# IMPACT OF FOLIAR MOLYBDENUM APPLICATION IN ACUTELY DEFICIENT VINEYARD ON WINE QUALITY

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By

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# DEDICATIONS

I dedicate this thesis to my parents, Jaime Gonzalez Navarro (Jimmy) and Maria Antonieta Andrade Vallejo (Tonchis). Both departed before I graduate, but I know my words will find you in the stars and heavens. I promise to never give up and live as you taught me, to always do my best and be humble.

You graduate together, grew up together, raised us together, traveled together, soared together and eventually, departed together.

**TOGETHER FOREVER**.

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IMPACT OF FOLIAR MOLYBDENUM APPLICATION IN ACUTELY DEFICIENT VINEYARD ON WINE QUALITY

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## ABSTRACT

Molybdenum is a trace element and micronutrient found in soils involved in plant growth crucial for nitrogen metabolism. From 2017 to 2020, a 5% sodium molybdate solution was foliarly applied on *Vitis* interspecific hybrid cv. Vignoles vines in commercial production after grapevine health and wine quality problems were reported and diagnosed as molybdenum deficiency (in the absence of other nutrient issues). Among grape juice parameters: pH, Brix and titratable acidity, in 2017 samples, Brix was higher in molybdenum-treated vines compared with the untreated control, but no difference was found in the rest of the juice parameters. In 2018 and 2020 only titratable acidity showed significant difference between treated and untreated vines, while pH and Brix differences were not significant. Molybdenum leaf concentration after treatment was significantly higher in treated vines across the four years of experiment. In 2017, treated vines molybdenum content was 6.56±3.52 mg/Kg and untreated vines was 0.938±2.02 mg/Kg on leaves sampled at harvest. In 2018, molybdenum deficient vines showed a concentration of 0.067±0.006 mg/Kg while vines treated for two consecutively years showed a molybdenum concentration of 5.04±1,67 mg/Kg in samples collected pre-veraison. In 2019, vines treated three years consecutively showed 4.12±2.47 mg/Kg, vines treated in 2017 & 2019 showed 3.41±1.57 mg/Kg, and untreated vines showed 0.224±0.05 mg/Kg

of molybdenum on leaves sampled pre-veraison. At the same sample time point in 2020 vines treated four years consecutively exhibit a molybdenum content of 4.71±1.41 mg/Kg while untreated vines exhibit 0.472±0.101 mg/Kg. Ionomic analysis showed difference in leaves elemental composition where boron, sodium, magnesium, phosphorus, sulfur, potassium, calcium, nickel, arsenic, zinc, strontium and cadmium showed significant difference between treated and untreated vines from 2018 to 2020 samples. Vignoles leaf Nitrogen percentage was determined in 2019 and 2020 treated and untreated vines. In 2019 no significant difference was found on N% between vines treated 3 years consecutively, vines treated in 2017 & 2019, and untreated vines. In 2020 N% samples, significant difference was found at veraison and harvest between the different treatments. Wines produced from grapes harvested from the treated and the untreated vines were analyzed via GC-MS to determine the effect of foliar molybdenum application on wine aroma profile. Across the four years of experiment, a diversity of aromas showed difference in concentration between treated and untreated vines. In 2017 Ethyl Hexanoate (fruity aroma), D-Limonene (citrus aroma), Terpinolene (floral aroma), and Linalool (floral - lavender aroma) were significantly different. In 2018 only Ethyl Hexanoate was found different between treatments. In 2019, Terpinolene, 1-Hexanol and Linalool showed significant difference, and in 2020, Terpinolene and Damascenone presented significant difference between samples.

# CHAPTER 1

# INTRODUCTION

Molybdenum is a micronutrient essential for plant growth and development, although macronutrients deficiencies are the first to impact growth and yield of crops, and their symptoms are well studied, the efficiency of macronutrient take up into the plant is directly affected by micronutrients like molybdenum (Tejada-Jimenez, Chamizo-Ampudia, Llamas, Galvan, & Fernandez, 2018). Molybdenum insufficiency is generally ignored and because its deficiency affects the nitrate reduction on plants and crops, it is normally confounded with nitrogen deficiency. Plants with very low amount of molybdenum become pale, their growth is restricted, flower formation and development is stunted, and they eventually wither (Kovacs, Puskas-Preszner, Huzsvai, Levai, & Bodi, 2015). In grapevines, the bunch disorder development called "Millerandage" or "hen and chicken", has been attributed to molybdenum deficiency in Australian *Vitis vinifera* cv. Merlot, this phenomenon is characterized by unevenly matured berries (Kaiser, Gridley, Ngaire Brady, Phillips, & Tyerman, 2005).

In 2017, in response to a commercial vineyard and winery that reported problems with grapevine health and wine quality problems, and after the vines were diagnosed as molybdenum deficient (in the absence of other nutrient issues), the present research was designed. The producer reported that in every variety, vines exhibited poor fruit set, growth, yield, and their response to macronutrient application was ineffective. At the same time, the producer observed an "off" character in the wine aromas made from grapes harvested from the deficient vines.

Wine quality is directly link to grape health, and berry quality is dependent of vine nutrition, therefore, nutrients deficiency negatively impacts grapes health. From 2017 to 2020 the same block of *Vitis* interspecific hybrid Vignoles was used to study the effects of foliar molybdenum application to deficient grapevines, on a range of parameters. In basic berry chemistry, the pH, sugar content (Brix) and titratable acidity (TA) were measured, and only Brix and TA showed difference between treated and untreated vines in at least one of the four years of experiment.

Through ICP-MS, twenty elements, including molybdenum were quantified in Vignoles leaves, and besides molybdenum, concentration of fourteen other elements were found significantly different between treatments. Leaf nitrogen content obtained by Total Kjeldahl Nitrogen analysis, showed statistical difference only in 2020 samples. By GC-MS, wines elaborated with the treated and untreated vines were analyzed, and results showed concentration differences in the aromatic groups: terpenes, esters, $C_{13}$ norisoprenoids and $C_6$ alcohols.

# CHAPTER 2

# LITERATURE REVIEW

## 1. Grapevine cultivars

Wine, one of the oldest alcoholic beverages known to man, has a recorded history of nearly 6,000 years, however, archeological evidence date wine back wine to 5500 b.c. It is thanks to a pottery jar recovered from a mudbrick house in Hajji Firutz, Tepe, in the northern of Zagros Mountains of Iran, that the chemical residues of wine or grape juice were found. The identification of calcium salts of tartaric acid, one of the two major acids found in grapes, and the identification of terebinth tree resin (*Pistacia terebinthus)*, that was usually used as a preservative, flavorant and bacteria growth inhibitor, suggested that the content of the ancient jar could be wine. Researchers place the development of wine making and the domestication of the wine grape *Vitis vinifera* in the southern Caucasus, because it is there where the natural distribution of *Vitis vinifera* closely approaches the distribution that occurs in Western viticulture, suspecting that wine grapes spread from the Caucasus (Jackson, 2014; Sandler & Pinder, 2003).

Around the seventeenth century, the use of sulfur in barrel treatment became more wide-spread in wine production and its acceptances increased. North Americans are relatively new to winemaking, regardless the large-scale plantation of vines by the missionaries, it was after the Prohibition that the United States started to produce wine. An AVA (American Viticulture Area) is a delimited grape-growing area, characterized by its climatic features that have an effect in the grape and how it grows, and at the same time is

different from the surrounding regions. The first AVA in the country was Augusta, Missouri in 1980(Soleas, Diamandis, & Goldberg, 1997).

All grapevines are classified in the genus *Vitis,* this genus is one of the sixteen genera that compose the family Vitaceae. Members of this family are woody plants with climbing habit, their leaves develop alternately on shoots and possess swollen or joined nodes. Some generate flowers or tendrils. Predominantly, the plants in the Vitaceae family that contains approximately 900 species, are sub-tropical, however, the *Vitis* genus, has a primarily temperate zone distribution, and its origin has been traced to have occurred in the northern hemisphere (Jackson, 2014).

The genus *Vitis* has been comprised into two distinct sections: Muscadinia and Euvitis. The Muscadinia section comprises three species: *Vitis rotundifolia*, indigenous from southeastern United States and domesticated by European settlers. *Vitis rotundifolia* has been used as a rootstock due European grapevines high sensitivity to *Phylloxera* disease. Muscadinia also includes *Vitis munsoniana* native to Florida and Bahamas, and *Vitis popenoei* from Mexico, Belize, and Guatemala. The Euvitis section includes most of the grapevines used for table consumption, wine making or rootstocks grafting, among the most used *Vitis* are: *Vitis labrusca* (originally from North America), *Vitis aestivalis*, (native to the southeastern United States), *Vitis riparia* (widely distributed in the northern United States), *Vitis berlandieri* that is used for rootstocks (common in southwestern Texas and Mexico), *Vitis rupestris*, (native to Arkansas, southern Missouri, and Tennessee) normally used for hybrid wine grapes making and rootstock, *Vitis cinerea* and *Vitis vinifera* (Fortes & Pais, 2016; Jackson, 2014).

## 1.1. *Vitis vinifera*

*Vitis vinifera* is the primary cultivated *Vitis* species that produces the most famous quality wines. It is native to Asia near the Caspian Sea and it has been imported to Europe since before recorded history. It is generally believed that *Vitis vinifera* domestication began around Transcaucasia near Anatolia about 4000 B.C., where its greatest genetic diversity has been found. It is also believed that *Vitis vinifera* domestication may have occurred nearby Zagros Mountains where the earliest archeological evidence of wine production was found, and other theories suggested that domestication could have occured in Southern Spain. Whether from Transcaucasia or Zagros Mountains, it is hypothesized that wine making spread eventually westwards around the Mediterranean (Arroyo-Garcia et al., 2006). Through Greek colonization, *Vitis vinifera* was transplanted in Italy and southern France, whereas in the rest of France, Spain and Germany grapevine transplantation was done by Roman settlers. From the fourth century, with the expansion of Christian faith to Europe, viticulture experienced a geographical growth. In North America, the first *Vitis vinifera* varietals successfully planted were the ones brought by Spanish priest from Mexico in 1680, when they stablished their missions in New Mexico a century after the discovery of the Americas (Jackson, 2014; Terral et al., 2010).

*Vitis vinifera* grapevine cultivars (unique grape species) also known as varietals, are important for viticultural production in their native countries like Portugal, Spain, France, Germany, Italy and Croatia; and in New World wine producing countries like New Zealand, Australia, South Africa, Argentina, Chile, Mexico, United States and Canada. Some of the most famous traditional varietals are: Cabernet Sauvignon, Chardonnay,

Gewürztraminer, Grenache, Nebbiolo, Pinot Noir, Merlot, Malbec, Tinta Roriz, Riesling and Sauvignon Blanc (Fortes & Pais, 2016; Jackson, 2017).

## 1.2. Hybrid cultivars

Due to disease tolerance, grapevine hybridization was implemented in North America, however, some of resulting *Vitis* interspecific hybrid grapes varieties were accidental. Hybrid grapes are divided in two categories: American hybrids and French-American hybrids. These firsts are the pioneer examples of crosses between *Vitis vinifera* and indigenous *Vitis* spp, although their parentage is speculative because there is not much information about them. The varieties Concord, Niagara, Ives, Isabella and Catawba, are the most important hybrids between *Vitis vinifera* and *Vitis lambrusca*. Herbemont and Lenoir vine grapes are likely the result of the crossing of *Vitis aestivalis*, *Vitis cinerea* and *Vitis vinifera.* Cynthiana and Norton grapes are likely a *Vitis aestivalis* and *Vitis vinifera* crossing. These early cultivars are termed American hybrids, to distinguish them from hybrids developed in France (Slegers, Angers, Ouellet, Truchon, & Pedneault, 2015; Soleas et al., 1997).

The French-American hybrids are cultivars derived from crosses made originally in France between *Vitis vinifera* and *Vitis riparia, Vitis lincecumii, Vitis aestivalis and Vitis rupestris*. This designation comes from the ability to grow ungrafted in phylloxera-infested soils. French-American hybrids possess complex parentage, based on subsequent crossing with one or more *Vitis vinifera* varieties. Only a few French-American cultivars have *Vitis lambrusca* parentage, as it is common to find the compounds, methyl anthranilate and O-

aminoacerophenone, both identified as the main causal for the peculiar Foxy smell in lambrusca varieties and hybrids (Soleas et al., 1997; Sun, Gates, Lavin, Acree, & Sacks, 2011). By 1955 they were very popular on French vineyards, however, their grape overproduction and nontraditional aromas led to a general ban in the appellation-controlled wines; therefore, French-American hybrids were gradually rejected in the land of their origin. Meanwhile at the East coast of the United States and Canada, these hybrids cultivars were broadly accepted due their disease tolerance, high enological potential and cold-hardiness. Cultivars like Aromella, Brianna, Frontenac, La Crescent and Marquette are some of the recent introductions from North American grapevine breeding programs (Atucha, Hedtcke, & Workmaster, 2018; Jackson, 2014; Soleas et al., 1997).

## 2. Vine growth

Grapevines are perennial plants that undergo characteristic morphological changes with the seasons. There are two clearly distinguishable periods in the yearly cycle of the vine: winter dormancy that extends from leaf fall until bud burst during which no morphological changes occur, and active growth, which begins with bud burst and ends with leaf fall. During the annual active growth period of a fruit giving, and mature grapevine, the organs are constructed, the seeds and berries are formed, and the materials necessary for survival accumulates into the plant. Under normal conditions, the grapevine active growth cycle generally occurs from March/April through October/November in the Northern Hemisphere, and from September/October through April/May in the Southern Hemisphere. For viticulture practices, the active growth cycle is often divided into two cycles: vegetative and a reproductive cycle, that at the same time are also divided into two

phases each. The vegetative cycle phases are vegetative growth where the grapevine develops its vegetative organs (shoots, leaves, tendrils, and roots), and withering, where the accumulation of reserves occurs before entering winter dormancy. The reproductive cycle phases are growth and development of reproductive organs, which occurs from bud burst until fruit set and involves the flowers, and berries formations; and ripening or fruit maturation which occurs from veraison until leaf fall (Keller, 2020; Moreno & Peinado Rafael., 2012). The complete grapevine annual cycle on the Northern Hemisphere is presented in figure 1.

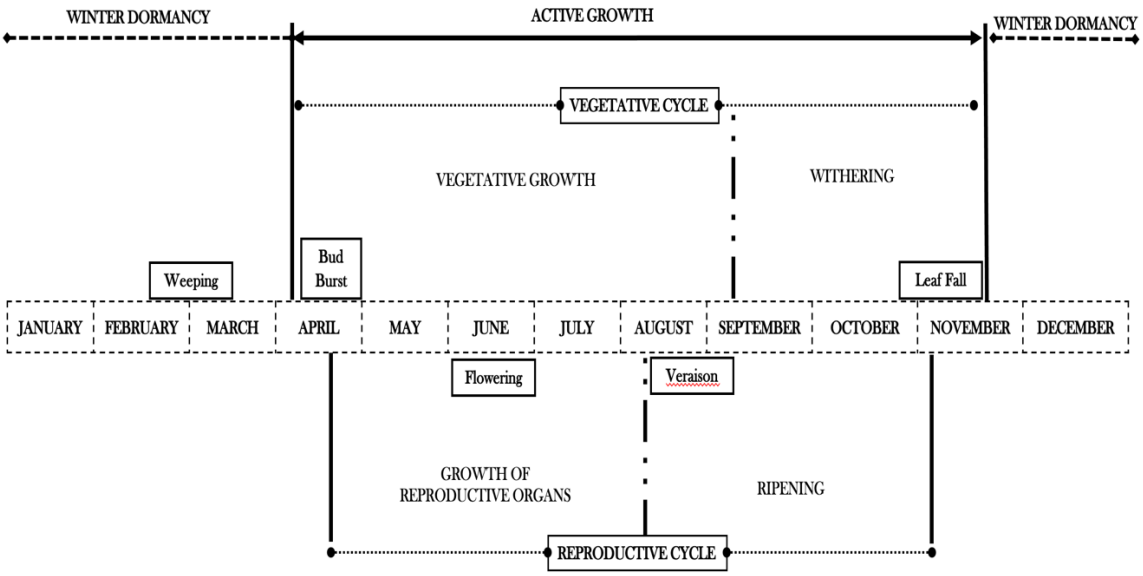


Figure 1. Grapevine cycle in the Northern Hemisphere (*modified from Moreno & Peinado Rafael., 2012*).

The several distinct and developmental stages that have been identified in grapevine, have been given a variety of names. The most known stages are dormancy (rest), budbreak (budburst), bloom (anthesis, flowering), fruit set (berry set, setting), veraison (French verer=to change; color change), harvest (ripeness, maturity), and leaf senescence and subsequent leaf fall (abscission). All these phenological stages happen in chronological

order that started with weeping, bud burst, flowering, fruit set, ripening, and leaf fall (Keller, 2020).

## 2.1. Vegetative cycle

**Bleeding or weeping**:

In late winter or early spring, with the return of warm weather the grapevines often exude sap from pruning wounds. This is known as weeping or bleeding by viticulturists and can last for days, marking the transition from winter dormancy to active growth. Weeping is influenced by soil temperature, moisture, and rootstock, but on average it starts when soil temperature reach approximately 7°C. In bleeding, hydrolysis of stored starch generates the hydrostatic pressure that induces the upward flow of sugars, amino acids growth regulators and trace amounts of other organic compound contained in the sap(Keller, 2020; Moreno & Peinado Rafael., 2012).

**Bud break or bud burst**:

When temperatures rise above a critical value, which depends on the variety, buds begin to burst, marking the beginning of vegetative growth in spring. Bud burst is the process by which the protective scabs covering the buds break open. Buds that burst in the spring do so because a latent bud will have formed during the previous growth cycle. Budbreak starts in the distal and progresses downward from the tips of cane or vine toward the base. Often, budbreak of the distal buds inhibits outgrowth of the basal buds on the same cane. Typically, only the primary bud in the overwintered bud develops. The

secondary and tertiary buds remain inactive unless the primary bud has been killed or severely damaged. Although temperature increment in spring induces budbreak, the buds are generally unresponsive to the warming effect unless they have previously experienced a period of cool temperatures also called “chilling requirement”. This requirement is often defined as the period of low temperature that is necessary to permit 100% of the buds to break under favorable temperatures. Longer duration of chilling and lower temperatures down to about 3°C typically accelerate the rate of budbreak and enhance the uniformity of budbreak once warmer temperatures return. The buds on a single vine usually break within a few days in areas with cold winters, however, budbreak may take longer in areas with mild winters and tends to be erratic in subtropical regions. For this reason, vine growers often induce budbreak by applying hydrogen cyanamide, usually in the form of calcium cyanamide between 2 and 4 weeks before the intended time of budbreak(Jackson, 2014; Keller, 2020).

**Shoot growth and Foliation**:

Foliation involves the appearance and development of the leaves, and this phenomenon occurs parallel to the growth of the shoots. Once initiated, shoot growth rapidly reaches its maximum with a new leaf appearing every few days as the climate continues to warm. In many grape cultivars, growing leaves, and often the emerging inflorescences have a reddish color before turning green. As growth continues, the shoot differentiates new leaves and tendrils. Simultaneously, new buds begin to form, and those that form early may give rise to lateral shoots. Early shoot growth is entirely dependent on nutrient reserves mainly carbohydrates, such as starch and sugars, and nitrogen-containing

components, such as proteins and amino acids stored in the permanent structure of the vine, until new leaves become photosynthetically competent and begin to produce and export sugar. Once shoots have developed enough, they are normally tied or restrained by a support system. Shoots developing from old wood on the trunk are removed. Shoot growth tends to slow down somewhat during the bloom period but may resume after fruit set if the availability of water and soil nutrients allows it. Shortly before fruit ripening, the shoots begin to form a periderm and turn from green to yellowish or reddish brown. Vigor is the viticultural terminology used to refer to the rate of shoot growth or shoot elongation over time. Vigor declines with increasing vine size; therefore, shoots of small, young vines often grow more vigorously than those of large, old vines (Jackson, 2014; Keller, 2020; Moreno & Peinado Rafael., 2012).

**Withering:**

Withering begins at the end of veraison and continues until leaf fall. During this process the accumulation of reserves, particularly of starch in the trunk and shoots occurs. In autumn, leaf senescence marks the end phase of the shoot growth cycle. Senescence is followed by leaf shedding, dehydration, and cold acclimation of the aboveground woody organs. Before they are shed, the senescing leaves develop their characteristic yellow, orange, or red autumn colors, and at this point the grapevine can be considered to have entered to winter dormancy. The decreasing day length triggers leaf senescence, however, temperature, has no effect on the onset of senescence, although lower temperature may accelerate the rate of senescence once initiated(Jackson, 2014; Moreno & Peinado Rafael., 2012).

## 2.2. Reproductive cycle

**Growth of reproductive organs**:

Reproductive growth is very similar in the different *Vitis* species and begins with flower formation. Flower clusters exist in embryonic form in the fertile buds formed during the previous growth cycle. Flowering involves the opening of the corolla of the flower and is linked to fertilization. It is difficult to separate these two processes in time since the same vine will carry flowers that have yet to open and others that are already been fertilized. Flower development in the spring progresses from the outermost ring of flower parts (the sepals) inward to the pistil followed by fertilization, embryo, and berry development commencement. The flower number is highly variable, even on the same shoot, approximately 2 weeks after the ovules have been formed and 5–10 weeks after budbreak, anthesis marks the beginning of bloom, exposing the male and female floral organs. Anthesis theoretically refers to the release of pollen but is commonly regarded as the opening of a flower, which occurs by shedding of the calyptra, or cap fall. Like budbreak, anthesis generally begins in the shoots growing from distal buds and progresses basipetally toward the trunk (Keller, 2020).

After flowering, the inflorescence is termed a raceme or cluster. It is made up of a principal axis, together with secondary axes formed by the stalks or stems that support the fruit or berries. The structure of the raceme and the number and volume of berries are determined by the inflorescence. Unlike their wild relatives and the few cultivars with female flowers, cultivated grapevines are typically self-pollinated, whereby pollen originates from the flower's own anthers. Wind appears to be responsible for only

occasional cross-pollination, where pollen originates from a flower on a different plant than the pollinated one (Jackson, 2014; Moreno & Peinado Rafael., 2012).

**Berry development:**

In varieties containing seeds, the berry begins to develop after fertilization. At this stage the fruit is said to be set. Under favorable conditions for production, this type of growth and formation of the fruit generates berries of the maximum size for each variety. The proportion of flowers that develop into berries following anthesis is typically in the range of 30 to 50%. Fruit set is dependent on the interplay between two hormones, auxin responsible of cell division, and gibberellin that induces cell expansion. These two hormones thereby induce the pistil to develop into the fruit and to differentiate in exocarp (skin) and mesocarp (flesh or pulp). Fruit set is highly variable among cultivars and is strongly modulated by environmental conditions and rootstocks(Keller, 2020; Moreno & Peinado Rafael., 2012).

Berry development has three distinctive stages: The first stage is characterized by the initial rapid increase in size of the seeds and pericarp (exocarp & mesocarp), both remain green and hard. In this stage the cluster behaves as a green organ, it contains chlorophyll and contributes to photosynthesis. Their sugar content is low, but acids begin to accumulate and reach their maximum concentration when the grapes are close to veraison. In the second stage the pericarp growth slows down to very low rates, but the seeds enter their maturation stage growing rapidly, and between 10 to 15 days before veraison, the seeds reach their final size, maximum fresh weight, and maximum tannin content. The third stage of berry development is the ripening period better known as

veraison. During veraison, the berries become softer, more elastic due to changes in the cell walls, they lose chlorophyll and change color due to the formation of pigments; white grapes become translucent, and some develop a yellowish color, the red grapes change their skin color from green to red, purple, or blue in dark skinned cultivars. Not all grapes change color at the same time and the process takes approximately two weeks. During veraison, the pulp rapidly begins to accumulate sugars while the acidity is considerably reduced. Once ripeness has been achieved, the grapes are left to overripe, during this time, external physical factors have a greater influence and the berries become fragile. The grape receives almost no contribution from the plant and there is a partial evaporation of water from the pulp that leads to concentration of the sugars and reduction of acids. Overripening is essential to obtain wines with high alcohol content since the concentration of alcohol in the final product is proportional to the sugar content of the grapes from which it was produced(Jackson, 2017; Keller, 2020; Moreno & Peinado Rafael., 2012).

Poor fruit its due to an excessive abortion of flowers and ovaries, the terms used are shatter, shedding, or “coulure” (to leak or to fall off in French). The loss of ovaries can occur for up to 4weeks after anthesis. Less than 30% of fruit set can lead to clusters with few berries or clusters with significant berry variability, this phenomenon is called “millerandage” and results in clusters having the appearance of “hens and chicks”. The term refers to large, normal berries with at least one viable seed, along with small berries with tiny, degenerated seeds that often lack an endosperm (Keller, 2020). The grape vine phenological growth stages in chronological order are presented in figure 2.

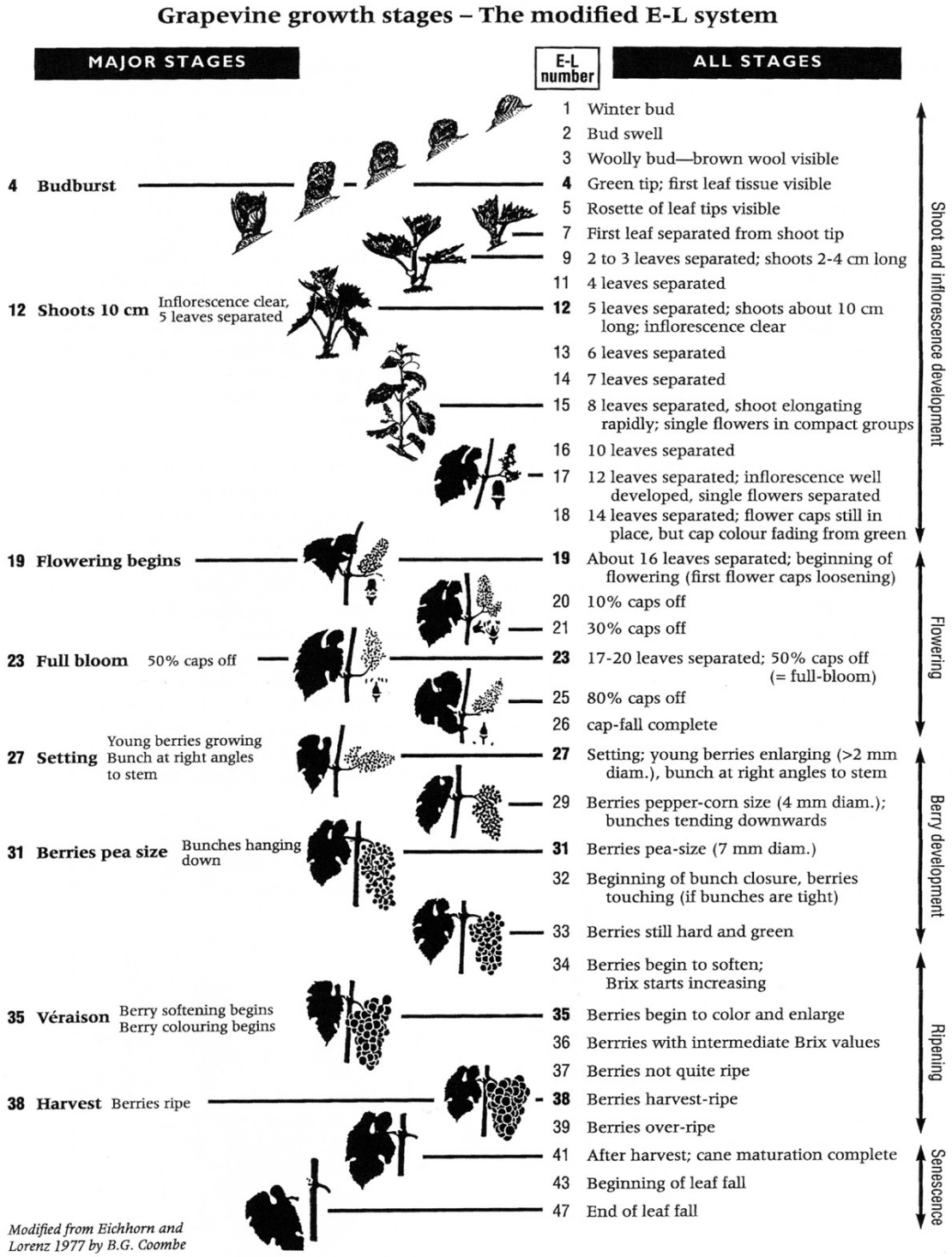


Figure 2. Grapevine phenological growth stages – the modified E-L system by (Coombe, 1995).

## 3. Grapevine nutrition

Various nutrients are essential for grapevine growth, crop yield, berry composition and ultimately, must and wine quality, these nutrients are required in certain quantities to ensure healthy growth and good grapevine performance. From the sixteen nutrients required by the grapevine, carbon, hydrogen, and oxygen are obtained as water by the roots or as gases by the leaves, the rest of the nutrients are classified in macronutrients and micronutrients, depending on the quantity of the plant element requirement. Macronutrients include nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur that occur at high levels in plant tissue. Micronutrients occur at lower levels in plant tissue, these are iron, manganese, molybdenum, copper, zinc, and boron (Ibacache, Verdugo-Vásquez, & Zurita-Silva, 2020; Jackson, 2014).

Nutrient availability, uptake, requirement, and accumulation often vary considerably throughout the season. They can also differ significantly among cultivars. Below a certain value, the effects of deficiency become increasingly severe. If an element is not available in adequate amounts, then vine performance is limited by the supply of that one element. The potential for storage and mobilization of nutrients within the vine, makes assessing the actual need of a vine nutrient complicated, which may explain the occasional delay in vine response to fertilization. When vine tissues are suffering a deficiency, response is usually rapid, but may decline in subsequent years, and in excess, nutrient accumulation can become toxic (Jackson, 2014; Wolf, 1993).

## 3.1. Macronutrients

### Nitrogen

Nitrogen is the most abundant soil-derived macronutrient in grapevine and is involved in almost every metabolic process occurring in the growth of grapevines, including the development of berries. It is associated with the quality of wine or must because of the amount of nitrogen that can be used by fermentative microorganisms. Nitrogen is an essential component of functioning proteins and chlorophyll in leaves and therefore, photosynthesis. Although nitrogen is required in larger quantities than any other soil nutrient, grapevine needs are considerably less than those of most other agricultural crops. The availability of soil nitrogen depends on the level of organic matter in the soil and with continual harvesting of fruit and removal of pruning, nitrogen fertilization may be necessary (Ashley, 2011; Brunetto, Melo, Toselli, Quartieri, & Tagliavini, 2015; Ibacache et al., 2020).

Vines low in nitrogen generally display low vigor and poor production, because of reduced protein synthesis and photosynthesis. Vines with nitrogen deficiency will display a reduced shoot growth, yellowing of all leaves and green tissue. Yellowed leaves may defoliate mid-season, which can lead to delayed ripening and in extreme cases defoliation and loss of bunches. Smaller bunches with few and small berries are also an effect of nitrogen deficiency. A decrease in fruit yield is one of the first consequences of marginal nitrogen deficiency. During vinification, nitrogen deficiency in the fruit can result in stuck fermentation (Bell & Henschke, 2005; Jackson, 2014).

The application of nitrogen on grapevines should be undertaken with caution because of its conflicting effects on vegetative growth, yield, and chemical composition of the grapes, must and wine. High rates of nitrogen fertilizers, may stimulate vegetative growth hence reducing solar radiation within the plant canopy, favoring the incidence of fungal diseases on the leaves and fruits, reducing the number of pollinated flowers, producing a lower number of berries per bunch and, in the end, delaying leaf senescence and plant dormancy (Brunetto et al., 2015; Proffitt & Campbell-Clause, 2012).

## Phosphorus

Phosphorus is involved in the energy transfer within plant cells, this facilitates metabolism and is a constituent of the fatty portion of cell membranes, nucleic acids and of compounds involved with assimilation and metabolism of carbohydrates. Phosphorus deficiency is rare in grapevines; however, its deficiency has been detected in grapevines grown on acidic soils and weathered, low-phosphorus, hillside soils. Deficiency symptoms include the formation of dark-green leaves, down turning of leaf edges, reduced shoot growth, and purple discoloration in the main veins of older leaves, as well as interveinal discoloration, pre-mature fruit ripening, and reduced yield. These symptoms may be confused with leafroll virus, but phosphorus symptoms occur earlier at the growing of reproductive organs phase, around flowering. Poor bud initiation and fruit set may also be observed. Because phosphorus deficiency is uncommon, fertilization should be used only if deficiency has been clearly demonstrated in order to avoid potential interference with potassium, manganese, magnesium, and iron uptake. Excessive phosphorus has not been shown to be a direct problem for grapevines; however, in excess it may limit the uptake of

other essential elements, such as zinc (Ibacache et al., 2020; Jackson, 2014; Proffitt & Campbell-Clause, 2012).

## Potassium

Potassium functions in several regulatory roles in the vine biochemical processes. It constitutes up to 3% of the dry weight of a grapevine and is an important component of grape juice and wine. Potassium is the only macronutrient that is not used as a structural component of cellular macromolecules. However, its presence is vital to cellular osmotic and ionic balance, electrochemical processes, neutralization of organic acids, regulation of stomatal function, cell division, enzyme activation, protein synthesis, and the biosynthesis and translocation of sugars. In red varieties, potassium is important for berry color development (Ashley, 2011; Jackson, 2014).

Potassium deficiencies is expressed as marginal leaf yellowing in white varieties and marginal leaf reddening in red varieties, followed by marginal leaf burn, marginal leaf curling and defoliation of all varieties in severe cases. Potassium is readily mobilized in vines as symptoms move from basal leaves to younger leaves, as the vine grows. Other less common symptoms include reduced bunch weight, uneven berry ripening and blackening of leaves. Like phosphorus, high levels of potassium do not directly affect the vine or fruit but may limit calcium and magnesium uptake and increase grape juice pH levels. Vines low in potassium are also more drought-prone and less cold-tolerant. Excess on potassium in the soil can result in high levels in berry juice which will increase the pH of the must and may cause problems with malolactic fermentations. Potassium in excess may also

produce poor color stability in the resulting wines (Ibacache et al., 2020; Proffitt & Campbell-Clause, 2012).

## Calcium

Calcium plays a crucial role in the grapevine structure as a constituent of plant cell walls. When reacted with pectin, calcium makes it relatively water insoluble and rigid. Calcium is also important in the regulation of cell membrane permeability, in ion and hormone transport, and in a range of enzyme functions. Calcium deficiency typically occurs only on strongly acidic quartz gravel. It is expressed as a narrow zone of necrosis along the edge of leaves, which may progress toward the petiole. Minute, brown, slightly raised regions may develop in the bark. Clusters may show dieback from the tip. When in excess, as in calcareous soils, it can cause lime-induced chlorosis with sensitive rootstocks (Jackson, 2014).

## Magnesium

Magnesium is a vital component in the absorption of light energy by chlorophyll, thus contributes to carbohydrate production in leaves through photosynthesis. It stabilizes ribosome, nucleic acid, and cell-membrane structures, and is involved in the activation of phosphate-transfer enzymes. Deficiencies are frequently experienced on sandy soils in high-rainfall regions, poorly drained sites, and high-pH soils. Symptoms first begin to develop in basal leaves, due to magnesium's translocation to growing points under deficient conditions. Interveinal regions develop a straw yellow chlorotic discoloration, while bordering regions remain green. If deficiency appears early in the season, small

brownish spots may be found next to the leaf margins. Magnesium deficiency may also be involved in the physiological disorder termed bunch stem necrosis. Magnesium deficiency symptoms are usually confided to older leaves, except in cases of severe deficiency Excessive concentration of magnesium levels may limit uptake of potassium by the vine (Ashley, 2011; Jackson, 2014).

### Sulfur

In soils, Sulfur is found as sulphates of nitrogen, potassium, calcium, and magnesium. It is an integral component of the amino acids, cysteine, and methionine, thus it is present in proteins and chlorophyll and plays a role in energy metabolism. Sulfur deficiency symptoms are similar to nitrogen deficiency yet are rare given the widely adopted use of sulfur-based sprays for fungicide management and sulfate with potassium or magnesium fertilizers around the world. Sulfur is also an integral component of the two vitamins, thiamine and biotin. Sulfur is not known to occur naturally at toxic levels in soils. It also is incorporated as sulfur dioxide as an antimicrobial and antioxidant in most wines (Jackson, 2014; Singh et al., 2013).

## 3.2. Micronutrients

### Iron

Iron as a micronutrient, plays a role in chloroplast development. It is essential in the synthesis of chlorophyll, acts as a cofactor in redox reactions, and is a constituent in enzymes such as catalase and peroxidase. As with other nutrients closely associated with photosynthesis, deficiency of iron is observed as stunted growth and diffuse yellowing of

young leaves and shoot tips. In severe cases, the whole leaf becomes chlorotic (bleached appearance), whereas leaf veins remain green with mild deficiency. Fruit yield and berry size are also severely affected. Although the high pH of calcareous soils may limit iron solubility, this by itself does not fully explain iron-deficiency chlorosis. To date, iron toxicity is not known to occur in vineyards (Ashley, 2011; Jackson, 2014).

## Manganese

Manganese plays an important role in the synthesis of chlorophyll and nitrogen metabolism and is present in soil as exchangeable manganese or manganese oxide. Manganese is directly involved in the synthesis of fatty acids, in the neutralization of toxic oxygen radicals, and in the reduction of nitrates to ammonia. Manganese deficiency is expressed as yellowing of the interveinal area of older leaves and may be mistaken for zinc or iron deficiency. However, manganese deficiency neither modifies leaf-vein angles nor induces ‘little leaf.’ These symptoms may be found in vines on sandy, calcareous soils or in areas of high rainfall. On poorly aerated acidic soil, manganese may reach toxic levels due to increased solubility of its reduced oxides form. Toxicity of manganese is rare but can be seen as black spots on the leaves, shoots and bunch stems (Ashley, 2011; Jackson, 2014).

## Copper

Copper acts primarily as a cofactor in respiratory oxidative reactions and the synthesis of proteins, carbohydrates, and chlorophyll. Deficiency symptoms are not common, probably due to the use of copper based fungicidal also known as Bordeaux mixture. When copper deficiency occurs, the leaves are dwarfed with a pale green color,

shoots develop short internodes, cane bark has a rough texture, and root development is poor. Toxicity most commonly occurs in soils where copper has accumulated following prolonged use of copper-containing fungicides and presents as leave chlorosis (Ibacache et al., 2020; Jackson, 2014).

## Zinc

Although zinc is required in small amounts, its solubility can be suppressed in alkaline soils leading to deficiency. Zinc deficiency is common in Australian viticultural regions and is involved in reduced protein synthesis, some plant hormone production and fruit set. Deficiency of zinc can result in stunted growth and development of small, undersized leaves with mottling between veins, clawed margins and widened petiolar sinus. Poor fruit set and bunches of variable sized berries may occur even when leaf symptoms are not observed. Foliar application of zinc before flowering is a common practice in vineyard (Ashley, 2011; Singh et al., 2013).

## Boron

Boron is required for nucleic acid synthesis, in the maintenance of cell-membrane integrity, and in calcium use. Although boron is required in trace amounts, its deficiency can be observed as stunted growth with shortened internodes displaying a “zig-zag” pattern, death of shoot tips and interveinal chlorosis of older leaves. Boron deficiency also induces poor fruit set (due to retarded germ tube elongation) and the development of many small berries. Deficiency symptoms tend to develop on sandy soils in high-rainfall areas, on soils irrigated with water low in boron, or on strongly acidic soils. Application of borax to counteract deficiency can lead to toxicity if not evenly distributed. Toxicity symptoms

begin with the development of brown to black specks on the tips of leaf serrations. The necrotic regions spread and become continuous. The inhibition of growth may result in leaves wrinkling and puckering along the margin (Jackson, 2014; Wolf, 1993).

## 4. Molybdenum requirements and deficiencies

Molybdenum (Mo) is a trace element found in the soil and required for growth of most biological organisms including plants and animals. The metal itself is biologically inactive unless it is complexed by a special cofactor. Except for bacterial nitrogenase, where Mo is a constituent of the FeMo-cofactor, Mo is bound to a pterin, thus forming the molybdenum cofactor (Moco) which is the active compound at the catalytic site of all other Mo-enzymes. The four prominent Mo-enzymes involved in plant nutrition are: Sulfite oxidase, which catalyzes the final step in the degradation of sulfur-containing amino acids and is involved in detoxifying excess sulfite. Xanthine dehydrogenase, which is involved in purine catabolism and reactive oxygen production. Aldehyde oxidase, which oxidizes a variety of aldehydes and is essential for the biosynthesis of the phytohormone abscisic acid, and in autotrophic organisms also. And nitrate reductase, which catalyzes the key step in inorganic nitrogen assimilation. (Kaiser et al., 2005)

Molybdenum is an essential micronutrient for biological nitrogen fixation (BNF), and its deficiency in soil can decrease this process leading to nitrate accumulation in plants. Molybdenum deficiency may induce symptoms similar to those of nitrogen deficiency, which is the reason why Mo deficiency can be misidentified, as molybdenum is required for the assimilation of nitrate taken up by the plant. Such symptoms would include a general pale green color, stunted growth with small leaf size, and possible reddening of veins on

the young leaves. The pollen of molybdenum deficient plants will usually be less viable than that of healthy plants, so grain and fruit production is often reduced (Kovacs et al., 2015).

The availability of molybdenum for plant growth is strongly dependent on soil pH, concentration of adsorbing oxides (e.g. Fe oxides), extent of water drainage, and organic compounds found in the soil colloids. In alkaline soils, molybdenum becomes more soluble and is accessible to plants mainly in its anion form. To the contrary, in acidic soils (pH <5.5) molybdenum availability decreases as anion adsorption to soil oxides increase (Tejada-Jimenez et al., 2018).

Molybdenum deficiencies have been documented in many plant species where phenotypes range in severity and appearance. However, it hasn't been researched extensively in grapevines, where molybdenum deficiency has been suggested as the primary cause of a bunch development disorder called Millerandage or 'hen and chicken'. Millerandage is characterized by grapevine bunches that develop unevenly, where fully matured berries are present in a bunch alongside a large number of fertilized underdeveloped berries as well as unfertilized swollen green ovaries and less nitrogen assimilation by the grapevine (Williams, Maier, & Bartlett, 2004)

In the Brassicaceae family (broccoli, cauliflower, brussels sprouts, cabbage, turnips, kale, etc.), molybdenum deficiencies are pronounced and reproducible amongst many of its members. Visual effects in young plants include mottling, leaf cupping, grey tinting, and flaccid leaves which are often found on seedlings that remain dwarfed until

dying (Kaiser et al., 2005). Deficiency symptoms can also be masked by the indirect effect of molybdenum on nitrogen assimilatory enzymes (i.e. NR). Many horticultural, cereal and legume crops growing at deficient molybdenum levels will develop pale green leaves and, at times, necrotic regions at leaf margins with accompanied decreases in overall plant growth (Tejada-Jimenez et al., 2018).

Molybdenum-deficient oat and wheat develop necrotic regions on leaf blades, and seeds are poorly developed. In maize, molybdenum deficiency shortens internodes, decreases leaf areas and causes the development of chlorotic leaves. In reproductive tissues in maize, molybdenum deficiency can alter the phenotypes in developing flowers, including delayed emergence of tassels, small anthers, poorly developed stamens, and reduced pollen grain development. In maize seedlings, molybdenum along with sulfur and iron, play an important role in the nitrate reduction, because they are necessary for the function of the nitrate reductase enzyme (Kovacs et al., 2015)

Liu et al. (2017) reported that in strawberries, molybdenum deficiency affects photosynthesis and photosynthate accumulation in the plant, therefore also affecting fruit quality and nutritional content. This deficiency has been related with excessive or inappropriate fertilizer application that leads to acidification of orchard soil (pH<5.5), thereby decreasing molybdenum availability. Also (Hippler et al., 2017) mentioned that the recent increase in citrus tress areas grown under intensive nitrogen fertilization and the utilization of rootstocks with superior nutritional demand, are leading to a greater need of molybdenum.

Molybdenum is closely linked to nitrogen nutrition, when the grapevine has insufficient Mo, the nitrogen accumulates in the leaves and the vine cannot use it to make proteins. The result is a vine that produce low quality berries with low bunch yield and weight; where berries develop unevenly and fully matured berries are present in a bunch alongside a large number of fertilized underdeveloped berries as well as unfertilized swollen green ovaries, with symptoms similar to those of N deficiency (Kaiser et al., 2005; Williams et al., 2004). Nitrogen is one of the most influential mineral nutrients in the physiology of the grapevine and has major implications for winemaking. Nitrogen is involved in almost every metabolic process occurring in the growth of grapevines, including the development of berries. Grapevine nitrogen nutrition has the potential to influence quality components in the grape and, ultimately, the wine. The effect of nitrogen assimilation in the vineyard on grape berry quality components is an increase in the concentration of the major nitrogenous compounds, such as total nitrogen, total amino acids, arginine, proline, and ammonium, and consequently yeast-assimilable nitrogen (YAN). In addition, nitrogen status of the must plays an important role in fermentation kinetics and formation of flavor-active metabolites (Gobert, Tourdot-Marechal, Sparrow, Morge, & Alexandre, 2019)

## 5. Grape chemistry

The chemical composition of harvested grapes has a strong influence in the composition of the must that will be later reflected in the wine quality. The berry composition is influenced by factors such as climate, genotype, management, and soil type. In the world of winemaking, there is a universal term called vintage quality, and it is directly correlated with optimal grape maturity. Sugars, pH, and titratable acidity (TA) are

the key compounds in berry chemistry that are used for determining the quality and ripeness of the berry (Barnuud, Zerihun, Gibberd, & Bates, 2014; Castellarin et al., 2016).

## 5.1. Sugars

The main sugars found in *Vitis vinifera* grapes are the hexoses, glucose and fructose, and the ratio between the two sugars may vary over the berry set phase or between cultivars. Saccharomyces cerevisiae, the principal wine yeast, derives essentially all of its metabolic energy from glucose and fructose; in *Vitis* interspecific hybrids, trace amount of sucrose has been also measured. Sucrose, whether natural or added to must, is rapidly split into glucose and fructose by yeast during fermentation. Sugar content depends on different parameters such as vine vigor, grape maturity level, crop load, vine water status and berry health. In North America, sugar content (roughly equivalent to total soluble solids) is measured in °Brix. Brix is a good indicator of berry sugar content at levels above 18°, when sugars become the predominant soluble solid. In finished wines, unfermented sugars are called residual sugars, in dry wine is generally less than 1.5 g/liter. The perception of sweetness varies from person to person; however, a sugar content above 10 g/L will be perceived as sweet (Jackson, 2014; Moreno & Peinado Rafael., 2012; Reboredo-Rodríguez, González-Barreiro, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015).

## 5.2. pH and titratable acidity

The pH and titratable acidity (TA) are the two most commonly measured aspects of juice and wine acidity, and both are important parameters to determine ripening in berries. In general, wine grapes are harvested around an ideal pH of 3.5, with a titratable

acidity of 5g/L at the lower end, and sugar accumulation of 20-24 °Brix. The titratable acidity has no known effect on chemical or enzyme reactions or microbial activity and is of primary importance only to the sensory perception of finished wines. pH affects the equilibrium of tartrate salts, determines the effectiveness of sulfur dioxide and enzyme additions, influences the solubility of proteins and effectiveness of bentonite, affects red wine color and oxidative and browning reactions as certain pigments will also take on different conformations depending on the pH of a solution, again altering the perception of the depth, stability, or brightness of color in a wine; and influences microbiological stability since many spoilage bacteria such as *Pediococcus* and Lactobacillus cannot thrive below pH values of 3.5(Boulton, Singleton, Bisson, & Kunkee, 1999; Jackson, 2014).

Fruit in which the exchange reactions have been more extensive can have pH values between 3.5 and 4.0 and even higher pH values are sometimes observed in extreme conditions, particularly overripe grapes or in regions with an extended growing season due to cool conditions. For consumers, pH can impact the sensory perception of a wine since odor activity values (OAVs) of volatile compounds in wines are highly dependent on the pH of the solution. If the winemaker intends to allow secondary fermentation by malolactic bacteria, pH can influence the kinetics of the fermentation; the use of potassium metabisulfite or sulfur dioxide as an antimicrobial is dependent on pH, and sulfur additions become less effective as pH rises and values approach 4 (Boulton et al., 1999; Moreno & Peinado Rafael., 2012).

Titratable acidity is determined by titrating the juice or wine to an end point with a strong base and expressing the number of protons recovered as an equivalent concentration of some chosen acid. In the United States the end point chosen is pH = 8.2 and the acid for reference is tartaric acid. The titratable acidity of grape juice, like most fruit juices, is always less than expected from the organic acid concentrations. This is because the total acidity is the sum of all the organic acid anions in solution, while the titratable acidity measures the total available hydrogen ions in solution. This is because total acidity analysis measures both the dissociated and undissociated forms of each individual acid. As an example, if a solution of 1 g/L tartrate, as potassium bitartrate (KHT), is analyzed for titratable acidity, the result will be 0.5 g/L expressed as tartaric acid. However, if the solution is analyzed for total acidity, using HPLC for example, the result will be 1 g/L as tartrate (AWRI, 2017; Boulton et al., 1999).

## 5.3. Aromas

In contrast with the major chemical constituents in wine that generate the gustatory sensations: alcohol, acids, sugars, and phenols, the aroma characters are the minor or trace constituents that donate the distinctive, fine, complex, and unique character to wine (Jackson, 2017). Bouquet and flavor are obviously related to the expertise of the winemaker and the techniques used, but the aromas primarily reflect the grape composition. Although wines possess hundreds of aromatic compounds, less than 50 may occur at concentrations sufficient to directly influence their fragrance Wine aroma is a complex mixture of volatile organic compounds of a wide range of concentrations. The aromas derived from the grapes are called varietal aromas or primary aromas, the fermentation aromas that are derived

during fermentation are called secondary aromas; and the aging aromas, also known as tertiary, are the aromas that develop during chemical reactions happening during aging (Ebeler & Thorngate, 2009; Thibon, Darriet, & Dubourdieu, 2012). Aroma compounds in grape berries can be found in either free volatile or odorless glycosidically bound form. Glycosidically bound compounds are non-volatile aromas linked to a sugar moiety. Although they do not contribute directly to wine aroma, they form a precursor pool that is released as volatiles during the fermentation process and hydrolytic activities during winemaking and by enzymatic hydrolysis in the mouth. Studies have reported that mature grapes show higher levels of glycosylated volatiles that constitute mostly of terpenes, C13-norisoprenoids, and aromatic phenols, when compared with their free counterparts. The aroma glycosides are found to be present in higher proportion on the skin of the grapes in comparison to the pulp and juice, especially monoterpenes (Ferreira & Lopez, 2019; Ghaste et al., 2015; Hjelmeland & Ebeler, 2015).

## Methoxypyrazines

Alkyl-methoxypyrazines (MPs) are nitrogen heterocyclic compounds belonging to the pyrazine group Among the various methoxypyrazines, some such as 2-methoxy-3-isobutylpyrazine (IBMP), 2-methoxy-3-sec-butylpyrazine and 2- methoxy 3-isopropylpyrazine (IPMP) are extremely volatile, with very low odor thresholds in the nanogram per liter range in water. Methoxypyrazines possess a vegetable green aroma reminiscent of green bell peppers, pea pods and depending on the concentration and the compound, earthy nuances. MPs have been identified in bell peppers, pea pods, potatoes, and carrots. At concentrations between 8 to 20 ng/L, MPs may be desirable, however, when

present above these values, it starts to generate an overpowering vegetal, herbaceous aroma (Robinson et al., 2014; Thibon et al., 2012).

Among these compounds, IBMP have been identified in various grape varieties such as Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc, Merlot, Carmenere and Verdejo. IPMP has also been found in Pinot Noir, Chardonnay, Riesling, Chenin Blanc, Gewürztraminer, Syrah, and Pinotage, but in very low concentrations. Most studies have addressed the management of MPs through viticultural practices with particular emphasis on cluster light exposition and grape over ripeness. The exception to this proposition has been in the study of ladybug taint, which is the contribution of 3-isopropyl-2-methoxypyrazine (IPMP) in red wines (Botezatu, Pickering, & Kotseridis, 2014; Jackson, 2017).

## Terpenes

Terpenes are important secondary metabolites in plants for disease and pest resistance. As volatile constituent in grape berries, they are mostly present in the skin in free and bound form. Free terpenes are volatile and odorous monoterpenes (C10) and sesquiterpenes (C15). The first are the major compounds that impart floral, citrus, and sweet notes to the wines made from high terpenic cultivars like Gewurztraminer, Riesling and Muscat due to their low sensory threshold. Terpenes attracts appropriate pollinators in order to ensure reproductive success. Also, the volatile terpene emissions from the fruit attract seed dispersers (Ebeler & Thorngate, 2009; Thibon et al., 2012).

Approximately 90% of the terpenes in grapes, are present as nonvolatile glycosides that can be hydrolyzed (enzymatically or chemically) to the free form during fermentation

and aging. Free monoterpene concentrations in grape berries generally range from 0 to <1000 μg/kg. Among the 70 monoterpenes identified in grapes and wines, linalool, geraniol, nerol, α-terpineol, citronellol, rose oxide, nerol oxide are the most important aroma compound, present in both free and glycosidic forms (Ebeler, 2001; Ebeler & Thorngate, 2009).

The glycoside terpenes are hydrolyzed during fermentation by yeast glycosidase enzymes and by the acidic fermentation conditions. Their composition in grapes can be influenced by climate and viticultural conditions. Generally, in cool climates and shade, terpenes tend to decrease in concentration by mechanisms that are not well understood yet. Although terpenes are liberated by fermentation, the aromatic compound remains unaffected by fermentation, however in grape infection by *B. cinerea*, grapes reduce and modify their terpene content, playing a major role in the minimal varietal character of most botrytized wines. During aging, terpenes change significantly, some increase in sensory impact, as they are liberated from their glycosidic bondage, but losses due to oxidation are more common. In the latter reactions, most monoterpene alcohols are converted to terpene oxides. These have sensory thresholds approximately 10 times higher than their precursors (Ebeler, 2001; Jackson, 2017).

## Norisoprenoids

$C_{13}$-Norisoprenoids are a diverse aroma group derived from grape carotenoids and its abundance can be influenced by the carotenoid profiles of berries. Although more than 600 carotenoids and xanthophylls have been isolated from natural sources, only a few have been identified in grapes and wines, with β-carotene, lutein, neoxanthin, violaxanthin, and

zeaxanthin being the most abundant. Carotenoids accumulate prior to veraison in the grape exocarp (skin) (Ebeler & Thorngate, 2009; Robinson et al., 2014).

Similar to terpenes, many norisoprenoids occur in grapes as non-volatile glycoside precursors and the formation of the $C_{13}$-norisoprenoids occurs from the biodegradation of the parent carotenoid, followed by enzymatic conversion to the aroma, and finally the acid-catalyzed conversion to the aroma-active compound. Once formed, these compounds are then subject to further acid reaction during wine aging (Ebeler, 2001; Jackson, 2017).

There are three major norisoprenoid groups, each containing various odoriferous compounds. The oxygenated magastigmane group (first group) includes compounds such as beta-damascenone, ethyl hexanoate, linalool, ß-ionone, 3-oxo-alpha-ionol, 3-hydroxy-betadamascone and ß-damascone. The second group consists of non-oxygenated megastigmane compounds, with 1-(2,3,6-trimethylphenyl) buta-1,3-diene (TPB) as a major representative. The third group is composed by non-megastigmanes, this group includes some odorous compounds as TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), which smells like kerosene and has a detection threshold of 20 μg/L (Thibon et al., 2012).

## Esters

Numerous acetate esters and ethyl esters of fatty acids contribute characteristic fruity aromas to wines. These esters are formed by yeast during fermentation. Subsequently, slow acid hydrolysis of esters occurs during the aging of wines. More than 160 esters have been isolated from wine. Because some esters have a fruity fragrance, they can contribute significantly to the bouquet of young wines. Their influence tends to be

comparatively short-lived as they hydrolyze back to their acid and alcoholic moieties during aging (Ebeler, 2001; Jackson, 2017).

There are three main categories of vinous esters: those formed between ethanol and short-chain fatty acids, acetic acid and various short-chain alcohols, and nonvolatile acids and ethanol. Ethyl acetate, formed between ethanol and acetic acid, is the most significant wine ester and it may contribute to aroma complexity in wines. Ethyl acetate can be either a byproduct of microbial activity (yeast or bacterial) or form abiotically by a reaction between acetic acid and ethanol. Other than ethyl acetate, the major ethanol-based esters are those formed with higher alcohols, such as isoamyl and isobutyl alcohols. These are often termed fruit esters, because of their fruit-like fragrances. Isoamyl acetate (3-methylbutyl acetate) has a banana-like scent, whereas benzyl acetate has an apple-like aspect. The concentrations of ethyl hexanoate and ethyl octanoate are positively correlated to concentrations of their precursors, hexanoic acid, and octanoic acids, respectively (Robinson et al., 2014; Sumby, Grbin, & Jiranek, 2010).

## 6. Analysis Methods

In the last three decades, a series of methods that will detect, identified, and quantified the different aroma compounds in grapes and wines have been developed. The analysis of volatile compounds in wine is usually performed by gas chromatography (GC) coupled to different detectors like flame ionization (FID) or mass spectrometry (MS). The first methods for sample preparation were done by liquid – liquid extraction using solvents, however, because many volatile and semi-volatile compounds in wine are present in trace amounts, the use and development of more sensitive separation and analytical methods

were designed to measure odor concentrations ranging from ng/L to mg/L. Solid - phase microextraction (SPME) of wine was developed for both headspace (HS) and liquid - phase sampling as a solvent-free pre-treatment. In SPME the analytes are absorbed into a fiber phase until the equilibrium is reached between sample matrix and fiber coating. After equilibrium is reached, the absorbed compounds are thermally desorbed by exposing the fiber into the injection port of a GC (Flamini & Traldi, 2010; Xu et al., 2016).

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# CHAPTER 3

# IMPACTS OF MOLYBDENUM APPLICATION ON BASIC BERRY CHEMISTRY, LEAF ELEMENTAL CONTENT AND WINE AROMA

## ABSTRACT

Molybdenum is a trace element and micronutrient found in soils involved in plant growth crucial for nitrogen metabolism. From 2017 to 2020, a 5% sodium molybdate solution was foliarly applied on *Vitis* interspecific hybrid cv. Vignoles vines in commercial production after grapevine health and wine quality problems were reported and diagnosed as molybdenum deficiency (in the absence of other nutrient issues). Among grape juice parameters: pH, Brix and titratable acidity, in 2017 samples, Brix was higher in molybdenum-treated vines compared with the untreated control, but no difference was found in the rest of the juice parameters. In 2018 and 2020 only titratable acidity showed significant difference between treated and untreated vines, while pH and Brix differences were not significant. Molybdenum leaf concentration after treatment was significantly higher in treated vines across the four years of experiment. In 2017, treated vines molybdenum content was 6.56±3.52 mg/Kg and untreated vines was 0.938±2.02 mg/Kg on leaves sampled at harvest. In 2018, molybdenum deficient vines showed a concentration of 0.067±0.006 mg/Kg while vines treated for two consecutively years showed a molybdenum concentration of 5.04±1,67mg/Kg in samples collected pre-veraison. In 2019, vines treated three years consecutively showed 4.12±2.47 mg/Kg, vines treated in

2017 & 2019 showed 3.41±1.57 mg/Kg, and untreated vines showed 0.224±0.05 mg/Kg of molybdenum on leaves sampled pre-veraison. At the same sample time point in 2020 vines treated four years consecutively exhibit a molybdenum content of 4.71±1.41 mg/Kg while untreated vines exhibit 0.472±0.101 mg/Kg. Ionomic analysis showed difference in leaves elemental composition where boron, sodium, magnesium, phosphorus, sulfur, potassium, calcium, nickel, arsenic, zinc, strontium and cadmium showed significant difference between treated and untreated vines from 2018 to 2020 samples. Vignoles leaf Nitrogen percentage was determined in 2019 and 2020 treated and untreated vines. In 2019 no significant difference was found on N% between vines treated 3 years consecutively, vines treated in 2017 & 2019, and untreated vines. In 2020 N% samples, significant difference was found at veraison and harvest between the different treatments. Wines produced from grapes harvested from the treated and the untreated vines were analyzed via GC-MS to determine the effect of foliar molybdenum application on wine aroma profile. Across the four years of experiment, a diversity of aromas showed difference in concentration between treated and untreated vines. In 2017 the terpenes aroma compounds, D-Limonene, Terpinolene and Linalool, and the ester aroma compound Ethyl Hexanoate were significantly different. In 2018 only the ester Ethyl Hexanoate was found different between treatments. In 2019, the $C_6$ aromatic alcohol 1-Hexanol, and the terpenes Terpinolene and Linalool showed significant difference. In 2020, Terpinolene and $\beta$ −Damascenone presented significant difference between wines made from treated and untreated vines.

# 1. INTRODUCTION

High quality grapes are crucial to produce high quality wines. Wine produced without healthy grapes (free of pathogens, optimal pH, acidity, soluble solids, size and ripens) will result in low quality and unsatisfactory wines, that can affect the economics of the winery and eventually the wine region. Various nutrients influence the berry health and must composition which determines the taste and aroma profile of the wine. These impacts can be due to berry development and vine health; and precursor concentration in the fruit that will be released during fermentation or impact yeast health which, in turn, can manifest in different flavor active metabolites being produced (Jackson, 2014). Other nutrients impact must and wine quality such as potassium (K), that in the vine, can affect grape sugar production, water uptake and color formation in red varieties. In the must, it stimulates fermentation rates by enhancing yeast glucose metabolism, and at unbalanced concentrations causes a stuck fermentation (Schmidt, Dillon, Kolouchova, Henschke, & Chambers, 2011). Phosphorus (P), can affect vine growth and impacts berry composition, ergo must and wine quality (Jackson (2014). Magnesium (Mg), in an elevated ratio with calcium (Ca), can affect the Mg-yeast uptake, increasing the content of acetic acid and acetaldehyde (Birch, Ciani, & Walker, 2003). Vines low in nitrogen (N) generally display low vigor, poor production and slow-ripened fruit due to insufficient photosynthetically active leaf area. During winemaking, yeast requires N in form of free amino nitrogen and ammonia nitrogen in order to perform a complete and desirable fermentation, in low concentrations, yeast metabolism can be impaired resulting in a slow or stuck fermentation and the production of unwanted compounds (Gobert, Tourdot-Marechal, Sparrow, Morge,

& Alexandre, 2019). Far less information is available about micronutrients like molybdenum.

Molybdenum deficiencies have been documented in many plant species where phenotypes range in severity and appearance. However, it hasn't been researched extensively in grapevines, where molybdenum deficiency has been suggested as the primary cause of a bunch development disorder called Millerandage or 'hens and chickens'. This disorder is characterized by grapevine bunches that develop unevenly, where fully matured berries are present in a bunch alongside a large number of fertilized underdeveloped berries, as well as unfertilized swollen green ovaries and less nitrogen assimilation by the grapevine (Williams, Maier, & Bartlett, 2004). Molybdenum deficiency may induce symptoms similar to those of nitrogen deficiency, which is the reason why Mo deficiency can be easily misidentified as nitrogen deficiency, because molybdenum is required for the assimilation of nitrate taken up by the plant. Such symptoms would include a general pale green color, stunted growth with small leaf size, and possible reddening of veins on the young leaves. (Kaiser, Gridley, Ngaire Brady, Phillips, & Tyerman, 2005; Kovacs, Puskas-Preszner, Huzsvai, Levai, & Bodi, 2015)

The availability of molybdenum for plant growth is strongly dependent on the soil pH, concentration of adsorbing oxides (e.g. Fe oxides), extent of water drainage, and organic compounds found in the soil colloids. In alkaline soils, molybdenum becomes more soluble and is accessible to plants mainly in its anion form. Conversely, in acidic soils (pH <5.5) molybdenum availability decreases as anion adsorption to soil oxides increase (Tejada-Jimenez, Chamizo-Ampudia, Llamas, Galvan, & Fernandez, 2018).

In 2017, this research started in response to a commercial vineyard and winery that reported problems with grapevine health, after analysis, vines were diagnosed as molybdenum deficient (in the absence of other nutrient issues). In every variety, vines exhibited poor fruit set, growth, yield and did not responded to nitrogen application. Additionally, the producer reported an "off" character, described as a subdued intensity in the wine aromas across deficient grape varieties.

Molybdenum (Mo) deficiency in grapevines is poorly understood, yet a potentially widespread viticultural problem. In molybdenum deficient grapevines, previous research has shown that treating the deficiency with a Mo pre-flowering foliar application, was effective in terms of producing a significant yield increase compared to the control (Williams et al., 2004). However, no investigations have been made into Mo deficiency and its relationship with wine flavor.

# 2. MATERIALS AND METHODS

## 2.1. Vineyard treatments

The study was conducted for four years (2017-2020) in a commercial vineyard in Ste. Genevieve, Missouri (37°46'43.9"N 90°11'23.4"W), at 680 feet ASL with Fourche silt loam, 3% slope average. Grafted six years old (at the time of the first-year treatment) *Vitis* hybrid Vignoles on 3309C rootstock were trained to high bilateral cordon, top wire at 1.73 meters (68 inches) approximately and planted in southwest/ northeast-oriented rows with vine/row spacing of 2.13 x 3.05 meters (7 x 10 feet). Both the untreated control vines and the treated vines received normal commercial management for the region across the four years of experiment.

In the first year 2017, treated vines received two foliar applications of 1 gallon per acre (gal/A) of potassium acetate ($CH_3CO_2K$) and 1.5 pint per acre (pint/A) of 5% sodium molybdate ($Na_2MoO_4$); The first application was performed in mid-July and the second in late July. In 2018 the same treatment dose and mix as the previous year (1 gal/A of $CH_3CO_2K$ and 1.5 pint/A of 5% $Na_2MoO_4$) was applied two times at vine flowering stage: mid-May and late May (between 18 and 21 E-L stages), 2018 application was performed earlier as this fit better with literature and treatment supplier recommendations. With the interest to observe if 2017 treatment had any carryover effect on molybdenum content on treated vines, two 2017 treated rows were left untreated, with this design, 2018 treatment conditioned were: Treated 2017 & 2018, treated only 2017 and untreated. In 2019, potassium. In 2019, potassium acetate was not included in the foliar application of molybdenum as poor uptake was observed in 2018, while it had initially been included due

to supplier instructions, a change in the treatment was made, only 2 pint/A of 5% sodium molybdate was foliarly applied two times at vine flowering stage: mid-May and late May (between 18 and 21 E-L stages) except for the untreated (control) vines. As in 2018, three conditions were stablished: Treated 3 years consecutively (2017, 2018 & 2019), treated 2017 & 2019 (untreated on 2018) and untreated. In 2020, the commercial vineyard allowed the experimental treatments to extend to more rows within the Vignoles block previously used since 2017, with this expansion, a new set of treatments were stablished in the interest of observe the effects of molybdenum application at different time points along with the addition of potassium acetate as it was used in 2017 and 2018 treatments. A total of five treatments plus the untreated (control) vines were performed on 2020 vines: Treatment 1A and 1B consisted in the application of 2 pint/A of 5% sodium molybdate two times at mid veraison that in 2020 happened at late July (same as the 2017 treatment time points), the only difference between treatment 1A and 1B was the addition of 1gal/A of potassium acetate (as previously used in 2017 and 2018 treatments). Treatment 2A and 2B consisted in the application of 2 pint/A of 5% sodium molybdate two times at vine flowering stage late May and early June (between 18 and 21 E-L stages), the only difference between treatment 2A and 2B was the addition of 1gal/A of potassium acetate (as previously used in 2017 and 2018 treatments). Treatment 3 consisted in two applications of only 2 pint/A of 5% sodium molybdate at two time points, the first dose was applied at vine flowering stage on late May and the second dose was applied at mid veraison on late July. A resume of number of treatments and their conditions is presented in table 1.

Table 1. Vignoles vines treatments, application times and rows used from 2017 to 2020.

| Year | Treatments | Applications | Time points | Rows |
|---|---|---|---|---|
| 2017 | Untreated | - | Dose 1: Mid July<br>Dose 2: Late July | 15 & 16 |
| | Applied | 1 gal/A potassium acetate and 1.5 pint/A 5% sodium molybdate | | 9, 10, 12, 13 & 18 |
| 2018 | Untreated | - | | 15 & 16 |
| | Treated 2017 only | - | | 9 & 10 |
| | Treated 2 years | 1 gal/A potassium acetate and 1.5 pint/A 5% sodium molybdate | Dose 1: Mid May<br>Dose 2: Late May | 12, 13 & 18 |
| 2019 | Untreated | - | | 15 & 16 |
| | Treated 2017 & 2019 | 2 pint/A 5% sodium molybdate | Dose 1: Mid May<br>Dose 2: Late May | 9 & 10 |
| | Treated 3 years | | | 12, 13 & 18 |
| 2020 | No treatment | - | - | 9 & 10 |
| | Treatment 1A | 1 gal/A potassium acetate and 2 pint/A of 5% sodium molybdate | Dose 1: Late July<br>Dose 2: Late July | 3 & 4 |
| | Treatment 1B | 2 pint/A of 5% sodium molybdate | | 6 & 7 |
| | Treatment 2A | 2 pint/A of 5% sodium molybdate | Dose 1: Late May<br>Dose 2: Early June | 12 & 13 |
| | Treatment 2B | 1 gal/A potassium acetate and 2 pint/A of 5% sodium molybdate | Dose 1: Late May<br>Dose 2: Early June | 15 & 16 |
| | Treatment 3 | 2 pint/A of 5% sodium molybdate | Dose 1: Late May<br>Dose 2: Late July | 18 |

## 2.2. Sampling

From 2017 treated and untreated vines, leaves samples and fruit for winemaking were collected at harvest. From the three conditions set on 2018 (treated 2017 & 2018, treated only 2017 and untreated), leaves samples were taken at three time points: Pre-veraison on June 22$^{nd}$, veraison on July 20$^{th}$ and harvest on August 21$^{st}$. Fruit for winemaking were collected at the same day as harvest leaves sampling. From 2019 three conditions set: treated 3 years consecutively (2017, 2018 & 2019), treated 2017 & 2019 (untreated on 2018) and untreated, leaves samples were taken at four time points: 2 weeks after first dose (late May), Pre-veraison on June 20$^{th}$, veraison on August 8$^{th}$ and harvest on August 22$^{nd}$. Fruit for winemaking were collected on the same day that harvest leaves were sampled.

From the 2020 six conditions, treatment 1A & 1B, treatment 2A & 2B, treatment 3 and untreated (control), leaves samples were taken at three time points: Pre-veraison on June 20$^{th}$, veraison on July 30$^{th}$ and at harvest on September 1$^{st}$. Fruit for winemaking was collected at the same day as harvest leaves sampling. Across the four years of experiment, leaves and grape samples were collected manually, stored in zip top plastic bags, labeled, and kept at 4 °C until analysis.

## 2.3. Juice analysis: pH, Brix & Titratable Acidity

Brix were measure on the grape juice after pressing, using a benchtop digital refractometer. The pH was measure on the grape juice after pressing, using a benchtop pH meter. Titratable Acidity was measured using a Mettler Toledo™ G20 Potentiometric

Titrator for Food, where 2 ml of juice were added to 48 ml of distilled water and titrated with 0.1N NaOH until a pH of 8.2 was reached. The amount of NaOH given by the Potentiometric Titrator was then multiplied by the stablished factor of 3.77 in order to obtain the titratable acidity expressed as grams of tartaric acid per liter of juice.

## 2.4. Leaf molybdenum quantification

The early season, veraison and harvest leaves samples were placed between paper towel sheets and let dried in a drying oven at 60°C for 24hrs. Once dried, leaves were ground and storage in labeled plastic cups until analysis. For digestion, approximately 0.4g of dried leaf were placed in a microwave digestion tube along with 10mL nitric acid. The tubes were tightly screwed and placed into the microwave digestion rack following the proper arrangement. The CEM Mars 5 Digestion Microwave Oven was calibrated and edited to perform the digestion under the next conditions: 300W for 30 min & 1200W for 30 min. After digestion, tubes were allowed to cool down overnight, the resulting digestion was flask up to 50mL on a grad cylinder, decanted into a 50mL falcon tube and stored at 4.0°C until analysis.

Molybdenum quantification in 2017 was done using an Agilent 4200 MP-AES (Microwave Plasma-Atomic Emission Spectroscopy) equipped with autosampler SPS4, nebulizer and a single-pass glass cyclonic spray chamber and controlled by the Agilent MP Expert Software . A standard solution of 5% nitric acid was used as a blank, along with a molybdenum six-point calibration curve from 0.0156 ppm to 5 ppm. Mo wavelengths used for quantification were 379 and 386 nm.

## 2.5. Leaf elemental quantification

Molybdenum content in 2018 and 2019 leaves samples were lower than the MP-AES was able to detect as concentration fell below the detection limit, therefore, it was decided to send all the extracts available to the Baxter Ionomics lab at the Donald Danforth Plant Science Center, St. Louis, MO, where an elemental analysis was done on 2018, 2019 and 2020 leaves' extract using an ICP-MS

## 2.6. Leaf nitrogen quantification

The leaf nitrogen quantification was performed by the Soil and Plant Testing Laboratory of the University of Missouri, using the Total Kjeldahl nitrogen (TKN) method. From each sampled vine, one gram of dried and grounded leaves previously described for the molybdenum quantification method, were sent to the Soil and Plant Testing Laboratory in a 15 mL centrifuge tube.

## 2.7. Wine making

Fruit was harvested at the above dates for each of the four years. In 2017, 2018 and 2019 fruit were hand harvested by treatment block, homogenized and then processed. In 2020 sub-samples were taken, frozen and held at -80 °C until micro-fermentations could be performed due to logistical challenges of the pandemic. In all years, fermentations were conducted in triplicate, in 15L columns for the first three years and in a 50ml volume for 2020.

For the first three years, grapes from various treatments were destemmed and crushed, then pressed in a small 15L hydraulic bladder press. The juice was cold settled (5$^{O}$C) for 24 hours, racked, and inoculated with *Saccharomyces cerevisiae* strain DV10 yeast (Lallemand, Petaluma, CA) previously rehydrated in 260mg/L GoFerm (Lallemand) according to manufacturer's guidelines. Fermentation was conducted at 13$^{O}$C until dry, which was determined using Clinitest (Bayer). In 2020 random samples from treatment panels were crushed in plastic bags by hand, juice extracted through cheese cloth and clarified by centrifuge. Fermentations were conducted as above, with wines re-centrifuged and racked into a new sample tube at the end of fermentation.

## 2.8. GC-MS wine aroma analysis

A 65 µm polydimethylsiloxane (PDMS)/divinylbenzene (DVB) 1cm solid phase microextraction fiber (SPME), 23 gauge (Supelco) was used for volatile extraction. Fibers were conditioned before use according to the manufacturer's recommendations. The samples (grape extracts and wines), in a 15 ml amber glass vials, were preincubated for 15 minutes at 45 °C to ensure consistent temperature during extraction. The fiber was exposed for 45 min at 45 °C in the headspace above the sample prior to GC-MS analysis. All samples were agitated at 500 rpm during extraction.

The HS-SPME GC-MS system consisted of a PAL autosampler (Varian) mounted on an Agilent 7890B gas chromatograph (Santa Clara, CA, USA) coupled with an Agilent 5977A mass selective detector (MSD). The 65 µm PDMS/DVB SPME fiber was desorbed in the inlet at 250 °C for 2 min in spitless mode (inlet glass liner/SPME direct, 0.75mm I.D,

Supelco), after which the spit flow was turned on (50mL/min) for the remainder of the GC-MS run; the SPME fiber was conditioned in the inlet for 14.7 min before it was inserted into the next sample. A DB-WaxETR column (30m x 0.25mm I.D, 0.25 μm film thickness; Agilent, Santa Clara, CA, USA) and helium carrier gas at a flowrate of 1.2mL/min was used for all analyses. The GC oven temperature program was as follows: the initial temperature was 40 ˚C for 1.0 min then was increased to 200 ˚C at 5 ˚C /min followed by a second increase at 12 ˚C /min to the final temperature of 240 ˚C, which was held for 10min. the mass selective detector was operated in SIM/Scan mode, including SIM parameters for allowing adequate β-damascenone detection limits (121 m/z;190m/z, range 40-250 m/z; 6.4 scans/s), and the MS transfer line was maintained at 240 ˚C.

## 2.9. Statistical Analysis

All data was analyzed with Minitab 18® Statistical Software and GraphPad Prism version 9.0 using one-way ANOVA followed by Tukey's HSD.

## 3. Results & Discussion

The effects of foliar molybdenum application on deficient vines found across the four years of treatments and experiments, are not limited only to be a consequence of the presence or lack of molybdenum. Application time points, yearly accumulation effect and usage of the mixture sodium molybdate and potassium acetate (recommended by supplier), were other variables analyzed in this research project. The results and discussions are presented in four influence factors: treatment impact, potassium acetate impact, annual application impact and application timing impact.

Treatment impact: Molybdenum deficiency in grapevines has not been extensively researched since is a rare phenomenon, although, when encountered, it has damaging consequences. When applied to deficient vines, molybdenum deficient *Vitis* vinifera cv. Merlot grapes, yield, bunch weight and berry weight increased significantly when compared with the poor growth deficient Merlot grapes (Longbottom, Dry, & Sedgley, 2010; Williams et al., 2004). This treatment impact is the first and most direct approach to observe the effect of foliar sodium molybdite application.

Potassium acetate impact: In 2017 and 2018 treatments, potassium acetate was mix with sodium molybdate and foliarly applied selected vines at fertilizer supplier recommendations. After observing no late season molybdenum leaf concentration on treated vines in 2018, the idea of comparing the effect of molybdenum application with and without potassium acetate in the mix, was planned for 2019 treatments. For logistical reasons in 2019, it was only possible for the grower cooperator to do the treatments without potassium acetate, however, in 2020 treatments, direct comparison were possible.

Treatment 1A and Treatment 2B consisted in the application of sodium molybdate plus potassium acetate at two different time points each (veraison and flowering respectively), while Treatment 1B and Treatment 2A consisted in only sodium molybdate at to different time points each (veraison and flowering respectively) also.

Annual application impact: With the aim to determine if there was a carryover effect of foliar molybdenum application, a block treated in 2017 did not receive any molybdenum treatment in 2018 but was sampled and analyzed. In 2019 the same block was treated with the purpose to observe if molybdenum application had an impact between continuously year application (block treated 2017, 2018 and 2019) and annual application (block treated 2017, untreated 2018 and treated 2019). And in 2020 the same block was left untreated so a comparison could be made between treatment intermittent application and four consecutively years of application.

Application timing impact: Because there is not much research regarding foliar molybdenum application on grapevines, it is still not clear if there is a specific timepoint within vine growth when the best results for molybdenum application can be obtained. While one study done with *Vitis vinifera* cv. Merlot, applied molybdenum at flowering stage (Longbottom et al., 2010), another study done with *Vitis vinifera* cv. Sangiovese, applied one block with molybdenum at early flowering, while a second block was provided with three applications respectively in early flowering, early fruit set and early veraison (Masi, 2011). In 2017, the first year of study, the molybdenum application was done around mid veraison (mid and late July), in 2018, after observing significant difference between treated and untreated samples, and with more time for planning, it was stablished

that the application in the consecutive years should be at flowering. As it happened with potassium acetate, for logistical reasons with grower cooperator, it was until 2020 when a direct comparison between application timepoints could be tested. Treatments 1A and 1B were applied at late July (mid veraison), while Treatments 2A and 2B were applied at late May (flowering), Treatment 3 received half the treatment at late May and the other half at Late July.

## 3.1. Juice analysis: pH, Brix & Titratable acidity

### 3.1.1. Treatment impact

Among the parameters measured on grape juice, the application of molybdenum on the deficient vines had generally improved or had no impact on quality. In 2017 samples, Brix was higher in molybdenum-treated vines (22.98 $\pm$ 0.71), when compared with the untreated control (21.69 $\pm$ 0.45), however no difference was found in pH or titratable acidity. Although sugar concentration (Brix) on molybdenum treated and deficient *Vitis* hybrids vines has not been previously reported, there is research that showed an significant increment of Brix on *Vitis vinifera* cv. Sangiovese previously applied with molybdenum (Masi, 2011), and in strawberry grown in a soilless system without molybdenum (Liu et al., 2017). Masi in 2011 reported that Sangiovese treated grapes contained 23.9 Brix, while untreated grapes contained 22.6 Brix. Liu et al., 2017 reported that the total sugar content on treated strawberries was 90.034 $\pm$ 3.39 g/Kg and untreated strawberries was 70.30 $\pm$ 3.05 g/Kg fresh weight. Research performed in four tomato genotypes treated with molybdenum, presented that Brix were different not only between treatments but also between genotypes treated with the same amount of molybdenum (Sabatino et al., 2018).

However in Red Jonaprince apples, the application of molybdenum on deficient apple trees was not reflected in changes in Brix concentration between untreated and treated trees, nevertheless, difference was found on starch index (Wójcik, 2020).

In 2018 only titratable acidity showed significant difference between treated vines with 3.49 $\pm$ 0.34 g/L tartaric acid, and untreated vines with 3.04 $\pm$ 0.29 g/L tartaric acid; pH and Brix were not significant. Research done in *Vitis vinifera* cv. Merlot (Longbottom et al., 2010) and *Vitis vinifera* cv. Sangiovese (Masi, 2011), both treated with molybdenum, don't report significant changes in titratable acidity between untreated and treated vines. In contrast, Liu et al., 2017 reported that strawberries treated with molybdenum showed a significant increment on titratable acidity when compared with untreated strawberries.

Grape juice samples from 2019 and 2020 didn't show significant difference in pH, soluble solids (Brix) and titratable acidity related with treatment impact, despite significant differences and deficiencies observed in some of the treatments (see below).

### 3.1.2. Potassium acetate impact

Molybdenum treatments designed in 2020, were the only ones where a direct comparison between the effect of molybdenum treatment with and without potassium acetate could be observed, however, no significant difference was found between treated and untreated vines regarding the combined application of molybdenum and potassium acetate. This comparison was made as a consultant working with the grower-cooperator, initially suggested potassium acetate was needed for adequate foliar uptake of molybdenum, a combination which also has been used in several studies. However, as the

two are not always used in conjunction and limited evidence has been published supporting the synergistic effects, potassium acetate was not included in 2019 treatments after 2018 treatments did not improve late season molybdenum content.

### 3.1.3. Annual application impact

No carryover effect (i.e. treatment from a previous year increasing the following year's molybdenum) was observed in any of the four years of analysis regarding the juice measured parameters. This absence of carryover effect in annual molybdenum application has been also observed by Longbottom et al., 2010, where it is reported that across four years of molybdenum foliar application on deficient *Vitis vinifera* cv. Merlot grapes, no significant difference was found on basic berry chemistry. This may in part be to the method of application, and if soil applied molybdenum was used rather the foliar application so lasting impact could occur.

### 3.1.4. Application timing impact

Significant difference was found only in titratable acidity between two blocks treated at different timepoints, Treatment 1B block (applied at mid veraison) presented $9.52 \pm 0.88$ g/L tartaric acid and Treatment 2B block (applied at flowering) presented 11.02 $\pm 0.84$ g/L tartaric acid, Treatment 1A, 2A, 3 and untreated block were not significantly different.

Mean and standard deviation for pH, Brix and titratable acidity (TA) values from 2017 to 2020 juice are presented in table 2.

Table 2. Grape juice pH, soluble solids (Brix) and titratable acidity of treated and untreated vines from 2017 to 2020

| | pH | | Brix | | TA (g/L tartaric acid) | |
|---|---|---|---|---|---|---|
| **2017** | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| Applied | **3.19** | 0.04 | **22.98**a | 0.71 | **7.93** | 0.66 |
| Untreated | **3.17** | 0.04 | **21.69**b | 0.45 | **7.76** | 0.35 |
| **2018** | | | | | | |
| Untreated | **3.61** | 0.04 | **20.54** | 0.65 | **3.04**b | 0.29 |
| Treated 2017 only | **3.61** | 0.03 | **20.61** | 0.83 | **3.58**a | 0.42 |
| Treated 2 years | **3.60** | 0.04 | **20.61** | 0.60 | **3.49**a | 0.34 |
| **2019** | | | | | | |
| Untreated | **3.43** | 0.05 | **18.55** | 0.84 | **8.64** | 0.60 |
| Treated 2017 & 2019 | **3.44** | 0.04 | **18.06** | 0.94 | **8.80** | 0.53 |
| Treated 3 years | **3.46** | 0.05 | **17.68** | 1.51 | **8.63** | 0.47 |
| **2020** | | | | | | |
| Treatment 1A | **3.36** | 0.07 | **21.43** | 0.84 | **10.33**ab | 0.41 |
| Treatment 1B | **3.05** | 0.05 | **21.05** | 0.56 | **9.52**b | 0.88 |
| No treatment | **3.39** | 0.06 | **20.08** | 2.06 | **10.28**ab | 1.00 |
| Treatment 2A | **3.42** | 0.02 | **20.97** | 0.35 | **10.58**ab | 0.57 |
| Treatment 2B | **3.38** | 0.07 | **21.22** | 0.65 | **11.02**a | 0.84 |
| Treatment 3 | **3.45** | 0.05 | **21.43** | 0.67 | **10.01**ab | 0.17 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$)

## 3.2. Leaf molybdenum quantification

### 3.2.1. Treatment impact

From the first year of the study, the impact of molybdenum application could be observed between the deficient vines and the foliarly treated vines. In 2017, the treated vines leaves sampled at harvest presented a molybdenum content of 10.3 $\pm$ 5.31 ppm and

the untreated vines a content of 1.88 ± 3.59 ppm when read at 379nm (figure 1) and 6.56 ± 3.52 ppm and the untreated vines a content of 0.938 ± 2.02 ppm when read at 386nm via MP-AES. The critical deficiency or marginal concentration levels of molybdenum in plant tissue varies between crops, cultivars and parts being analyzed. In poinsettia, deficiency critical level has been reported at <0.5 mg/Kg (Cox, 1992). However, in fruit and crops a molybdenum deficiency occurs at concentrations below 0.05 mg/Kg or ppm, and low or marginal molybdenum in concentrations have been consider to happened between 0.06 and 0.19 mg/Kg or ppm, while optimum molybdenum values are above 0.2 mg/Kg or ppm (Kadyampakeni & Morgan, 2020; Longbottom et al., 2010) . Although molybdenum content results in deficient leaves samples at values above the deficient limit (<0.05 mg/Kg), and a larger standard deviation, there was no doubt that the studied vine presented molybdenum deficiency symptoms. The larger variation in the untreated vine leaves values occurred because many of the results, fell below the lower point of the calibration point (0.0156 ppm of molybdenum) and the MP-AES detection limit. However, it is possible to observe that the treated vine leaves values increased with the foliar application of molybdenum.

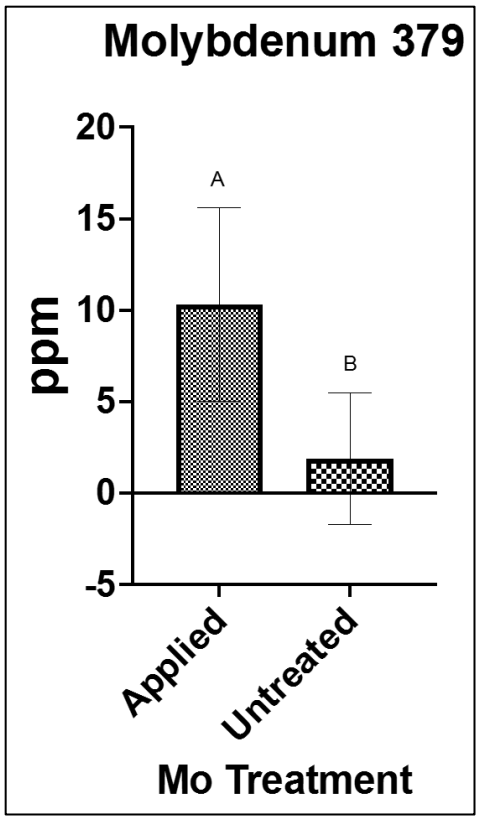


Figure 3. Molybdenum quantification on treated and untreated 2017 dried vine leaves sampled at harvest, analyzed through MP-AES at 379 nm. Means with different letters indicate statistical difference between treatments (unpaired T-test, $p<0.05$)

In the second year of research, significant differences (p value<0.05) were found between the three treatment conditions, on vine leaves sampled at late June (late-berry formation stage). Leaves from vines treated for 2 consecutive years presented a molybdenum concentration of $5.04 \pm 1.67$ mg/Kg while leaves from vines treated in 2017 only and untreated vines presented a concentration of $0.133 \pm 0.005$ mg/Kg and $0.067 \pm 0.006$ mg/Kg respectively, vines treated only in 2017 and untreated were not significantly different between them (figure 2).

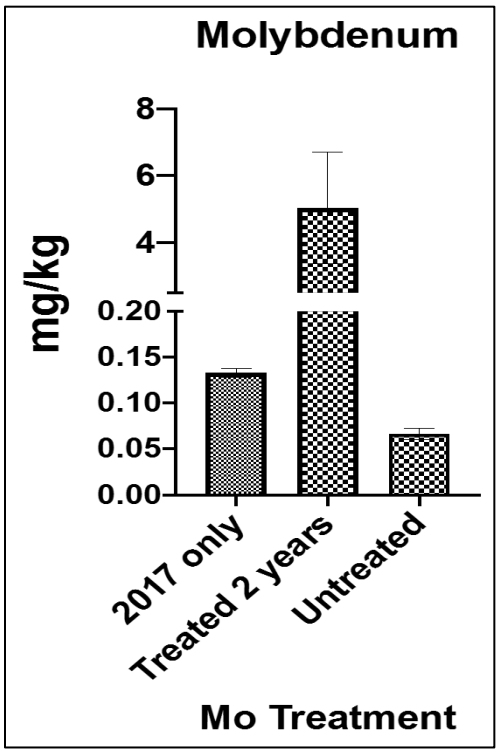


Figure 4. 2018 dried leaves molybdenum quantification from vines treated 2 years, treated only 2017 and untreated sampled in June (early season) before treatment application, analyzed by ICP-MS. Means with different letters indicate statistical difference between treatments ($p<0.05$)

Veraison and Harvest molybdenum quantification did not present significant difference between conditions, however, the leaf samples from the two years of consecutive treatment had slightly more molybdenum that the other two treatment conditions. The leaf molybdenum content from the three treatments at the three sample timepoints is presented in table 3. It is possible to observe that, in 2018 untreated leave vines samples, molybdenum amounts fell inside the marginal range previously mentioned (0.06 - 0.19 mg/Kg).

Table 3. Molybdenum concentration on mg/Kg measured by ICP-MS in three collection timepoints from 2018 vine treatments

| Molybdenum | June | | Veraison | | Harvest | |
|---|---|---|---|---|---|---|
| | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| Treated 2 years | **5.04**a | 1.67 | **0.275** | 0.208 | **0.162** | 0.091 |
| Treated 2017 only | **0.133**b | 0.005 | **0.092** | 0.033 | **0.090** | 0.040 |
| Untreated | **0.067**b | 0.006 | **0.102** | 0.055 | **0.079** | 0.051 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$). Only presenting elements that showed significant difference in at least one timepoint measurement.

In the third year of study, samples collected at all timepoints presented significant difference between treated and untreated vines (table 4). In 2019 vines that had been treated for 3 consecutively years showed a molybdenum concentration of 4.12± 2.47 mg/Kg, 1.09 ± 0.45 mg/Kg and 1.01 ± 0.90 mg/Kg in June, veraison and harvest respectively. Vines treated in 2017, left untreated in 2018 and treated again in 2019, presented a molybdenum concentration of 3.41± 1.57 mg/Kg at June, 1.97 ± 2.46 mg/Kg at veraison and 1.04 ± 0.84 mg/Kg at harvest. Both conditions (treated 3 years and treated 2017 & 2019) were significantly higher when compared with untreated vines leaves that presented a molybdenum concentration of 0.22± 0.05 mg/Kg at June, 0.09 ± 0.03 mg/Kg at veraison and 0.08 ± 0.03 mg/Kg at harvest. Similarly to 2018 untreated samples, the molybdenum concentration in 2019 untreated vines fell into the low-marginal range mentioned on references (Kadyampakeni & Morgan, 2020).

Table 4. Molybdenum concentration on mg/Kg measured by ICP-MS in three collection timepoints from 2019 vine treatments

| **Molybdenum** | **June** | | **Veraison** | | **Harvest** | |
|---|---|---|---|---|---|---|
| | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| 3 years | **4.119**a | 2.47 | **1.087**ab | 0.452 | **1.007**a | 0.904 |
| 2017 & 2019 | **3.407**a | 1.57 | **1.970**a | 2.479 | **1.038**a | 0.837 |
| Untreated | **0.224**b | 0.05 | **0.093**b | 0.029 | **0.080**b | 0.032 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$). Only presenting elements that showed significant difference in at least one timepoint measurement.

In the fourth and last year of study, molybdenum concentration difference was also found between treated and untreated vines. In June samples, vines treated at flowering presented a molybdenum content range between 3.06 and 9.29 mg/Kg, while untreated vines presented a range between 0.472 and 0.0576 mg/Kg. At veraison, vines treated two weeks before sampling exhibited a concentration range between 12.51 and 31.38 mg/Kg, the vines treated at flowering exhibited a range between 1.87 and 3.32 mg/Kg, while untreated vines exhibited $0.046 \pm 0.14$ mg/Kg. At harvest, the vines treated at flowering, showed a molybdenum concentration between 1.32 – 1.81 mg/Kg, the vines treated at veraison showed a concentration between 5.52 – 12.75 mg/Kg, and the untreated vines showed $0.34 \pm 0.14$ mg/Kg of molybdenum.

Williams et al., 2004 reported that deficient *Vitis vinifera* cv. Merlot presented a petiolar molybdenum concentration in a range between 0.05 – 0.09 mg/Kg at flowering and 0.07 – 0.14 mg/Kg at veraison, at the same time, foliarly treated Merlot vines presented a petiolar concentration range of 2.10 – 25.04 mg/Kg at flowering and 1.44 – 28.04 mg/Kg at veraison. Longbottom et al., 2010 reported also in Merlot a petiolar molybdenum content in a range of 0.058 – 0.186 mg/Kg on deficient vines, and in a range of 5.43 – 28.47 mg/Kg

of molybdenum from samples collected at 80% flowering, the treatments were applied on early stages of inflorecence development.

### 3.2.2. Potassium acetate impact

From 2017 to 2018, due to fertilizer supplier recommendations, potassium acetate was applied along with the molybdenum treatments. However, in 2018 samples, molybdenum concentration was very low even in treated vines. A study made on eggplants, reported that when potassium supply is increased, a lower molybdenum concentration in leaf blades can be observed, while at 0.5 mM of potassium sulfate ($K_2SO_4$), molybdenum concentration in eggplant leaf reached 1.9 mg/Kg and 1.3 mg/Kg in petioles, at 2 mM of potassium, molybdenum decreased to 1.3 mg/Kg on leaf and to 0.6 mg/Kg on petioles (Villora, Moreno, & Romero, 2002). With that observation, it was decided to test in 2019 treatments, how potassium acetate affects molybdenum concentration in molybdenum deficient vines, but for logistical reasons, the grower cooperator could only perform one condition among the already decided treatments (treated 3 years consecutively, treated 2017 & 2019 and untreated), therefore all the treatment in 2019 were done without potassium acetate. It was until 2020 when a direct comparison between applied molybdenum and applied potassium along with molybdenum could be tested.

Significant difference in molybdenum concentration was found between 2020 samples treated with and without potassium acetate (table 5). In June samples, treatment 2A without potassium showed a molybdenum concentration of 4.71 $\pm$ 1.41mg/Kg and treatment 2B, with potassium, showed a higher concentration of 9.29 $\pm$ 1.44 mg/Kg, simultaneously, treatment 2A and treatment 2B, which application was performed at late

May and early June, were significantly higher than untreated vines. In veraison samples, treatment 1B without potassium, showed a concentration of 12.51 ± 3.81 mg/Kg, while treatment 1A with potassium showed 31.38 ± 14.07 mg/Kg, both significantly difference between themselves and the untreated vines.

Table 5. Molybdenum concentration on mg/Kg measured by ICP-MS in three collection timepoints from 2020 vine treatments

| **Molybdenum** | | **June** | | **Veraison** | | **Harvest** | |
|---|---|---|---|---|---|---|---|
| | | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| 1A | Treatment | **0.538**c | 0.136 | **31.38**a | 14.07 | **12.75**a | 3.8 |
| 1B | Treatment | **0.576**c | 0.097 | **12.51**b | 3.81 | **5.524**b | 2.20 |
| 2A | Treatment | **4.711**b | 1.410 | **1.868**bc | 1.250 | **1.322**c | 0.58 |
| 2B | Treatment | **9.290**a | 1.437 | **3.321**bc | 1.124 | **1.807**c | 1.18 |
| 3 | Treatment | **3.057**b | 1.436 | **4.73**bc | 1.76 | **2.564**bc | 0.98 |
| | Untreated | **0.472**c | 0.101 | **0.459**c | 0.137 | **0.342**c | 0.143 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$). Only presenting elements that showed significant difference in at least one timepoint measurement.

Contrary to the potassium effect on molybdenum absorption in eggplant reported by Villora et al., 2002, other researchers have published a different relationship between potassium and molybdenum. Low supplies of potassium reduce the uptake of molybdenum from soil, leading to an insufficient nitrogen utilization (Ranade-Malvi, 2010). In *Brassica napus*, low concentrations of potassium have been proved to decrease the uptake of molybdenum along with other micronutrients (Maillard et al., 2016; Tejada-Jimenez et al., 2018).

### 3.2.3. Annual application impact

Although no impact regarding yearly application was observed, the outcome is common among yearly molybdenum treatments. Williams et al., 2004, after three years of study, reported variability on molybdenum petiolar content in each year of study. In each year, the amount of molybdenum quantified on treated and untreated vines were different but nevertheless different between treatments within each year. Longbottom et al., 2010 made a more direct tests regarding the carryover effect of previously treated vine; vines in a block that had been treated in 2003-04, were not treated in 2004-05, and those vines presented a non-significant low concentration, similar to the untreated vines. Wójcik, 2020, did not found difference between two years of treatemnt on molybdenum among treated and untreated apple trees.

### 3.2.4. Application timing impact

Similar to potassium acetate impact, application timing impact was determined only on 2020 samples. treatments 2A and 2B received the foliar molybdenum application at flowering (1$^{st}$ does in late May, 2$^{nd}$ dose Early June), both treatments presented higher molybdenum concentration in June samples when compared to treatments 1A, 1B and untreated, because treatment 3 received the first molybdenum dose in late May and the second dose in late July (same time as treatment 1A and 1B 1$^{st}$ dose), treatment 3 molybdenum content was also higher than treatment 1A, 1B and untreated, but lower that treatment 2B. At veraison, Treatment 1A and 1B showed higher molybdenum content

when compared with the other treatments, this happened because treatments 1A and 1B received the two doses of molybdenum application in late July, both treatments had more molybdenum content that the one quantified on treatments 2A and 2B in June. Treatment 3 showed a similar molybdenum content as the one quantified in June samples. At harvest treatments 1A and 1B remained highest in molybdenum content, while treatments 2A and 2B presented molybdenum concentrations similar to the ones quantified in veraison samples.

## 3.3. Leaf elemental quantification

The elemental analysis performed by the Baxter Ionomics lab at the Donald Danforth Plant Science Center returned with the twenty elements quantified in 2018, 2019 and 2020 leaves samples. The elements were: boron, sodium, magnesium, aluminum, phosphorus, sulfur, potassium, calcium, iron, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, rubidium, strontium, molybdenum, and cadmium. The results obtained on molybdenum were presented in section 3.2 of this chapter. In this section, presented are only the elements that showed significant difference in at least one collection time point between the samples in each year of experiment.

### 3.3.1. Treatment impact

In 2018 samples, magnesium only showed a significant difference in harvest, where untreated vines presented higher content of magnesium, untreated vines were significantly higher that vines treated 2 years consecutively but no significant difference was found between treated 2017 only and untreated or between treated 2017 only and treated 2 years.

Phosphorus showed higher content in untreated vine when compared to vines treated for 2 years and vines treated only in 2017 in June samples, this behavior is opposite to the one found in *Brassica napus*, five rice cultivars where phosphorus shoot uptake increased with molybdenum addition when phosphorus was sufficient(Hongen et al., 2010; H Zakikhani, Khanif, Anuar, Radziah, & Soltangheisi, 2014; Hamed Zakikhani, Yusop, & Soltangheisi, 2014), however, Williams et al., 2004, reported a decreased petiolar phosphorus concentration in deficient *Vitis vinifera cv.* Merlot treated with molybdenum. The decreased phosphorus content when molybdenum has been applied, was also found in mustard plant (Chatterjee, Nautiyal, & Agarwala, 1985). No difference was found in phosphorus content between treatments in veraison and harvest samples.

Sulfur showed a significant difference only in harvest samples but not in June and veraison samples. Sulfur did not present a clear pattern in its treatment concentration, untreated vines and vines treated 2 years consecutively possessed the higher value compared with vines treated only in 2017. Arsenic concentration was only significantly different among treatment in June samples, where vines treated only in 2017 were significantly higher than untreated vines and vines treated 2 years. The metalloid arsenic is considered one of the most dangerous environmental pollutants with serious health repercussions if consumed in high doses. The arsenic concentration in plants is usually less than 1.0 mg/Kg, and when presented in more that 1% of the plant dry weight, it is considered dangerous, like grapevines with an arsenic mean concentration of $464.1 \pm 23$ mg/Kg in Turkey (Topal, Arslan Topal, & Öbek, 2020). A table with 2018 concentration values of elements found with significant difference among treatments is presented in table 6.

Table 6. Leaf elemental content in mg/Kg measured by ICP-MS in three collection timepoints from 2018 vine treatments

| | **Magnesium** | | **Phosphorus** | | **Sulfur** | | **Arsenic** | |
|---|---|---|---|---|---|---|---|---|
| **June** | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| Untreated | **752.4** | 54.7 | **404.74**a | 13.03 | **674.9** | 39.2 | **0.0099**b | 0.0007 |
| Treated 2017 only | **643.4** | 13.5 | **356.86**b | 4.91 | **622.7** | 94.7 | **0.0131**a | 0.0003 |
| Treated 2 years | **645.4** | 26.0 | **349.68**b | 2.22 | **512.40** | 13.49 | **0.0086**b | 0.0005 |
| **Veraison** | | | | | | | | |
| Untreated | **523** | 287 | **475.7** | 149 | **602.2** | 144.8 | **0.247** | 0.577 |
| Treated 2017 only | **507.8** | 232.4 | **456.4** | 170.8 | **547.9** | 152.3 | **0.0043** | 0.002 |
| Treated 2 years | **419** | 342 | **279.7** | 170.7 | **472.6** | 162.8 | **0.0043** | 0.002 |
| **Harvest** | | | | | | | | |
| Untreated | **686.3**a | 61.0 | **349.9** | 115.1 | **743.1**a | 49.3 | **0.0055** | 0.002 |
| Treated 2017 only | **473.2**ab | 219.3 | **311.9** | 105.3 | **525.6**b | 87.5 | **0.0076** | 0.005 |
| Treated 2 years | **421.1**b | 127.2 | **379.5** | 60.2 | **661.8**a | 107.2 | **0.0057** | 0.003 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$). Only presenting elements that showed significant difference in at least one timepoint measurement.

Samples from 2019 presented more significant difference on elemental composition. Sodium presented difference in June where vines in 2017 & 2019 showed higher sodium content that untreated vines, but not different from vines treated 3 years consecutively. In veraison samples, vines treated 3 years showed higher concentration of sodium, significantly different form vines treated in 2017 and 2019 but not from untreated vines. Sulfur showed significant difference only in June samples, untreated vines possessed significantly more sulfur that vines treated in 2017 & 2019 but not more that vines treated 3 years, as it happened in 2018 harvest samples, sulfur concentration between treatments did not present a consistent effect regarding molybdenum application. Williams et al., 2004 reported an increment in sulfur concentration in grapevine petioles after molybdenum application, Kaiser et al. 2005 mentioned that when sulfate is present in nutrition medium, it will inhibit the molybdenum uptake in tomatoes. Calcium had a significant difference only in June samples, where the untreated vines presented significantly more concentration when compared with vines treated 3 yeas and vines treated in 2017 & 2019. William et al., 2004 reported the same effect in calcium concentration between treated and untreated vines.

Iron & Strontium presented a significant difference only in June samples, the untreated vines had significantly more iron, compared with vines treated 3 yeas and vines treated in 2017 & 2019. The link between iron and molybdenum is not yet fully understood, in *Vitis vinifera* cv. Merlot vines (Williams et al., 2004), in rice shoots (H Zakikhani et al., 2014) and in *baby leaf* var. Mimosa lettuce plants (Rocha et al., 2020) the application of molybdenum decreased iron concentration as it happened in this study, however, in tomato

plants (Bittner, 2014), and strawberries (Liu et al., 2017) the application of molybdenum increase the capacity of the plants to absorb iron.

Nickel presented significant difference in veraison and harvest samples, in both, vines treated 2017 & 2019 showed a higher amount of nickel in comparison with untreated vines and vines treated 2017 & 2019. Zinc also presented difference only in veraison samples, untreated vines showed a higher amount of nickel in comparison with vines treated in 2017 & 2019, however, no difference was found in nickel concentration between untreated vines and treated 3 yeas. Cadmium showed a significant difference in veraison and harvest samples; in veraison, vines treated 3 years had the highest amount of cadmium significantly different that untreated vines and vines treated 2017 & 2019, in harvest the pattern changed, and the untreated vines presented the highest and significantly different cadmium concentration in comparison to vines treated 3 years and vine treated 2017 & 2019. Two tables with 2019 concentration values of elements found with significant difference among treatments are presented in table 7.1 and table 7.2.

Table 7.1. Leaf elemental content in mg/Kg measured by ICP-MS in three collection timepoints from 2019 vine treatments

| | **Boron** | | **Sodium** | | **Sulfur** | | **Potassium** | | **Calcium** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **June** | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| 3 years | **17.43** | 1.18 | **50.30**ab | 14.66 | **2596**ab | 2285 | **13124** | 1132 | **5491**b | 395 |
| 2017 & 2019 | **16.92** | 1.38 | **61.62**a | 12.42 | **2269**b | 3199 | **12292** | 1186 | **5727**b | 655 |
| Untreated | **18.83** | 1.46 | **39.66**b | 2.35 | **2726.8**a | 2167 | **13393** | 1104 | **6901**a | 632 |
| **Veraison** | | | | | | | | | | |
| 3 years | **12.86**a | 2.16 | **56.56**a | 11.82 | **2346.8** | 208.0 | **12301**a | 986 | **12420** | 1216 |
| 2017 & 2019 | **11.92**ab | 0.79 | **44.44**b | 6.66 | **2216.7** | 204.6 | **11350**ab | 1546 | **11298** | 1834 |
| Untreated | **10.89**b | 0.67 | **48.47**ab | 10.57 | **2308.1** | 89.2 | **10625**b | 612 | **11970** | 1381 |
| **Harvest** | | | | | | | | | | |
| 3 years | **10.34** | 1.32 | **37.05** | 13.12 | **1931.4** | 214.9 | **11170** | 1377 | **12286** | 1837 |
| 2017 & 2019 | **10.69** | 0.83 | **40.39** | 16.90 | **1981.8** | 225.6 | **11300** | 863 | **13253** | 1913 |
| Untreated | **9.83** | 1.21 | **38.02** | 11.21 | **1977.8** | 115.4 | **10586** | 1416 | **12733** | 1315 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$). Only presenting elements that showed significant difference in at least one timepoint measurement.

Table 7.2. Leaf elemental content in mg/Kg measured by ICP-MS in three collection timepoints from 2019 vine treatments

| | Iron | | Nickel | | Zinc | | Strontium | | Cadmium | |
|---|---|---|---|---|---|---|---|---|---|---|
| **June** | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| 3 years | **66.94**b | 7.11 | **0.342** | 0.096 | **42.93** | 6.70 | **12.45**b | 1.58 | **0.0326** | 0.033 |
| 2017 & 2019 | **65.76**b | 8.29 | **0.477** | 0.237 | **43.10** | 7.35 | **13.05**b | 2.83 | **0.0341** | 0.006 |
| Untreated | **78.18**a | 7.83 | **0.294** | 0.019 | **47.26** | 4.32 | **15.21**a | 1.33 | **0.0291** | 0.007 |
| **Veraison** | | | | | | | | | | |
| 3 years | **59.86** | 7.61 | **0.442**ab | 0.091 | **437.2**ab | 117.1 | **32.71** | 8.43 | **0.0189**a | 0.007 |
| 2017 & 2019 | **56.40** | 7.20 | **0.493**a | 0.099 | **345.5**b | 66.3 | **29.02** | 4.50 | **0.0172**b | 0.008 |
| Untreated | **55.65** | 5.75 | **0.365**b | 0.047 | **486.7**a | 58.4 | **29.54** | 2.54 | **0.0114**b | 0.003 |
| **Harvest** | | | | | | | | | | |
| 3 years | **49.74** | 7.49 | **0.413**b | 0.097 | **181.5**b | 65.2 | **33.69** | 7.25 | **0.0162**b | 0.006 |
| 2017 & 2019 | **56.03** | 9.87 | **0.574**a | 0.088 | **275.4**a | 93.0 | **36.12** | 5.12 | **0.0392**b | 0.025 |
| Untreated | **51.98** | 12.34 | **0.343**b | 0.091 | **201.6**ab | 66.8 | **36.25** | 2.28 | **0.0086**a | 0.003 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$). Only presenting elements that showed significant difference in at least one timepoint measurement.

Besides molybdenum, other six elements were found with significant difference in 2020 samples: magnesium, phosphorus, calcium, cobalt, zinc and cadmium. Magnesium concentration was found different only in harvest samples, there, treatment 1A (applied at veraison) showed the highest concentration of magnesium when compared with the rests of the treatments and the untreated vines, no significant difference was found between the rest of treatments, William et al., 2004 reported that in terms of main effects, the application of molybdenum to deficient *Vitis vinifera* cv. Merlot also showed an increased concentration of Magnesium. Phosphorus also was found with significantly high values only at harvest samples, treatment 1A and 2A were different from untreated vines, but not from the other treatments, the differences found, agreed with the results reported by Hongen et al., 2010, where *Brassica napus* shoots presented more phosphors concentration when molybdenum was added as nutrient. Calcium presented significant difference in veraison and harvest samples, in veraison treatment 1A vines possessed the highest amount of calcium, and was significantly different from treatment 2A, treatment 3 and untreated vines, but not different from treatment 1B and treatment 2B vines, at the same time, treatment 1B and 2B vines were not significantly different from treatment 2A, treatment 3 and untreated vines. In harvest remain with the highest value in treated 1A vines that were significantly different from only 2A vines, the rest of the treatments were not found different between them, although significant concentrations were found, they were not consistent between sampling time point and treatments.

Zinc presented difference in June and veraison samples; in June, treatment 1B presented higher zinc content when compare with the rest of the treatments, but only was significantly different from treatment 2B, the remaining treatments did not present any

significant difference. In veraison, zinc content in treatment 3 and untreated vines were the highest among the treatments but only significantly different form treatment 1A, no difference was found between the rest of the treatments. Cadmium was the only element that showed significant difference in the three sample time points. In June, treatment 2A presented the highest cadmium concentration, that was significantly different from the rest of the treatments, following in significance was treatment 2B that showed a cadmium concentration lower that treatment 2A but higher when compared with the rest higher in June and veraison when moly applied, at harvest high in all applied. In veraison samples treatment 1A presented the highest cadmium concentration and was significantly different in comparison with the rest of the treatments, no significant difference was found between the rest of the treatments in veraison. In harvest, treatment 1A was the only significantly different from the untreated vines. A table with 2020 concentration values of elements found with significant difference among treatments is presented in table 8.

### 3.3.2. Potassium acetate impact

With 2020 treatments, it was possible to observe if the conjunct addition of potassium acetate with molybdenum had any effect in other elements concentration. In the case of cobalt, it only changed in harvest samples, however, treatment 1A, that was applied with potassium acetate, presented the highest concentration of cobalt, even significantly higher that the treatment 1B that had no potassium acetate.

Table 8. Leaf elemental content in mg/Kg measured by ICP-MS in three collection timepoints from 2020 vine treatments

| | Magnesium | | Phosphorus | | Calcium | | Cobalt | | Zinc | | Cadmium | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **June** | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| Treatment 1A | **2282** | 301 | **2329.5** | 121.9 | **5452** | 1165 | **0.056** | 0.031 | **44.04**ab | 4.51 | **0.0094**d | 0.002 |
| Treatment 1B | **2325.4** | 189.4 | **2361.4** | 220 | **5365** | 585 | **0.038** | 0.008 | **49.95**a | 5.71 | **0.0094**d | 0.002 |
| Treatment 2A | **2286.1** | 226.5 | **2257.9** | 188.8 | **5050** | 1087 | **0.029** | 0.009 | **42.01**ab | 3.52 | **0.0189**b | 0.003 |
| Treatment 2B | **2134** | 271 | **2205** | 252 | **4867** | 852 | **0.037** | 0.004 | **40.96**b | 6.39 | **0.0262**a | 0.004 |
| Treatment 3 | **2260** | 196 | **2425.6** | 168.7 | **5171** | 564 | **0.036** | 0.003 | **48.66**ab | 1.11 | **0.0167**bc | 0.004 |
| Untreated | **2125** | 185.5 | **2152.3** | 79.1 | **4520** | 619 | **0.031** | 0.012 | **46.86**ab | 4.66 | **0.0099**cd | 0.002 |
| **Veraison** | | | | | | | | | | | | |
| Treatment 1A | **3778** | 521 | **1882** | 332 | **13492**a | 1617 | **0.102** | 0.051 | **302.5**b | 25.0 | **0.2426**a | 0.123 |
| Treatment 1B | **3332.6** | 234.1 | **1830.1** | 135.9 | **12977**ab | 1521 | **0.069** | 0.012 | **362.4**ab | 64.4 | **0.1087**b | 0.031 |
| Treatment 2A | **3341** | 466 | **1771.9** | 126.0 | **10454**b | 1252 | **0.097** | 0.042 | **398.3**ab | 46.6 | **0.0556**b | 0.116 |
| Treatment 2B | **3520.6** | 174.8 | **1717.5** | 108.8 | **1174**ab | 1248 | **0.088** | 0.031 | **374.0**ab | 68.9 | **0.0435**b | 0.023 |
| Treatment 3 | **3137** | 287 | **1793.4** | 79.2 | **10264**b | 1497 | **0.071** | 0.019 | **435.4**a | 15.01 | **0.0456**b | 0.024 |
| Untreated | **3798** | 825 | **1857.4** | 118.8 | **11337**ab | 1605 | **0.078** | 0.017 | **440.2**a | 66.3 | **0.0119**b | 0.019 |
| **Harvest** | | | | | | | | | | | | |
| Treatment 1A | **4088**a | 471 | **1985.9**a | 149.8 | **19365**a | 1733 | **0.143**a | 0.052 | **336.6** | 47.7 | **0.094**a | 0.038 |
| Treatment 1B | **3209**b | 518 | **1867.5**ab | 83.7 | **16325**ab | 3201 | **0.083**b | 0.027 | **255.1** | 68.6 | **0.050**ab | 0.025 |
| Treatment 2A | **2765**b | 338 | **1916.8**a | 171 | **11801**c | 2160 | **0.066**b | 0.014 | **275.1** | 127.3 | **0.030**ab | 0.041 |
| Treatment 2B | **3363**ab | 574 | **1856.4**ab | 111 | **15412**abc | 1657 | **0.089**b | 0.024 | **274.2** | 49.5 | **0.039**ab | 0.059 |
| Treatment 3 | **2908**b | 207 | **1781.4**ab | 90.8 | **15545**abc | 1356 | **0.091**ab | 0.009 | **274.9** | 54.3 | **0.025**ab | 0.001 |
| Untreated | **3231**b | 319 | **1654.7**b | 73.2 | **14283**bc | 2715 | **0.090**b | 0.019 | **276.5** | 23.9 | **0.021**b | 0.018 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$). Only presenting elements that showed significant difference in at least one timepoint measurement.

### 3.3.3. Annual application impact

No carryover effect was clearly observed in any of the years of analysis regarding the elemental composition of leave samples. Although it was observed that in 2018, magnesium showed a decrement on vines treated 2 years consecutively when compared with vines treated only in 2017, and as is presented in table 6, the highest magnesium concentration was found in untreated vines, followed by vines treated only in 2018 (the previous year), finishing with vines treated 2 years (2017 and 2018)

Boron in 2019 presented difference only in veraison, however, vines treated 3 years consecutively possessed significantly more boron than the untreated vines, but not significantly more that vines treated 2017 & 2019. This result is opposite to the reported by William et al., 2004, where a decreased petiolar boron concentration in Merlot grapes was found after molybdenum application, however, in chickpea (*Cicer arietinum* L.) an increase in boron was found after molybdenum application (Valenciano, Boto, & Marcelo, 2011). Potassium in 2019 samples (present in table 7.1), only showed significant difference at veraison, where vines treated 3 years consecutively possessed more potassium when compared with untreated vines, but not when compared with vines treated in 2017 & 2019. William et al., 2004 mentioned that an increment in potassium concentration was found at flowering, but a decrement at veraison only in two of the three years of molybdenum addition to deficient grapevines. It has been reported that low supplies of potassium can reduce the intake of molybdenum un plants, and that an increment in molybdenum content may have the same effect in potassium intake. Often with potassium deficiency symptoms,

the plant will show nitrogen deficiency symptoms due to molybdenum intake decrement(Maillard et al., 2016; Ranade-Malvi, 2010; H Zakikhani et al., 2014).

### 3.3.4. Application timing impact

No application timing impact was observed in 2020 analysis regarding the elemental composition of leave samples.

## 3.4. Leaf nitrogen quantification

Total Kjeldahl Nitrogen (TKN) is total concentration of organic nitrogen plus ammonia contained in the sample, and it commonly reported as the percentage if nitrogen found in the original sample as Nitrogen percentage (N%). No significant difference was found in 2019 between treatments and sampling time points. In 2020, significant difference was observed in two sampling time point, at veraison, Treatment 1B presented the highest amount of N% of 2.36 $\pm$ 0.16 when compared with the rest of the treatments, however, significance was only found between Treatment 1B and Treatment 3 (N% 2.01 $\pm$ 0.09), the remaining treatments did not show significant difference among themselves and between Treatment 1B and Treatment 3. At harvest significant difference was found between Treatment 1A with the highest N% concentration at 2.38 $\pm$ 0.08, and Treatments 2A (2.07 $\pm$ 0.10) & untreated (2.10 $\pm$ 0.08) both with the lowest N% concentration. The rest of the treatments did not present significant difference among themselves and the highest and lower N% concentrations. The Nitrogen percentage concentration found in 2019 and 2020 samples fell between the normal range of N% reported in other grapevines. *Vitis vinifera* cv. Sangiovese leaves presented a mean N% of 2.2 at veraison and 2.0 at

harvest (Poni, Intrieri, & Silvestroni, 1994), Cabernet Sauvignon and Xinomavro had a mean N% of 2.14 & 2.07 respectively (Taskos et al., 2015), while Chardonnay and Pinot Noir grapevine leaves had a mean N% of 2.18 & 2.37 respectively (Walker et al., 2021).Even when significant changes were found, the were not consistent between treatments and sampling timepoints. N% values are presented in Table 9.

Table 9. Vine leaves N% means and standard deviations of June, Veraison and Harvest samples from 2019 and 2020

| | **June** | | **Veraison** | | **Harvest** | |
|---|---|---|---|---|---|---|
| **2019** | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| Untreated | **2.79** | 0.21 | **2.43** | 0.13 | **2.45** | 0.16 |
| Treated 2017 & 2019 | **2.55** | 0.15 | **2.51** | 0.13 | **2.41** | 0.53 |
| Treated 3 years | **2.67** | 0.14 | **2.52** | 0.25 | **2.43** | 0.17 |
| **2020** | | | | | | |
| Treatment 1A | **2.53** | 0.17 | **2.18**ab | 0.09 | **2.38**a | 0.08 |
| Treatment 1B | **2.65** | 0.24 | **2.36**a | 0.16 | **2.23**ab | 0.13 |
| Treatment 2A | **2.45** | 0.19 | **2.18**ab | 0.19 | **2.07**b | 0.10 |
| Treatment 2B | **2.55** | 0.19 | **2.12**ab | 0.22 | **2.26**ab | 0.19 |
| Treatment 3 | **2.65** | 0.14 | **2.01**b | 0.09 | **2.31**ab | 0.22 |
| Untreated | **2.40** | 0.23 | **2.18**ab | 0.11 | **2.10**b | 0.08 |

Means with different letters within the column indicate statistical difference between treatments ($p \leq 0.05$).

## 3.5. GC-MS wine aroma

From the treated and untreated vines, wines were produced each year, and every year the aroma profile showed significant difference between the treated and untreated grapes. In 2017 the wine made from treated vines showed higher concentration of D-Limonene, Ethyl Hexanoate, Terpinolene and $\beta$-Linalool, calculated values of all the aroma compound quantified in 2017 are presented in table 10.

Table 10. Means and standard deviations on µg/L for key aromas compounds on wines produced from molybdenum treated and untreated vines on 2017, analyzed through GC-MS

| Aromas | Applied | | Untreated | |
|---|---|---|---|---|
| | **Mean** | SD | **Mean** | SD |
| **D-Limonene** | **17.90**a | 3.82 | **12.29**b | 3.15 |
| **Ethyl Hexanoate** | **408.1**a | 51.7 | **322.1**b | 86.6 |
| **Terpinolene** | **2.60**a | 0.56 | **1.80**b | 0.45 |
| **1-Hexanol** | **388.5** | 83.9 | **331.4** | 102.8 |
| **$\beta$-Linalool** | **33.64**a | 3.01 | **29.41**b | 5.04 |
| **TDN** | **3.58** | 1.52 | **3.29** | 0.53 |
| **$\beta$-Phenethyl acetate** | **286.52** | 36.57 | **241.3** | 80.1 |
| **$\beta$-Damascenone** | **118.27** | 31.51 | **137.23** | 26.54 |
| **2-Phenylethyl alcohol** | **3376** | 655 | **3176** | 1428 |

Means with different letters within each row, indicates statistical difference between treatments (unpaired T-test, $p \leq 0.05$)

Among four of the aromas that showed a significant difference, three belong to the Terpene aroma group: D-Limonene (citrus aroma), Terpinolene (floral-herbal aroma) and β-Linalool (sweet orange-lavender aroma). These aromatic compounds supply the characteristic floral, fruity, and citrusy fragrance to wines. Chemically, they are composed of two or more five-carbon isoprene skeleton called monoterpenes, sesquiterpenes, diterpenes and triterpenes respectively (Jackson, 2014, 2017). There are fifty main monoterpenes that have been isolated from wines and they contribute to the aroma of white wines made from Muscat variety grapes like Muscat of Alexandria or Muscat de Frontignan, aromatic *Vitis vinifera* varieties like Gewürztraminer and Riesling, and *Vitis* interspecific hybrids like Traminette and Vignoles (Robinson et al., 2014). Monoterpene's biosynthesis occurs during grape ripening, they are located mainly in the skin as free and bound compounds. Ripening conditions and especially temperature and light exposure

during the maturation accounts for different levels of terpenes in grapes, where non-excessive exposure has been reported as favorable to increase volatile monoterpenes at grape ripeness (Thibon, Darriet, & Dubourdieu, 2012). The difference found in terpenes concentration between treated and untreated vines could be related to the increment of molybdenum observed in leaves, as molybdenum is directly correlated with nitrogen intake, and at the same time, nitrogen is related with vine vigor, it could be assumed that grapes from treated vines were exposed to less light exposure that grapes from untreated vines, allowing a better terpene biosynthesis and accumulation.

Esters are synthesized by yeast during juice fermentation or appear after fatty acid esterification with ethanol and acetic acid through fermentation and wine aging. They contribute the floral, fruity sweet notes to wine, and the most important esters and acetates in wines include ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate and isobutyl acetate (Robinson et al., 2014). Ester formation during fermentation is influence by grape variety, yeast strain and in certain instances, the ability of must to support ester formation due its constituents like sugar and amino acid content (Jackson, 2014; Sumby, Grbin, & Jiranek, 2010). As it happened with terpenes, the difference found in wine's ethyl hexanoate concentration made with treated and untreated grapes, could be attributed to the grape's maturity. 2017 treated grapes showed a higher sugar concentration in comparison with untreated grapes. With higher sugar content and in general, a better ripping thanks to molybdenum application, the must obtained from treated grapes possessed better constituents that ended in higher ethyl hexanoate concentration.

Difference in the aroma profile on wines produced in 2018 were less than the previous year, among the aromas that were quantified on 2018 wines, only Ethyl hexanoate

showed a significant difference, but, contrary to what was found in 2017, ethyl hexanoate was found in higher concentration in untreated wines. Calculated values of all the aroma compound quantified in 2018 are presented in table 11.

Table 11. Means comparison in µg/L for key aromas compounds found in wines produced from molybdenum treated and untreated vines on 2018, analyzed by GC-MS

| **Aromas** | **2017 only** | | **2017 & 2018** | | **Untreated** | |
|---|---|---|---|---|---|---|
| | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| **D-Limonene** | **20.3** | 8.29 | **16.3** | 5.01 | **15.3** | 3.41 |
| **Ethyl Hexanoate** | **357**b | 145 | **401**ab | 153 | **575**a | 140 |
| **Terpinolene** | **6.19** | 0.92 | **6.24** | 0.97 | **6.48** | 0.89 |
| **1-Hexanol** | **576** | 118 | **489** | 111 | **599** | 56.2 |
| ***β*-Linalool** | **93.7** | 15.2 | **86.8** | 18.7 | **84.4** | 8.06 |
| **TDN** | **5.46** | 1.76 | **7.41** | 1.55 | **6.31** | 1.32 |
| ***β*-Phenethyl acetate** | **43.8** | 22.5 | **127** | 116 | **156** | 131 |
| ***β*-Damascenone** | **322** | 26.3 | **362** | 57.1 | **320** | 28.1 |
| **2-Phenylethyl alcohol** | **4392** | 447 | **4787** | 577 | **4459** | 254 |

Means with different letters within each row, indicates statistical difference between treatments ($p \leq 0.05$)

Wines elaborated in 2019 presented fewer quantifiable aromas, but significant difference was found between treatments in those few aromas when compared with only one significantly different aroma found in 2018. In 2019 wines, terpinolene was found in higher amounts in vines treated 3 years consecutively and in untreated vines, both conditions were significantly different when compared with wines made from vines treated 2017 & 2019. 1-Hexanol showed significant higher values in treated 2017 & 2019 wines when compared with 3 years and untreated wines. 1-Hexanol is an aromatic $C_6$ alcohol product of yeast fermentation that possess an herbaceous scent, that at concentrations below 300 mg/L may contribute complexity to wine bouquet (Oliveira, Faria, Sá, Barros,

& Araújo, 2006; Waterhouse, Sacks, & Jeffery, 2016) $\beta$-Linalool content was higher in wines produced from vines treated 2017 & 2019 and only significantly different in comparison with wines made from untreated vines. Calculated values of all the aroma compound quantified in 2018 are presented in table 12.

Table 12. Means and standard deviations on µg/L for key aromas compounds on wines produced from molybdenum treated and untreated vines on 2019, analyzed through GC-MS

| **Aromas** | **3 years** | | **2017 & 2019** | | **Untreated** | |
|---|---|---|---|---|---|---|
| | **Mean** | SD | **Mean** | SD | **Mean** | SD |
| **Terpinolene** | **2.59**a | 1.09 | **1.74**b | 0.42 | **2.50**a | 0.63 |
| **1-Hexanol** | **392**b | 85.6 | **583**a | 114 | **437**b | 64.1 |
| **$\beta$-Linalool** | **29.9**ab | 12.4 | **33.8**a | 9.65 | **23.7**b | 5.97 |
| **$\beta$-Damascenone** | **2.28** | 0.84 | **2.41** | 0.365 | **2.27** | 0.35 |
| **$\beta$-Ionone** | **2.65** | 2.26 | **4.02** | 4.20 | **1.85** | 1.80 |

Means with different letters within each row, indicates statistical difference between treatments ($p \leq 0.05$)

In 2020 wines only four aromas were quantifiable, and only two showed significant difference between treatments. One aroma that showed difference was terpinolene, this aroma was found in significantly higher concentrations in wines made from treatment 1B, 2A and untreated vines, when compared to treatment 3 that showed the lowest terpinolene concentration, treatment 1A and treatment 2B wines were not significantly different from treatment 3 wines, nor the highest terpinolene wines (figure 5)

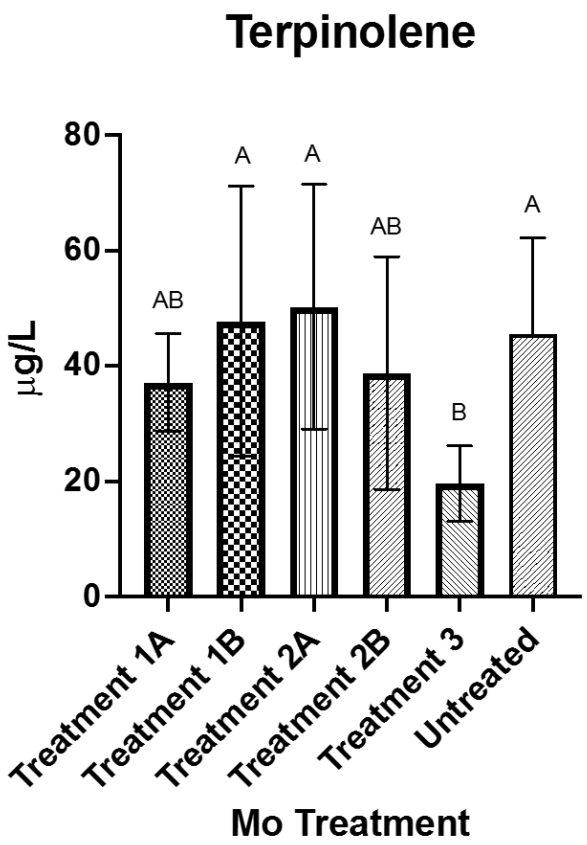


Figure 5. Means and standard deviations for Terpinolene concentrations on wines produced from molybdenum treated and untreated vines on 2020, analyzed through GC-MS. Means with different letters indicate statistical difference between treatments ($p \leq 0.05$)

$\beta$- Damascenone is an aroma compound classified as $C_{13}$-Norisoprenoind, this compounds are mainly contained in the grape skin, and originates from carotenoid degradation, grape exposure to sunlight has been reported to increase carotenoid degradation (Alakaam, Castellanos, Bodzio, & Harrison, 2015; Ebeler & Thorngate, 2009; Thibon et al., 2012). $\beta$- Damascenone is characterized by a rose-apple sauce aroma. It presented significant difference between wines; treatment 2A, 2B and untreated wines, presented the highest concentration of $\beta$- Damascenone, being significantly different from treatment 1A wines only. Treatment 1B and treatment 3 did not presented significant different in $\beta$- Damascenone concentration when compared with treatment 1A nor

treatment 2A, 2B and untreated wines (figure 6). Linalool and 1-Hexanol (figure 7) did not showed significant difference between wines

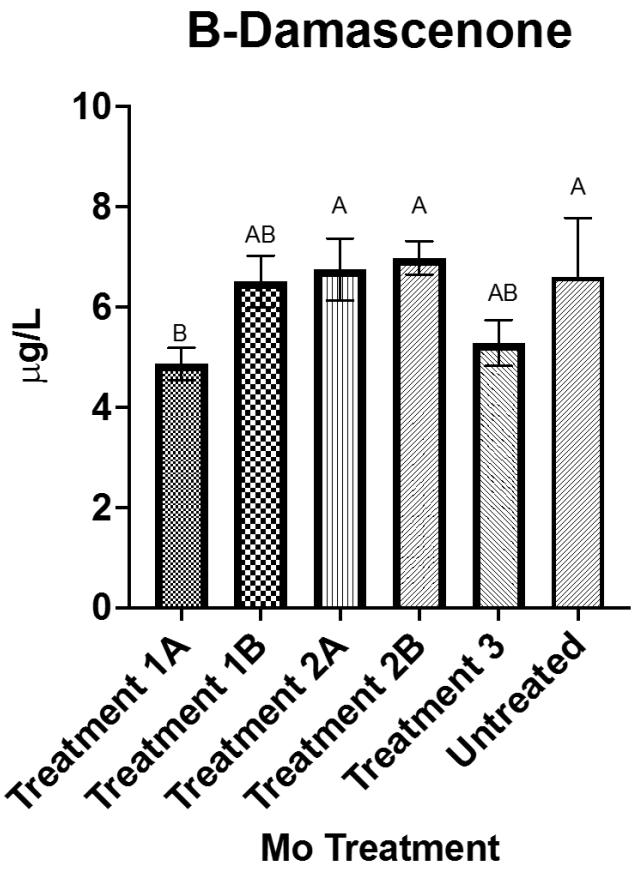


Figure 6. Means and standard deviations for $\beta$-Damascenone concentrations on wines produced from molybdenum treated and untreated vines on 2020, analyzed through GC-MS. Means with different letters indicate statistical difference between treatments ($p \leq 0.05$)

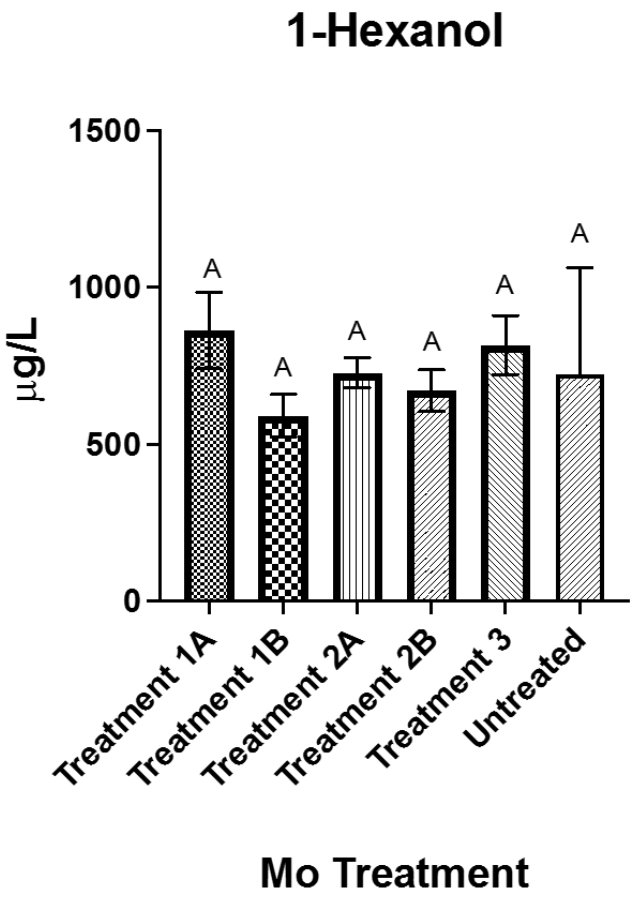


Figure 7. Means and standard deviations for 1-Hexanol concentrations on wines produced from molybdenum treated and untreated vines on 2020, analyzed through GC-MS. Means with different letters indicate statistical difference between treatments ($p \leq 0.05$)

Although significant difference was found in $\beta$-Linalool, 1-Hexanol in 2019 wines, in $\beta$- Damascenone in 2020 and in Terpinolene in 2019 and 2020 wines, the changes were not consistent between treatments.

## 4. Conclusions

There is only a few papers related with molybdenum deficiency in *Vitis vinifera* grape vines, and none related with *Vitis* interspecific hybrid grapevines. Previous studies have only focused on vegetative and reproductive structures, and yield effects of molybdenum treated-deficient grapevines, however, any research has traced the impact of

molybdenum deficiency on wine aromas. This study showed basic grape juice parameters as Brix and Titratable acidity changes between treated and untreated vines. Foliar application of molybdenum to deficient grapevines had an impact in leaves molybdenum concentration, it could be observed the synergic effect of potassium acetate in molybdenum intake on 2020 samples. Also, it was possible to observe the effect of treatment application time on foliar molybdenum intake and concentration. Elemental analysis showed changes in magnesium, phosphorus, sulfur, arsenic, boron, sodium, potassium, calcium, iron, nickel, zinc, strontium, cadmium and cobalt content in treated and untreated vine leaves at least in one of the three sampling time points in the three years of leaves sampling. A change in the aroma groups: Terpenes, Esters, $C_{13}$-Norisoprenoids and $C_6$ Alcohols could be found in wines elaborated with grapes harvested from treated and untreated grapevines.

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