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Research Article

NO_3^-, K^+, and chlorophyll index in fertigated grapevines in the semi-arid region of Brazil

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ABSTRACT: Evaluating the effects of N and K supply on grapevines (*Vitis vinifera* L.) and the techniques for nutritional diagnosis is of great importance for fertigation management of this crop. This study evaluated the effects of N and K fertigation on the soluble concentrations of NO_3^- and K^+ in the petiole sap and on the leaf chlorophyll index in drip irrigated 'Syrah' grapevine (from 17 June 2013 to 25 Nov 2014). The treatments consisted of five levels of N (0, 15, 30, 60 and 120 kg ha^{-1}) and K_2O (0, 15, 30, 60 and 120 kg ha^{-1}), combined in an incomplete 5^2 factorial scheme in 13 combinations and arranged in randomized blocks with four replications. We determined NO_3^- and K^+ concentrations in petiole sap, leaf chlorophyll index, grapevine cluster mass and number per plant, mean grapevine cluster mass, and phenolic composition. High NO_3^- concentrations contributed positively to grapevine yield; however, increased K+ concentrations caused a negative response of sap. For 120 kg N ha^{-1} rate, NO_3^- in the sap and chlorophyll index showed higher values at the flowering stage, while high values for K^+ were observed during the grape-ripening stage.

Keywords: *Vitis vinifera* L., fertigation, petiole

Introduction

The São Francisco Valley is located in the semi-arid region of Northeastern Brazil and wine production in that region has grown since the 2000s as a result of the establishment of wineries and governmental support, making it a major wine-producing area in Brazilian with the advantage of obtaining two grape harvests per year (Dias et al., 2016). Wine grape growers have widely used localized irrigation to supply plant water requirements in this area and it has also been used to apply fertilizer through water irrigation (fertigation) to grapevines (*Vitis vinifera* L.) (Silva et al., 2016).

Nitrogen (N) is the nutrient most likely to be deficient in grapevines; nevertheless, it is most applied to vineyards to increase yield (Loulakakis and Roubelakis-Angelakis, 2001). N plays a role in grapevine related to protein synthesis, leaf area increase, chlorophyll formation and photosynthate production, and increase of the number of organs (roots, cane, and trunk), which allow the plant to store N and carbohydrates. N also influences grape composition, wine quality, yield, and its components (Bell and Henschke, 2005). The rate of 60 kg N ha^{-1} promoted better grape composition and increased yield (Canoura et al., 2018). For wine grapes, timely and accurate regulation of N levels is essential, and a simple and effective means to monitor N and chlorophyll is urgently needed (Yang et al., 2021).

Potassium (K) is a highly phloem-mobile macronutrient, which is also required in large amounts by grapevines since it is the most abundant cation in grape berry and influences cell growth, berry sugar accumulation, mitigating senescence and must acidity. However, high K additions may negatively affect the overall grapevine status and wine quality (Leibar et al., 2017; Rogiers et al., 2017). An increase of K in leaf content and must composition has been reported due to different K levels (132 to 222 kg K ha^{-1}) in soil cultivated with grapevine (Ciotta et al., 2016).

The petiole and leaf blade analyses have been widely used to quantify the concentrations of N and K in plants (Tecchio et al., 2011; Kondi et al., 2018). The analysis of the petiole extract is more sensitive than leaf analysis to determine nitrate (NO_3^-) and K levels, while the leaf analysis better shows the plant response to fertilization with phosphorus (P) and K (Souza et al., 2012). Conversely, the evaluation of grapevine N content with handheld meters to measure relative chlorophyll index (RCI) and to measure sap NO_3^- concentration has been demonstrated (Martinez et al., 2017). RCI correlates the chlorophyll content with N levels in the leaves, indicating plant N status and quantifying N fertilization (Taskos et al., 2015).

This study evaluated the effect of N and K fertigation on NO_3^- and K^+ concentrations in the petiole sap and leaf chlorophyll contents measured by handheld meters in 'Syrah' grapevines in São Francisco Valley, Northeastern Brazil.

Materials and Methods

Experimental site

A vineyard was established in the municipality of Petrolina, Pernambuco State, Brazil (09°08'08.09" S, 40°18'33.6" W, altitude 373 m). According to Köppen classification, the climate is BSh type, that is, semi-arid climate, low latitude, and altitude (Alvares et al., 2013). Grapevine 'Syrah' (*Vitis vinifera* L.) grafted onto '1103 Paulsen' rootstock was planted on 30 Apr 2009, at a

spacing of 1 m between plants and 3 m between rows. The training system was a bilateral cordon trellised to a three-wire vertical system. The soil was classified as Aridic Fragiustalf (Soil Survey Staff, 2014). The vineyard formation lasted until Apr 2010. Plants were pruned on branches with six spurs (three per branch) and two buds per spur, and three consecutive growing cycles (from pruning to harvesting) were evaluated: from 17 June to 8 Oct 2013; from 7 Feb to 9 June 2014; and from 6 Aug to 25 Nov 2014, lasting 113, 122, and 111 days, respectively.

Prior to the experiment, 36 soil samples were collected within the plant rows in 0-0.20, 0.20-0.40 and 0.40-0.60 m layers using an auger. The samples were analyzed at the Soil and Leaf Analysis Laboratory of Embrapa Semi-Arid to determine soil physical and chemical attributes following the methodologies described by Teixeira et al. (2017). The average values of soil attributes throughout the three soil layers were: soil with 74.11 % sand, 16.37 % silt and 9.52 % clay and was classified as sandy-loam texture; and bulk density of 1.23 kg cm^{-3}; electrical conductivity = 0.50 dS m^{-1}; pH = 7.1; organic matter content = 14.97 g dm^{-3}; NO_3^- = 3.63 mg kg^{-1}; $NH4^+$ = 4.53 mg kg^{-1}; P = 124.86 mg dm^{-3}; K = 0.63 $cmol_c$ dm^{-3}; Ca = 3.15 $cmol_c$ dm^{-3}; Mg = 1.16 $cmol_c$ dm^{-3}; cation exchange capacity = 6.33 $cmol_c$ dm^{-3}; and base saturation = 79.26 %.

Five levels of N (0, 15, 30, 60 and 120 kg ha^{-1}) and five levels of K_2O (0, 15, 30, 60 and 120 kg ha^{-1}) were combined into an incomplete 5^2 factorial scheme (Littell and Mott, 1975), totaling 13 combinations in randomized blocks with four replications. Each experimental unit (EU) comprised 17 plants to minimize the sampling error.

Drip irrigation was used with emitters adjusted to a flow rate of 3.5 L h^{-1}, where each EU presented a 17 m long tubing with a plastic valve for controlling each fertigation event. The pumping system comprised a 6 m long pipe for water suction from a reservoir, a hydraulic pump with a flow of 15 m^3 h^{-1} at 100 KPa, a disc filter, an injection pump with a capacity of 300 L $h^{-1}$and a 60 L reservoir for the fertilizer solution. Fertigation was carried out once a week (periodic application). The fertilizers used were potassium sulphate (50 % K_2O), potassium chloride (60 % K_2O), potassium nitrate (12 % N and 45 % K_2O) and urea (46 % N). The accompanying ions were balanced using complementary fertilization.

Irrigation management was based on the crop evapotranspiration (ETc) estimate. For that purpose, the reference evapotranspiration (ETo, mm d^{-1}) was estimated using the Penmam-Monteith FAO method (Allen et al., 1998), with data collected from an automatic weather station installed 60 m from the experimental area, from where rainfall data and the mean, minimum, and maximum temperatures were also obtained to first (Figure 1A), second, and third growing cycles (Figure 1B). The crop coefficients (kc) used for the 'Syrah' grapevine were obtained by Bassoi et al. (2007) in the same site as that of the experiment. The ETc during the growing cycles under study was 543.17 mm for the first cycle, 475.17 mm in the second cycle, and 580.0 mm in the third cycle.

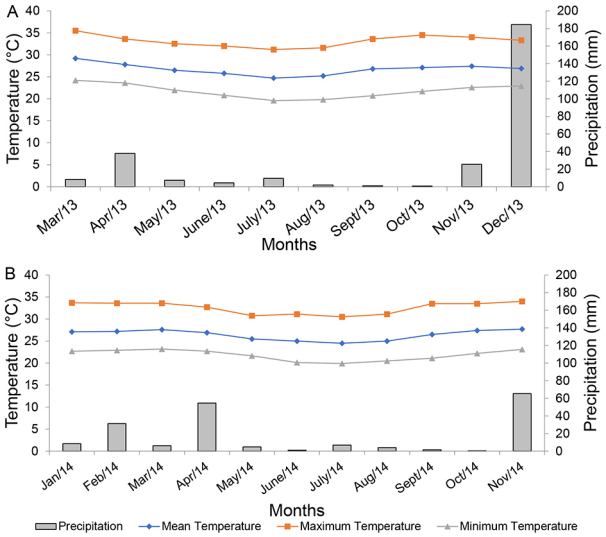

Figure 1 – Weather data throughout the growing cycles of 'Syrah' grapevines from Mar to Dec 2013 (A), and from Jan to Nov 2014 (B).

Sap analysis

During grapevines flowering and ripening stages, 30 leaf petioles located opposite the flowers and clusters were collected per experimental unit (Figure 2A). The petioles were packed into plastic bags, kept under refrigeration in a polystyrene container with ice and taken to the laboratory, where they were cleaned with cotton wool and distilled water, and dried with filter paper. The samples were then cut into small pieces of 1 to 2 cm and stored in identified plastic pots (Figure 2B). They were later placed in an ethyl ether solution and frozen at –10 °C to interrupt the metabolism and extract the sap (Souza et al., 2012). After two weeks, the samples were thawed and the sap extracted by pipetting with the aid of falcon tubes, where the sap, as it is denser, decanted to the tube bottom (Figure 2C). The sap was not filtered due to the small amount extracted. The choice for analyzed phenological phases and position of the sampled petiole were based on Tecchio et al. (2011), Martinez et al. (2017), Romero et al. (2010) and Pradubsuk and Davenport (2010).

Nitrate (NO_3^-) concentration was determined using a nitrate card meter, with no dilution and potassium concentration (K^+) was determined by flame photometry, using 500-fold dilution. This method of sap extraction was adapted for the grapevine from the method proposed by Souza et al. (2012) for citrus plants.

Figure 2 – Procedure to extract sap from grapevine: cutting the petiole from the plant (A), sample preparation (B), sap (yellow color) on the bottom, and ether (green color) after extraction (C).

Leaf chlorophyll index

To evaluate the chlorophyll content (*a*, *b* and total) in the grapevine leaves, non-destructive measurements were made in the field using an electronic chlorophyll meter on the useful plants of each experimental unit during the flowering stage and as the ripening stage (changing of color berries). Healthy leaves opposite the first cluster starting from the base of the branch were chosen. The mean value of two readings was used as the relative chlorophyll index for each leaf.

Production parameters

At the end of each growing cycle, grape clusters were harvested, counted and weighed on a scale with a precision of 0.01 g to estimate plant yield (Mg ha^{-1}). The number of clusters (NC) was obtained by counting, while the mean grapevine cluster mass (MCM) was obtained by the ratio between the total grapevine mass and NC. The values were correlated with the concentration of NO_3^- and K^+ in the sap of the leaf petiole.

Phenolics, flavonoids, and anthocyanins content

The total polyphenol content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The absorbance reading was performed on a UV-VIS spectrophotometer and a standard curve was prepared using gallic acid. The concentration of flavonoids and anthocyanins was determined by the method described by Lee et al. (2005).

Statistical analyses

The data obtained were previously subjected to Kolmogorov-Smirnov tests to check their normality and subsequently to the analysis of variance (ANOVA) at 1 % ($p < 0.01$) and 5 % ($p < 0.05$) significance levels by F test. The isolated variables with significant results were subjected to the regression analysis, where linear or quadratic regression models were fitted, and their equations were obtained. When the interactions were significant ($p < 0.05$), response surface with regression models of the first and second order were explored and used as a criterion of the significance of equation parameters and determination coefficient to observe the sensitivity of the response variable in relation to the factors as well as the optimal levels of the studied factors and saddle points.

Results and Discussion

NO_3^- concentration in plant sap

The results showed an effect ($p < 0.05$) of the treatments with N and K on the NO_3^- concentration on the petiole sap during the first (Figures 3A and 3B), second (Figures 3C and 3D), and third (Figures 3E and 3F) growing cycles. The evaluations made at the flowering stage showed that N fertilizers altered ($p < 0.01$) NO_3^- concentrations in the petiole sap during the three cycles, with a further interaction ($p < 0.05$) between N and K_2O during the second growing cycle. During the ripening stage, N fertilizers also altered ($p < 0.01$) NO_3^- concentrations in the petiole sap during the three cycles, with a further effect from the interaction between N and K_2O fertilization ($p < 0.05$) during the second growing cycle. In the third cycle, there was an effect only on N factor ($p < 0.05$).

During the flowering stage of the first cycle, the highest levels of N (120 kg ha^{-1}) provided the highest concentrations of NO_3^- in the petiole sap (4494.67 mg L^{-1}), with the data adjusted to a quadratic polynomial model (Figure 3A). During the ripening stage, there was a linear increase in NO_3^- concentration in the petiole sap due to the N dose, with 14.452 mg L^{-1} NO_3^- for each unit increase in the applied dose of N (Figure 3B).

At the flowering stage during the second growing cycle, the data for NO_3^- concentration in the petiole sap were adjusted to a response surface in relation to the applied levels of N and K_2O (Figure 3C), with the highest values obtained at a rate of 120 kg ha^{-1} N and 120 kg ha^{-1} K_2O, equal to a concentration of 11002.1 mg L^{-1}. At the ripening stage, there was a reduction in NO_3^- concentration in the plant sap, equivalent to 6846.90 mg L^{-1}, obtained at 75.22 kg ha^{-1} N and 120 kg ha^{-1} K_2O (Figure 3D).

At the flowering stage during the third growing cycle (Figure 3E), the adjusted regression model was linear, increasing NO_3^- concentration in the petiole sap by 174.89 mg L^{-1} for each unit increase in N rate. At the ripening stage (Figure 3F), the increase in NO_3^- was 164.19 mg L^{-1} for each unit increase in N rate via fertigation.

'Müller-Thurgau' grapevines supplied with levels from 0 to 100 kg ha^{-1} N showed an increase in the translocation of glutamine and glutamate, and of the NO_3^-, NH_4^+, K^+, Ca_2^+, Mg_2^+ and $PO4_3^-$ ions, with a reduction in the ratio of organic to inorganic N in the xylem sap, which was collected when the berries had a pea-size and changed their color (veraison). The N application promoted approximately a three-fold increase in the concentration of amino acids in the xylem, while NO_3^- increased more than 20 times and NH_4^+ doubled in concentration (Keller et al., 2001).

^{15}N absorption increased during the vegetative growth, becoming more intense after flowering, and reaching around 1 mg of N per g of root per day between flowering and berry growth (Zapata et al., 2004). This result was confirmed by the values in Figure 3A, in which NO_3^- is the primary anion in the xylem sap of the grapevine at the flowering stage (Peuke, 2000).

There was a significant reduction in NO_3^- concentration during the grape ripening stage compared

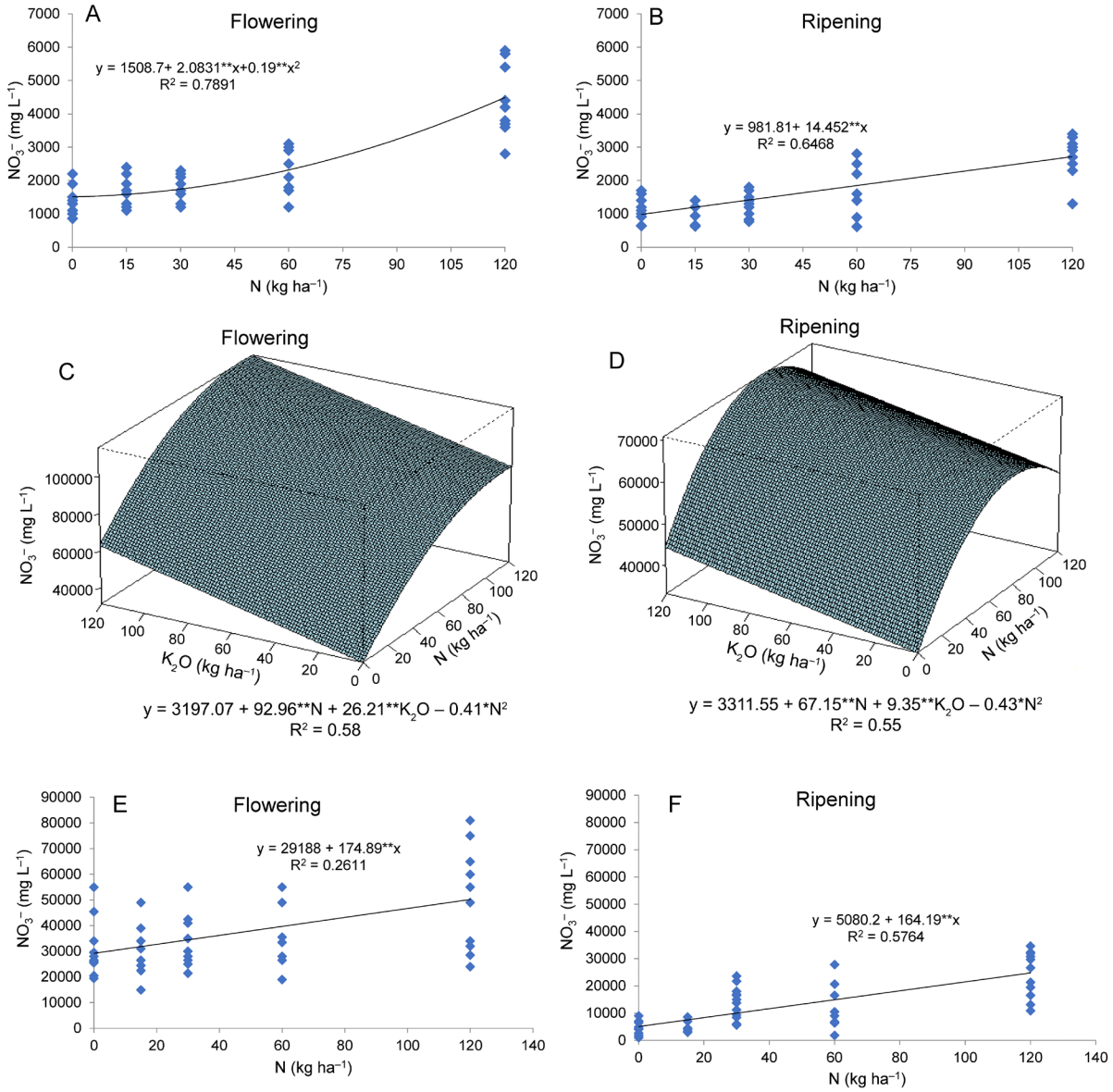

Figure 3 – Effect of N and K_2O on NO_3^- concentration in petiole sap in the 1st (A and B), 2nd (C and D) and 3rd growing cycles (E and F) of 'Syrah' grapevine. ** and * significant at 1 and 5 % probability by the F-test, respectively.

to the flowering stage, confirming the reduced demand for N. However, solute concentrations in the xylem decreased during the fruit growth stage and increased again during the closed-cluster stage, with glutamine (0.30-7.96 mM N) and glutamate (0.03-0.09 mM N) as the main components of the xylem sap, followed by NO_3^- (0.00-1.95 mM N) and NH4$^+$ (0.00-0.65 mM N) at a lower concentration (Keller et al., 2001).

The NO_3^- levels in the petiole sap during the flowering stage were more significant than those reported by other authors, who obtained 1243 mg L^{-1} NO_3^- in the 'Sultana' grapevine (Nagarajah, 1999), 1989 mg L^{-1} NO_3^- in the 'Niagara Rosada' grapevine (Tecchio et al., 2011), and 1859 mg L^{-1} of NO_3^- in the 'Thompson Seedless' grapevine (Martinez et al., 2017).

The amount of N applied via fertigation greatly influences the NO_3^- concentration and N addition should be controlled, as it drains into the berries (Gutiérrez-Gamboa et al., 2017). Other factors should also be considered. Time of sap collection, before or after berry color change (veraison), and water supply, had a significant influence on the NO_3^- concentration in the xylem sap of the 'Semillon' and 'Riesling' grapevines, since water stress increased NO_3^- concentration in grapes of 'Riesling' (Müller et al., 2016).

The decrease in N concentration after flowering can be attributed to N translocation from new and old leaves to permanent woody organs. N concentrations ranging from 40 to 160 kg ha^{-1} decreased N concentration in petioles due to the mobilization of old leaves to meristems, young leaves, and fruit (Romero et al., 2010).

The rate and the source of the fertilizer applied also influence the nutritional status of plants, and their concentration in the soil. This fact was observed in the present experiment and was also demonstrated by Lang et al. (2018), evaluating four sources of N (calcium nitrate, ammonium sulphate, urea, and arginine) applied at increasing levels (0, 0.5, 1.0 and 3.0 g per plant) in 'Regente' grapevine grafted onto SO4. The most significant response in berry and cluster production was obtained at 0.5 and 1.0 g N rates per plant. The 1.0 g N per plant rate provided the most significat biomass production; however, the highest N levels were obtained with 3.0 g N per plant. Conversely, the highest rate favored vegetative growth to the detriment of grape production. Calcium nitrate, ammonium sulphate and urea fertilizers provided more significant vegetative growth than arginine.

K^+ concentration in plant sap

The results for K^+ at the flowering stage showed an effect of the treatments with K_2O ($p < 0.01$) on the K^+ concentration in the petiole sap for the first and third growing cycles. During the ripening stage, N influenced the K^+ values in the petiole sap during the first ($p < 0.05$) and second ($p < 0.01$) growing cycles.

At the flowering stage during the first growing cycle, there was an increase of 11.58 mg L^{-1} for each unit increase at the K_2O rate, with the data adjusted to a linear model (Figure 4A). At the ripening stage, the increase was 78.61 kg ha^{-1} for each unit increase at the applied rate of N (Figure 4B), higher than that obtained at the flowering stage, although there was no interaction between the levels of N and K_2O. At the ripening stage during the second growing cycle (Figure 4C), using a linear model, the value of 5670.8 mg L^{-1} was obtained for the 120 kg N ha^{-1} rate. During the third growing cycle, at the flowering stage (Figure 4D), there was a linear increase of 7.68 mg L^{-1} for each unit increase in the K_2O rate.

The evaluations carried out during the flowering and ripening stages showed that the highest K^+ concentrations were obtained during the ripening stage. Studies on K concentrations in the petiole sap of the 'Sultana' grapevine, showed K^+ concentrations of between 8500 and 36900 mg L^{-1} (Nagarajah, 1999), and from 1751 to 2742 mg L^{-1} in the 'Niagara Rosada' grapevine (Tecchio et al., 2011) during the flowering stage.

The highest K^+ concentrations obtained during the reproductive phase were due to the relationship between this cation and the nitrate, since high concentrations of this anion in the soil lead to high concentrations of metal cations in the xylem sap (Keller et al., 2001). Therefore, the inorganic N in the xylem must be positively correlated with K^+.

The K is one of the nutrients most absorbed by the grapevine, acting in various physiological and biochemical processes, such as water relation, resistance to disease, production, and fruit quality (Rogiers et al., 2017). K^+ is highly mobile in the plant, in the phloem and the xylem (Romero et al., 2010), and is the predominant cation in the xylem sap (Peuke, 2000) as well as the most abundant in grape berries at all developmental stages (Rogiers et al., 2017).

The results of K^+ concentrations of this research differ from those obtained under a temperate climate, not only for methodological reasons but also due to the greater intensity of physiological processes in tropical climates. In regions of temperate climate, starting with budding and continuing to the end of flowering, K remobilization from permanent grapevine organs provides most of the K to the growing organs, which include leaves and inflorescences (Pradubsuk and Davenport, 2010), possibly due to a deficiency in the growth of new thin roots and the slow uptake by the roots in cold soils (Clarke et al., 2015). Remobilization of K^+ occurs from new leaves and petioles to the berries, considering that ripe berries are a robust K^+ drain (Romero et al., 2010). K^+ uptake from soil occurs from flowering to harvest, but it becomes slower after the berry color change (veraison) (Pradubsuk and Davenport, 2010). At this stage, xylem contribution to berry growth is reduced, but the causes of this behavior are still unclear. Several nutrients presented high concentrations in the experimental soil, such as K, which can be explained by the successive grapevine cultivations in this area with fertilizer application and may have influenced the result of the present study.

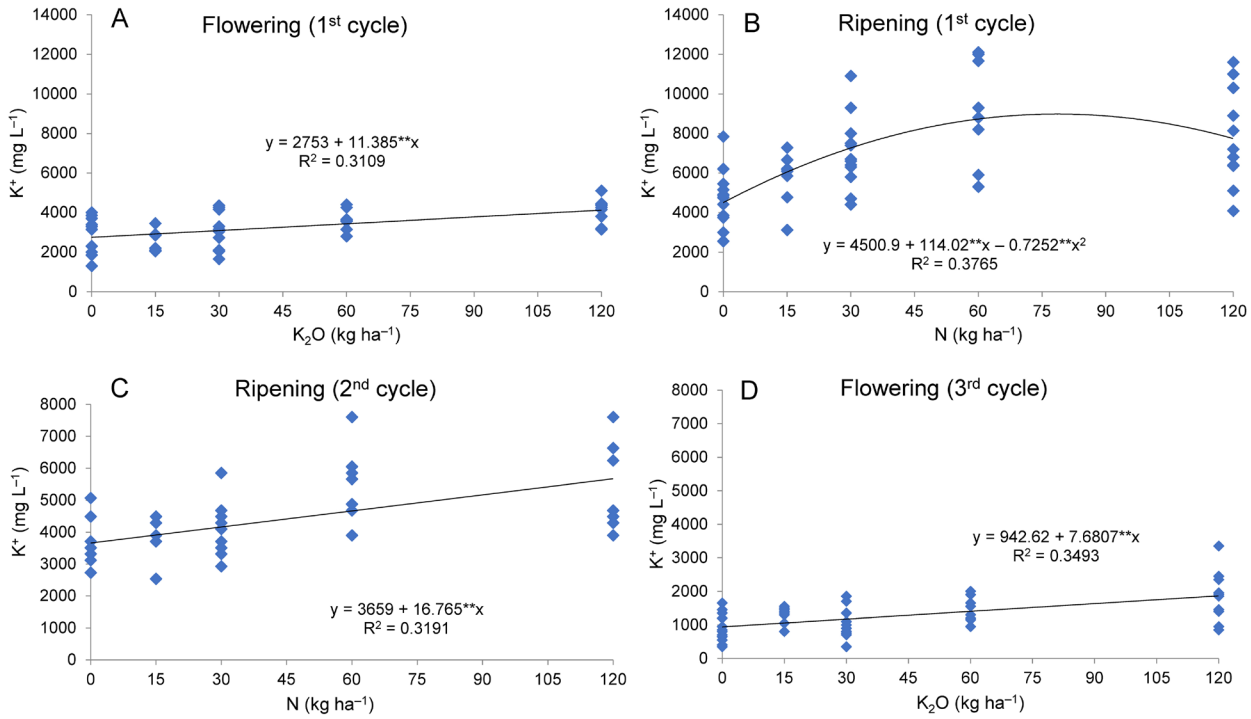

Figure 4 – Effect of N and K_2O on K^+ concentration in petiole sap of 'Syrah' grapevine: 1st growing cycle at flowering (A) and ripening (B) stages, 2nd growing cycle at flowering stage (C), and 3rd growing cycle at ripening stage (D). **Significant at 1 and 5 % probability by the F-test.

Relative chlorophyll index (RCI) in the leaves

The result of the analysis of variance for the chlorophyll *a* index at the flowering stage was influenced ($p < 0.01$) by the N rate during the first and third growing cycles and at the ripening stage, during the third growing cycle only ($p < 0.01$).

The chlorophyll *b* index at flowering (1st and 3rd cycle) and ripening (3rd cycle) stages was influenced by the N rate, respectively, at $p < 0.01$ and $p < 0.05$.

The total chlorophyll index at the flowering stage showed an effect ($p < 0.01$) on the N levels during the first and third cycles, while at the ripening stage, this variable showed a response to the levels of K_2O ($p < 0.05$) during the second growing cycle, but there was no adjustment to the regression analyses. However, during the third growing cycle, the N levels had a significant response ($p < 0.01$). N accumulation in the soil during the three cycles (Silva et al., 2016) probably increased N concentration in the plant, increasing the chlorophyll index.

At the flowering stage of the first growing cycle (Figure 5A), the adjustment was linear with an increase of 0.0429, 0.0275 and 0.0182 for each unit increase in N in the chlorophyll *a*, chlorophyll *b*, and total chlorophyll indices, respectively.

During the third growing cycle, the flowering stage (Figure 5B) showed a linear adjustment between the applied levels of N and the index for chlorophyll *a*, chlorophyll *b*, and total chlorophyll, with an increase of 0.0149 (chlorophyll *a*), 0.0255 (chlorophyll *b*), and 0.0404 (total chlorophyll) in the index for each unit increase in the N rate applied to the soil. At the ripening stage (Figure 5C), the proposed linear adjustment showed an increase of 0.2084 (chlorophyll *a*), 0.3024 (chlorophyll *b*), and 0.5108 (total chlorophyll) for each unit increase in the applied rate of N.

The N levels provided the best plant responses for the chlorophyll index during the two phenological stages under study. This response has been reported in various crops (Taskos et al., 2015).

Studies compared two techniques to measure chlorophyll in grapevine leaves and showed that a relative meter shows satisfactory sensitivity for leaf chlorophyll content with an index lower than 300 mg m^{-2} chlorophyll (Steele et al., 2008). Regarding the response obtained in our experiment concerning the N levels applied to the soil, Chavarria et al. (2012) obtained mean values for chlorophyll *a* (19.36), chlorophyll *b* (4.22) and total chlorophyll (20.61) below the values reported here that were 34.01 (chlorophyll *a*), 9.01 (chlorophyll *b*), and 43.13 (total chlorophyll).

Studies in Northern Greece used chlorophyll meters to estimate the N status and productivity of Cabernet Sauvignon and Xinomavro (*Vitis vinifera* L.) grapevines with 0.60 and 120 kg ha^{-1} N (Taskos et al., 2015). These authors obtained values close to our study, where the highest levels of N (120 kg ha^{-1})

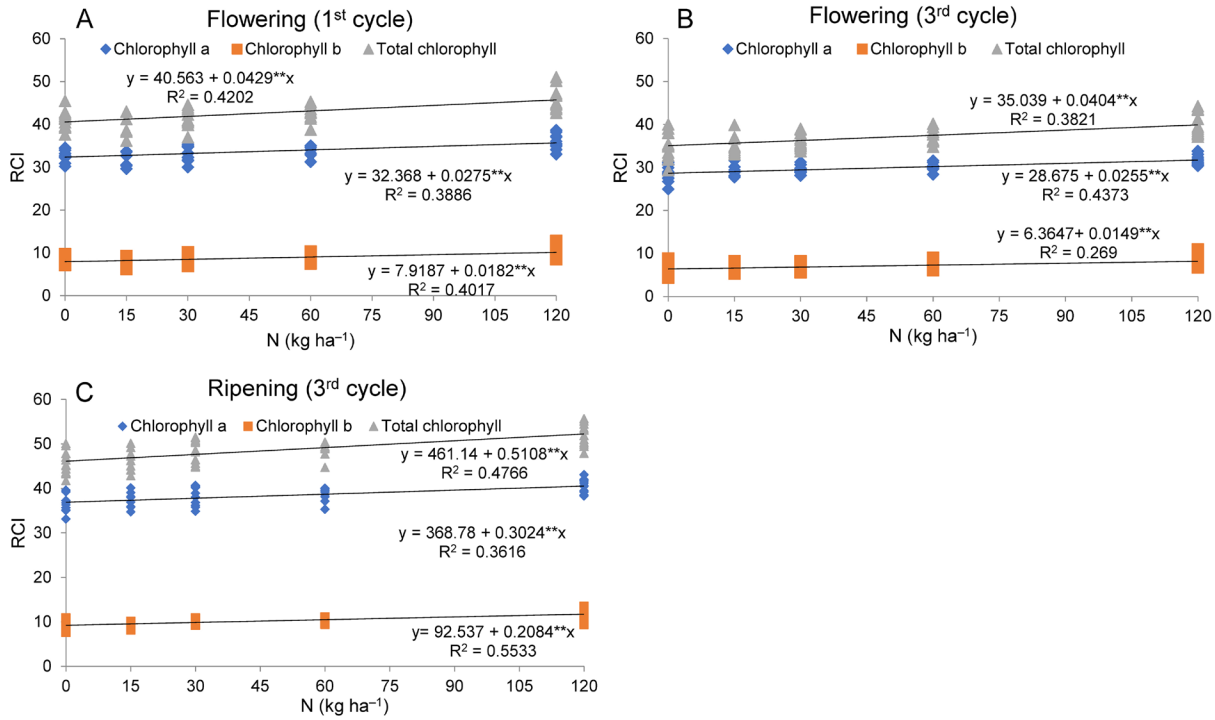

Figure 5 – Adjustment equations for the relative chlorophyll content index (RCI) in leaves of the 'Syrah' grapevine for levels of N applied at the following stages: flowering in the 1st growing cycle (A), and flowering (B) and ripening (C) in the 3rd growing cycle. ** and * significant at 1 and 5 % probability by the F-test, respectively.

showed a relative chlorophyll content index of 40.1 (Cabernet Sauvignon) and 38.1 (Xinomavro) and also found a steady rise in the relative index for increases in the applied rate of N. Despite the response observed in the present experiment, it is necessary to consider the low increment obtained with the rates of N applied.

Production parameters and relationship with sap ion concentration

The yield (Mg ha^{-1}) of wine grape was correlated with nitrate (NO_3^-) and potassium (K^+) concentrations in the petiole during both phenological stages ($p < 0.01$) with adjustment of the response surface. In the flowering stage (Figure 6A), high NO_3^- concentration increased linearly grape yield to a maximum value (6.4 Mg ha^{-1}) while yield reduced to a minimum value (0.2 Mg ha^{-1}) due to the increase of K^+ concentration. For the ripening stage (Figure 6B), NO_3^- and K^+ in the sap concentrations presented similar behavior to the flowering stage with yield increase at high NO_3^- concentration (3,400 mg L^{-1}) and a reduction of K^+ concentration (12,100 mg L^{-1}).

According to the regression analysis, the mean grapevine cluster mass (MCM) correlated with nitrate concentration in the petiole during both phenological stages ($p < 0.01$). For the flowering stage, a linear adjustment occurred with an increase of 0.0012 g for each unit increase in NO_3^- in the petiole sap (Figure 7A). For the ripening stage, this increase was 0.0028 g (Figure 7B). For the K^+ concentration in the petiole, a low correlation was found between the adjusted models. Nevertheless, there was a reduction in grapevine cluster mass for each unit increase in K^+ in the petiole sap of 0.0078 g (Figure 7C) and 0.0064 g (Figure 7D).

The number of clusters (NC) in the plants showed a relationship ($p < 0.01$) with the NO_3^- concentration of the petiole at both the flowering (Figure 8A) and fruit ripening stages (Figure 8B), with an increase of 0.0011 and 0.0018 per plant for each unit increase in NO_3^- concentration in the sap. For the K^+ concentration, the number of clusters was also influenced ($p < 0.01$) and the regression adjustment at the flowering stage (Figure 8C) showed a reduction of 0.0026 in NC for each unit increase of K^+ in the petiole. However, at the ripening stage (Figure 8D), the correlation for the adjusted model was low, with a reduction of 0.01 per plant for each unit increase in K^+ concentration in the petiole sap.

The amount of available N is an important production indicator (Keller et al., 2001), where the NO_3^- concentration in the petiole can provide information on the plant nutritional status and yield prospects (Hidayatullah et al., 2018), unlike the K^+ concentration, since this nutrient may have a poor relationship with plant yield (Ciotta et al., 2016).

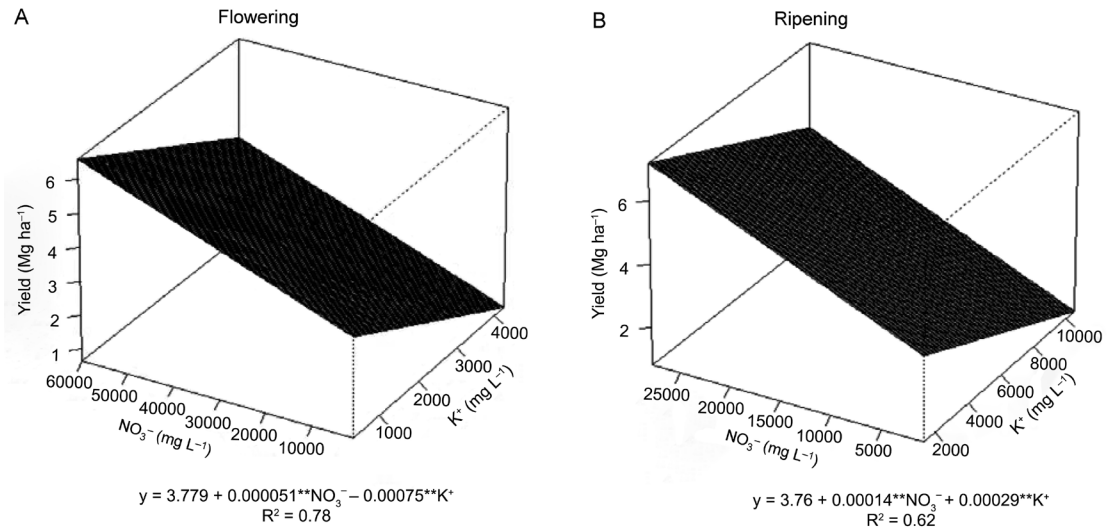

Figure 6 – Yield of 'Syrah' grapevine in relation to NO_3^- and K^+ concentrations in petiole sap at flowering (A) and ripening (B) stages. **Significant at 1 % probability by the F-test, respectively.

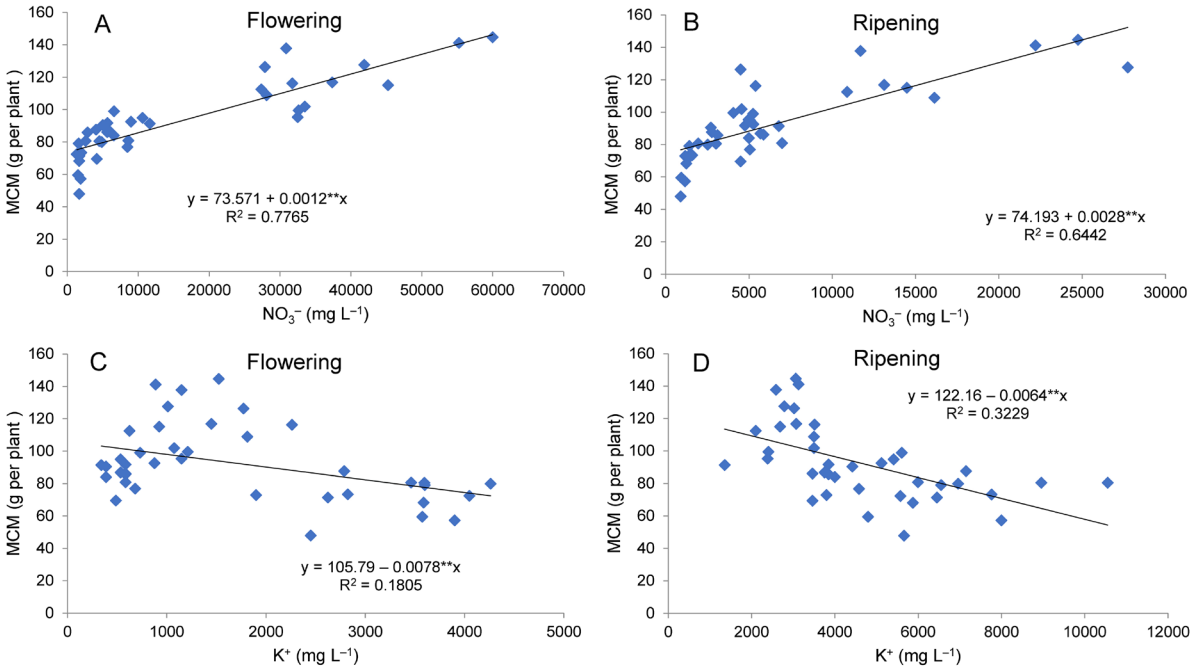

Figure 7 – Mean grapevine cluster mass (MCM) in the 'Syrah' grapevine in relation to NO_3^- and K^+ concentrations in petiole sap at the flowering (A and C) and ripening (B and D) stages. **Significant at 1 % probability by the F-test, respectively.

Studies have demonstrated that an increase in N concentration in the leaf petiole can increase yield in orange and grapevine (Souza et al., 2012; Hidayatullah et al., 2018, respectively). However, these authors did not report any negative effects of the increase in K concentration in the petiole, as the levels of K^+ at flowering was not determined in either study. Increases in K fertilization at the flowering stage may have undesirable effects on production. The K shows no relationship with yield, but with aspects related to grape quality, such as titratable acidity, pH, soluble solids, among others (Ciotta et al., 2016).

Phenolic composition and relationship with sap

Total polyphenols were correlated with nitrate (NO_3^-)

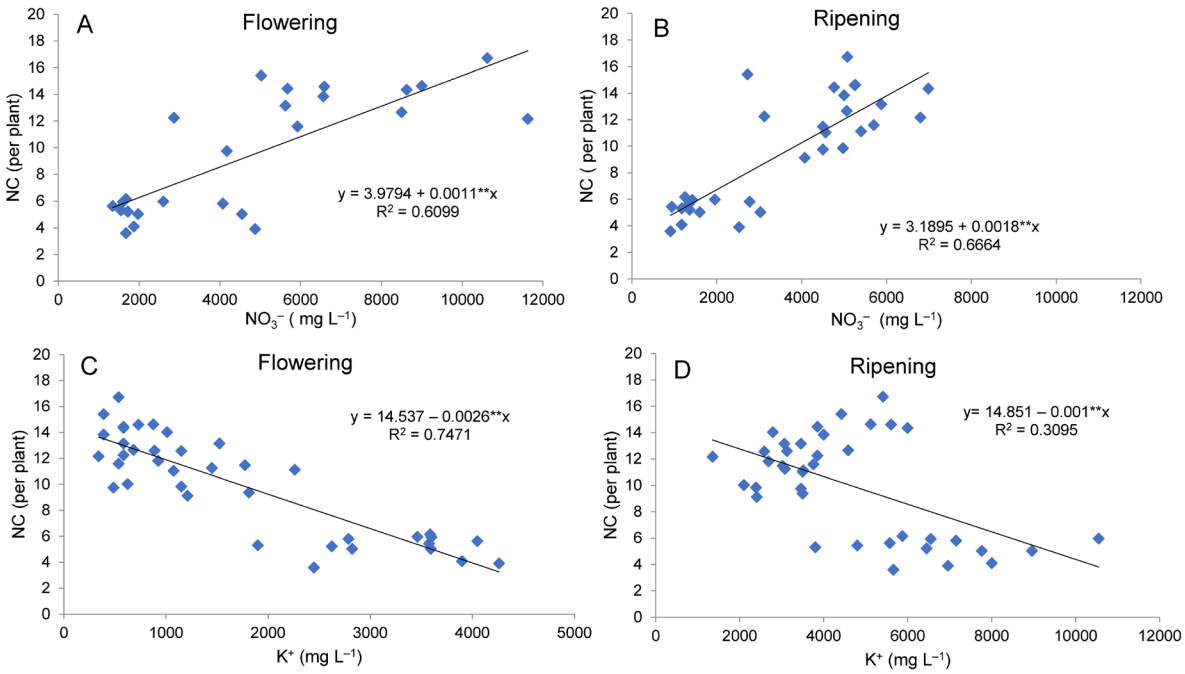

Figure 8 – Number of grapevine cluster (NC) in the three growing cycles of the 'Syrah' grapevine in relation to NO_3^- and K^+ concentrations in petiole sap at flowering (A and C) and ripening (B and D) stages. **Significant at 1 % probability by the F-test, respectively.

and potassium (K^+) concentrations in the petiole during both phenological stages ($p < 0.01$) with the adjustment of the response surface. The flowering stage (Figure 9A) presented a higher value in total polyphenols (411.2 mg 100 g^{-1}) with a linear increase of NO_3^- and K^+ concentrations. The ripening stage (Figure 9B) presented similar behavior to the flowering stage, with a higher value of total polyphenols (485.1 mg 100 g^{-1}) for each unitary increment of NO_3^- and K^+ concentrations.

Anthocyanins presented a linear adjustment in the flowering and ripening stages ($p < 0.01$) with an increase of 0.0021 (Figure 9C) and 0.0036 mg 100g^{-1} (Figure 9D) for each unitary increment of NO_3^-. A linear adjustment ($p < 0.01$) was obtained to flavonoids in the flowering stage (Figure 9E) with an increase of 0.0284 mg 100 g^{-1} for each unitary increment of NO_3^- in the sap concentration. In the ripening stage (Figure 9F), the response surface presented better adjustment ($p < 0.01$) with an increase of 0.010 and 0.021 mg 100g^{-1} for each unitary increment of NO_3^- and K^+ sap concentrations, respectively.

Several authors have investigated changes in the grape chemical composition with the use of fertigation (Ciotta et al., 2016; Canoura et al., 2018; Vilanova et al., 2019). Higher values of total polyphenols and anthocyanins were related to exchangeable soil K^+ (Ciotta et al., 2016). However, no relation between the N supply and the anthocyanin content was observed in 'Syrah' (Canoura et al., 2018), opposite to the results reported here.

Conclusions

Extracting sap from grapevine petioles using ether allows monitoring NO_3^- and K^+ concentrations measured by card meters.

The increase of N and K_2O influenced the NO_3^- and K^+ concentrations in grapevine sap, with higher NO_3^- values at the flowering stage and higher K^+ values at the ripening stage. High concentrations of NO_3^- contributed positively to increasing grape yield, but the response of sap K+ increasing was opposite. Then, K^+ increments in the sap are not recommended.

For the flowering stage, 120 kg N ha^{-1} rate positively influenced NO_3^- and K^+ values in the sap, the chlorophyll index, yield and phenolic composition. For the K_2O dose, only K+ in the sap (ripening stage) and the phenolic composition showed a positive response with 120 kg K_2O ha^{-1} rate.

The high NO_3^- concentration in the flowering stage was due to the fertigation applied, which increased the response of the leaf chlorophyll index. Therefore, the flowering stage is the recommended period of the grapevine growing cycle to perform measurements to monitor N using a handheld meter.

Further research on the variation of NO_3^- and K^+ concentration on petiole sap throughout the grapevine phenological stages should give a better understanding to the growers, facilitating their decision-making and consequently improving the fertigation practice.

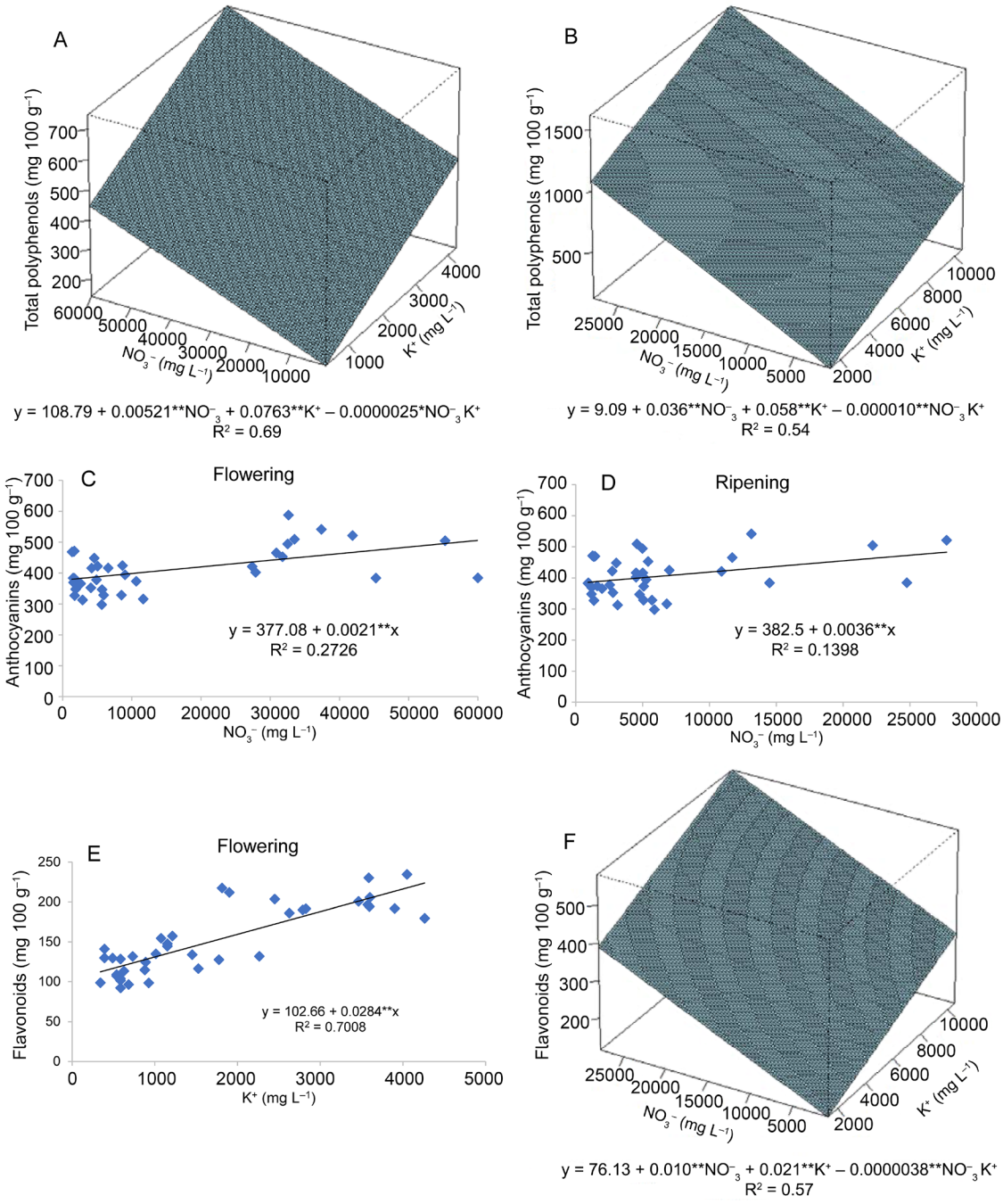

Figure 9 – Regression analysis for total polyphenols (A, B), anthocyanins (C and D) and flavonoids (E and F) in relation to NO_3^- and K^+ concentrations in petiole sap at flowering and ripening stages of 'Syrah' grapevine. * * Significant at 1 % probability by the F-test, respectively.

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D.J.; Bassoi, L.H. **Project administration:** Silva, D.J. **Supervision:** Bassoi, L.H. **Writing – original draft:** Silva, A.O. **Writing – review & editing:** Silva, A.O.; Bassoi, L.H.; Silva, D.J.; Chaves, A.R.M.

References

Allen, R.G.; Pereira, L.S.; Raes, D. 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO, Rome, Italy. (Irrigation and Drainage Paper, 56).

Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.M.; Sparovek, G. 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22: 711-728. https://doi.org/10.1127/0941-2948/2013/0507

Bassoi, L.H.; Dantas, B.F.; Lima Filho, J.M.P.; Lima, M.A.C.; Leão, P.C.S.; Silva, D.J.; Maia, J.L.T.; Souza, C.R.; Silva, J.A.M.; Ramos, M.M. 2007. Preliminary results of a long-term experiment about RDI and PRD irrigation strategies in winegrapes in São Francisco Valley, Brazil. Acta Horticulturae 754: 275-282. https://doi.org/10.17660/ActaHortic.2007.754.35

Bell, S.J.; Henschke, P.A. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research 11: 242-295. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x

Canoura, C.; Kelly, M.T.; Ojeda, H. 2018. Effect of irrigation and timing and type of nitrogen application on the biochemical composition of Vitis vinifera L. cv. Chardonnay and Syrah grapeberries. Food Chemistry 241: 171-181. https://doi.org/10.1016/j.foodchem.2017.07.114

Chavarria, G.; Santos, H.P.; Castro, L.A.S.; Marodin, G.A.B.; Bergamaschi, H. 2012. Anatomy, chlorophyll content and photosynthetic potential in grapevine leaves under plastic cover. Revista Brasileira de Fruticultura 34: 661-668. https://doi.org/10.1590/S0100-29452012000300003

Ciotta, M.N.; Ceretta, C.C.; Silva, L.O.S.; Ferreira, P.A.A.; Sautter, C.K.; Couto, R.R.; Brunetto, G. 2016. Grape, yield and must compounds of 'Cabernet Sauvignon' grapevine in sandy soil with potassium contents increasing. Ciência Rural 46: 1376-1383. https://doi.org/10.1590/0103-8478cr20150472

Clarke, S.J.; Lamont, K.J.; Pan, H.Y.; Barry, L.A.; Hall, A.; Rogiers, S.Y. 2015. Spring root-zone temperature regulates root growth, nutrient uptake and shoot growth dynamics in grapevines. Australian Journal of Grape and Wine Research 21: 479-489. https://doi.org/10.1111/ajgw.12160

Dias, M.; Santos, H.; Pezzella, M.; Fernando, M. 2016. São Francisco Valley: Vitivini culture activities in the Brazilian unthinkable semiarid climate and its challenges. International Journal of Business and Management Review 4: 1-13.

Gutiérrez-Gamboa, G.; Garde-Cerdán, T.; Portu, J.; Moreno-Simunovic, Y.; Martínez-Gil, A.M. 2017. Foliar nitrogen application in Cabernet Sauvignon vines: effects on wine flavonoid and amino acid content. Food Research International 96: 46-53. https://doi.org/10.1016/j.foodres.2017.03.025

Hidayatullah; Khan, A.; Mouladad; Mirwise; Shah, S.A.; Zainullah. 2018. Nitrogen management in grapes as a function of rate and time of application. Indian Journal of Science and Technology 11: 1-10. https://doi.org/10.17485/ijst/2018/v11i15/119930

Keller, M.; Kummer, M.; Vasconcelos, M.C. 2001. Reproductive growth of grapevines in response to nitrogen supply and rootstock. Australian Journal of Grape and Wine Research 7: 12-18. https://doi.org/10.1111/j.1755-0238.2001.tb00188.x

Kondi, A.E.; Meti, S.; Champa, B.V.; Nagaraja, M.S. 2018. Comparison of petiole nutrient contents of different table and wine grape varieties. International Journal of Current Microbiology and Applied Sciences 7: 447-453. https://doi.org/10.20546/ijcmas.2018.701.052

Lang, C.P.; Merkt, N.; Zörb, C. 2018. Different nitrogen (N) forms affect responses to N form and N supply of rootstocks and grafted grapevines. Plant Science 277: 311-321. https://doi.org/10.1016/j.plantsci.2018.10.004

Lee, J.; Durst, R.W.; Wrolstad, R.E.; Barnes, K.W.; Eisele, T.; Giusti, M.M.; Haché, J.; Hofsommer, H.; Koswig, S.; Krueger, D.A. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. Journal of AOAC International. 88: 1269-1278. https://doi.org/10.1093/jaoac/88.5.1269

Leibar, U.; Pascual, I.; Aizpurua, A.; Morales, F.; Unamunzaga, O. 2017. Grapevine nutritional status and K concentration of must future expected climatic conditions texturally different soils. Journal of Soil Science and Plant Nutrition 17: 385-397. http://dx.doi.org/10.4067/S0718-95162017005000028

Littell, R.C.; Mott, G.O. 1975. Computer assisted design and analysis of response surface experiments in agronomy. Soil and Crop Society of Florida Proceedings 34: 94-97.

Loulakakis, K.A.; Roubelakis-Angelakis, K.A. 2001. Nitrogen assimilation in grapevine. In: Roubelakis-Angelakis, K.A., eds. Molecular biology & biotechnology of the grapevine. Springer, Dordrecht, the Netherlands. https://doi.org/10.1007/978-94-017-2308-4_3

Martinez, E.A.; Ribeiro, V.G.; Vilar, P.F.I.; von Hausen, L.J.O.; Bezerra, E.D. 2017. Evaluation of nitrogen monitoring, bud fertility and 'Thompson seedless' grapevine production on different rootstocks. Revista Brasileira de Fruticultura 39: 1-10. https://doi.org/10.1590/0100-29452017950

Müller, M.C.; Smith, J.P.; Clark, A.C.; Holzapfel, B.P. 2016. Effect of N and water supply on xylem composition in "Semillon" and "Riesling". Acta Horticulturae 1136: 185-192. https://doi.org/10.17660/ActaHortic.2016.1136.25

Nagarajah, S. 1999. A petiole sap test for nitrate and potassium in Sultana grapevines. Australian Journal of Grape and Wine Research 5: 56-60. https://doi.org/10.1111/j.1755-0238.1999.tb00152.x

Peuke, A.D. 2000. The chemical composition of xylem sap in *Vitis vinifera* L. cv. Riesling during vegetative growth on three different Franconian vineyard soils and as influenced by nitrogen fertilizer. American Journal of Enology and Viticulture 51: 329-339.

Pradubsuk, S.; Davenport, J.R.; 2010. Seasonal uptake and partitioning of macronutrients in mature 'Concord' grape. Journal of the American Society for Horticultural Science 135: 474-483. https://doi.org/10.21273/JASHS.135.5.474

Rogiers, S.Y.; Coetzee, Z.A.; Walker, R.R.; Deloire, A.; Tyerman, S.D. 2017. Potassium in the grape (*Vitis vinifera* L.) berry: transport and function. Frontiers in Plant Science 8: 1-19. https://doi.org/10.3389/fpls.2017.01629

Romero, I.; García-Escudero, E.; Martín, I. 2010. Effects of leaf position on blade and petiole mineral nutrient concentration of Tempranillo grapevine (*Vitis vinifera* L.). American Journal of Enology and Viticulture 61: 544-550. http://dx.doi.org/10.5344/ajev.2010.09091

Silva, A.O.; Silva, D.J.; Bassoi, L.H. 2016. Nitrate and potassium concentration in fertigated soil cultivated with wine vines. Ciência e Agrotecnologia 40: 305-316. https://doi.org/10.1590/1413-70542016403042215

Singleton, V.L.; Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

Soil Survey Staff. 2014. Keys to Soil Taxonomy. 12ed. USDA-Natural Resources Conservation Service, Washington, DC, USA.

Souza, T.R.; Bôas, R.L.V.; Quaggio, J.A.; Salomão, L.C. 2012. Nutrients in the sap of fertigated citrus plants. Revista Brasileira de Fruticultura 34: 482-492 (in Portuguese, with abstract in English). https://doi.org/10.1590/S0100-29452012000200022

Steele, M.R.; Gitelson, A.A.; Rundquist, D.C. 2008. A comparison of two techniques for nondestructive measurement of chlorophyll content in grapevine leaves. Agronomy Journal 100: 779-782. https://doi.org/10.2134/agronj2007.0254N

Taskos, D.G.; Koundouras, S.; Stamatiadis, S.; Zioziou, E.; Nikolaou, N.; Karakioulakis, K.; Theodorou, N. 2015. Using active canopy sensors and chlorophyll meters to estimate grapevine nitrogen status and productivity. Precision Agriculture 16: 77-98. https://doi.org/10.1007/s11119-014-9363-8

Tecchio, M.A.; Moura, M.F.; Paioli-Pires, E.J.; Terra, M.M.; Teixeira L.A.J.; Smarsi, R.C. 2011. Nutrients levels, relative chlorophyll index and concentration of nitrate and potassium in the petiole of grapevine 'Niagara Rosada'. Revista Brasileira de Fruticultura 33: 649-659 (in Portuguese, with abstract in English). https://doi.org/10.1590/S0100-29452011005000058

Teixeira, P.C.; Donagemma, G.K.; Fontana A.; Teixeira, W.G. 2017. Soil Analysys Methods = Manual de Métodos de Análise de Solo. 3ed. Embrapa, Brasília, Brazil (in Portuguese).

Vilanova, M.; Fandiño, M.; Frutos-Puerto, S.; Cancela, J.J. 2019. Assessment fertigation effects on chemical composition of *Vitis vinifera* L. cv. Albariño. Food Chemistry 278: 636-643. https://doi.org/10.1016/j.foodchem.2018.11.105

Yang, Z.; Tian, J.; Feng, K.; Gong, X.; Liu, J. 2021. Application of a hyperspectral imaging system to quantify leaf-scale chlorophyll, nitrogen and chlorophyll fluorescence parameters in grapevine. Plant Physiology and Biochemistry 166: 723-737. https://doi.org/10.1016/j.plaphy.2021.06.015

Zapata, C.; Deléens, E.; Chaillou, S.; Magné, C. 2004. Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). Journal of Plant Physiology 161: 1031-1040. https://doi.org/10.1016/j.jplph.2003.11.009