.60	54.1	2275	1429
2019	T	14.4	6.68	3.196	0.62	97.2	1.65	51.02	2078	1354
	P	1.00	0.707	0.49	0.587	0.533	0.741	0.463	0.16	0.463

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