

# Foliar application of molybdenum: effects on yield quality of the grapevine Sangiovese (*Vitis vinifera* L.)

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**Abstract:** Field experiments with Sangiovese vines were carried out in the Chianti Classico region over a period of two years to examine the effect of molybdenum (Mo) foliar sprays on nutrient composition of leaves, petioles and berries, leaf gas exchanges, must composition, total yield, bunch size and pruning weight. Two Mo foliar doses and time sprays (Mox1: one application in early flowering; Mox3: three applications in early flowering, early fruit set and veraison) were applied. Basal sample of petioles, leaves and berries collected at fruit set (except berries), veraison and harvest for mineral analyses showed not relevant interactions between Mo and the main macro and micronutrients. Leaf gas exchanges monitored after the applications, as well as SPAD units, showed a higher activity in the Mox3-treated vines. Increased vigour was also confirmed by the slightly higher total yield, bunch size and pruning weight, as well as the delay in fruit maturation (lower sugar and polyphenol contents at harvest). No relevant discrepancy between Mox1 and the control was found, except for higher soluble solid and yeast-assimilable nitrogen contents (YANC) in the treated vines. YANC was positively influenced also in the Mox3 vines, however with no significant differences towards the Mox1 treatment. The application of Mo as a useful tool to stimulate nitrogen metabolism, as well as indications about dose and time of Mo application, are discussed.

## 1. Introduction

The transition element molybdenum (Mo) is a very rare but essential micronutrient for all organisms (Bortels, 1930; Fortescue, 1992), and understanding of its role and function in plants is progressing rapidly. Its importance for plants has been known for a long time (Coughlan, 1980), even though Mo itself seems to be catalytically inactive in biological systems until it is complexed by a special cofactor, the pterin (Mendel and Hänsch, 2002) which binds to diverse apoproteins. This latter compound is a unique pterin named molybdopterin or metal-containing pterin. In this form, it occurs in more than 40 enzymes catalysing many redox reactions, four of which have been found in plants (Hille, 1996; Kappl *et al.*, 2002). One of these is nitrate reductase (NR) that catalyses the first step in nitrate assimilation, a pathway of key importance for plant nutrition. Nitrate reductase is the key-enzyme for nitrate assimilation while nitrogenase is found in nitro-

gen-fixing bacteria inside nodules of symbiotically growing species. The last step of abscisic acid biosynthesis is catalyzed by the molybdenum-enzyme aldehyde oxidase, and sulfite oxidase protects the plant against toxic levels of sulfite (Hänsch and Mendel, 2009).

Nitrogen (N) is assimilated into the cell as a fully reduced form, ammonia (NH<sub>3</sub>). NH<sub>3</sub> may be obtained in free form (e.g. by fertilizers) and from the degradation of amino acids. If these sources are not available, then ammonia must be produced *via* nitrate (NO<sub>3</sub>) reduction. When NR is a limiting factor, plant growth, development and protein synthesis by plants are reduced (Solomonson and Barber, 1990). Hence a shortage of Mo in the soil, even though plants' requirement for it is very low, or a mutational block of the cellular ability to use Mo leads to the loss of essential metabolic functions and can cause the death of the plant. Mo deficiency has been reported for many plant species including herbs, crops and trees (Gupta, 1997) and could determine poor NR activity (Hewitt, 1983), causing an inability to utilize N, with visible symptoms of chlorosis or yellowing of the leaves.

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In grapevine, N is essential in overall vine establishment and maintenance, fruit quality, and the conversion of grape juice to wine. In grape berries, N is found primarily as ammonium cations and organic compounds such as amino acids (proline, arginine, glutamine, glutamate, etc.), hexose amines, peptides, nucleic acids and proteins. There are two phases of intense N incorporation in the fruit: the first takes place during the two weeks before the “pea-size” stage of the berries; the second starts one month later at veraison and lasts an additional two weeks (Löhnertz, 1991). The amount of N in the clusters at harvest is approximately 40-44% of total available N of the entire plant (Alexander, 1957; Conradie, 1991; Weinbaum *et al.*, 1984).

Furthermore, N compounds are required by yeast for the production of cell biomass and the synthesis of proteins and enzymes necessary for the biochemical process of fermentation. The readily (easily assimilable) fermentable N compounds in juice and must consist primarily of NH<sub>3</sub> and N available from the alpha amino acids present at harvest, particularly arginine (Bisson, 1991). Low levels of yeast-assimilable N content (YANC) in grape juice and must at harvest have been associated with sluggish and stuck fermentation and consequent undesirable levels of residual sugar in wines (Kunkee, 1991; Jiranek *et al.*, 1995).

Slow and stuck fermentations are sometimes associated with grapes from vineyards composed of not vigorous plants or with high planting density, clearly characterized by scarce availability of N (Masi and Boselli, 2007). In some cases, despite plants having a sufficient level of N in their tissues, plant growth and crop yield show a clear N deficiency making it possible to hypothesize that most of the N in the plant is stored in non available forms. However, fertilizations with N are considered to be difficult, especially concerning choice of the right dose for optimum plant and yeast growth: even a low dose of N frequently causes excessive canopy growth and an unacceptable delay in fruit maturation being, at the same time, not sufficient in order to assure an optimal assimilable N concentration of the juice (Spayd *et al.*, 1995). Assuming the inadequacy to reach optimal levels of available forms of N by chemical fertilization, Mo supply could be a possible solution since its key role in the activation of N metabolism could be compared to a N fertilization itself. Moreover, Mo application has been shown to increase fruit quality, sugar content (Rus’-Ko, 1979; Strakhov, 1988) and qualitative and quantitative composition of free amino acids (Veliksar, 1977) in grapevine. Mo is important in nitrogen nutrition of vines and has been suggested as a primary cause of millerandage in Merlot vines (Longbottom *et al.*, 2004; Williams *et al.*, 2004). It is thought that Mo directly affects the development of reproductive structures. Molybdenum is necessary for successful pollen tube growth, ovule penetration, and fertilization (Longbottom *et al.*, 2004). Williams *et al.* (2004)

showed that Mo increased the percent of coloured berries with one or more functional seeds and decreased the proportion of green berries, thus suggesting that Mo application affected pollination and/or fertilization, and thereafter berry development.

The present study, comprising two different levels and time of Mo supply, was undertaken to determine the effect of Mo on N metabolism by studying fruit and vegetative development in the grapevine Sangiovese (*Vitis vinifera* L.).

## 2. Materials and Methods

### Plant material

The study was carried out in a six-year-old vineyard of Sangiovese *Vitis vinifera* (L.) cultivar, grafted onto *Berlandieri* x *Riparia* 420A, located in the Chianti Classico DOCG region (elevation about 380 m asl; weather was characterized by mild winter, hot and dry season during the summer and average yearly rainfall of 700 mm, with the highest levels in November and March). The vines were spaced 0.8 m within the row and 2.4 m between rows and pruned to a single cordon system with four spurs, with an average of eight buds growing off the cordon. Soil was rich in clay and “skeletal” material. Pre-planting studies showed that the soil chemical fertility was good for most of the elements except for N (Table 1). For that reason, every year, soil fertilizations rich in N (400 kg of NPK-fertilizer 12+10+20) had been executed since the vineyard was established; although tissue analysis did not show any mineral deficiency, neither for microelements nor macroelements (Fig. 1), light symptoms of N-deficiency were present on the vines at the moment the experiment was performed, consisting in low vigour and poor YANC must content in the previous vintages.

### Treatments

During two consecutive years (2002-2003), the vineyard was divided into three homogeneous areas, of six rows each, for two Mo treatments and a control

Table 1 - Soil chemical analysis

Soil parameter	
pH (H <sub>2</sub> O)	8.2
Electrical Conductivity	126.5 μS · cm <sup>-1</sup>
Total Lime (CaCO <sub>3</sub> )	7.6%
Active Lime (CaCO <sub>3</sub> )	0.7%
Cation Exchange Capacity	18.5 mE·100g <sup>-1</sup>
Organic Matter	0.9%
N	0.4%
Ca	1157 mg·kg <sup>-1</sup>
Mg	134 mg·kg <sup>-1</sup>
K (K <sub>2</sub> O)	146 mg·kg <sup>-1</sup>
P (P <sub>2</sub> O <sub>5</sub> )	12.5 mg·kg <sup>-1</sup>
Fe	78.3 mg·kg <sup>-1</sup>
Mn	85.7 mg·kg <sup>-1</sup>
B	2.8 mg·kg <sup>-1</sup>

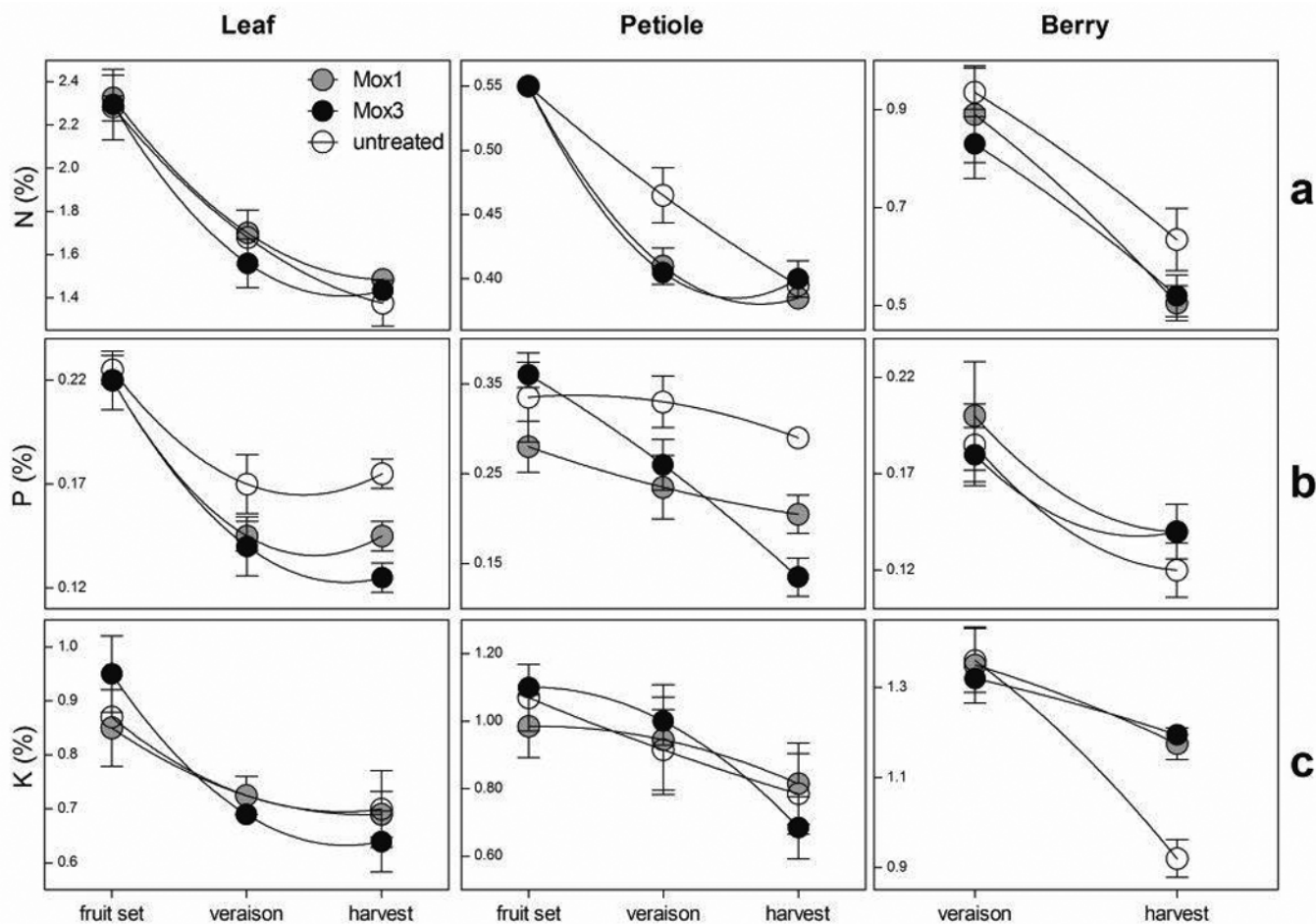


Fig. 1 - Primary macronutrient (N, P, K) concentrations in basal leaf, petiole and berry at fruit set, veraison and harvest in Mo-treated and untreated grapevine plants. Error bars reflect the LSD at  $p \leq 0.05$ . Mox1= Mo treatment in early flowering; Mox3= Mo treatments in early flowering, early fruit set and early veraison.

(untreated vines). Foliar applications were sprayed using  $350 \text{ g}\cdot\text{ha}^{-1}$  of a custom-made Mo fertilizer (12% w/w) dissolved in 200 l of water. The first treatments provided a single application in early flowering (Mox1); the second provided three applications, respectively in early flowering, early fruit set and early veraison (Mox3). Recommended cultural practices of the vineyard were applied.

#### Data collection

Three basal leaf and petiole samples were collected (at fruit set, veraison and harvest); two berry samples were also collected from veraison to harvest. Each sample was the result of 50 to 60 organs harvested from random positions among the six rows of each treatment area. The material was rinsed with distilled water in order to take away any pesticide or fertilizer traces, then oven dried at about  $80^\circ\text{C}$  until thoroughly dry. Each dried sample was then ground and sent to the laboratory for chemical analysis of macro and microelements; the analyses were performed three times and results averaged.

Gas exchange measurements were taken after veraison on 25 replications obtained by selecting five

leaves on five plants per treatment: the leaves were of the same age, spatial orientation and light exposure. Net photosynthetic rate ( $P_n$ ) and transpiration rate ( $E$ ) were recorded with a portable photosynthesis system (CIRAS-I, PPSsystems, Hertfordshire, UK). Leaves were enclosed in a ventilated leaf cuvette and exposed to saturating irradiance ( $\text{PAR} \geq 1200 \mu\text{mol}\cdot\text{m}^{-2}$ ); concentration of  $\text{CO}_2$  used for the measurements was  $350 \text{ mg}\cdot\text{l}^{-1}$ . In addition, water user efficiency (WUE) was calculated as the rate of net photosynthesis per unit of transpired water.

According to the same statistical design, after veraison (and 30 days later) on the same plants and leaves leaf chlorophyll and nitrate content (Westerveld *et al.*, 2003) was estimated non-destructively using a portable chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, N.J., U.S.).

At fruit set, veraison and harvest, berry samples were picked randomly from 10 plants per treatment, crushed, pressed and the juice analyzed for the yeast fermentable nitrogen content. The YANC is expressed as  $\text{mg}\cdot\text{l}^{-1}$  of N as the sum of the assimilable nitrogen from ammonia plus the assimilable nitrogen from alpha amino acids.



At harvest, 300 berries were collected, weighed and then squeezed by hand and filtered through a strainer. Samples were harvested randomly from 10 plants (30 berries per plant) per treatment. The expressed juice was measured to determine total soluble solids (Brix) with a hand refractometer and titratable acidity by titration with 0.1 N NaOH to pH 7. Titratable acidity was expressed by  $\text{g}\cdot\text{l}^{-1}$  of tartaric acid. Anthocyanin content of the must was performed *via* Glories indices (Glories, 1984). Indices evaluated were: total anthocyanins  $A_1$  ( $\text{mg}\cdot\text{l}^{-1}$ ); extractable anthocyanins  $A_{3,2}$  ( $\text{mg}\cdot\text{l}^{-1}$ ); extractability assay  $E_A$  (based on the ratio of the above two anthocyanin values); and extractability of the tannins contained in the seed  $M_p$ .

During the season, five plants per treatment were labelled; they were harvested separately in order to calculate average yield per vine and their pruning materials were weighed as an indicator of the vegetative vigour. The ratio of total yield to total pruning weight per vine, referred to as the Ravaz Index (Champagnol, 1984) was used as an indicator of balance between fruit and vegetative growth.

Statistical analysis of variance on the obtained data was performed with separation of the means by LSD test at 5%. No year effect was observed, thus data shown are the average of the measurements of two years.

### 3. Results

Tissue analysis showed in general no deficiency or excess in the levels. The effect of applied Mo on the concentration of the nutrients in basal petioles, leaves and berries was small and of little practical importance. Nitrogen (N) content of leaf, petiole and berry compared with control treatment was not influenced by Mo (Fig. 1 a). Leaf and petiole phosphorous (P) content tended to decrease in leaf and petiole after Mo supply, especially in the Mox3 treatment (Fig. 1 b), while berry content was not influenced. On the contrary, potassium (K) level was significantly higher in berries of Mo-treated plants (Fig. 1 c) at harvest. Calcium (Ca) content was not affected by any kind of treatment in all analyzed organs (Fig. 2 a), while petiolar and leaf manganese (Mn) tended to increase (Fig. 2 b), especially at harvest time and for Mox3 treatment. Moreover, for the same treatment, a higher and significant level of Mn was found in berries during both measurements (at veraison and harvest time). No significant differences were detected for the level of Iron (Fe) (Fig. 2 c).

$\text{CO}_2$  assimilation rate of Mox3-treated vines was significantly higher than those of the Mox1-treated and untreated vines (Fig. 3 top). Mox3 showed also relevant difference towards the other treatments in transpi-

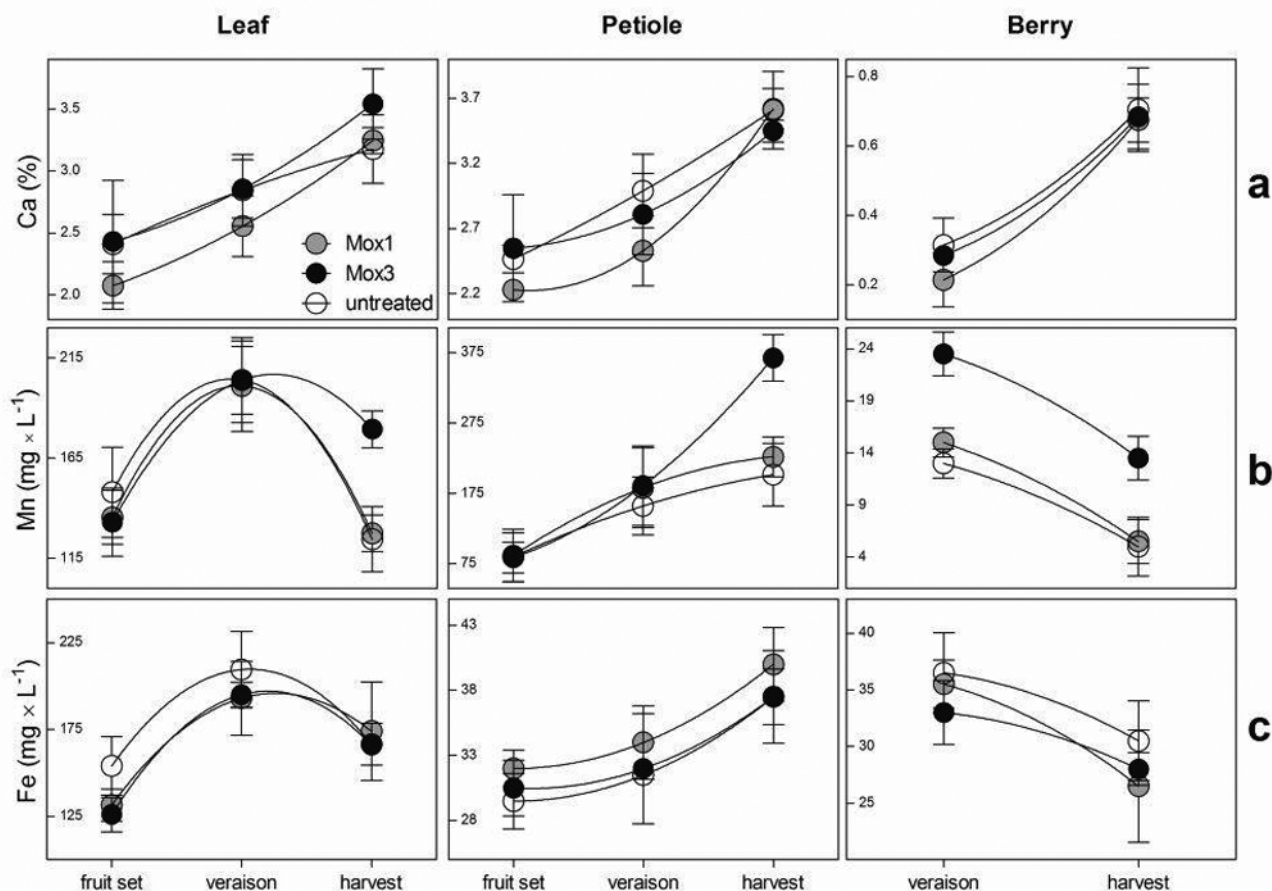


Fig. 2 - Secondary macro (Ca) and micronutrient (Mn, Fe) concentrations in basal leaf, petiole and berry at fruit set, veraison and harvest in Mo-treated and untreated grapevine plants. Error bars reflect the LSD at  $p \leq 0.05$ . Mox1= Mo treatment in early flowering; Mox3= Mo treatments in early flowering, early fruit set and early veraison.

ration rate (Fig. 3 middle); as a result of the higher transpiration rate, however, the Mox3 vines showed more critical water use efficiency values (Fig. 3 middle).

Accordingly, the SPAD unit measurements were significantly influenced by Mo treatments (Fig. 3 bottom). Mox3 showed the highest values in both sampling data; the values decreased for Mox1 treated vines after veraison, although remained higher than those of the control plants.

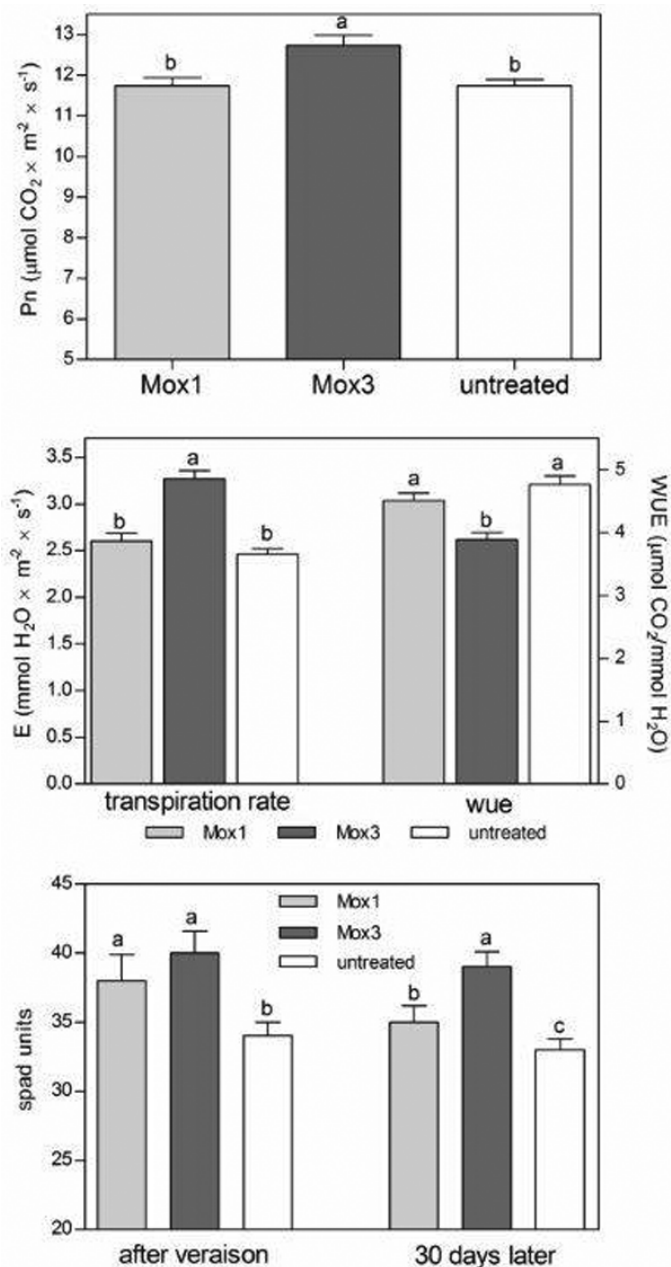


Fig. 3 - Top: assimilation rates measured after veraison in Mo-treated and untreated grapevine plants. Middle: transpiration rates and water use efficiency measured after veraison in Mo-treated and untreated grapevine plants. Bottom: SPAD units measured after veraison and a month later in Mo-treated and untreated grapevine plants. Error bars reflect the LSD at  $p \leq 0.05$ . Different letters correspond to significant differences ( $p \leq 0.05$ ). Mox1= Mo treatment in early flowering; Mox3= Mo treatments in early flowering, early fruit set and early veraison.

The YANC content increased significantly after the applications showing the positive role of Mo on NR activity to provide an available form of N for the plant (Table 2). Furthermore, at veraison, after more than a month from the first (and single, for Mox1) application, both Mox1 and Mox3 berries had higher levels of YANC compared to the control ones. Surprisingly, no significant difference was found between the two Mo treatments, not showing a linear correlation between Mo dose and NR activity. In any case at harvest time, no effect was found for either of the Mo treatments: the difference tended to decrease during berry ripening, disappearing at harvest when both the treated and untreated vines had quite the same amount of available N.

Significant differences in must soluble solids (SS) analysis at harvest were observed between Mox1 treatment (higher value) and both Mox3 and control treatments (lower values). Titratable acidity (TA) and pH levels were similar for treated and untreated vines (Table 3). Total anthocyanin content was significantly greater in the untreated vines compared to the treated ones while extractable anthocyanins in Mox1-treated and control plants were similar and higher than in Mox3-treated vines (Table 3). Mox1-treated vines must extractability assay and  $M_p$  value suggest a better polyphenolic evolution during ripening. Mox3 treatment negatively influenced anthocyanin extractability and polyphenol evolution as confirmed by the highest value of  $M_p$ .

Table 2 - Yeast assimilable nitrogen content (YANC)

YANC (mg·l <sup>-1</sup> N)	Fruit set	Veraison	Harvest
Mox1	96 a	66 a	68 NS
Mox3	92 a	62 a	64 NS
untreated	74 b	34 b	55 NS

Different letters within the same column correspond to significant differences ( $p \leq 0.05$ ). Non-significant results are marked NS.

Mox1= Mo treatment in early flowering;

Mox3= Mo treatments in early flowering, early fruit set and early veraison.

Table 3 - Berry soluble solids (SS), pH, titratable acidity (TA), berry Glories indices ( $A_1$ : total anthocyanins;  $A_{3,2}$ : extractable anthocyanins;  $E_A$ : extractability assay;  $M_p$ : extractability of the tannins contained in the seed analysis results)

Technol. analysis	SS (Brix)	pH	TA (g·l <sup>-1</sup> )
Mox1	23.9 a	3.1 NS	7.3 NS
Mox3	22.1 b	3.0 NS	7.5 NS
untreated	22.6 b	3.0 NS	7.3 NS

Polyphenol. analysis	$A_1$ (mg·l <sup>-1</sup> )	$A_{3,2}$ (mg·l <sup>-1</sup> )	$E_A$ (%)	$M_p$ (%)
Mox1	1535 b	1007 a	34.4 b	29.6 b
Mox3	1437 b	706 b	50.9 a	40.5 a
untreated	2038 a	1005 a	50.7 a	32.0 b

Different letters within the same column correspond to significant differences ( $p \leq 0.05$ ). Non-significant results are marked NS.

Mox1= Mo treatment in early flowering;

Mox3= Mo treatments in early flowering, early fruit set and early veraison.

The different ripening development between Mox3 and untreated vines probably has to be linked to the influence of Mo on the fruit and vegetative activity of the plant, suggested also by the average bunch weights (Fig. 4 right) and the total pruning weight, which were higher in the Mox3 treatment (Fig. 4 left): plants treated with higher dose of Mo had greater difficulty completing the ripening process within the same time as the other vines. No significant differences were however revealed with regard to total yield per vine (data not shown) and Ravaz Index (Fig. 4 left).

#### 4. Discussion and Conclusions

The present study supports earlier suggestions that Mo affects the N metabolism in grape, whose unquestionable evidence is in the YANC analysis of the berry. The fact that the reaction of the plants to Mo applications was measurable, in terms of YANC, as early as a few days after the first treatments performed before fruit set gives interesting indications about its rate of speed. The absence of differences in YANC content between the two Mo treatments suggests that when the general plant availability of N is good (like in this case), higher doses of Mo result to be useless.

This hypothesis, therefore, is not confirmed by the analysis of the bunch weight and total yield per vine nor in the development of the ripening process, when relevant differences were found between the two Mo treatments, as well as a clearly positive correlation between the quantity of Mo supplied and the appearance of such phenomena, well known to be connected with an increased N-availability for the plant. In fact, Mo, especially when supplied at high rate, is able to increase plant activity (see results about gas exchange analysis) as the result of rendering the N of the plant more available for the metabolisms and, consequently, plants tend to be generally more vigorous.

Finally, important information about fertilization management can be underlined: a high dose treatment

(Mox3) seems to be inadequate and sometimes contrary to the quality of production. Mox1-treated vines, on the other hand, did not show an influence of the treatment on vegetative and fruit balance nor on phenolic evolution and, interestingly, differentiated positively from the control regarding the sugar content of the berry and the YANC, even though its level at harvest was not optimal. Assuming the Mox1 dose and time optimal for quality, further analysis should be aimed at correlating the role of Mo on NR activity towards N availability in order to understand how to increase must quality.

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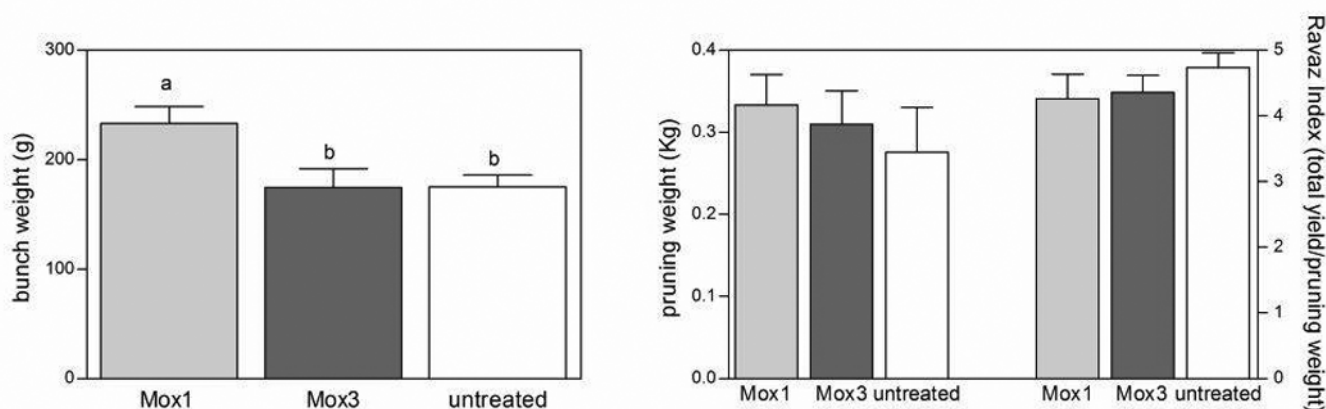


Fig. 4 - Bunch weight (left), total pruning weight and Ravaz index (right) measured in Mo-treated and untreated grapevine plants. Right: Error bars reflect the LSD at  $p \leq 0.05$ . Different letters correspond to significant differences ( $p \leq 0.05$ ). Non-significant results are marked NS.



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