

Effect of rootstock on levels of ammonium ions and yeast assimilable nitrogen in musts of the grapevine varieties 'Weißer Riesling' and 'Grüner Veltliner'

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This paper deals with the content of yeast assimilable nitrogen in grape musts originating from the winegrowing region South Moravia in the Czech Republic. Experimental data were collected in years 2007 and 2008 (rootstocks 'Kober 5BB' and 'SO4'; varieties 'Weißer Riesling' and 'Grüner Veltliner'). The obtained results showed that all musts contained high concentrations of both ammonium ions and yeast assimilable nitrogen (124.4 mg/l and 257.8 mg/l, respectively). A comparison of the two rootstocks indicated that average contents of ammonium ions were higher in the variant with the rootstock 'Kober 5BB', but these differences were not significant. Distinctive differences were found between both varieties under study: grape musts from the variety 'Grüner Veltliner' contained higher levels of ammonium ions and yeast assimilable nitrogen. It is well known that grapes of this variety contain high amounts of proteins.

Keywords: ammonium ions, yeast assimilable nitrogen, 'Weißer Riesling', 'Grüner Veltliner', 'Kober 5BB', 'SO4'

Einfluss von Unterlagen auf die Konzentrationen von Ammoniumionen und hefeverfügbarem Stickstoff in Keltermost der Rebsorten 'Weißer Riesling' und 'Grüner Veltliner'. Die Gehalte an hefeverfügbarem Stickstoff in Mosten aus der Weinbauregion Südmähren (Tschechische Republik) wurden untersucht. Die Daten (Unterlagen: 'Kober 5BB' und 'SO4'; Sorten: 'Weißer Riesling' und 'Grüner Veltliner') wurden in den Jahren 2007 und 2008 erhoben und zeigten, dass in allen analysierten Mosten die Konzentrationen von Ammoniumionen und hefeverfügbarem Stickstoff relativ hoch waren (124,4 mg/l bzw. 257,8 mg/l). Nach dem Vergleich beider Unterlagen wurde festgestellt, dass in der Variante mit 'Kober 5BB' die durchschnittlichen Konzentrationen von Ammoniumionen höher waren als in der Variante mit 'SO4', die Unterschiede waren aber nicht signifikant. Deutliche Unterschiede wurden zwischen den beiden Rebsorten festgestellt: Die Konzentrationen von Ammoniumionen und hefeverfügbarem Stickstoff waren im Most der Sorte 'Grüner Veltliner' höher. Es ist auch bekannt, dass die Trauben dieser Sorte hohe Proteinnengen enthalten.

Schlagwörter: Ammoniumionen, hefeverfügbarer Stickstoff, 'Weißer Riesling', 'Grüner Veltliner', 'Kober 5BB', 'SO4'

L'influence des porte-greffes sur les concentrations d'ions d'ammonium et d'azote disponible pour la levure dans le moût de cuve des cépages 'Weißer Riesling' et 'Grüner Veltliner'. Les teneurs en azote disponible pour la levure des moûts en provenance de la région viticole Moravie du Sud (République tchèque) ont été examinées. Les données (porte-greffes : 'Kober 5BB' et 'SO4'; cépages : 'Weißer Riesling' et 'Grüner Veltliner') ont été relevées au cours des années 2007 et 2008 et ont montré que les concentrations d'ions d'ammonium et d'azote disponible pour la levure étaient relativement élevées dans tous les moûts analysés (124,4 mg/l et 257,8 mg/l respectivement). Suite à la comparaison des deux porte-greffes, on a constaté que les concentrations moyennes d'ions d'ammonium étaient plus élevées dans la variante avec 'Kober 5BB' que dans la variante avec 'SO4', les différences n'étant pas significatives. Des différences importantes ont été constatées entre les deux cépages : les concentrations d'ions d'ammonium et d'azote disponible pour la levure étaient plus élevées dans le moût du cépage 'Grüner Veltliner'. Il est également connu que les raisins

de ce cépage contiennent de grandes quantités de protéines.

Mots clés: ions d'ammonium, azote disponible pour la levure, 'Weißer Riesling', 'Grüner Veltliner', 'Kober 5BB', 'SO4'

It is well known that nitrogen strongly influences the growth of grapevine (*Vitis vinifera* L.), it affects annual shoot formation as well as the colouration of its leaves (KRAUS et al., 1997). Nitrogen compounds stored in berries are very important for propagation and life activities (i.e. metabolism) of yeasts. Yeasts of the species *Saccharomyces cerevisiae* MEYEN ex E. C. HANSEN, which always predominate in the course of must fermentation, can utilise nitrogen either in the form of ammonium ions or of free amino acids (except proline under anaerobic conditions) and that is why the term "yeast assimilable nitrogen" (YAN) is generally used (HERNÁNDEZ-ORTE et al., 2006).

Concentration of ammonium ions in musts is getting more and more important especially in warm regions and in case of overripe grapes. Normal levels of ammonium range from tens to hundreds of milligrams. Within the Krebs cycle and during the degradation of sugars, great amounts of α -keto acids are produced and their amination results in the formation of many organic compounds containing nitrogen (above all amino acids). These compounds are very beneficial due to their antioxidative, antimicrobial, emulgative and surface-active properties and create a relatively great amount of sugar-free extract (up to 4 g/l) (RIBÉREAU-GAYON et al., 2006).

It is known that the content of YAN in must is not only dependent on fertilisation, weather, and length of the maceration of skins in must, but also on variety, year, origin and, last but not least, on the technology of wine making (SOUFLEROS et al., 2003; ETIÉVANT et al., 1998; ARVANITOYANNIS et al., 1999). In addition to all these factors, the utilisation of YAN also distinctively depends on the metabolism of yeasts and on conditions of fermentation. The YAN value of grape juices varies widely between 50 and up to 450 mg/l, the average value is approximately 200 mg/l. The published and generally accepted minimum level of YAN required to prevent stuck or sluggish fermentations, is considered to be 140 to 150 mg/l for a 21 °Brix clarified must (O'KENNEDY and REID, 2008).

Depending on the availability of nutritive substances and conditions of nitrogen utilisation, yeasts produce great amounts of substances that significantly influence the wine quality. Some of these metabolites show a positive effect on wine but some other may cause problems and organoleptic defects (JIRANEK et al.,

1995). It is known that a lack of YAN in must is the main cause for stuck and sluggish fermentations (BOULTON et al., 1996) as well as of production of sulphurous compounds (GIUDICI and KUNKEE, 1994; FERREIRA et al., 2009; JIRANEK et al., 1996). The total content of nitrogen in must may also affect wine aroma and influences the production of acetic acid (BELY et al., 2003), bioamines (MARCOBAL et al., 2005; COSTANTINI et al., 2009) and highly carcinogenic ethyl carbamate (OUGH et al., 1988; COULON et al., 2006). In practice a lack of YAN occurs most often in unripe grapes and in stressed ones originating from vineyards insufficiently fertilised with nitrogen or produced during dry and/or hot periods. In such cases, the lack of YAN must be compensated by the addition of nutritive salts into the must or, possibly, also vitamin B1 (thiamine). Furthermore grapes with a higher content of sugars require higher amounts of available nitrogen for a complete fermentation. The aim of this study was to monitor the effects of grapevine varieties ('Weißer Riesling' and 'Grüner Veltliner') and rootstocks ('Kober 5BB' and 'SO4') on ammonium ions and YAN levels in the viticultural region South Moravia (Czech Republic).

Material and methods

The experimental material originated from the viticultural sub-regions Mikulov (M) and Znojmo (Z) and was collected from vineyards with grass cover (G) or without grass cover (N) during the period from 2007 to 2008 (rootstocks 'Kober 5BB' and 'SO4', varieties 'Weißer Riesling' (RR) and 'Grüner Veltliner' (GV).

Samples

From 2007 to 2008 a total of 48 musts originating from the viticultural region Moravia were analysed. Nitrogen fertilisers were not applied in any of the experimental variants. To assure the objectivity of the results, grapes were sampled from more than 20 plants in each variant. After harvesting, grapes were destalked and crushed. The minimum weight of berries without stalks was 5 kg per variant. After a gentle pressing in a mechanical press, the must was treated with sulphur

dioxide to 40 mg/l SO₂ and spontaneously decanted (12 hours in cold). Thereafter all musts were frozen to -18 °C. The must samples for analyses were taken after defrosting.

Reagents and solvents

Formaldehyde (40 %), sodium hydroxide, sulphuric acid, and hydrochloric acid were all HPLC grade (Lach-Ner s.r.o, Czech Republic). HPMC (hydroxypropyl methylcellulose), 18-Crown-6 acid, MES (morpholinethansulphonic acid), and BTP (1,3-bis[tris(hydroxymethyl)methylamino] propane) were purchased from Sigma Aldrich Co. (USA).

Determination of YAN

The Formol titration method used in this study was described by GUMP et al. (2000) and FILIPE-RIBEIRO and MENDES-FAIA (2007).

The procedure is as follows: Pour 100 ml of the sample into a 200 ml beaker, then neutralise it to pH-value 8.0 using 1 M NaOH with a pH meter. The treated sample is transferred into a 200 ml volumetric flask. Bring to volume with deionised water and mix well. Filter the resulting solution through a filter paper. Thereafter transfer a 100 ml aliquot of the sample into a beaker, and readjust to a pH-value of 8.0 with 1 M NaOH (if necessary). Add 25 ml of neutralized formaldehyde (pH-value 8.0), mix the resulting solution and titrate it to pH-value 8.0 using 0.1 M NaOH.

Results can be calculated using the following equation:

$$\text{mg N/l} = \frac{[(\text{vol. NaOH}) \times (\text{conc. NaOH}) \times 14 \times (\text{dilution factor}) \times 1000]}{(\text{sample vol.})}$$

Determination of ammonium ions

Ammonium ions were determined by Capillary Isotachopheresis (CITP) using the apparatus Ionosep 2003 (Recman, Czech Republic). Leading electrolyte (LE): 5 mM H₂SO₄ + 7 mM-18-Crown-6 + 0.1 % HPMC; terminating electrolyte (TE): 10 mM BTP; initial current: 100 µA; final current 50 µA; mode of analysis: cationic.

The standard deviation of measured values was used as an indicator of significance of differences between obtained results.

Results

This paper is a continuation of the publication concerning the influence of different factors like year, sub-region and grass cover on the content of ammonium ions and yeast assimilable nitrogen (BAROŇ, 2011a). In the following further results are presented.

Contents of ammonium ions and YAN in 'Weißer Riesling' and 'Grüner Veltliner'

Contents of ammonium ions and YAN as determined in the varieties 'Weißer Riesling' and 'Grüner Veltliner' are presented in Table 1. A comparison of both varieties indicated that the average content of ammonium ions was higher in 'Grüner Veltliner' than in 'Weißer Riesling'. Also the content of total YAN was significantly and markedly higher in grapes of this variety. Not only the content, but also the variability of the determined values was higher in 'Grüner Veltliner' than in 'Weißer Riesling' (Fig. 1). Histograms elaborated for variants of the varieties 'Weißer Riesling' and 'Grüner Veltliner' (data not presented) indicated that the number of samples with concentrations of ammonium ions below 50 mg/l was equal to zero in both variants. The numbers of samples with YAN levels lower than 200 mg/l were eight and eight in variants of 'Weißer Riesling' and 'Grüner Veltliner', respectively. Numbers of samples containing more than 300 mg/l were 0 and 8 in variants with 'Weißer Riesling' and 'Grüner Veltliner', respectively.

Ammonium ions and YAN contents in rootstocks 'Kober 5BB' and 'SO4'

Values of ammonium ions and YAN, as determined for both rootstock variants (i.e. 'Kober 5BB' and 'SO4') are presented in Table 2. A comparison of both rootstocks indicated that average contents of ammonium ions and of YAN were higher in the variant with the rootstock 'Kober 5BB' than in the 'SO4' variant. However, these differences were not significant. Histograms elaborated for rootstocks 'Kober 5BB' and 'SO4' (data not presented) indicated that the number of samples with concentrations of ammonium ions below 50 mg/l was almost zero in both variants. The numbers of samples with YAN levels below 200 mg/l were two and six for rootstocks 'Kober 5BB' and

Table 1: Results (mg/l) of the statistical analysis (variants with 'Weißer Riesling' and 'Grüner Veltliner')

Variety	NH ₄ ⁺	Conf. Int.	Conf. Int.	AN	Conf. Int.	Conf. Int.
Weißer Riesling	123.65	110.70	136.60	215.47	198.18	232.76
Grüner Veltliner	125.14	117.93	132.36	300.13	275.50	324.76

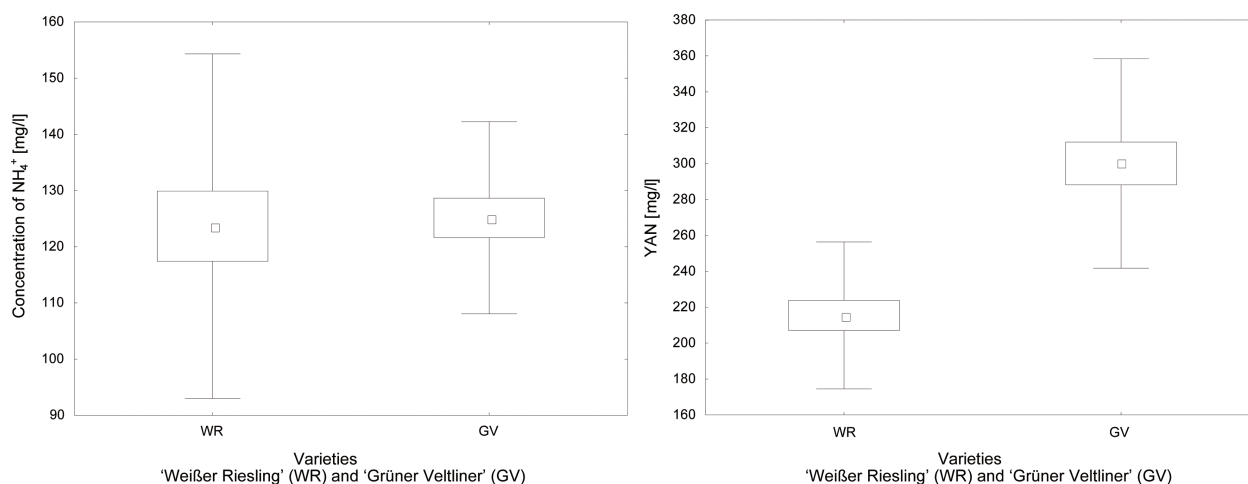


Fig. 1

'SO₄', respectively. Numbers of samples containing more than 300 mg/l were higher in case of the rootstock 'SO₄' (six) while in the variant with the rootstock 'Kober 5BB' only two samples contained more than 300 mg/l.

The following part of this paper presents results and interactions obtained by means of the variance analysis (ANOVA) method. Only the analysis of the 2nd degree was performed because higher degrees were considered as irrelevant regarding the number of determinations. The following interactions were calculated as significant:

Year x sub-region interaction (content of YAN)

As compared with the year 2008, the amount of YAN was significantly higher in grapes harvested in the Mikulov viticultural sub-region in 2007. On the other hand, in the Znojmo viticultural sub-region a quite opposite and more marked trend was identified (Fig. 3A).

Grass cover x rootstock interaction (content of YAN)

In grapes harvested from plants with the rootstock 'SO₄' in vineyards without grass cover the level of YAN was significantly higher than in those originating from vineyards with the grass cover (Fig. 3B).

Grass cover x variety interaction (content of YAN)

In 'Grüner Veltliner' grapes originating from vineyards without the grass cover, the content of YAN was significantly higher than in grapes from the variant with the grass cover. As far as the variety 'Weißer Riesling' was concerned, no interaction was found out (Fig. 3C).

Sub-region x rootstock interaction (content of YAN)

In grapes on rootstock 'SO₄' and originating from the Mikulov viticultural sub-region, the content of YAN

was significantly lower than in grapes harvested in the Znojmo sub-region. In case of the rootstock 'Kober 5BB' this interaction was not significant (Fig. 3D).

sively because it takes up mainly nitrogen and phosphorus from the soil. The uptakes of potassium and magnesium are only limited and this can result in an unbalanced nutrition of the plants. The rootstock

Table 2: Results (mg/l) of the statistical analysis (variants with 'Kober 5BB' and 'SO4')

Variety	NH ₄ ⁺	Conf. Int.	Conf. Int.	AN	Conf. Int.	Conf. Int.
K 5BB	129.27	117.83	140.70	260.98	233.54	288.42
SO4	120.92	111.41	130.42	255.53	227.90	283.17

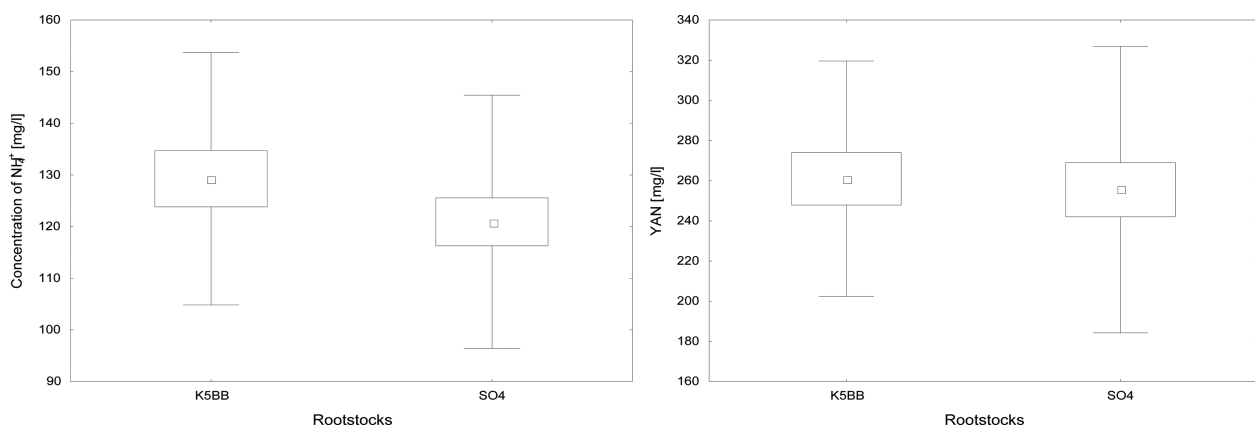


Fig. 2

Discussion

Because there are no data available about levels of YAN in the Czech Republic, it is necessary to compare results of this study only with data from literature and results obtained in other countries. Over the period 2007 to 2008 a total of 48 musts originating from the viticultural region Moravia were analysed. Regardless of influencing factors the mean content of ammonium ions and YAN was 124.4 mg/l and 257.8 mg/l, respectively (BAROŇ, 2011b). This fact indicates that musts from the viticultural region Moravia can start fermenting easily even without an addition of nutrients and that also the fermentation runs without problems. According to GUMP et al. (2002) and VILANOVA et al. (2007) these concentrations were higher than usual. However, it is possible that the contents of ammonium ions and YAN will be higher in regions with a colder climate and more rainfall.

'Kober 5BB' is a robust and vigorous rootstock, which is suitable for loamy and gravelly soils situated in wet regions with a lower content of active limestone. It is suitable above all for high-yielding varieties, which are not prone to blossom drop and do not grow too inten-

'SO4' shows an intermediate intensity of growth and accelerates ripening of both grapes and wood of grafted varieties. However, it is not very resistant to drought and for that reason it is suitable for loamy and uniformly wet soils; besides, it also tolerates well calcareous soils (up to 20 % of calcium). A comparison of results obtained with rootstocks 'Kober 5BB' and 'SO4' did not reveal any significant differences in YAN and ammonium content (Fig. 2). 'Weißer Riesling' is a typical grapevine variety in northern viticultural regions. It requires a balanced fertilisation and a careful application of nitrogen. Its most important advantages involve high resistance against frosts, regular good harvests, low expenditure of work when treating the vineyard, lower susceptibility to fungus diseases and excellent wine quality. Disadvantages involve late ripening, susceptibility to stalk diseases, and tendency to blossom drop.

'Grüner Veltliner' sprouts relatively early, is of intermediate vigour, and its grapes ripen lately. This variety requires very good sites and deep, loessial or loamy soils with a good water-holding capacity. A comparison of 'Weißer Riesling' with 'Grüner Veltliner' corroborated the assumption that 'Grüner Veltliner' is a

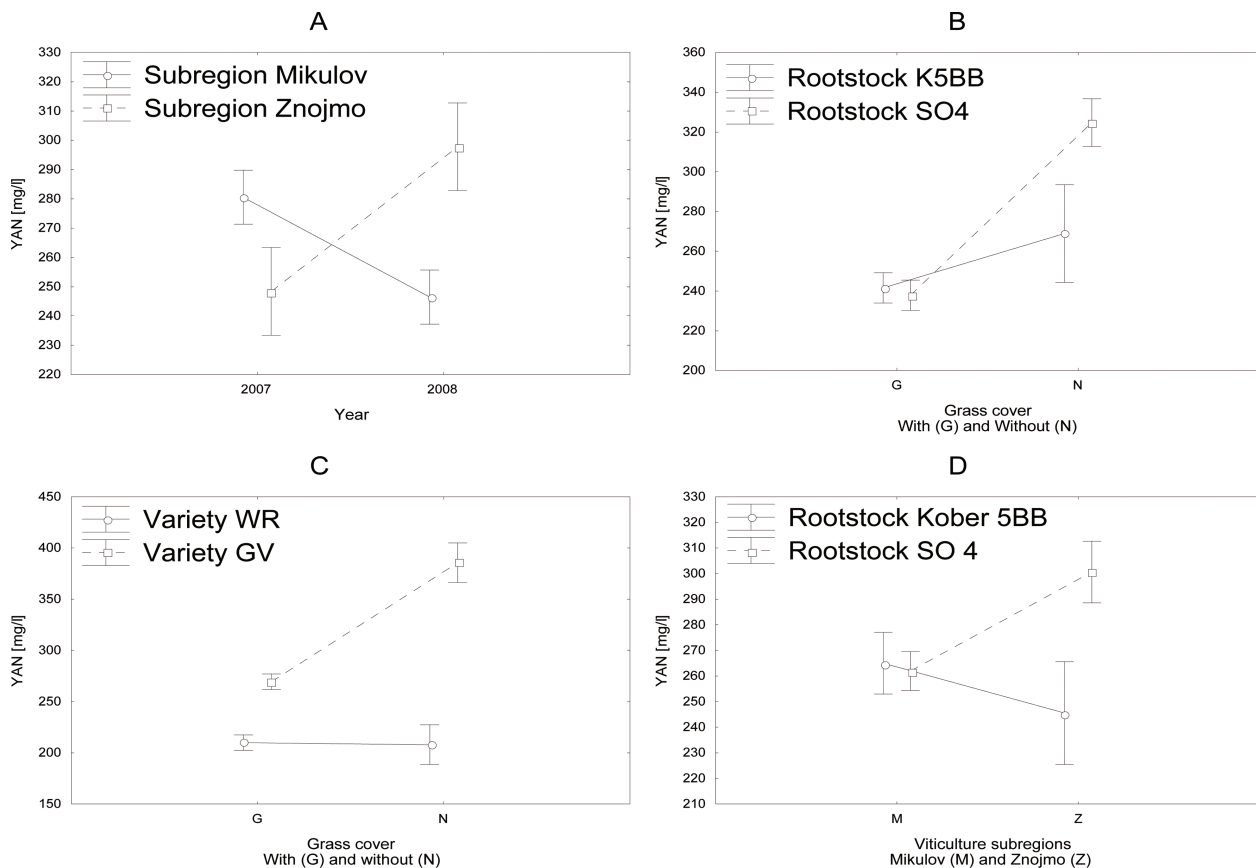


Fig. 3

variety requiring a high supply of nitrogen substances, both in the form of proteins and/or of YAN. In case of ammonium ions the observed differences were not significant (Fig. 1). The very wide interval of concentration of ammonium ions in the grapes of the variety 'Weißer Riesling' was probably caused by its late ripening. In case of YAN concentrations, however, the variability was higher in grapes of the variety 'Grüner Veltliner'. In this variety altogether eight samples showed values higher than 300 mg/l.

Interaction between individual factors

The significance of climatic differences on the content of YAN in grapes is shown in Fig. 3A, which illustrates that an interaction between the year and the sub-region exists. The content of YAN was correlated with the average sum of precipitations within the growing season in both sub-regions under study (Fig. 4). In 2007, a lower rainfall activity was recorded in Oblekovic (sub-region Znojmo), while in the extremely dry year 2008 low precipitations were registered in Břeží

(sub-region Mikulov). This weather characteristics markedly correspond with levels of YAN in grapes harvested in both sites.

Further interesting data concerned the content of YAN resulting from a proved interaction between grass cover and rootstock (Fig. 3B). In case of both rootstocks, YAN contents were higher in the variant without grass cover in inter-rows. Above all grapes produced on plants with the rootstock 'SO4' were markedly different. In both cases it was possible to demonstrate competitive relationships between grapevines and grass cover in the vineyard. However, the root system of 'SO4' is shallower and it can be expected that the N-competition with cover crops is more intensive. Nevertheless, it is necessary to repeat in this connection that the number of variants with inter-rows was markedly lower.

The interaction between grass cover and rootstock and variety also influenced the level of YAN (Fig. 3C). In case of 'Weißer Riesling' practically identical results were obtained in both variants of tillage while in case of 'Grüner Veltliner' the effect of inter-rows was very marked and extremely high contents of YAN were

achieved (in average nearly 400 mg/l). As compared with 'Weißer Riesling', the variety 'Grüner Veltliner' showed a markedly higher uptake of nitrogen substances from the soil and if the conditions were favourable for it (i.e. when the inter-rows were without grass cover), levels of YAN were much higher than the required concentrations.

The effect of the interaction between sub-region and rootstock on the level of YAN is illustrated in Fig. 3D. Based on these data it can be concluded that, in spite of a low amount of precipitations, soils in the Znojmo sub-region were more suitable for 'SO4' rootstocks than for 'Kober 5BB' because the achieved levels of YAN in grapes were higher.

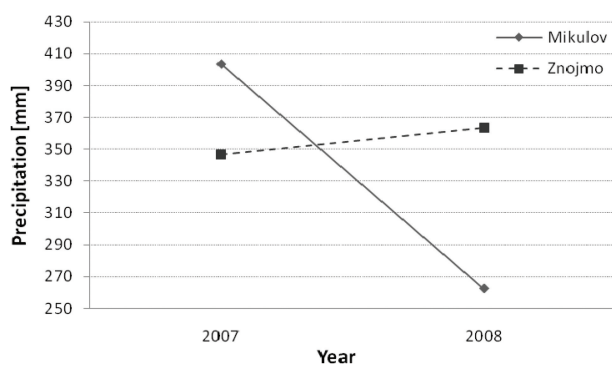


Fig. 4

Conclusions

In the viticultural region Moravia (Czech Republic), the contents of YAN and ammonium ions in musts are more than sufficient. Based on obtained results, it can be concluded that in the majority of cases it is not necessary to supplement musts with ammonium salts and that it is necessary to be rather cautious with the application of these salts. It was found that contents of nutrients and especially ammonium ions were higher than sufficient even in musts with increased sugar contents. If the addition of nutrients into fermenting musts is too high, there is a risk that the taste of the wine will be negatively influenced due to an easier utilisation of ammonium ions which can lead to a reduced formation of natural wine bouquet resulting from degradation of amino acids by yeasts.

When establishing vineyards, it is necessary to consider interactions and relationships existing between individual rootstocks, varieties, and types of grass cover of inter-rows to prevent the occurrence of extreme synergic effects. For example in vineyards with

bare inter-rows, rootstock 'Kober 5BB' and variety 'Grüner Veltliner', it can be expected that the amounts of N-substances and proteins will be much higher than required.

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