

## Photosynthetic activity and grape yield of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevines submitted to nitrogen supply methods and doses

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### Summary

The great majority of sandy soils present low nitrogen availability, generally below vines physiological demand, and therefore nitrogen fertilizer application, such as urea, is necessary. However, when urea is applied on the soil surface, the vine can use only a small amount of N. Thus, management strategies such as fertigation can increase N utilization by vine, reflecting in a greater photosynthetic activity and grape yield. The objective of this study was to evaluate the influence of nitrogen supply methods and doses on photosynthetic activity and grape yield of 'Alicante Bouschet' grapevines cultivated in sandy soil. The experiment was carried out in Santana do Livramento, in the southern region of Brazil, in a vineyard planted in 2011. The cultivar was 'Alicante Bouschet' grafted on '1103 Paulsen'. The experimental design was of randomized blocks in a bifactorial arrangement (dose x method). The N source used was urea (45 % N). The 0, 20, 60 and 100 kg N·ha<sup>-1</sup> doses were applied on the soil surface, without irrigation (NWI) and via fertigation (NF). Photosynthetic activity, N concentration in leaves and grape yield during two growing seasons were evaluated. The application of N doses in soil increased N concentration in grapevine leaves by up to 53 % in NF and 18 % in NWI methods but did not affect yield. Strong correlations between yield and water use efficiency were also observed, evidencing a certain synchronism between the physiological demand of the grapevine and the offer of the adequate N dose by a more efficient method.

**Key words:** fertigation; gas exchange; net photosynthesis; nitrogen fertilization; nutritional status; vineyards.

### Introduction

Agriculture advance over extensive livestock areas was intensified in southern Brazil after the Green Revolution, especially in important regions of Rio Grande do Sul (RS),

such as the "Campanha Gaúcha". Natural vegetation in this region is composed of a large variety of spontaneous species native to the Pampa Biome, which is also present in Argentina and Uruguay (DE CONTI *et al.* 2019). The low commercial value of land, ease conversion of native areas into vineyards, the mechanization possibility of management practices, large-scale productive potential, and adequate climatic conditions favored viticulture development in the region (FLORES and MEDEIROS 2013). Viticulture contributes approximately to 1 % of the Gross Domestic Product (GDP) of RS, and the state is responsible for more than 60 % of the planted area in the country (IBGE, 2019). Moreover, in this region, sandy soils predominate where the vineyards are planted (LORENSINI *et al.* 2014). These soils have low soil organic matter (SOM), which results in low nitrogen (N) availability native to the soil (STEFANELLO *et al.* 2020). Thus, to supply grapevine physiological demands leading to adequate development and yield, it is necessary to apply N sources to the soil (BRUNETTO *et al.* 2016).

The N application in vineyards usually occurs in solid form, onto the soil surface, and without incorporation. As vine is a perennial plant, physical damages to the root system can stimulate vascular diseases occurrence by infection, as (*Fusarium oxysporum f spp. herbemontis*), and black feet (*Cylindrocarpon destructans*), which may decrease vineyards useful life (COSTA *et al.* 2010). However, the N application on the soil surface, in tropical or subtropical regions, can stimulate losses to the atmosphere. These losses occur mainly by ammonia volatilization and they rise due to higher rainfall and relative air humidity common in these regions (VIERO *et al.* 2014, SILVA *et al.* 2017). It leads to a decrease in N availability and utilization by plants.

Thus, it is necessary to use alternative nitrogen fertilization management methods, such as fertigation, which allow the vine to obtain a better N fertilizer recovery (STEFANELLO *et al.* 2020). Water addition via irrigation combined with fertilizer may increase soil moisture content, favoring the movement of dissolution products from N granules into the soil, which, in turn, may favor N mineral forms increase in the profile (TEIXEIRA *et al.* 2011). An increase in N mineral forms concentration in soil solution, nitrate and ammonium

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can allow these ions a closer approximation to roots external surface, facilitating its absorption by plants (BARLOW *et al.* 2009; STEFANELLO *et al.* 2020). On the other hand, high water flow in the soil can also favor ion movement to rootless zones, stimulating nitrate leaching, resulting in a lower N uptake by vines, especially in sandy soils with low ion retention (LORENSINI *et al.* 2012, COLOMBO *et al.* 2015).

When vine roots absorb N, part of it can accumulate in the plant's own root system. But a great part of it tends to be transported to aerial parts in the form of asparagine and may have its increment detected inside the plant through leaf analysis (CARRANCA *et al.* 2018). During flowering, a high energy demand period, if the plant is well nourished as a consequence of nitrogen fertilization during budding, it will invest its carbohydrates in shoot and root growth, and this will result in higher N concentrations in leaves during this period, together with lower dry matter production compared to the cycle end (ARROBAS *et al.* 2014, BRUNETTO *et al.* 2016). However, for proper vine nutritional status monitoring, it is also recommended to collect leaves during veraison. Since in this period the plant will be investing in productive organs, such as bunches and berries, and a process of photoassimilates redistribution will take place at these growing points, which will imply N concentration reduction in leaves (METAY *et al.* 2015, THOMIDIS *et al.* 2015).

If N supply methods and doses, such as fertigation, provide lower atmosphere losses, it is expected that there will be some N increase within vines, which will be detected by leaf analysis (BRUNETTO *et al.* 2016, STEFANELLO *et al.* 2020). Besides, it may also be responsible for protein formation in leaf, as Rubisco synthesis, which in C3 plants leaves is equivalent to more than 50 % of soluble proteins (LANG *et al.* 2018). As a result of this nitrogen handling, an increase in plant photosynthetic rate is expected, due to higher carboxylation efficiency resulting from higher Rubisco enzyme concentration as well as other enzymes involved in the process (BASCUÑAN-GODOY *et al.* 2017). In addition, it can be argued that N-well-supplemented plants exhibit higher levels of photosynthetic pigments, and thus higher electron transport rates in chloroplasts, linked to higher production rates of NADPH and ATP (MORIWAKI *et al.* 2019). As N supply is related to these mentioned physiological reactions, an increase in productivity is expected in cultivars that respond to fertilization. 'Alicante Bouschet' has a highly productive capacity, since well supplied with N. Besides that, quality is also important, because the grapes of this cultivar are commonly destined to assemblage, due to its high anthocyanins contents which promote intense coloration to the wine (FALGINELLA *et al.* 2012, COSTA *et al.* 2015).

Considering the above, the authors raise the hypothesis that the application of intermediate N doses, especially *via* fertigation, may favor the N absorption in sandy soils. Thus, a greater amount of N would be accumulated and diagnosed in the leaves. However, in addition, it is necessary to monitor photosynthetic parameters, which allows correlating the nutritional status of the grapevine with its physiology. In addition, there is still little information on the physiological behavior of young grapevines in Southern America, from field experiments in repeated growing seasons. The aim of this study was to evaluate the photosynthetic activity

and grape yield of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevines submitted to nitrogen supply methods and doses.

## Material and Methods

**Description of the experimental area:** The study was conducted on a vineyard in the city of Santana do Livramento, Rio Grande do Sul State (RS), region of "Campanha Gaúcha", Southern Brazil (Latitude 30° 48' 31" S, Longitude 55° 22' 33" W, Altitude 200 m). The vineyard was installed in 2011, and evaluations were carried out during two growing seasons (2014/15 and 2015/16). The grapevine cultivar was 'Alicante Bouschet' (*Vitis vinifera* L.), grafted on '1103 Paulsen' rootstock. The conduction system of the vines was espalier in a double string on the first thread. The planting density was 2976 plants per hectare, with 1.2 m spacing between plants and 2.8 m between rows. The grapevines were subjected to Guyot hibernal pruning (stick and spur), and the same number of buds were maintained for all treatments. The climate is humid subtropical (Cfa) (ALVARES *et al.* 2013). The average annual rainfall is 1,600 mm. The hottest historic temperature was 23.8 °C in January, and the average in the coldest month was 12.4 °C, in July. Annual sunshine is approximately 2,500 h. From an automated meteorological station installed in the experimental area, it was possible to collect daily precipitation and air temperature data. These data were summed and values of observed accumulated precipitation and average monthly temperature were obtained (Fig. 1). The data for normal precipitation and average normal temperature are the climatological normals, that is, average values over an interval of 30 years monitored in the municipality of Santana do Livramento, RS, Brazil (Fig. 1). These data were obtained from the database of INMET (National Institute of Meteorology).

Soil was classified as Typical Hapludalf (SOIL SURVEY STAFF 2014) and its attributes in the 0-0.20 m layer were: 822 g·kg<sup>-1</sup> sand, 115 g·kg<sup>-1</sup> silt, 63 g·kg<sup>-1</sup> clay (pipette method) (EMBRAPA 1997); 11 g·kg<sup>-1</sup> organic matter (WALKLEY and BLACK 1934), pH 5.5 in water (1:1 ratio); P and K available, 5 and 72 mg·kg<sup>-1</sup>, respectively (both extracted by Mehlich<sup>1</sup>);

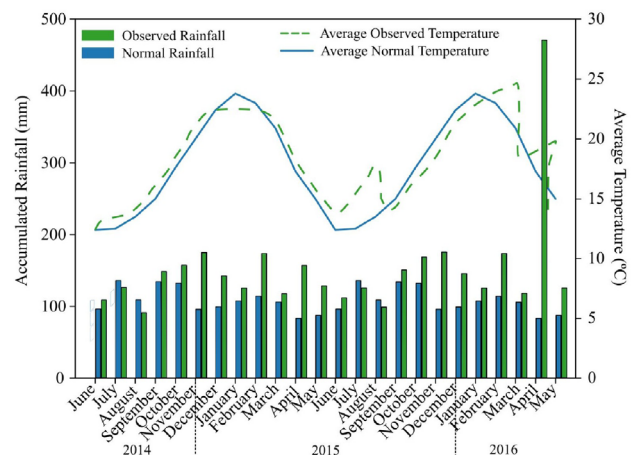


Fig. 1: Accumulated rainfall observed, normal accumulated rainfall, average observed temperature and average normal temperature in the experimental area in Southern Brazil during 2014/15 and 2015/16 growing season.

Al, Ca and Mg, 0.0, 1.99 and 0.923  $\text{cmol} \cdot \text{dm}^{-3}$ , respectively (both extracted by  $\text{KCl } 1 \text{ mol} \cdot \text{L}^{-1}$ ; TEDESCO *et al.* 1995). Before experiment installation, the vineyard was submitted to 45 kg  $\text{P}_2\text{O}_5 \cdot \text{ha}^{-1}$  application, using triple superphosphate (41 % P) and 45 kg  $\text{K}_2\text{O} \cdot \text{ha}^{-1}$  as the P source, and potassium chloride as the K source (60 % K). The P and K applications in later years were carried out according to the Chemistry Commission and Soil Fertility of Rio Grande do Sul and Santa Catarina States (CQFS-RS/SC-2004).

Along with the experiment, the vegetation in vines planting was desiccated with herbicide via directed jet. Invasive plants were monitored during winter with "Select" (Alkylbenzene + Cletodim) and "Finale" (Glufosinate) application, and during spring and summer with "Alion" (Indaziflam), both systemic herbicides, used at recommended dosages by each product leaflet and in a single application. The vegetation between rows present in the vineyard was composed of spontaneous species from the Pampa Biome as *Paspalum notatum*, *Paspalum plicatulum* and *Axonopus affinis*. Throughout the vine's cycle, cover plants between the planting rows were cut to approximately 10 cm of height and residues were deposited on the soil surface.

**Experimental design and treatments:** The experimental design was a bifactorial arrangement (N doses x N supply method) with a randomized block, with three replicates, each replicate being composed of five plants. The three central plants of each replicate were evaluated. The N doses were 0, 20, 60 and 100 kg  $\text{N} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ . Supplying methods were (i) superficial N application without irrigation (NWI) and (ii) N application via fertigation (NF). Irrigation was provided only in the NF treatment during fertilizer application, with no supplementary irrigation in the NWI treatment. The N source used was urea (45 % N). The N doses in the NWI supply method were applied to the soil surface, in the canopy projection area, without incorporation. Also, N doses application in NWI mode was carried out from September 25, in a single annual period in the vines budding phenological stage, as suggested by BRUNETTO *et al.* (2009). In the NF method, N doses were dissolved in a cistern with water and then the N-containing solution was applied via dripping in the canopy projection area. The Dripnet PC AS 16250 drip system from Netafim<sup>TM</sup> was used for fertigation and had a flow rate of 1.6  $\text{L} \cdot \text{h}^{-1}$  and a spacing of 0.6 m between drippers. The N application via fertigation occurred once a week, during four weeks, preferably at 9:00 am. This practice took approximately 30 min, and it was carried out following three steps: (I) during the first 10 min the irrigation hoses received only water; (II) after 10 min of water, the solution containing N previously dissolved in the cistern, was applied, which was injected into the hoses with previously described flow; (III) and as a last step, after applying the solution containing N, only water was applied again for 10 min. The NF mode used 1.6 L of water per plant in each application, totalizing 12.8 L of water per plant during the each growing season.

**Photosynthetic parameters:** Annually, at grapevines flowering (November), photosynthetic parameters were determined as  $\text{CO}_2$  net assimilation rate ( $A$ ), Rubisco carboxylation efficiency ( $A/C_i$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), transpiration rate ( $E$ ), stomatal conduc-

tance ( $G_s$ ) and water use efficiency ( $EUA$ ), obtained by the ratio between  $\text{CO}_2$  net assimilation rate and transpiration rate. Evaluations were carried out from 9 to 11 o'clock in the morning, using three vines of each treatment. For each plant, a completely expanded leaf was used from branches median position and sun-exposed. For this analysis an infrared gas analyzer (IRGA), model Li-COR<sup>®</sup> 6400 XT was used, equipped with a light source model LI-6400-2B to fix photosynthetic radiation intensity during evaluation. Initially, three photosynthetic radiation curves were performed, considering the following limits: 1800, 1500, 1000, 800, 600, 400, 200, 100 and 0  $\mu\text{mol}$  of photons  $\cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . From  $\text{CO}_2$  net assimilation evolution related to radiation variations, saturating intensity of 1000  $\mu\text{mol}$  of photons  $\cdot \text{m}^{-2} \cdot \text{s}^{-1}$  was determined, which was used for photosynthetic parameters point evaluations in different contrasts of treatments. During the analysis, environmental  $\text{CO}_2$  was used, which remained, on average, at 378  $\mu\text{mol} \cdot \text{mol}^{-1}$  concentration. Air and leaf temperatures were maintained between 25 and 28 °C and airflow velocity employed was 500  $\mu\text{mol} \cdot \text{s}^{-1}$  at 45 % relative humidity. In all measurements, data were recorded only when photosynthetic parameters reached 1 % or less variation.

**Leaf collection for N concentration analysis:** During flowering (November) and veraison (January), ten complete leaves opposite to the first bunch were harvested on branches of the year on each vine (counting from the top). Leaves were dried in a forced-air circulation oven at 65 °C until constant dry matter. For N concentration determination, leaves were milled in a Willey mill and tissue was subjected to sulfur digestion (TEDESCO *et al.* 1995). Subsequently, distillation was carried out, using micro-Kjeldahl N distiller (Te 0363, Tecnal, São Paulo, Brasil).

**Grape yield:** During the grape harvest (March), all bunches from plants were collected, counted and weighed, using a precision digital scale (Walmur, 50K, Brasil).

**Statistical analysis:** Obtained results were submitted to analysis of variance using Sisvar software version 5.6 (FERREIRA 2011), and when the effect was significant by F-test at 5 % error probability, data were submitted to Scott-Knott averages comparison test, and linear and polynomial regression models were adjusted. When the interaction between factors was significant, unfolding was performed, and when not significant each factor main effects were presented. Means that did not present significant differences were also presented. Principal component analysis (PCA) was performed using variable means (total N concentration in leaves at full bloom and veraison, grape production per plant,  $\text{CO}_2$  net assimilation rate ( $A$ ), Rubisco instantaneous carboxylation efficiency ( $A/C_i$ ),  $\text{CO}_2$  intercellular concentration ( $C_i$ ), transpiration rate ( $E$ ), stomatal conductance ( $G_s$ ), and water use efficiency ( $WUE$ ), using CANOCO software, version 4.5 (TER BRAAK and SMILAUER 2002).

## Results and Discussion

**Photosynthetic parameters:** In 2014/2015 growing season N doses and application methods, N applica-



tion via fertigation (NF) and N application without irrigation (NWI) did not affect net assimilation rate, transpiration rate, CO<sub>2</sub> intercellular concentration and stomatal conductance (Tab. 1). This might be due to well-distributed rainfall during most of the growing season (Fig. 1), especially in phenological stages along with the vegetative-productive period. It may have maintained soil moisture at adequate levels, which favors water and N absorption by vines. This possibly has reflected in similar values for photosynthetic parameters between plants grown on both N supply methods (TEIXEIRA *et al.* 2011, BASCUÑÁN-GODOY *et al.* 2017).

Water use efficiency (WUE) in the 2014/15 growing season presented a linear response, decreasing with the increase of N applied doses and did not show differences between N application methods (Tab. 1). Water use efficiency is a parameter that contrasts CO<sub>2</sub> absorption efficiency from the environment by leaf and water flow from leaf to external environment, allowing to evaluate treatment effect on water consumption and plant productivity (MEDEIROS *et al.* 2016, BASCUÑÁN-GODOY *et al.* 2017). Possibly, the increase in soil N availability increased vegetative growth vigor and, consequently, plants presented a higher water demand per unit

of fixed CO<sub>2</sub> (MORIWAKI *et al.* 2019). Rubisco carboxylation efficiency in vine leaves in the 2014/2015 growing season was not affected by N doses, but values were higher in leaves from plants submitted to NF than those of the NWI method (Tab. 1). This could be explained by the fact that in vines submitted to N application via NF, N concentration in leaves was greater when compared to plants from NWI application. The N is closely related to the carboxylation efficiency that occurs in leaves, as it is a constituent of the enzyme Rubisco (MARSCHNER 2012). Thus, factors such as the availability of water in the soil, and consequently, the movement of N to the roots, as well as the concentration of N forms in the soil solution, influence the rate of N absorption by the vines (THOMIDIS *et al.* 2015, STEFANELLO *et al.* 2020). In addition, how N plays a structural role in the formation of proteins, its proper concentration in tissues is vital (LANG *et al.* 2018). Thus, the proper functioning of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is maximized, as it is the most abundant protein in plant leaves, and accounts for up to 50 % of the total soluble proteins in most leaves (TAIZ and ZEIGER 2013). Thus, higher temperatures and less accumulated rainfall were observed for the 2014/15 growing

Table 1

Photosynthetic parameters, N concentration in leaves collected at flowering and veraison, and grape yield in vines submitted to N dose application via fertigation (NF) and without irrigation (NWI) in the 2014/15 growing season

Method	N doses (kg N·ha <sup>-1</sup> ·year <sup>-1</sup> )					Equation	R <sup>2</sup>
	0	20	60	100	CV %		
Net photosynthetic rate (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )							
NF	19.60	18.40	20.10	17.60	13.2	ns	ns
NWI	15.90	17.50	18.90	15.40	16.3	ns	ns
Stomatal conductance (mol H <sub>2</sub> O·m <sup>-2</sup> ·s <sup>-1</sup> )							
NF	0.316	0.298	0.316	0.277	21.3	ns	ns
NWI	0.234	0.305	0.288	0.274	28.7	ns	ns
Intercellular CO <sub>2</sub> concentration (μmol·mol <sup>-1</sup> )							
NF	239	234	230	229	4.8	ns	ns
NWI	234	244	225	244	6.8	ns	ns
Transpiratory rate (mmol H <sub>2</sub> O m <sup>-2</sup> ·s <sup>-1</sup> )							
NF	4.57	4.73	5.48	5.19	18.8	ns	ns
NWI	3.67	4.51	4.90	5.07	21.1	ns	ns
Water use efficiency (mol CO <sub>2</sub> ·mol H <sub>2</sub> O <sup>-1</sup> )							
	4.32	3.88	3.82	3.29	9.81	y = 4,24 – 0.009x	0.89
Instantaneous carboxylation efficiency of rubisco (μmol m <sup>-2</sup> s <sup>-1</sup> )							
NF			0.08 a			ns	ns
NWI			0.07 b			ns	ns
Leaf N concentration at flowering (%)							
NF	2.52a	2.99a	2.96a	2.87a	2.14	y = 2.598322 + 0.015388x – 0.000129x <sup>2</sup>	0.76*
NWI	2.45a	2.73b	2.76b	2.74b	2.13	y = 2.493950 + 0.009721x – 0.000074x <sup>2</sup>	0.88*
Leaf N concentration at veraison (%)							
NF	2.49a	2.49a	2.46a	2.38a	3.34	y = 2.507153 – 0.001103x	0,89*
NWI	2.04b	2.32b	2.41a	2.15b	2.91	y = 2.055879 + 0.014176x – 0.000133x <sup>2</sup>	0.98*
Grape production (kg·plant <sup>-1</sup> )							
NF	7.14	7.90	8.00	8.30	16.3	ns	ns
NWI	7.29	7.75	6.75	7.91	24.0	ns	ns

ns = not significant \* significant at 5 % probability of error.

season when compared to 2015/16 (Fig. 1), which allows us to conclude that the lower water availability occurred in growing negatively influenced photosynthetic parameters (Tab. 1). In the 2015/16 growing season, the net assimilation rate and Rubisco carboxylation efficiency were 21% lower in leaves from vines submitted to increasing N doses compared to the control (Tab. 2). On the other hand, transpiration rate values in leaves increased with N doses increment, as observed in the previous cycle. However, N supply methods (NWI and NF) did not affect the same. This might be due to the fact that an increase in transpiration stimulates stomatal partial closure and, consequently, CO<sub>2</sub> entry in the leaf is reduced, which may compromise both the photosynthetic rate and Rubisco carboxylation efficiency (THOMIDIS *et al.* 2015, BASCUÑAN-GODOY *et al.* 2017).

The 0 (control) and 60 kg N·ha<sup>-1</sup>·year<sup>-1</sup> doses provided a higher net assimilation rate and Rubisco carboxylation efficiency in vines (Tab. 2). While in vines subjected to the higher N dose, 100 kg N·ha<sup>-1</sup>·year<sup>-1</sup>, a 21 % decrease in these two parameters was observed, in addition to an increase in the transpiration rate. This may be a result of greater N mineral absorption from the soil by vines, which increases N concentration in foliar tissue, stimulating cellular division

and, consequently, aerial part dry matter increase (BRUNETTO *et al.* 2016, MORIWAKI *et al.* 2019). In this way, the higher growth stimulates self-shading, and shoots may be acting as a photoassimilate drain (ARROBAS *et al.* 2014). In addition, excess drainage induces tissue senescence, raising ethylene and abscisic acid (ABA) levels in the plant and, consequently, compromising the physiological functioning of the most exposed leaves (MORIWAKI *et al.* 2019). Besides, with higher cell division and, consequently, higher shoot growth, greater water loss may occur in vines subjected to higher N doses (THOMIDIS *et al.* 2015, SERMONS *et al.* 2017).

**N concentration in leaves:** The N concentration in leaves collected from vines at flowering in the 2014/15 and 2015/16 growing seasons increased with N doses applied by both NWI and NF methods (Tabs 1 and 2). At flowering, the interaction effect between dose and method was presented by the greater increase in N concentration in leaves from vines submitted to NF, when compared to leaves from vines with NWI application. The increase of N applied dose in soil via fertigation favors the rapid increase of N mineral concentration in the soil solution (BARLOW *et al.* 2009, STEFANELLO *et al.* 2020). Then, N forms approach the root system through mass flow and are preferentially ab-

Table 2

Method	N doses (kg N·ha <sup>-1</sup> ·year <sup>-1</sup> )				CV %	Equation	R <sup>2</sup>
	0	20	60	100			
Net photosynthetic rate (μmol CO <sub>2</sub> ·m <sup>-2</sup> ·s <sup>-1</sup> )							
	23.22	19.86	21.52	18.39	14.2	y = 22.315367 - 0.034212x	0.76*
Stomatal conductance (mol H <sub>2</sub> O·m <sup>-2</sup> ·s <sup>-1</sup> )							
	0.37	0.29	0.36	0.31	17.2	ns	ns
Intercellular CO <sub>2</sub> concentration (μmol·mol <sup>-1</sup> )							
NF	272	243	253	260	6.7	ns	ns
NWI	256	246	250	255		ns	ns
Transpiratory rate (mmol H <sub>2</sub> O·m <sup>-2</sup> ·s <sup>-1</sup> )							
	4.51	4.73	4.30	4.76	13.6	y = 4.707853 - 0.008499x	0.81*
Water use efficiency (mol CO <sub>2</sub> ·mol H <sub>2</sub> O <sup>-1</sup> )							
	5.21	4.24	5.07	4.88	11.91	ns	ns
Instantaneous carboxylation efficiency of Rubisco (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )							
	0.09	0.08	0.086	0.071		y = 0.088249 - 0.000137 x	0.56*
Leaf N concentration at flowering (%)							
NF	2.64a	2.72a	2.71a	2.66a	1.26	y = 2.650653 + 0.002947x - 0.000029x <sup>2</sup>	0.84*
NWI	2.50b	2.56b	2.60b	2.60b		y = 2.505296 + 0.002799x - 0.000019x <sup>2</sup>	0.99*
Leaf N concentration at veraison (%)							
NF	1.93a	2.39a	2.37a	2.96a	1.41	y = 2.003256 + 0.015066x - 0.000124x <sup>2</sup>	0.75*
NWI	1.87b	2.08b	2.19b	2.10b		y = 1.881859 + 0.010585x - 0.000084x <sup>2</sup>	0.98*
Grape production (kg·plant <sup>-1</sup> )							
NF	2.28	3.26	3.69	2.91	30.3	ns	ns
NWI	2.85	3.15	2.83	2.72		ns	ns

ns = not significant \* significant at 5 % probability of error.

sorbed by younger roots, which intensely grow during vines flowering (COMAS *et al.* 2010). Part of absorbed N fertilizer is transported to aerial organ parts of the grapevine, preferably to the leaves, which in flowering have intense division and cellular elongation and, therefore, become nutrient drains, such as N (THOMIDIS *et al.* 2015, BRUNETTO *et al.* 2016).

The N concentrations in leaves collected on the veraison period increased with N doses applied by both NWI and NF methods in the 2014/15 and 2015/16 growing seasons (Tabs 1 and 2). These results show that even in a period of less young root growth and lower vegetative crown growth (ARROBAS *et al.* 2014, BRUNETTO *et al.* 2016), especially leaves, part of applied N was absorbed and detected in leaves. We observed, as in flowering, an interaction between dose and supply method factors, and N concentrations in vines submitted to N doses applied *via* fertigation (NF) were also higher than those observed in leaves from vines submitted to N doses application without irrigation (NWI). However, it is worth taking into account that N concentrations in vine leaves collected in the veraison period were smaller than those observed in vine leaves collected in the flowering season. This happened due to part of present N, for example, N in amino acids and proteins from vine leaves collected in the veraison period, was redistributed, especially to other annual growing organs such as grape bunches (METAY *et al.* 2015, THOMIDIS *et al.* 2015). But also, part of N contained in leaves can be redistributed, probably in smaller quantities, to other annual organs, such as one year old branches, as growth is slow during this period (ZAPATA *et al.* 2004); and for perennial organs, such as for branches of more than one year, stem and especially roots (COMAS *et al.* 2010). Part of N accumulated in perennial organs can be redistributed to growing organs in the later cycle (BRUNETTO *et al.* 2014, PESCIÉ *et al.* 2018).

**Grape yield:** Doses and supply methods application (NWI and NF) in the 2014/15 and 2015/16 growing seasons did not affect grape yield (Tabs 1 and 2), even with N content increase in grapevines, diagnosed by N content in leaf analysis (Tabs 1 and 2). Part of absorbed N from absorbed fertilizer may have been redistributed to growing annual organs, mainly in larger amounts for leaves and branches of the year, possibly stimulating vegetative growth (ZAPATA *et al.* 2004, BALDI *et al.* 2017, PESCIÉ *et al.* 2018). In addition, it is important to note that vines cultivated on sandy soils with low organic matter, as in the present study, take advantage of a small N amount from applied fertilizer (BRUNETTO *et al.* 2006 and 2008), which can increase plant internal N reserves, justifying in part the lack of grape yield increase.

Most of the absorbed N by vines in this type of soil tends to come from sources other than mineral fertilizer. Among these sources, we can highlight the decomposition of organic residues from the leaves and branches of vines, or even, from the mowing of cover plants that cohabit the vineyards (BRUNETTO *et al.* 2014). Besides, although the organic matter is present in lower amounts in these soils and it has little physical protection, its mineralization can also contribute to the nutrition of vines (LORENSINI *et al.* 2014). Also, part of N derived from fertilizer applied in different doses and methods can be lost by leaching, in case of nitrate ( $\text{N-NO}_3^-$ ),

which has its mobility in soil favored by NF application, due to formation of external sphere complexes with functional groups from the soil surface (LORENSINI *et al.* 2012, BRUNETTO *et al.* 2014, STEFANELLO *et al.* 2020). Volatilization is another form of significant loss and is frequently observed in soils with surface urea application, such as NWI. In this case, the nitrogen fertilizer is exposed to high soil temperatures, air currents, which favor extracellular urease hydrolysis, releasing ammonium carbonate in water,  $\text{CO}_2$  and ammonia ( $\text{N-NH}_3$ ); these two potentially emitted in gas form into the atmosphere (VIERO *et al.* 2014). In addition, N losses may also be caused by denitrification in the form of nitrous oxide ( $\text{N-N}_2\text{O}$ ) if soil micropores remain filled with water for a certain time (WOLFF *et al.* 2018).

It should be noted that grape production varied between growing seasons. In the 2014/15 harvest, average grape production in all treatments was 7 kg per plant, approximately 2.5 times greater than that observed in the 2015/16 growing season, in which plants presented an average yield of 3 kg. Oscillatory behavior in yield can be explained by the fact that floral induction occurs in the first weeks after the node separation from the top (BASCUNÁN-GODOY *et al.* 2017). This primordial bunch develops in complexity and size for two to three months, coinciding with current cycle bunch development. At this stage, the total number of potential flowers for harvest in the following year is practically determined (PAULETTO *et al.* 2001). Thus, 2015/16 harvest productive potential was determined in the 2014/15 growing season. This behavior is characteristic of perennial species, which present high fruit yield in a given harvest, and reduce floral induction and differentiation in the next one (PAULETTO *et al.* 2001, BASCUNÁN-GODOY *et al.* 2017). In addition, the presence of many fruits per plant is influenced by the high concentration of gibberellin (MULLINS *et al.* 2007).

**Principal Component Analysis (PCA):** The principal component analysis (PCA) performed for 2014/15 growing season data demonstrates that the variability sum of the first two components explains 79.24 % of original results variability (Fig. 2a). Of these, 49.25 % were explained by principal component 1 and 29.99 % by principal component 2. Variables presented a high contribution in principal component 1 variation, and they have a strong relationship with N supply (NWI and NF). This led to the formation of two groups; one group was formed by 0, 20 and 100 kg  $\text{N}\cdot\text{ha}^{-1}$  doses of NWI method, where it was verified that variables that had the most influence on the group were *Ci* and *WUE*. The other group consisted of 60 kg  $\text{N}\cdot\text{ha}^{-1}$  dose of NWI mode and all N doses (0, 20, 60 and 100 kg  $\text{N}\cdot\text{ha}^{-1}$ ) provided by the NF method. This group has a greater influence on yield, N concentration in leaves at flowering and veraison, *E*, *G<sub>s</sub>*, *A*, *A/Ci*.

In the 2015/16 growing season, the sum of variability from the first two components explained 74.75 % of the variability of the original results (Fig. 2b). Principal component 1 accounted for 45.59 %, and principal component 2 for 29.16 % of the data variation explanation. Differently from what occurred in the 2014/15 and 2015/16 growing seasons, treatment and variables in principal component 1 were more influenced by N doses than by N supply methods. There were two groups formed, one was formed by 0 and 60 kg  $\text{N}\cdot\text{ha}^{-1}$

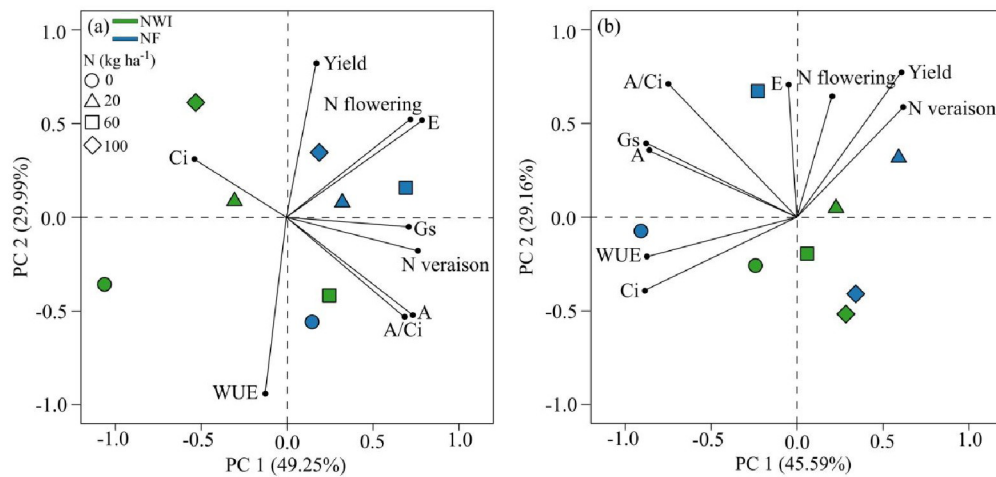


Fig. 2: Relationship between photosynthetic parameters ( $\text{CO}_2$  net assimilation rate -  $A$ , Rubisco carboxylation efficiency -  $A/C_i$ , internal  $\text{CO}_2$  concentration -  $C_i$ , transpiration rate -  $E$ , stomatal conductance -  $G_s$  and water use efficiency), grape yield and N concentration in leaves collected at flowering and veraison periods during 2014/15 (a) and 2015/16 (b) growing seasons, in vines submitted to N dose application via fertigation (NF) and without irrigation (NWI).

doses provided by the NF method and by  $0 \text{ kg N} \cdot \text{ha}^{-1}$  doses of NWI method. Under these conditions, photosynthetic variables  $A$ ,  $A/C_i$ ,  $C_i$ ,  $E$ ,  $G_s$ ,  $WUE$  had greater influence. The other group was formed by other doses in both supply methods (20, 60,  $100 \text{ kg N} \cdot \text{ha}^{-1}$  NWI and 20,  $100 \text{ kg N} \cdot \text{ha}^{-1}$  NF). This group had as greater influence variables grape production and N concentration in leaves in full bloom and veraison periods.

During the evaluated growing seasons,  $WUE$  presented a negative linear correlation with  $E$ , as the greater the water loss caused by high transpiration, the lower the water use efficiency (SCALON *et al.* 2015, THOMIDIS *et al.* 2015). The  $A$  and  $A/C_i$  presented a strong positive correlation with each other, and a negative correlation with  $C_i$ . In other words, with photosynthetic rate decrease, lower C fixation occurred, maintaining high  $\text{CO}_2$  concentration inside leaves (MACHADO *et al.* 2005).

The N concentration in leaves during flowering season showed a strong positive correlation with transpiration rate and grape yield, which was expected, since plant transpiration causes a negative pressure in xylem vessels responsible for upward movement of water flow, leading to N movement to roots surfaces, thus allowing N absorption (TAIZ and ZIEGER 2013, MORIWAKI *et al.* 2019). Grape yield showed a negative linear correlation with  $WUE$ , but there was no increase in grape yield with N dose. This means that more productive vines needed more water, which was directed to annual organ production, like bunches and berries (BRUNETTO *et al.* 2009, THOMIDIS *et al.* 2015).

### Conclusions

The application of intermediate N doses in the soil, such as  $20 \text{ kg N} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$  via fertigation or  $60 \text{ kg N} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$  without irrigation increased N concentration in leaves collected at flowering and veraison periods in the two growing seasons evaluated, but did not affect grape yield. We observed correlations between yield and  $WUE$ , and

N concentration in leaves at flowering, evidencing potential synchronism between plant physiological demand and N doses and supply methods.

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