COVER CROPPING IN *VITIS VINIFERA* L. CV. MANTO NEGRO VINEYARDS UNDER MEDITERRANEAN CONDITIONS: EFFECTS ON PLANT VIGOUR, YIELD AND GRAPE QUALITY

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Abstract

Aims: In temperate climates, cover crops are mainly used to reduce excess soil water and nutrient availability to grapevines, which otherwise could decrease grape quality. In Mediterranean climates, where water is a limiting factor, the use of cover crops is not as straightforward. However, in this scenario, summer senescent and self-seeding herbaceous cover crops could also help to decrease soil erosion as well as to reduce excessive early vegetative vigour, which could restrict grape water availability at later phenological stages. The aim of this experiment was to study the effects of particular cover crops in Mediterranean vineyards on grapevine vegetative growth, gas exchange, yield and grape quality.

Methods and results: The experiment was carried out over three consecutive years in an organic vineyard (cv. Manto Negro) in central Majorca, Spain. Three treatments (three cover cropping rows per treatment) were established: perennial grass and legume mixture (PM), no tillage, i.e., with permanent resident vegetation (NT), and traditional tillage or ploughed soil (TT). The grapevines were rain fed until veraison, and then drip irrigation was applied (30% potential evapotranspiration; ETP) until harvest. Plant water status was established according to a defined value of maximum daily leaf stomatal conductance (g_s). Cover crops reduced total leaf area (LA), g_s and grapevine vigour at early growth stages. g_s and net photosynthesis (A_N) were higher in cover crop treatments during the veraison and ripening stages, likely because of the reductions in LA. Intrinsic water use efficiency increased from flowering to veraison-maturity in all treatments. Yield was lower in the cover crop treatments (PM and NT) compared to TT for all years, but these differences were only significant in 2007. However, grape quality parameters slightly improved in the PM treatment.

Conclusion: The use of cover crops decreased LA, helping to avoid dramatic reductions of stomatal conductance in mid-summer, but decreased yield and only slightly increased grape quality.

Significance and impact of the study: This study showed that the use of specific cover crops in vineyards under Mediterranean climates helps to reduce vegetative vigour. Nevertheless, yield reduction and slight quality improvement suggest that cover crops should be adjusted in order to reduce competition for water and thus prevent these negative effects of water scarcity.

Key words: grape production, leaf gas exchange, must quality, total leaf area, vineyard cover crop

Résumé

Objectifs : Les cultures de couverture dans les climats tempérés sont principalement utilisées pour réduire d'une part l'excès de l'eau dans le sol et d'autre part la disponibilité des nutriments pour la vigne, qui autrement pourrait diminuer la qualité du raisin. Dans les climats méditerranéens, où l'eau est un facteur limitant, l'utilisation des cultures de couverture n'est pas aussi simple. Pourtant, dans ce scénario, la sénescence estivale et le propre réensemencement des cultures de couverture herbacée pourraient également contribuer à diminuer l'érosion des sols ainsi qu'à réduire la croissance végétative excessive aux stades précoces, ce qui pourrait limiter l'utilisation de l'eau par le raisin aux stades phénologiques suivants. L'objectif de cette expérience était d'étudier les effets de certaines cultures de couverture dans les vignobles méditerranéens sur la croissance végétative et les échanges gazeux de la vigne, ainsi que sur le rendement et la qualité du raisin.

Méthodes et résultats : L'expérience a été menée sur trois années consécutives dans un vignoble en culture biologique (cv. Manto Negro) dans le centre de Majorque, en Espagne. Trois traitements de trois lignes de cultures de couverture ont été établis : graminées vivaces et mélange de légumineuses (PM) ; sans labour, c'est-à-dire, avec végétation de résidence permanente (NT) et labour traditionnel ou travail du sol (TT). Les vignes ont été nourries par les pluies jusqu'à la véraison, puis la technique de l'irrigation goutte à goutte a été appliquée (30% évapotranspiration potentielle ; ETP) jusqu'à la récolte. L'état hydrique des plantes a été établi à partir d'une valeur définie de conductance stomatique maximum quotidienne (g_s). Les cultures de couverture ont réduit la surface foliaire totale, la conductance stomatique et la vigueur des plantes aux premières étapes de la croissance. La conductance stomatique et la photosynthèse étaient plus élevées dans les traitements de culture de couverture durant la véraison et la phase de maturation, probablement à cause de la réduction de la surface foliaire. L'efficacité d'utilisation de l'eau a augmenté de la floraison jusqu'à la phase «véraison-maturité» dans tous les traitements. Le rendement a été plus faible dans les traitements de culture de couverture (PM et NT) par rapport à TT pour toutes les années, mais ces différences n'ont été significatives qu'en 2007. Toutefois, les paramètres de qualité du raisin ont été légèrement améliorés dans le traitement PM.

Conclusion : L'utilisation de cultures de couverture diminue la surface foliaire, ce qui permet d'éviter une réduction drastique de la conductance stomatique durant l'été, mais avec une réduction du rendement et une légère augmentation de la qualité du raisin.

Signification et impact de l'étude : Cette étude a montré que l'utilisation de cultures de couverture dans les vignobles sous climat méditerranéen a contribué à réduire la vigueur végétative. Néanmoins, la réduction des rendements et une légère amélioration de la qualité suggèrent que les cultures de couverture devraient être ajustées afin de réduire la concurrence pour l'eau et de cette façon éviter ces effets négatifs par le manque d'eau.

Mots clés : production de raisin, échange gazeux foliaire, qualité du moût, surface foliaire totale, culture de couverture

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INTRODUCTION

Cover cropping is considered as the practical application of ecological principles such as diversity, crop interaction and other natural regulation mechanisms. Available resources, such as light, water and nutrients are more efficiently used by the intercrop than by the main crop. Management of these differences in competitive abilities between intercropping species and crops could lead to yield advantages and produce crops quality improvements. Furthermore, the multifunctional profile of cover cropping allows it to play many other roles in the agroecosystem, such as: improvement of soil structure, control of soil erosion (Gulick *et al.*, 1994; Le Bissonnais *et al.*, 2004), protection from pests and diseases (Valdés-Gómez *et al.*, 2008), greater competition with weeds (Porqueddu *et al.*, 2000), and reduction of the negative environmental impact of crops.

COVER CROPPING IN GRAPEVINES

Cover cropping in vineyards was a common practice in Europe. Nowadays, vineyard cover cropping is widely used in areas with frequent summer rainfall to remove excess water and nitrogen, but the benefits of cover crops in vineyards also include: soil erosion control, nitrogen and organic matter management (addition or removal depending on the intercrop specific composition), improved soil structure, increased water penetration and retention, decreased direct soil water evaporative losses, reduction of grapevine vegetative vigour, and grape and must quality enhancement (Winkler *et al.*, 1974; Hirschfelt, 1998; Pinamonti, 1998; Ingels *et al.*, 2005). Furthermore, using a cover crop system may help to reduce canopy leaf area (LA) and consequently to minimize transpiration losses (Dry and Loveys, 1998; Monteiro and Lopes, 2007). Several authors have related this effect on grapevine growth to cover crop competition for water (Caspari *et al.*, 1997; Maigre and Aerny, 2001; Afonso *et al.*, 2003). Finally, the use of cover crops could reduce mechanization by decreasing and/or avoiding soil ploughing and LA control practices. On the contrary, other studies have shown that the use of cover crops in vineyards has detrimental effects, such as yield reductions due to the competition for nutrients and water (Hirschfelt, 1998; Ingels *et al.*, 2005; Krohn and Ferree, 2005; Smart *et al.*, 2006). Taking into consideration all these interactions, it is required to have a balanced intercropping - vineyard system, selecting crop species accurately and carrying out a proper management to achieve maximum benefits.

COVER CROPPING IN VINEYARDS UNDER MEDITERRANEAN CONDITIONS

The use of cover crops remains hampered in Mediterranean areas, where low summer rainfall and high evaporative demand usually results in severe summer drought, because cover crops compete for soil water, leading to higher grapevine water stress (Celette *et al.*, 2009) and consequently to lower growth and yield (Williams and Matthews, 1990). Nonetheless, spring water use by cover crops could help to control grapevine canopy development, improving bunch microclimate (Dokoozlian and Kliewer, 1996), and thereby grape and wine quality (Matthews and Anderson, 1989; Ingels *et al.*, 2005). Therefore, the use of cover crops to control excessive vine vigour in spring could become an interesting agronomic tool for a more sustainable management of soil and water resources, that is, if reductions in yield are compensated by increases in grape and must quality (Matthews *et al.*, 1990).

In order to achieve the potential benefits of cover crops and to avoid the undesirable ones, it is a key point to select accurately the correct species and varieties. Under Mediterranean conditions, early senescent and self-seeding or perennial species can meet both these objectives by improving soil characteristics and by competing for water resources until mid spring (but not later).

In this sense, the present study aimed to assess the effects of a particular mixture of Mediterranean legumes and grasses as inter-row cover crop on LA development, leaf gas exchange, yield and grape quality. The following experimental questions were addressed: 1) How is vine vegetative growth and leaf gas exchange affected by the presence of a cover crop?, 2) How much is grape yield reduced by the cover crop competition? and 3) To what extent grape and must quality are enhanced by cover cropping?

MATERIALS AND METHODS

1. Site characteristics and experimental design

The experiment was carried out over three consecutive years (2006, 2007 and 2008) in a 5-year-old organic vineyard planted with a local variety "Manto Negro" (*Vitis vinifera* L.) grafted on Richter-110 rootstocks, in Consell, central Majorca (39º39'N, 2º48'E), Spain. Soil physical and chemical characteristics were determined before the establishment of treatments. The soil corresponds to a loamy soil containing 2.2% of organic matter and 14.1% of active calcium in the 0.3 m topsoil layer. After the three-year study period, soil chemical characteristics were analysed to determine the effects of cover crop on soil fertility. For that purpose, four soil samples (from 0.05 m to 0.30 m depth), each composed by four subsamples, were taken along the central row of each treatment. Soil organic matter content (%), total nitrogen content (%) and phosphorus content (ppm) were analysed by the Olsen method (Olsen *et al.*, 1954), and potassium content (ppm) was analysed by the ammonium acetate method.

The climate is typically Mediterranean, with a mean annual rainfall of about 575 mm (data from 1992 to 2008) usually concentrated from September to April. The drought period usually lasts from May to September, but its length is highly variable from year to year. Meteorological data were provided by an automatic meteorological station belonging to the « Centro Territorial of the Instituto Nacional de Meteorología » (INM).

The experimental design was a randomized complete block divided into three different plots (treatments). Each plot had three cover cropping strips of 200-m long. Grapevines were spaced 2.5 m (between rows) and 1.2 m (within rows). All grapevine measurements were performed on the south side of the middle row to ensure that plants had the same cover cropping system on both sides. The training system was a vertical shoot positioning with movable wires, and the vines were spur-pruned on a bilateral Royat Cordon system (leaving an average of eight nodes per plant). Shoots were trimmed twice a year, between bloom and veraison, at a height of about 1.0 m.

Three different treatments were established in the inter-rows: i) mixture of perennial grasses and legumes (PM), ii) no tillage (NT) (permanent resident vegetation cover of graminoids and leguminous plants, mainly Hordeum sp., *Medicago truncatula*, *Medicago orbicularis*, *Medicago polymorpha*, *Avena sterilis*, *Lotus ornithopodioides*, *Trifolium scabrum* and *Chrysanthemum coronarium*) and iii) traditional tillage (TT) (ploughed soil). The grass cover area between the vine rows was controlled by using a cultivator. PM was a mixture of self-seeding and/or perennial species: *Trifolium resupinatum* cvs. Prolific and Nitro plus (5 kg/ha); *Trifolium michelianum* cv. Frontier (3 kg/ha); *Medicago truncatula* cv. Parabinga (6 kg/ha); *Trifolium subterraneum* ssp. brachycalycinum cv. Davel (6 kg/ha); and *Dactylis glomerata* cv. Currie (2 kg/ha). Seeds from PM were sown only once in November 2005. In the following years, seed germination and seedling establishment occurred in early autumn.

All the grapevines were rain fed until veraison; from then on, the irrigation dosage was adjusted up to 30% ETP for all treatments, depending on precipitation distribution, and applied by a drip system from veraison to harvest (see Escalona *et al.*, 1999). This dosage and schedule were chosen based on previous studies (García Escudero 1991, *pers. comm.*) in order to maintain high crop yield without significant loss in grape quality and yield. Pest control by organic methods was common to all treatments. TT treatment included regular soil ploughing (tillage depth of 0.3 m) by using a cultivator to achieve a homogeneous treatment over the entire row. In the two cover cropping treatments, the resident (NT) and the sown vegetation (PM) were present from October, when biomass production sprouted, to mid to late spring, at the beginning of the drought period. No mowing was needed in any of the treatments since cover crop growth was moderate. Chemical fertilisation was absent from all treatments.

2. Vegetative growth: cover crop and grapevine

Aerial dry biomass production in the cover cropping strips (PM and NT treatments) was estimated in spring on six representative sample areas of 1 m^2 per plot. An additional dry biomass measurement was performed in early summer 2008 due to accumulated rain during late spring that year. All samples were taken in the central strip from each treatment to reduce the border effect. Samples from various grass, legume and unsown species were oven-dried at 60 ºC for three days and then weighted to determine the total dry biomass.

Vine vegetative growth was measured once a week from anthesis to « pea-size » stage on 12 shoots from 12 different vines per plot. Leaf elongation rate (increase of leaf length per unit of time) and main shoot length were measured on each shoot. Leaf elongation rate was estimated in 12 plants per treatment as an average from thee separate measurements per plant over the growing season. Each measurement was monitored in the youngest developed leaf from the main shoot apical meristem, from initial (youngest leaf) to final expansion, at five-to-eight day intervals. Total LA was measured using a leaf area meter (AM-100 Area Meter, Analytical Development Co., Hoddesdon, UK) over 10 shoots for PM and TT treatments. Statistically significant correlations were established between total LA per shoot and main shoot length ($r^2 = 0.56$ and 0.82 for PM and TT, respectively). As no statistical differences were obtained for the regression equation among the treatments, a single value was used for any of the three treatments that were previously established. Vine total LA was then estimated by measuring the number of shoots per plant and their length in late June, when vegetative growth was completed.

3. Grapevine water status, gas exchange, and carbon isotope analysis

In order to monitor vine water status, predawn leaf water potential (ψPD) was measured using a Scholander pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) on four replicates per treatment at five phenological stages: i) anthesis, ii) « pea-size », iii) veraison, iv) fruit maturity and v) post-harvest. On the same day, stomatal conductance (g_s) and net photosynthesis (A_N) were measured on six mature, healthy and sun-exposed leaves from six different plants per treatment with a portable open gas exchange system (Li-6400; Li-Cor Inc., Lincoln, NE, USA). Measurements were taken at mid-morning, from 10:00 am to 12:00 pm, on sunny days.

Grapevine water status was also monitored by using a defined value of daily maximum g_s measured with the same system above, as suggested by Flexas *et al.* (2002) and Medrano *et al.* (2002), where the general levels were as follows: (1) Mild water stress: g_s decreases from a maximum (typically 0.2-0.5 mol H_2O m-2 s^{-1}) to 0.15 mol H2O m^{-2} s^{-1}, (2) Moderate water stress: g_s ranges between 0.05 and 0.15 mol H_2O m^{-2} s^{-1} and (3) Severe water stress: g_s drops below 0.05 mol H_2O m^{-2} s^{-1}.

In order to highlight differences in water use efficiency (WUE) between treatments, the carbon isotope composition ($\Delta^{13}C$) in the dry matter of mostly expanded leaves was determined in 2007 and 2008 at fruit maturity on two leaves per plant and six plants per treatment (Farquhar and Richards, 1984). The samples were oven-dried at 70 ºC for 48 h, powdered and subsampled for C-isotope ratio analysis with an Isotope Ratio Mass Spectrometer (Thermo Finnigan Delta Plus, Bremen, Germany).

4. Grapevine yield and grape quality

Grape yield and quality were determined at harvest from 12 plants per treatment. Furthermore, a representative sample of 100 berries per vine was taken from the same plants, in order to determine average berry weight and must quality parameters, such as soluble solids (SS) (measured in ºBrix), pH, total polyphenols (TP), extractable anthocyanins and extractable tannins, according to the OIV official analysis methods (Organisation Internationale de la Vigne et du Vin; OIV, 1990). The Ravaz Index was also calculated by dividing total yield per vine by the pruning weight recorded during the annual growth cycle.

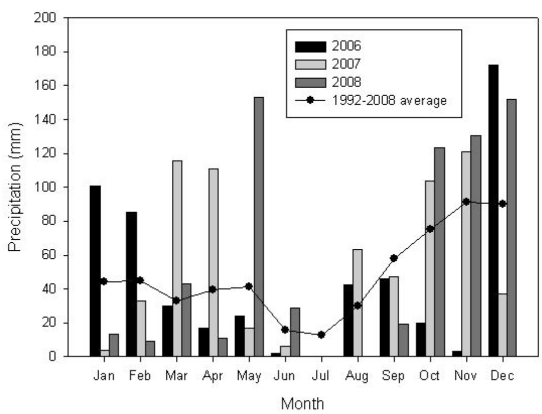

Figure 1. Average monthly precipitation (solid line) for the 1992-2008 period and monthly precipitation (bar graph) for the three experimental years (2006, 2007 and 2008).
Data were gathered from an automatic meteorological station belonging to the "Centro Territorial of the Instituto Nacional de Meteorología" (INM).

5. Statistical analysis

Correlations were obtained using the 8.0 Sigma Plot software package (SPSS; Chicago, IL, USA). ANOVA analysis and least square means were performed with the SPSS 16.0 software package for windows (SPSS Inc., 2008). In all the tables, treatment and year effects and interactions between both parameters were considered. Differences between means were assessed by Duncan test analyses ($P < 0.05$).

RESULTS

During the experimental period (2006 to 2008), the mean air temperature ranged from 7.7 ºC (mean daily temperature in January 2007) to 27.1 ºC (mean daily temperature in July 2006), while the mean annual rainfall was 543, 659 and 684 mm in 2006, 2007 and 2008, respectively (Figure 1). Spring precipitation (from March to June) varied to a great extent from year to year, leading to different irrigation levels between years: 10.3 L/m^2, 26.4 L/m^2 and no irrigation in 2006, 2007 and 2008, respectively. The precipitation recorded in spring 2006 (76 mm) was below the average (130 mm), while in subsequent years the accumulated precipitation was above the average (214 mm in 2007 and 236 mm in 2008). However, important differences in precipitation distribution were observed between these years. While March and April were the months of greatest precipitation in 2007, rainfall mainly occurred in late May and early June in 2008 (Figure 1).

Soil chemical parameters did not show large differences between treatments at the end of the experiment (Table 1). However, it is noticeable that the soil organic matter content significantly decreased in the NT plot, from 2.3% in December 2005 to 1.9% in December 2008. Moreover, the soil phosphorus content decreased, yet not significantly, in all plots after the three-year study, with a decrease ranging between 17 and 28%. By contrast, the soil nitrogen and potassium content did not show differences at the end of the experiment in any plot.

1. Cover crop vegetative growth

The cover cropping treatments were well established, completely covering the ground between rows throughout the three experimental years. At the initial stages of the vine cycle (late March to early April), the intercrop had already achieved a peak biomass in each experimental year. However, cover crop biomass production lasted for a longer period in 2008, compared to 2006 and 2007,

Table 1. Soil chemical characteristics of the three plots at the beginning (December 2005) and at the end (December 2008) of the experiment. Soil organic matter content (%), total nitrogen content (%) and phosphorus content (ppm) were analysed by the Olsen method, and potassium content (ppm) was analysed by the acetate method.

	Sampling date	Organic matter (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)
Perennial Mixture (PM)	dec-05	$2.4^{b} \pm 0.43$	$0.18^{a} \pm 0.040$	$15.4^{ab} \pm 2.65$	$373.3^{a} \pm 52.62$
	dec-08	$2.3^{b} \pm 0.08$	$0.19^{a} \pm 0.006$	$11.1^{a} \pm 1.41$	$363.8^{a} \pm 29.07$
No Tillage (NT)	dec-05	$2.3^{b} \pm 0.25$	$0.18^{a} \pm 0.030$	$16.5^{ab} \pm 3.51$	$379.6^{a} \pm 62.64$
	dec-08	$1.9^{a} \pm 0.10$	$0.17^{a} \pm 0.004$	$13.7^{a} \pm 1.51$	$363.8^{a} \pm 19.59$
Traditional Tillage (TT)	dec-05	$2.3^{b} \pm 0.28$	$0.21^{a} \pm 0.030$	$19.5^{b} \pm 4.25$	$363.8^{a} \pm 56.39$
	dec-08	$2.2^{ab} \pm 0.13$	$0.18^{a} \pm 0.008$	$15.5^{ab} \pm 1.73$	$372.8^{a} \pm 27.97$

Values are means of four replicates ± standard error, and each replicate is a complex sample of four subsamples. Different subscript letters within a column denote significant differences in treatment and year effect by a Duncan's multiple comparison test ($P < 0.05$).

as a consequence of the frequent rainfall events recorded in May and June that year.

There were no significant differences in cover crop biomass accumulation between NT and PM treatments within the same year (Table 2). Nevertheless, while biomass accumulation was slightly higher in the NT treatment in the first year (2006), PM showed a higher (but not statistically significant) total biomass than NT in 2007 and 2008. In 2006, dry conditions during spring led to the lowest accumulation of cover crop biomass (Table 2). By contrast, in 2008 the total cover crop biomass in NT and PM were 76% and 84% higher than the same treatments in 2006 and 80% and 71% higher than in 2007, respectively, in correspondence with the higher total rainfall during 2008 (684 mm) and most particularly to its distribution and intensity in late spring (Figure 1).

2. Grapevine water potential and vegetative growth

Ψ_{PD} decreased during the course of the growing season, ranging from -0.05 MPa (first sampling time) to -0.45 MPa, and showed a progressive decrease from May to August, which was evident for all years (data not shown). Differences between treatments were only evident at the anthesis stage in 2006 and 2007, when rainfall was scarce, but not in 2008, when the precipitation was more frequent during the late spring and cover crop biomass production lasted for a longer period. In general, these differences tended to decrease over the season and no statistical differences were found for ψPD among treatments during the experiment (data not shown).

Total LA showed significant differences between the experimental years, being highest in 2008 and lowest in 2007 (Table 3). No significant differences in total LA were found between PM and TT treatments in any of the three experimental years. However, it is worth noticing that in all cases the averaged grapevine LA was lower in PM compared to TT (average decreases of 14%) even though important interactions among treatments and year conditions were observed.

Leaf elongation rate and main shoot length were also significantly affected by the year effect (Table 4). In addition, significant differences in main shoot length among treatments were observed in 2006, with TT vines showing longer shoots compared to PM vines. By contrast, this treatment effect was not observed in 2007 and 2008 because of the higher spring rainfall. No differences between treatments were observed for leaf elongation rate within years.

Differences in the number of shoots per plant, which was significantly higher in 2008, determined the differences of LA between the three experimental years, but within each year, no differences in the number of shoots per plant were observed among treatments

Table 2. Total biomass production of the cover crop.

Year	Treatment	Total biomass (g/m^2)
2006	PM	$41.1^{a} \pm 3.62$
	NT	$49.5^{a} \pm 29.68$
2007	PM	$74.7^{a} \pm 15.19$
	NT	$40.6^{a} \pm 10.31$
2008	PM	$259.4^{b} \pm 13.41$
	NT	$204.7^{b} \pm 9.76$

Different subscript letters denote statistically significant differences in treatment and year effect by a Duncan's multiple comparison test ($P < 0.05$).

Table 3. Vine total leaf area.

Year	Treatment	Total leaf area (m^2)
2006	PM	$2.54^{bcd} \pm 0.19$
	NT	$2.96^{de} \pm 0.24$
	TT	$2.86^{cde} \pm 0.27$
2007	PM	$1.95^{ab} \pm 0.13$
	NT	$1.78^{a} \pm 0.18$
	TT	$2.24^{abc} \pm 0.24$
2008	PM	$3.23^{e} \pm 0.28$
	NT	$3.37^{ef} \pm 0.22$
	TT	$3.94^{f} \pm 0.27$

Different subscripts letters denote statistically significant differences by a Duncan's multiple comparison test ($P < 0.05$). Treatment, year, and interactions between both parameters were analysed.

according to the vine grower's usual practices (ANCOVA, $p < 0.05$, data not shown).

3. Leaf gas exchange and carbon isotope discrimination

Although the differences in vegetative growth were not statistically significant (Table 3 and 4), PM vines showed lower leaf transpiration rate at early growing stages (44 %, 30 % and 22 % lower in 2006, 2007 and 2008, respectively) compared to TT vines. Similarly, g_s was also consistently lower in PM vines compared to TT vines at early growing stages, until the « pea-size » stage (DOY (day of year) 180) (Figure 2). On the contrary, differences in A_N among treatments at those stages were much less important. Nevertheless, g_s and A_N were slightly higher in the PM vines during the veraison and ripening stages, specially in 2007 (DOY 200 and 230, respectively). Although most differences between treatments were not statistically different, the values of g_s and A_N in TT vines were closer to those in NT than in PM vines (Figure 2).

A similar tendency was observed between treatments in the three years of the experiment, but in general the lowest values of A_N and g_s were obtained in 2006, when the lowest average annual rainfall was recorded. In this sense, minimum values of about 3-4 μmol CO_2 m^{-2} s^{-1} and 0.025 mol H_2O m^{-2} s^{-1} for A_N and g_s, respectively, were reached in 2006 (as compared to 7-10 μmol CO_2 m^{-2} s^{-1} and 0.07 mol H_2O m^{-2} s^{-1} in 2007 and 9-12 μmol CO_2 m^{-2} s^{-1} and 0.07 mol H_2O m^{-2} s^{-1} in 2008).

Intrinsic WUE_{leaf} (A_N/g_s) did not show significant differences among treatments over the three experimental years. However, as a consequence of the lower values of A_N and g_s in 2006, increments of WUE (14% and 18% higher than in 2007 and 2008, respectively) were observed. The corresponding values of intrinsic WUE_{leaf} were 134.38, 116.13 and 110.16 °mol CO_2 mol^{-1} H_2O in 2006, 2007 and 2008, respectively. Slight differences in WUE between 2007 and 2008 were also reflected in carbon isotope discrimination ($\Delta^{13}C$) in the dry matter of grapevine leaves. In this sense, lower values of $\Delta^{13}C$ in 2007 (-26.46, -26.57 and -26.56‰ for PM, NT and TT, respectively) than 2008 (-27.13, -27.16 and -27.64‰ for PM, NT and

Table 4. Vine vegetative growth: main shoot length and leaf elongation rate.

Year	Treatment	Main shoot length (m)	Leaf elongation rate (cm/day)
2006	PM	$1.76^{cd} \pm 0.08$	$0.22^{a} \pm 0.03$
	NT	$1.94^{de} \pm 0.11$	$0.23^{a} \pm 0.03$
	TT	$2.07^{e} \pm 0.11$	$0.24^{a} \pm 0.03$
2007	PM	$1.05^{a} \pm 0.04$	$0.46^{b} \pm 0.02$
	NT	$0.99^{a} \pm 0.06$	$0.48^{b} \pm 0.03$
	TT	$1.15^{a} \pm 0.07$	$0.48^{b} \pm 0.03$
2008	PM	$1.45^{b} \pm 0.06$	$0.50^{b} \pm 0.02$
	NT	$1.41^{b} \pm 0.06$	$0.48^{b} \pm 0.02$
	TT	$1.58^{bc} \pm 0.07$	$0.48^{b} \pm 0.01$

Different subscript letters within a column denote statistically significant differences by a Duncan's multiple comparison test ($P < 0.05$). Treatment, year, and interactions between both parameters were analysed.

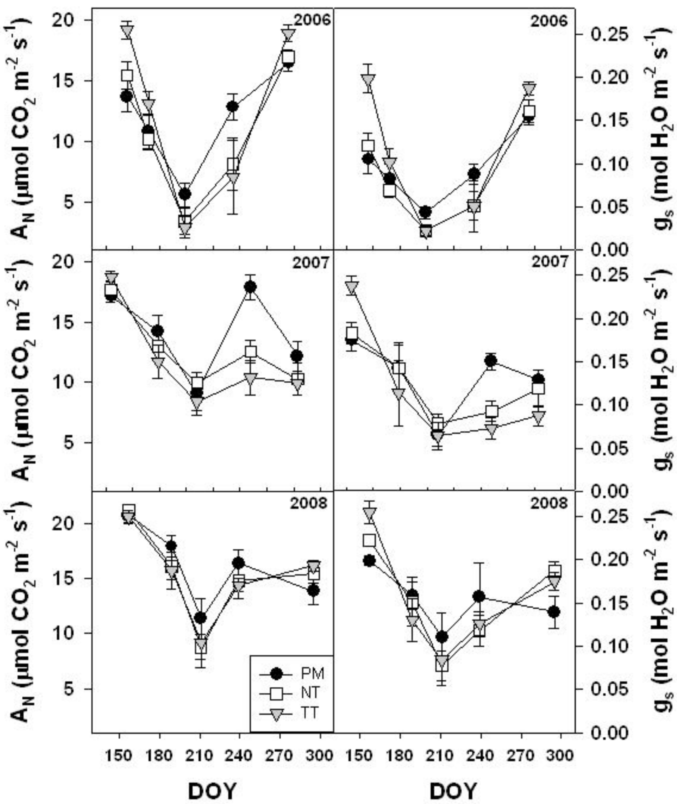

Figure 2. Seasonal pattern of net photosynthesis (A_N) and stomatal conductance (g_s) of grapevines over the three experimental years for the three treatments (PM: perennial mixture; NT: no tillage; TT: traditional tillage).
Vertical bars represent ± SE. DOY: day of year. DOY 180 ("pea-size"), DOY 200 (veraison) and DOY 230 (ripening).

TT, respectively) were found. By contrast, the treatment did not affect $\Delta^{13}C$ values in the dry matter of leaves.

4. Yield and fruit composition

Grape yield was significantly reduced in 2006 when compared to 2007 and 2008 data. The number of clusters per vine and mean cluster weight were the main yield components that were lower in this particular year with respect to 2008, whereas only the number of clusters per vine was lower with respect to 2007 (Table 5).

Grape yield (kg grapes/vine) was lower in the cover crop treatments (both PM and NT) compared to the TT treatment for the three experimental years, however, these differences were only significant in 2007 (Table 5). Similarly, the number of clusters per vine and the mean cluster weight were significantly lower in PM vines than in TT vines only in 2007. NT vines also showed significant lower cluster weight than TT ones in 2007. In 2006 and 2008, both the number of clusters per vine and the mean cluster weight did not show statistically significant differences among treatments.

The Ravaz Index presented a large variation between years and treatments. In 2006 and 2008, this index was higher in PM vines compared to TT vines (Table 6). Such differences were obtained partially as a result of differences in pruning weight, which were up to two-fold higher in TT vines than in PM ones in the three years, with intermediate values for NT vines (Table 6).

Table 5. Yield characteristics: mean cluster weight, grape yield and number of clusters per vine.

Year	Treatment	Mean cluster weight (g)	Grape yield (kg /vine)	Clusters/vine
2006	PM	$188.6^{ab} \pm 25.08$	$1.03^{a} \pm 0.20$	$5.1^{a} \pm 0.66$
	NT	$208.1^{abc} \pm 29.89$	$1.13^{a} \pm 0.16$	$5.6^{a} \pm 0.45$
	TT	$225.2^{abc} \pm 19.00$	$1.37^{ab} \pm 0.29$	$5.6^{a} \pm 0.59$
2007	PM	$147.6^{a} \pm 27.43$	$1.12^{a} \pm 0.31$	$6.6^{a} \pm 0.90$
	NT	$160.5^{a} \pm 23.05$	$1.61^{abc} \pm 0.32$	$9.3^{b} \pm 0.97$
	TT	$276.3^{c} \pm 36.50$	$3.11^{d} \pm 0.49$	$11.0^{b} \pm 0.05$
2008	PM	$250.2^{bc} \pm 26.26$	$2.64^{cd} \pm 0.46$	$10.0^{b} \pm 0.10$
	NT	$223.5^{abc} \pm 24.16$	$2.57^{cd} \pm 0.42$	$11.0^{b} \pm 0.11$
	TT	$233.9^{abc} \pm 27.45$	$2.92^{bcd} \pm 0.68$	$11.3^{b} \pm 0.14$

Different subscript letters within a column denote statistically significant differences by a Duncan's multiple comparison test($P < 0.05$). Treatment, year, and interactions between both parameters were analysed.

Table 6. Grape yield to pruning weight ratio (Ravaz Index).

Year	Treatment	Pruning weight (kg/vine)	Ravaz Index
2006	PM	$0.28^{a} \pm 0.04$	$4.05^{abc} \pm 0.83$
	NT	$0.50^{bc} \pm 0.05$	$2.31^{a} \pm 0.30$
	TT	$0.56^{c} \pm 0.06$	$2.46^{a} \pm 0.35$
2007	PM	$0.41^{b} \pm 0.04$	$3.48^{ab} \pm 0.58$
	NT	$0.44^{bc} \pm 0.04$	$3.01^{ab} \pm 0.45$
	TT	$0.70^{de} \pm 0.06$	$5.51^{c} \pm 0.52$
2008	PM	$0.48^{bc} \pm 0.04$	$5.50^{c} \pm 0.90$
	NT	$0.58^{cd} \pm 0.05$	$4.63^{bc} \pm 0.71$
	TT	$0.82^{e} \pm 0.05$	$3.37^{ab} \pm 0.69$

Ravaz Index is fruit yield (kg grapes per vine; see Table 5 for data) to pruning weight ratio. Different subscript letters within a column denote statistically significant differences by a Duncan's multiple comparison test ($P < 0.05$). Treatment, year, and interactions between both parameters were analysed.

Berry composition differed among years (Table 7). The SS, i.e., sugar content, TP, anthocyanin and tannin contents were usually lower in 2008 compared to 2006 and 2007, which can be directly related to the higher precipitation recorded during that particular year at late spring. Significant differences among treatments within the same year were only observed in 2007, when TP were higher in PM than in TT vines (Table 7). No significant differences among treatments were observed in other quality parameters that year.

DISCUSSION

1. Cover crop growth

Due to the significant biomass increments reached by the NT and PM treatments, cover crops can be considered as potential vine competitors for resources such as nutrients and water availability (Table 1 and Figure 2). However, the soil organic matter, nitrogen and potassium contents did not show significant differences at the end of the experiment when compared with initial contents (Table 1). By contrast, the reduction in phosphorus content was slightly higher in the PM treatment than in the TT one (28% and 20%, respectively), probably as a consequence of cover crop uptake. Soil covering over the three experimental years was as expected under Mediterranean climate conditions. PM and NT showed high soil cover from October to May, and in summer the sward was dry and remained on the soil, decreasing direct soil water evaporative losses. Furthermore, an important increase in biomass production was observed for PM from the first to the third experimental year. This could be a consequence of the higher spring rainfall in 2007 and 2008 compared to 2006, but could also be due to the fact that the PM was mainly composed of perennial species, with a medium or low growth capacity and a low establishment ability during the first year. On the other hand, these species usually show a high reseeding capacity and « perenniality ». It is noteworthy that soil cover by the cover crops did not decline after three years in the PM treatment, showing a higher « perenniality » than other species or species mixtures used as cover crops under Mediterranean conditions (Porqueddu *et al*., 1996; Volaire *et al*., 1992). In this sense, the reduction of vineyard mechanization, i.e., no ploughing, could be an interesting benefit from both an economical and ecological point of view, since the use of permanent mixtures as cover crops implies reduced costs, energy use and CO_2 emissions.

2. Cover cropping effects on vine growth, leaf gas exchange and carbon isotope discrimination

Studies of competition for water resources between grapevines and cover crop have generated contradictory results. Some studies reported greater water stress in grapevines grown with a cover crop (Morlat, 1987), whereas others showed that cover cropped vineyards do not always exhibit higher water stress than those with uncovered soil (Celette *et al*., 2005). Nevertheless, cover cropping has been suggested to induce a reduction of grapevine vigour, which is translated into smaller canopy due to reduced LA and less pruning weight (van Huyssteen, 1990). Several other authors have reported this effect of sward treatments on grapevine growth as a consequence of cover crop competition for water (Loveys,

Table 7. Must quality characteristics.

Year	Treatment	SS (°Brix)	pH	Total polyphenols (A_{280})	Anthocyanins (mg/L)	Tannins (g/L)
2006	PM	$24.1^{bcd} \pm 0.89$	$4.0^{c} \pm 0.05$	$26.5^{bcd} \pm 3.84$	$248.1^{bcd} \pm 27.03$	$4.50^{c} \pm 0.65$
	NT	$24.7^{d} \pm 0.74$	$4.04^{c} \pm 0.08$	$25.0^{d} \pm 2.07$	$288.5^{d} \pm 27.10$	$4.11^{c} \pm 0.37$
	TT	$24.9^{cd} \pm 0.56$	$4.13^{c} \pm 0.04$	$24.7^{bcd} \pm 2.56$	$242.2^{cd} \pm 32.69$	$2.95^{ab} \pm 0.41$
2007	PM	$21.5^{a} \pm 0.71$	$3.45^{a} \pm 0.07$	$24.6^{cd} \pm 2.91$	$173.4^{abc} \pm 30.26$	$3.22^{abc} \pm 0.31$
	NT	$22.7^{abc} \pm 0.42$	$3.32^{a} \pm 0.04$	$19.1^{abc} \pm 1.67$	$102.3^{a} \pm 11.70$	$2.77^{a} \pm 0.56$
	TT	$22.1^{ab} \pm 0.66$	$3.36^{a} \pm 0.05$	$16.8^{a} \pm 1.22$	$158.4^{ab} \pm 54.63$	$2.69^{a} \pm 0.25$
2008	PM	$20.6^{a} \pm 1.11$	$3.81^{b} \pm 0.08$	$17.6^{a} \pm 1.22$	$115.3^{a} \pm 10.27$	$2.21^{a} \pm 0.21$
	NT	$21.7^{a} \pm 0.77$	$3.77^{b} \pm 0.04$	$18.6^{ab} \pm 0.71$	$98.8^{a} \pm 16.16$	$3.89^{bc} \pm 0.37$
	TT	$22.0^{ab} \pm 0.50$	$3.75^{b} \pm 0.04$	$16.7^{a} \pm 1.08$	$93.3^{a} \pm 13.45$	$2.23^{a} \pm 0.21$

Different subscript letters within a column denote statistically significant differences by a Duncan's multiple comparison test ($P < 0.05$). Treatment, year, and interactions between both parameters were analysed.

1984; Loveys *et al*., 2000; Monteiro and Lopes, 2007). In accordance with these results, the cover cropping treatment reduced the canopy growth, being higher in TT compared to PM and NT grapevines, presumably due to its higher soil water availability at early growing stages (when irrigation was not applied), as estimated by g_s (Figure 2). Reductions in shoot length and LA by cover cropping, although not always statistically significant, suggest that vines and cover crops were competing for soil water resources at early stages of the vine cycle. Such a competition provoked an early mild water stress on PM and NT vines, reflected by a lower Ψ_{PD} and a g_s decrease at the anthesis and « pea-size » stages (Figure 2). Furthermore, A_N showed to be lower in PM vines, although this reduction was not as large and consistent as the g_s one, suggesting that the competition for nutrients between vines and cover crop was not so important. In this sense, the lower soil phosphorus content observed in the PM plot did not seem to exert a great influence on grapevine photosynthetic capacity, which rules it out as a key parameter to explain grapevine vegetative growth reduction. By contrast, a reduction of soil water content was presumably the main effect of cover cropping, since g_s has been largely described as one of the first parameters to be affected by a reduction of soil water content (Hsiao, 1973). Moreover, a significant relationship has been reported between soil water content and g_s in grapevine (Pou *et al*., 2008).

Contrary to what was observed at early stages, g_s and A_N in PM vines were higher during the veraison and ripening stages, which could be a consequence of LA reduction. In this sense, deeper root development could be expected in cover cropped vines due to the reduction of water in soil surface as a consequence of cover crop water consumption from the upper soil layers.

Although vegetative growth was not significantly different between treatments, a LA reduction was observed for cover cropping treatments, which is in accordance with the lower gas exchange rates showed in PM vines at early growing stages in relation to TT treatment.

In general, A_N and g_s values were in the same range than that reported by other authors for grapevine in Mediterranean conditions (Escalona *et al*., 1999; Lopes *et al*., 2008; Kondouras *et al*., 2008).

Higher WUE is often found under water stress. Such increments in WUE are related with $\Delta^{13}C$ measured in leaf dry matter via its effect on Ci/Ca ratio (Farquhar *et al*., 1984). Higher Ci/Ca ratio is positively related with $\Delta^{13}C$, which is a reliable, long-term assessment of WUE in grapevines (Gibberd *et al*., 2001). More recently, the carbon isotope composition ($\Delta^{13}C$) in leaf dry matter was used as a long-term indicator of WUE_{leaf} (Condon *et al*., 2004). Here, intrinsic WUE (A_N/g_s) and $\Delta^{13}C$ of grapevine leaves differed significantly between years with higher A_N/g_s in 2006, which was a drier year than 2007 and 2008, and lower $\Delta^{13}C$ in 2007 than in 2008. The lower reduction of $\Delta^{13}C$ in PM (-0.67‰) and NT (-0.59‰) than in TT grapevine leaves (-1.08‰) between both years implies a less sensitive response to variations in water availability of cover cropped plants than control ones. This led to an increase in A_N/g_s for treated grapevines (NT and PM), i.e., a near optimization of carbon assimilation in relation to water supply (Chaves *et al*., 2002).

3. Intercropping effects on yield and fruit composition

The Ravaz Index represents the ratio of reproductive to vegetative growth. In balanced vines, the values range from 3 to 10 (Cavagnaro *et al.*, 1997; Main *et al.*, 2002), with optimal values between 5 and 7 (Ravaz and Sicard, 1903, cited by Vasconcelos and Castagnoli, 2001), while values greater than 10 indicate a lack of vigour and those below 3 indicate an excess of vigour, at the expense of yield. Considering such approximation, the cover cropping system (PM treatment) significantly increased the Ravaz index to 4.05 in 2006 and to 5.50 in 2008 (Table 6). Therefore, PM offered a better balance between vegetative and reproductive plant growth in comparison to NT and TT treatments these years, although the opposite was observed in 2007, when yield reduction in cover cropped vines was the highest and counteracted their lower pruning weight. This growth reduction can be beneficial to grape health and berry composition, especially in high vigour environments, allowing a more open canopy and consequently a better cluster microclimate (Dokoozlian and Kliewer, 1996).

It was remarkable that both cover crop treatments presented higher yield (kg grapes vine^{-1}) in 2008 compared to 2007 and 2006 (Table 5), which is related to greater water availability as a consequence of unusual late spring rainfalls that year. As an exception, TT showed similar values in 2008 compared to 2007 (Table 5). The year effect, which is probably due to precipitation differences between years (2008 being the year with the highest and 2006 the year with the lowest rainfall), determined those differences in the averages of almost each parameter (Table 5 and 7). The most interesting results concern yield and number of clusters per vine. Cretazzo *et al.* (2007) described similar effects on quality parameters and grape yield in Manto Negro cv. as a consequence of higher precipitations.

Furthermore, differences among treatments were only observed in 2006 and 2007 and not in 2008, when those late rainfalls probably counteracted the effects of cover crop water consumption during spring.

In 2006 and specially in 2007, PM and NT vines showed lower yield than TT ones, as well as both a lower number of clusters per vine and a lower mean cluster weight, although differences were only significant in 2007. This yield reduction was probably due to the mild water stress experienced by PM and NT vines at the early stages of the cycle in 2006 and 2007. By contrast, Monteiro and Lopes (2007) did not report any significant yield reduction in cover cropped vines under similar Mediterranean conditions.

In contrast to what was reported by Celette *et al.* (2005) and Morlat and Jacquet (2003), the measured must quality parameters (see M&M) did not show significant differences among treatments over the three experimental years. However, significantly higher concentrations of TP were observed in the PM treatment in 2007, but not in 2006 and 2008 (Table 7). This could be related to its lower grape yield that year. In this sense, the expected berry quality improvement by the increment in the content of anthocyanins and TP as a consequence of vigour reduction (dos Santos *et al.*, 2003) was not observed. Other studies also showed no cover crop effect on berry quality under Mediterranean conditions (Monteiro and Lopes, 2007).

CONCLUSIONS

The interest of using cover crops under Mediterranean conditions can arise from the benefits of vigour reduction on berry quality. Indeed, the increase of must and wine quality could counteract the yield reduction provoked by the mild and early water stress imposed by cover crop water consumption in the spring. Nevertheless, this study has shown that these benefits are not always achieved since vigour and yield reduction can be recorded without berry quality improvement. On the other hand, the LA reduction showed by cover cropped vines may reduce the vine water consumption at later stages (veraison and ripening), which can be of interest to ensure grape yield during the dry years or to reduce irrigation necessities.

Inter-annual variability of rainfall pattern, which tends to be high in Mediterranean climate areas, can be highlighted as one of the main factors determining cover crop effects on vines. But the species composition of the cover crop, as well as their management, are also of major interest to achieve the objectives of using cover crops. In this sense, the use of perennial mixtures (with grass and legume species) as cover crops in Mediterranean vineyards may exert a moderate competition for water resources, specially under rain fed conditions, since these kinds of species usually show a low growth capacity. Furthermore, the lower vineyard mechanization achieved by using perennial mixtures as intercropping can provide economical and ecological benefits that should be taken into account when globally assessing the use of cover crop.

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