Chemical Bonds of Cations in Grapevine Berries and Stems of Some Important Cultivars of *Vitis vinifera* (L.) and Their Relevance for Viticulture and Enology

Klaus SCHALLER* and Ruth LEHNHART

Geisenheim University, Department of Applied Biology, P.O.B. 1154, D-65366 Geisenheim, Germany

*)Corresponding author, e-mail: klaus.schaller@hs-gm.de

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Abstract

Cations are essential for plant growth, tissue build-up and its stabilization. Divalent cations are necessary for cell wall stability and other interstitial tissues. They may be cross-linked with pectins or form looser ionic bindings. This is of importance for grape processing during the technical operations of juice extraction. Monovalent ions are more involved in vacuolar homeostasis and pH stabilization. With a specific extraction procedure H₂O-soluble, 1N NaCl-soluble, 2% CH₃COOH-soluble, and 5% HCl-soluble fractions as well as the total content of K, Ca, and Mg were analyzed in grapevine berries and stems. In berries as well as in stems the monovalent K is only found in the water and the NaCl fraction. But only 50-60% of the total K can be extracted with both solutes. The divalent cation Ca is found in all four fractions with differing amounts, but water soluble Ca is in most cases highest. Ca may also have an indicator value for BSN, because the water soluble fraction is lowest in the most susceptible variety Riesling. Mg is not found in HCl fraction. An analysis of variance shows that the varieties have a significant influence on nutrient distribution in different tissues. It seems that the relation Ca/Mg (NaCl fraction) in stems is a good indicator for BSN because susceptible varieties show lower ratios than resistant ones. Some more research is necessary to clear up this possible relationship.

Keywords: bunches, bunch stem necrosis (BSN), grapevine, nutrient fractions, stems

INTRODUCTION

Cations have different functions in plant cells and tissues: maintain vacuolar homeostasis, stabilize cell pH, activate diverse enzymes, stabilize cell walls via cross-linking of pectins etc. which is well described in all important textbooks for plant nutrition (Marschner 2011; Mengel and Kirkby, 2007).

Fruits are important sources for mineral nutrients (MN) in our daily diet as well as vitamins and other valuable compounds used for direct human consumption and also processed foods (Souci-Fachmann-Kraut, 2008). For plants it is a given that MN are essential for their growth and are constitutive elements of organelles, cell walls, enzymes etc. (Epstein-Bloom 2005; Marschner

2011; Mengel and Kirkby 2007). MN can improve the resistance of plants to fungi diseases. Most promising results exist for potassium and calcium (Datnoff *et al.* 2007). Both elements are discussed concerning the incidence of bunch stem necrosis (BSN) in susceptible cultivars like Riesling, Mueller-Thurgau and Pinot Noir (Hiffny and Alleweldt 1972; Stellwaag 1967; Theiler and Müller 1986).

In case of grape processing into must (juice) and wine, cations are partly responsible for the wines' buffer system, which guaranties an undisturbed fermentation. Screening the most widely used textbooks of wine chemistry concerning mineral composition of berries and stems the information is not as good as expected.

Tab. 1. Total potassium and its fractions in berries of different cultivars (mmole K/kg dm)

Fraction	Riesling	Silvaner	Ehrenfelser	Reichensteiner	Mueller-Thurgau
H ₂ O-soluble	24.62a	33.06be	29.41cd	28.97d	32.93e
1N NaCl-soluble	2.81	2.88	3.45	3.07	3.77
Total	42.20a	45.65b	42.71ac	37.82d	42.97ace

Note: Different letters between cultivars denote significant differences (LSD test, $p < 0.05$).

There exist some good compilations about MN in wines and their ranges (Blouin and Cruège 2013; Usseglio-Tomasett 1989; Würdig and Woller 1989). In all publications only total MN contents are reported.

MN may also have some sensorial effects in a finished wine; but it is up to now not completely proven how the interaction with other organic compounds e.g. acids, may lead to such sensations. Sometimes is reported that higher potassium content give wines a creamy sensation. Following the processing line for winemaking it is important which steps are used for juice extraction: mashing or whole bunch pressing, maceration time, pressing programs including differing pressure steps. Hence, by varying these operational steps, MN may be extracted in variable amounts into the must. Influence of cultivars may be also important. No scientific papers exist about the fractionation about mono- and divalent cations in berries and stems of grapevines.

Taking into account these above mentioned problems a study was initiated to get more information about chemical bonding of cations in berries and stems and cultivar's behavior.

MATERIALS AND METHODS

For the experiment 5 white wine cultivars, all grafted on rootstock 5 C, were used: 1. Riesling (clone 239) [RI], 2. Silvaner [SI], 3. Ehrenfelser (crossbred RI x SI) [EHR], Reichensteiner (crossbred Mueller-Thurgau x (Madeleine Angevine x Calabreser Frölich)) [REI], Mueller-Thurgau (crossbred Riesling x Madeleine Royale) [MUE]. RI, SI, EHR and REI were cultivated in test field G and MUE in test field D of the Department of Grapevine Breeding and Grafting. All varieties were planted in 4 replications. Every replication contained 12 grapevines. Soil in both fields is a deep loess derived "Parabraunerde" (chromic luvisol) with high fertility status.

Sampling: bunches were picked at technological ripeness, transferred to the lab, separated

in stems and berries, shock frozen with liquid nitrogen and stored in a deep freezer until analysis.

Sample preparation: Prior to analysis berries were cut with a scalpel into halves and kernels removed from the pulp. Stems were cut into small pieces.

Extraction of cations: Extraction of cations from the different tissues follows the scheme according shown in fig 1. Potassium (K) was determined by flame photometry: Calcium (Ca) and magnesium (Mg) by Atomic Absorption Spectrophotometry (AAS). Concentrations are reported in mmole/kg dm. Results for the different nutrients and their nutrient fractions were analyzed after testing for homogeneity with a one way respectively two way Anova.

RESULTS AND DISCUSSIONS

1. Potassium (K)

Berries: K is not covalently bound in any organic structures in cells and organelles i.e. after rupture of tissues and cells it will leak into the surrounding fluids. In general K increases the permeability of cell walls and tissues because of its hydrations effects. According to our proposed extraction procedure and the general findings in the literature K should be nearly complete soluble in water (Marschner 2011). Table 1 shows the distribution of K in the water soluble and NaCl soluble fraction.

Highest amounts are found in the H₂O-fraction ranging from 24.6 mmole K/kg dm in RI to 33.1 mmole K/kg dm in SI. The remaining cultivars EHR, REI and MUE are grouped in between both extremes. K in the NaCl-fraction is remarkably lower in all cultivars. They range from 2.81 mmole K/kg dm in RI to 3.77 mmole K/kg dm in MUE. This fraction accounts only for 8-12% of the H₂O-soluble K. No significant difference between cultivars could be found. The remaining fractions acetic acid and HCl did not contain any K. In contrast to the assumption that K should be completely found in the first two fractions only 65-85% of total K can be extracted with the solutes. If the remaining

Fig. 1: Scheme for the extraction of cations with differing solubility from plant material

Tab. 2. Total potassium and its fractions in stems of different cultivars (mmole K/kg dm)

Fraction	Riesling	Silvaner	Ehrenfelser	Reichensteiner	Mueller-Thurgau
H ₂ O-soluble	32.29a	47.64b	39.90c	33.38ad	35.67d
1N NaCl-soluble	7.10a	16.11b	8.18cd	8.18d	8.89d
Total	56.08a	63.75b	48.08c	49.36cd	58.31e

Note: Different letters between cultivars denote significant differences (LSD test, $p < 0.05$).

Tab.3. Total calcium and its fractions in berries of different cultivars (mmole Ca/kg dm)

Fraction	Riesling	Silvaner	Ehrenfelser	Reichensteiner	Mueller-Thurgau
H ₂ O-soluble	2.03a	2.18ab	2.50bc	1.79d	2.20ac
1N NaCl-soluble	0.59a	0.54b	0.49bcd	0.41d	0.55c
2% Acetic acid	1.01a	0.84ab	0.99abc	0.78acd	1.66e
5% HCl	0.53	0.48	0.54	0.52	0.49
Total	4.14a	4.04abd	4.52abce	3.55ad	4.90e

Note: Different letters between cultivars denote significant differences (LSD test, $p < 0.05$).

K may be complexed or bound in some structures which may prevent a complete extraction stays up to now un-answered. The assumption seems not conclusive because K should be found in the following extractants with a stronger extraction and exchange capacity.

Significant differences between cultivars are indicated in table 1 with exception of MUE vers. SI and REI vers. EHR all varieties are significantly different. NaCl extracted K is not significant for all cultivars. Total K content in berries is probably a varietal characteristic (table 1). Lowest content is found in REI (37.8 mmole K/kg dm) and highest in SI (45.7 mmole K/kg dm). Differences between RI, SI and REI are significant. Percentage of water-soluble K on total K demonstrates that the lowest amount is found in RI (58%) and the highest in SI (82%); the remaining cultivars EHR, REI and MUE range from 66.5 – 76.7%.

Stems

Stems have no influence on quality in red wine processing because prior to fermentation and crushing bunches are de-stemmed. In contrast, during white wine production it is possible that they can exert influence on must composition. In a more positive sense they will improve the drainage of must out of the press cake but then stems are immersed in the drain-off must and possibility exists that cations from stems can leak or diffuse in the surrounding solute. The probability for such a process increases when stems are broken down during transport and pumping into the press.

Besides these technological facts mineral nutrient content of stems may also have an important effect on the physiological disorder called “bunch stem necrosis” (BSN), “dessèchement de la rafle” or “Stiellaehme” (Hiffny 1972, Stellwaag-Kittler 1967, Theiler 1986). Stems are getting necrotic and the transport of sugars and solutes into berries is restricted. As a consequence berries will shrivel, having a low sugar and high acidity content. Expected wine quality may be minor. Table 2 presents the K content of the different fractions in stems of the tested cultivars.

Lowest H₂O-soluble K has RI (32.3 mmole/kg dm), followed by REI (33.8 mmole K/kg dm) and MUE (35.7 mmole K/kg dm). RI is significantly different from vers. SI, EHR and MUE. Other research reveals that RI and MUE are strongly prone to BSN (Hiffny and Alleweldt 1972, Theiler and Müller 1986). If K is solely responsible for the incidence of BSN is not yet proven. In the fraction of NaCl-soluble K RI has the lowest content whereas that of SI is more than doubled. If K alone may act as an indicator for BSN is still strongly called into question.

On the other site stems may also contribute to the K load of must depending on their mechanical stress during must extraction especially in white wine processing.

2. Calcium (Ca)

Berries

Calcium is an important nutrient because it is responsible for stability of cell walls, integrity of

Tab.4. Total calcium and its fractions in stems of different cultivars (mmole Ca/kg dm)

Fraction	Riesling	Silvaner	Ehrenfelser	Reichensteiner	Mueller-Thurgau
H ₂ O-soluble	4.14a	7.17b	5.48bc	4.73abcd	5.14bcd
1N NaCl-soluble	0.60	0.68	0.59	0.47	0.57
2% Acetic acid	1.75a	2.10b	1.68ac	1.51d	1.59ac
5% HCl	1.66abd	1.87bdc	1.94adc	1.46de	1.15e
Total	8.18a	11.82b	9.69ace	7.81ad	8.45a

Note: Different letters between cultivars denote significant differences (LSD test. $p < 0.05$).

Tab.5. Total magnesium and its fractions in berries of different cultivars (mmole Mg/kg dm)

Fraction	Riesling	Silvaner	Ehrenfelser	Reichensteiner	Mueller-Thurgau
H ₂ O-soluble	1.74a	2.84bc	2.61c	2.09d	2.53ce
1N NaCl-soluble	0.29	0.25	0.22	0.25	0.27
2% Acetic acid	0.17abe	0.17bce	0.16cba	0.14abcd	0.20e
Total	2.20a	3.25b	2.93c	2.48ad	3.00bce

Note: Different letters between cultivars denote significant differences (LSD test. $p < 0.05$).

membranes, as well as their permeability. Therefore one can expect in contrast to K that it is not only available in a water- or NaCl-soluble form. Table 3 demonstrates that Ca is found in four different fractions.

As with K, the main fraction for Ca is the water soluble one. Nearly 50% of the total Ca is located in that fraction; the range is 44.9 - 55.3%. Second place holds acetic acid soluble Ca; NaCl- and HCl-soluble have similar contents ranging between 0.41 mmole and 0.59 mmole Ca/kg dm. Ca content in berries represent roughly one tenth of their total K content. The tested cultivars differ significantly between each other, which is indicated in table 3. HCl-soluble fraction shows no significant differences.

Stems

Total Ca content in stems shows a great span from 7.81 mmole in REI till 11.82 mmole Ca/kg dm in SI. RI has also a low Ca content with 8.18 mmole Ca/kg dm. Comparing stems' Ca content to berries it is 1.7 – 2.9 times higher (Tab. 4).

The water soluble fraction is lowest in RI (4.14 mmole Ca/kg dm); except the cultivar REI, all other are significantly different to Riesling. NaCl-soluble Ca shows no differentiation between the tested cultivars, but then in the acetic acid fraction clear differences exists. Again SI has the highest values with 2.10 mmole Ca/kg dm. Both REI and MUE range lowest. In the HCl-fraction RI, SI and EHR are close together (1.66 – 1.94 mmole Ca/kg

dm), whereas REI and MUE are in the low range with 1.15-1.46 mmole Ca/kg dm. Water soluble Ca in stems may also have an indicator value for BSN because the most sensitive cultivar RI has the lowest concentration in stems.

3. Magnesium (Mg)

Berries

The total Mg content in berries is found for RI (2.20 mmole Mg/kg dm). With exception of REI all other cultivars are significantly different from RI (table 5). The water soluble fraction is again the lowest in RI (mmole Mg/kg dm) and significantly different from all other tested cultivars.

The ratio between total K in berries/total Mg in berries varies from 14.0 in SI to 19.2 in RI. The ration of water soluble K in berries/water soluble Mg in berries is widest in RI with 19.2 whereas SI, EHR, REI and MUE are very close together; the span is from 14.0 to 15.3.

Stems

Total Mg content in stems varies from 3.70 to 5.98 mmole Mg/kg dm dependent on cultivar (Tab.6.).

Both, RI and EHR rank lowest whereas SI, REI and MUE are significantly higher. The water soluble fraction shows a similar distribution: RI, EHR and MUE rank lowest (2.75 – 3.16 mmole Mg/kg dm). SI and REI are significantly higher: 4.54-4.99 mmole Mg/kg dm. In the NaCl-fraction RI, REI and MUE are highest.

Tab.6. Total magnesium and its fractions in stems of different cultivars (mmole Mg/kg dm)

Fraction	Riesling	Silvaner	Ehrenfelser	Reichensteiner	Mueller-Thurgau
H ₂ O-soluble	2.75a	4.54b	3.03ac	4.99cad	3.16ce
1N NaCl-soluble	0.70ab	0.64b	0.46c	0.77ad	0.70ace
2% Acetic acid	0.26a	0.40b	0.22ac	0.21acde	0.25ace
Total	3.72 ac	5.51 b	3.70 c	5.98 d	4.16 ce

Note: Different letters between cultivars denote significant differences (LSD test. $p < 0.05$).

Tab.7. Nutrient ratios in stems and its different fractions of different cultivars

Fraction	Riesling	Silvaner	Ehrenfelser	Reichensteiner	Mueller-Thurgau
K/Mg					
H ₂ O-soluble	7.8ac	6.9 ab	7.5bc	7.1b	7.0b
1N NaCl-soluble	10.1a	25.2 b	17.4c	9.2a	12.7a
Total	15.5a	12.1b a	13.4ca	8.4b	14.6a
K/ $\sqrt{\text{Ca}+\text{Mg}}$					
H ₂ O-soluble	12,3a	14.0b	12.8ab	10.7c	12.5a
1N NaCl-soluble	6.2a	14.0b	7.9ac	7.3ca	7.9c
Total	16.3a	15.4bc	13.2c	13.3c	16.5a
Ca/Mg					
H ₂ O-soluble	1.55ab	1.59ab	1.85ac	0.98a	1.69c
1N NaCl-soluble	0.87a	1.07b	1.30c	0.64d	0.83a
Total	2.26a	2.16a	2.69b	1.34c	2.09a

Note: Different letters between cultivars denote significant differences (LSD test. $p < 0.05$).

Water soluble and NaCl-fraction together combine more than 90.5% of the total Mg content. The ratio of total K/Mg (table 7) is highest in RI and MUE, 15.5 respectively 14.6. In SI, EHR, and REI are all lower viz. 12.1, 13.4 and 8.4, that means in RI and MUE is a strong dominance of K over Ca and Mg, in other words the tissue has probably a higher permeability. For instance cations may leach out of tissues if adverse weather conditions favor permanent moistening of stems. It is also possible that oxygen diffuses in the tissues and can initiate adverse oxidation processes with the help of phenol-oxidases resulting in its damage.

In the water soluble K/Mg fraction only RI differ vers. REI and MUE and the differentiation of the measured values is not very strong. In contrast K/Mg ratios in the NaCl-fraction differ very clearly. RI together with REI and MUE form a group with low K/Mg values in contrast to SI and EHR with very high values.

In soil science exists a formula in order to describe the interaction of K to Ca + Mg as a flocculating and/or dispersing agent which was also trans-

ferred to plant physiology. It shall characterize the status of tissues concerning their permeability.

The term
$$\frac{K}{\sqrt{\text{Ca} + \text{Mg}}}$$

was calculated for both water soluble and NaCl fractions including the total contents in stems. Results are shown in table 7. The calculation for the total elements values show that the highest amounts are found in RI, MUE and SI with 16.3, 16.5 and 15.4 respectively. High values indicate that K dominates over (Ca+Mg) and is a measure for the stability of membranes and/or tissues (Marschner and Mengel 1966). The findings for the water soluble fraction as well as the total amounts are not really compelling, because a differentiation between susceptible cultivars (RI and MUE) and non-susceptible ones is not possible.

Comparing both the characteristics of Ca and Mg in physiology there exist some major differences: Ca stabilizes mainly cell walls, middle lamellas and other supporting tissues and is involved to a minor, but very important part in

Fig.2. Breakdown of total variance for mineral nutrients in berries and stems and their partitioning on cultivar, nutrient fraction, interaction, and unexplained variance (%).

signaling stress e.g. drought, light, pathogens etc. (Tuteja, 2007). Mg has functional tasks as central ion in chlorophyll and can also overtake some unspecific functions of Ca i.e. stabilizing cell walls.

Taking these thoughts into account it should be promising to form a ratio between Ca/Mg because changes could probably indicate if Ca is lost from its main functions i.e. strengthening the cell wall system. As can be seen in table 7, these interactions between both nutrients seem to be a criterion in order to discriminate between cultivars. The water soluble fraction, which represent nutrients found in the cytosol and also the free space of the tissues indicates no clear differentiation as well as the ratios calculated with the total content. However, the NaCl fraction, which represents the exchangeable parts of both nutrients differentiate the cultivars very clearly. In both susceptible cultivars RI and MUE the ratio is very low, i.e. dominance of Mg over Ca and in SI and EHR the more robust cultivars, it is significantly higher.

Assessing the complete system of nutrients in berries and stems with help of a two way ANOVA is shown in fig. 2. The cultivars' influence on K, Ca, and Mg content in berries is significant but small; the main part of variance is loaded in the fraction. The interaction as well as the error term as also very small. It is therefore the technological process, which is responsible for the transfer of the main cations into the must. This is important when musts or wines are examined for inorganic constituents by official authorities.

The composition of stems may have a minor influence on must nutrients, except by strong mechanical stresses during transport and handling prior to pressing. In that case the accumulation of K, Ca, and Mg is a little bit more influenced by cultivar in comparison to berries, but the main load of variance is in the factor "fraction".

That means that handling can exert some influence on the cation transfer into musts. As stated above, mineral nutrients in stems may also have an influence on stems' health. Bunch stem necrosis is a much feared disease which may be responsible for yield and quality losses. Fig. 3 shows a bunch of Italian Riesling with severe symptoms of BSN. In the lower part of the bunch the berries shrinkle and are partly infected by Botrytis. It is obvious that such grape material is not very well suited for wine making.

From these findings it may be argued that Ca uptake, its transport and localization in different organs is genetically controlled. This is in agreement with Pooviah (1979) and Dodd et al. (2010). In ripening fruits an interchange of bound and soluble Ca in pericarp tissue of tomatoes occurs. A lot of scientific studies exist about the development and ripening of fruits (Ulrich 1952). In addition Solomos and Laties (1973) studied avocado, Suwwann and Pooviah (1978) tomatoes, Fils-Lycaon and Buret (1990) cherries and Hallet et al. (1992) kiwis. Early studies about grapevine ripening dealt only with sugars, acids and phenolic compounds (Kriedemann 1969, Hardy 1967, Pirie and Mullins 1986).

Fig.3. Bunch stem necrosis in Italian Riesling (by courtesy of Dr. G. Brendel, Geisenheim University)

Similar processes may also happen during bunch development and ripening of grapes. Further research seems necessary, on a time axis to verify these assumptions.

CONCLUSION

The evolution of cations in berries and stems was hardly in the focus of studies, because the common idea was that there exists no direct influence on the vinification process. Nevertheless, cation absorption is directly connected with organic acid synthesis which are important for vinification and conservation of wines. Only the accumulation of potassium got some notice, because of its influence on pH and its interaction with tartaric acid.

In a field test with 5 varieties (Riesling, Silvaner, Mueller-Thurgau, Ehrenfelser and Reichensteiner) the cations K, Mg and Ca were analyzed in berries and stems. It could be demonstrated that the tested varieties differ in their ability to accumulate the above mentioned cations in both organs. Much

stronger is the differentiation between varieties when nutrient fractions are compared. It can be seen that the chemical bonds of K, Mg and Ca differ in a wide range in the fractions. It is possible that with varying extraction or pressure techniques the mineral composition of must may vary and with the variety in use. An influence on wine quality may be expected but has to be proven in future investigations. The slightly exchangeable fractions of K and Mg have probably a strong effect on the incidence of BSN in susceptible varieties. There is no doubt about the importance of mineral nutrient on berry and stem health and their importance for wine quality as well as the yield and quality potential.

Mineral nutrients in different organs of grapevine bunches should attract more notice in order to get more information about their influence on bunch soundness and the final wine quality. Research on these topics needs to be intensified.

REFERENCES

1. Alleweltdt G and Hifny H A A (1972). Studies on grape stalk necrosis. II. Analysis of the causes of the disease. *Vitis* 11: 10-28.
2. Blouin J and Cruège J (2013). *Analyse et Composition des Vins*. Dunod, Paris.
3. Datnoff L E, Rodrigues F A, Seebold K W (2007). Silicon and plant disease. In: *Mineral nutrition and plant disease*. Eds. Datnoff, L.E., Elmer, W.H. and Huber D.M.. St. Paul, MN, USA: The American Phytopathological Society.
4. Dodd A N, Kudla J and Sanders D (2010). The language of calcium signaling. *Annu. Rev. Plant Biol.* 61, 539-620.
5. Epstein E and Bloom A J (2005). *Mineral Nutrition of Plants: Principles and Perspectives*. 2nd ed. Sinauer Associates, Inc. Publishers. Sunderland, MA, USA.
6. Fils-Lycaon B and Buret M (1990). Loss of firmness and changes in pectic fractions during ripening and overripening of sweet cherry. *HortScience* 25 (7): 777-778.
7. Hallett I C, Macrae E A and Wegrzyn T F (1992). Changes in kiwifruit cell wall ultrastructure and cell packing during postharvest ripening. *Int. J. Plant Sci.* 153 (1): 49-60.
8. Hardy P J (1967). Sucrose breakdown and synthesis in the ripening grape berry. *Aust. J. Biol. Sci.* 20: 465-470.
9. Hifny H A A (1972). Untersuchungen zur Stielähme der Reben. I. Die Symptomataologie der Krankheit. *Vitis* 10: 298-313.
10. Kriedemann P E (1969). Sugar uptake by grape berry: a note on the absorption pathway. *Planta* 85: 111-117.
11. Marschner P (ed.) (2011). *Marschner's Mineral Nutrition of Higher Plants*. Academic Press. ISBN: 978-0-12-384905-2.
12. Marschner H and Mengel K (1966). Der Einfluß von Ca- und H-Ionen bei unterschiedlichen Stoffwechselbedingungen auf die Membranpermeabilität junger Gerstenwurzeln. *Z. Pflanzenern. und Bodenkunde* 112, 39-49.
13. Mengel K and Kirkby E A (2007). *Principles of Plant Nutrition*. International Potash Institute, Worblaufen-Bern, Switzerland. 3rd ed.
14. Pirie A J G and Mullins M G (1980). Concentration of phenolics in the skin of grape berries during fruit development and ripening. *Am. J. Enol. Vitic.* 31: 34-36.
15. Pooviah B W (1979). Role of calcium in ripening and senescence. *Comm. Soil Sci. Plant Anal.* 10, 83-88.
16. Solomos T and Laties G G (1973). Cellular organization and fruit ripening. *Nature*, 245: 390-391.
17. Souci S W, Fachmann W, Kraut H. (2008). *Food Composition and Nutrition Tables*. 7th Ed., Wissenschaftliche Verlagsgesellschaft Stuttgart.
18. Stellwaag-Kittler F (1967). Die Stielähme der Trauben. *Dt. Weinbau* 18: 1048.
19. Suwwan M A and Pooviah B W (1978). Association between elemental content and fruit ripening in rin and normal tomatoes. *Plant Physiol.* 61: 883-885.
20. Theiler R (1986). Beziehungen zwischen Klimafaktoren und dem Stielähmebefall bei Riesling x Sylvaner. *Vitis* 25: 8-20.
21. Tuteja N, Mahajan S (2007). Calcium signaling network in plants. *Plant Signaling & Behavior* 2: 79-85.
22. Ulrich R (1952). *La vie des fruits*. Masson Ed. Paris.
23. Usseglio-Tomasset L (1989). *Chimie Oenologique*. TecDoc-Lavoisier, Paris.
24. Würdig G and Woller R (1989). *Chemie des Weines*. Verlag Eugen Ulmer Stuttgart.