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GABA in Grapevines - Is It Only a Compound for Nitrogen Storage and/or an Import Stress and Quality Indicator?

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Abstract. Taking into account all information about GABA in different plants we tried to find out some of the fundamental roles of GABA in grapevines: Its dynamic in different plant organs including vegetative and generative ones as well as dependencies on exogenous factors. AA dynamic in one year old wood was confirmed according former findings; N nutritional status of the vine has an important influence on stored amounts of Arg, Gln, Glu and GABA. Shoots shows relatively low contents of all determined AAs; highest amounts are found with Ala, Gln and Glu. Fertilization increases Gln and Glu. Highest amounts of al AAs are found in emerging and developing leaves; GABA has highest amounts besides Glu. The influence of N fertilization is obvious. In developing fruits main AA is Gln; role of GABA is just unclear. GABA synthesis seems to be dependent on GDD and on N nutritional status of the grapevine. In further research work more emphasis has to be laid on the influence of exogenous factors like water, light and also diseases. The relationship on wine quality is still open but the by-products of GABA-shunt should be shifted in the focus of wine makers and wine microbiologists.

Keywords: *Vitis vinifera (c.v.White Riesling)*, GABA, alanine, glutamine, glutamic acid, vegetative, generative organs

INTRODUCTION

γ-Amino butyric acid (GABA) is an ubiquitous four-C, non-protein, amino acid, widely distributed about biological world, first identified in potatoes (Stewart *et al*., 1949), however, its functional significance is still not fully understood. The pathway starts with the decarboxylation of glutamate (Glu) to produce GABA and CO_2 in the cytosol. GABA is then presumably transported to the mitochondria by an as yet unidentified GABA transporter, where it is converted to succinic semialdehyde (SSA). Subsequently, SSA is converted either to succinate or 4-hydroxybutyrate. GABA is proposed to be involved in a legion of cellular processes ranging from neuronal inhibition in animals (Bowery, N. *et al*. 2004) to pollen-tube development in *Arabidopsis thaliana* (Palanivelu *et al.* 2003). Hypotheses on its role in plants have flourished, largely owing to its rapid accumulation in response to biotic and abiotic stresses (Kinnersley and Turano, 2000) and its high concentration in various tissues (Kato-Noguchi and Ohashi, 2005; Bown *et al.*, 2002; Fait *et al.*, 2006; Reggiani *et al.*, 2000). Assumptions to explain such alterations in GABA metabolism include roles in signalling, herbivore deterrence, pH regulation, redox regulation, energy production and maintenance of carbon/nitrogen (C/N) balance (see Bouché and Fromm, 2004 and references therein). However, when changes in GABA are looked at in the context of broader changes of metabolism, interesting patterns emerge. For example, the GABA shunt has been shown to be activated by light, during developmental phases and in an N status-dependent manner (Fait *et al.*, 2006; Fait *et al.*, 2005; Allan and Shelp, 2006; Masclaux-Daubresse *et al.*, 2002), as well as in parallel to other changes that occur in central metabolism during plant growth (Stitt *et al.*, 2002). GABA is linked via the GABA shunt to TCA cycle and my influence the production of succinic acid, which may also alter wine quality (Baron and Fiala, 2012). Aim of our research was, to elucidate the dynamics of GABA and its related amino acids in grapevines respectively different vegetative and generative organs during two growing seasons as well as the influence of a differing nitrogen nutrition status.

MATERIALS AND METHODS

In a long lasting fertilizer trial (start in 1985 until 2015) plant organs (one year old wood, stems, leaves and generative organs) were harvested during two growing seasons (1990 and 1991) at distinct stages of phenological development:

1. One year old wood (DOY: 11, 53, 74,102, 117,124, and 128);
2. Leaves inserted above bunches at “flowers fully developed”, “full bloom”, “after bloom”, “fruit set”, “berries like peas”;
3. Shoots: corresponding part of the shoot belonging to the leaves with plastochrone index 9-12;
4. Flowers including berries

Samples were taken in the control plot (no nitrogen) and that treated with 30 units N at budburst and 60 units N after bloom (4 replicates).

Amino acids were extracted from tissues and determined after derivatisation with HPLC. Main focus was on alanine (Ala), glutamic acid (Glu) glutamine (Gln), and GABA.

RESULTS AND DISCUSSION

It is a well known fact that in perennial parts of grapevines large amounts of Arg are stored during autumn and winter and subsequently metabolized and transferred to newly emerging organs (Schaller *et al*., 1989). Fig 1 a and b show the dynamic of Arg including Gln, GABA and Pro. The influence of N fertilization is obvious. Generally, the content of all soluble AA is higher in the fertilized plots (1b). The decay of Arg during early plant development follows the same pattern in both treatments: Shortly before bud break (DOY 117) Arg and Gln show a maximum. Arg peaks some days earlier than Gln; earliest max is with GABA at DOY 102.

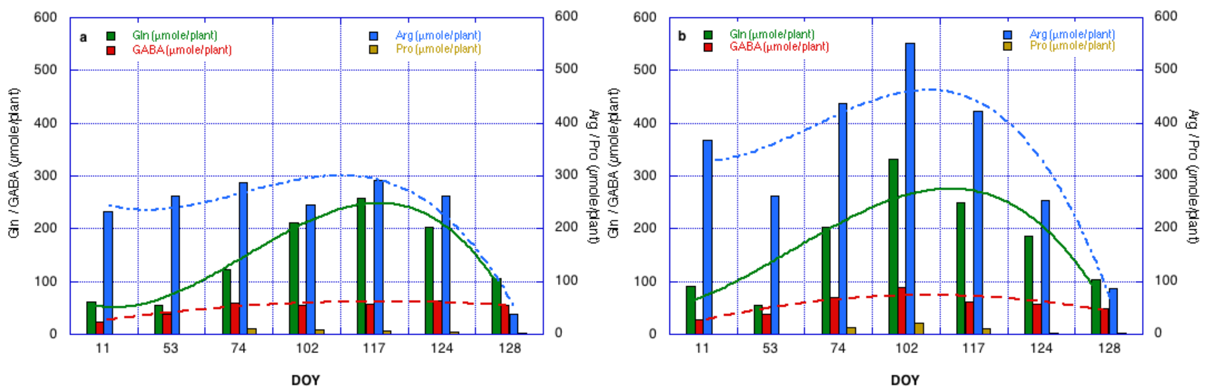

Fig 1: Dynamic of soluble AAs (**Gln, GABA, Arg and Pro**) in 1 year old wood from DOY 11 to 128 (µmole/plant organ). 1a: control plot; 1b: fertilized with 30 units N at “5-6 leaves unfolded” and 60 units N “after bloom”.

After emergence of leaflets (DOY >117) Arg is metabolized very rapidly and Gln content is higher than Arg. Pro does not reach important amounts in wooden parts.

Besides Arg as a dominating AA in the wooden part of grapevines, also AAs belonging to the Gln and GABA shunt play a significant role (Fig. 2 a and b).

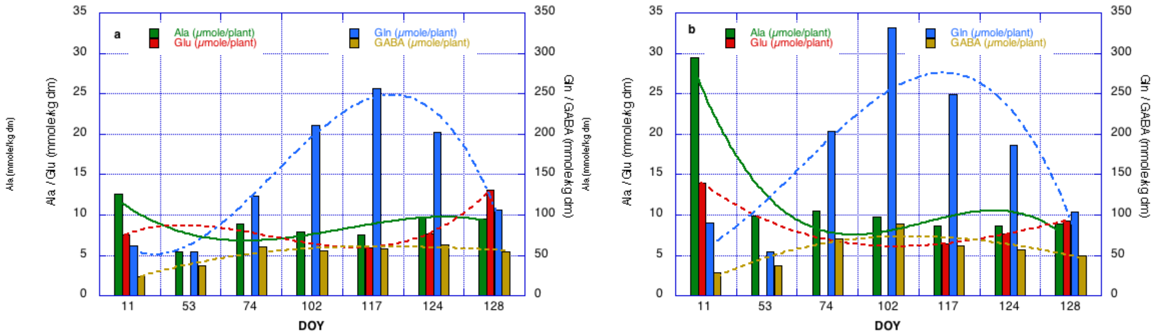

Fig 2: Dynamic of soluble AAs (**Ala, Glu, Gln and GABA**) in 1 year old wood from DOY 11 to 128 (µmole/plant organ). 2a: control plot; 2b: fertilized with 30 units N at "5-6 leaves unfolded" and 60 units N "after bloom".

As stated already above, nitrogen fertilization increases the amount of soluble AAs in the 1-year old wood of grapevines. In the case of Ala the content is tripled and GABA nearly doubled. In both cases during early development (DOY 11 to 74) Ala declines and subsequently Gln increases. GABA and Gln have a similar max at DOY 117, which corresponds to bud break/first leaflets.

Basis for emerging leaves and the shoot system are stems; they are the carrier system for solutes and also nitrogenous compounds. The composition may give also some insights in the dynamic of the root and woody system, which nourish growing stems and leaves (Fig. 3 a and b). Control plots as well as fertilized ones have similar contents of all AAs at DOY 128 (bud break/first leaflets). But already 10 days later Ala and Glu have doubled in the treated plot. At DOY 150 (5-6 leaves unfolded) contents of Ala, Glu and Gln have nearly tripled in fertilized plots. Also GABA shows a steady increase until DOY 200 ("berries like peas"). In the control plot all soluble AAs, especially Glu and Gln have a very low level, indicating an overall low level of nitrogen nutrition of grapevines.

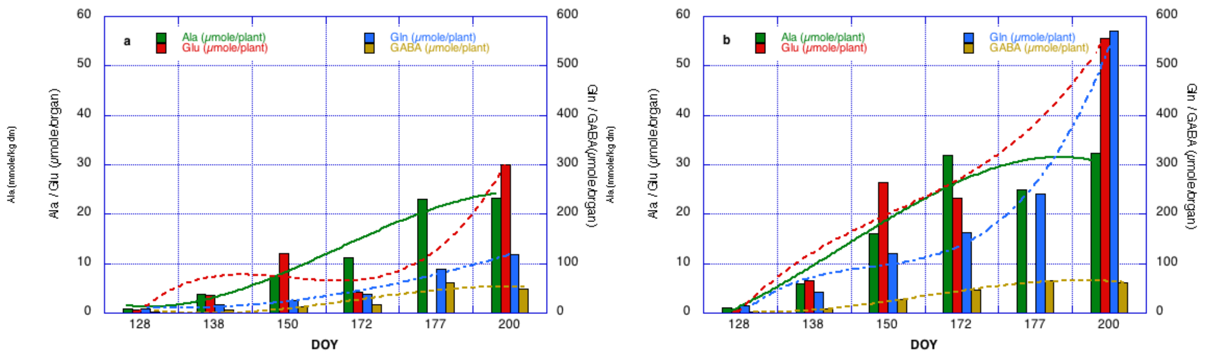

Fig. 3: Dynamic of soluble AAs (Ala, Glu, Gln and GABA) in shoots DOY 128 to 200 (µmole/plant organ). 3a: control plot; 3b: fertilized with 30 units N at "5-6 leaves unfolded" and 60 units N "after bloom".

Leaf material shows a high dynamic between the important phenological stages "leaves emerging until pea sized berries" (Fig. 4 a and b). Indeed, it can be stated again, that in fertilized plots the level of all AAs is higher after DOY 138 until 200.

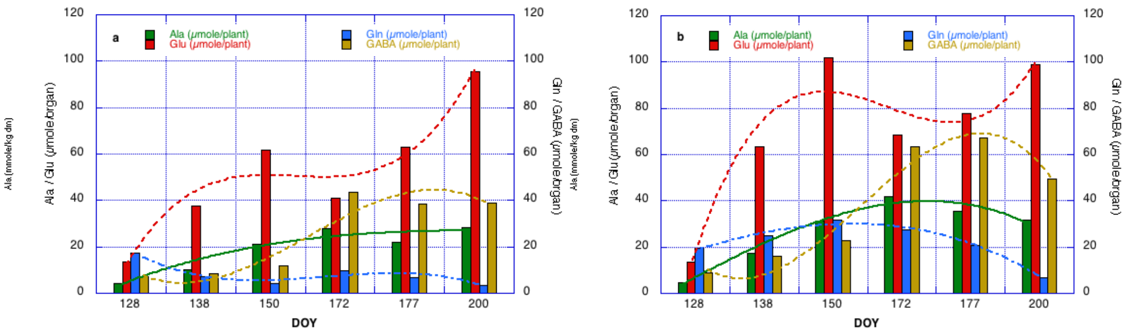

Fig. 4: Dynamic of soluble AAs (Ala, Glu, Gln and GABA) in leaves from DOY 128 to 200 (µmole/plant organ). 4a: control plot; 4b: fertilized with 30 units N at "5-6 leaves unfolded" and 60 units N "after bloom".

By a direct comparison of both plots (Fig 4 a and b) one can see that all AAs follow the same pattern of accumulation and decline. However, the level of Ala, Gln and GABA is always higher during DOY 138 until 200 in the fertilized plots. Only Glu reaches similar amounts in the control plots.

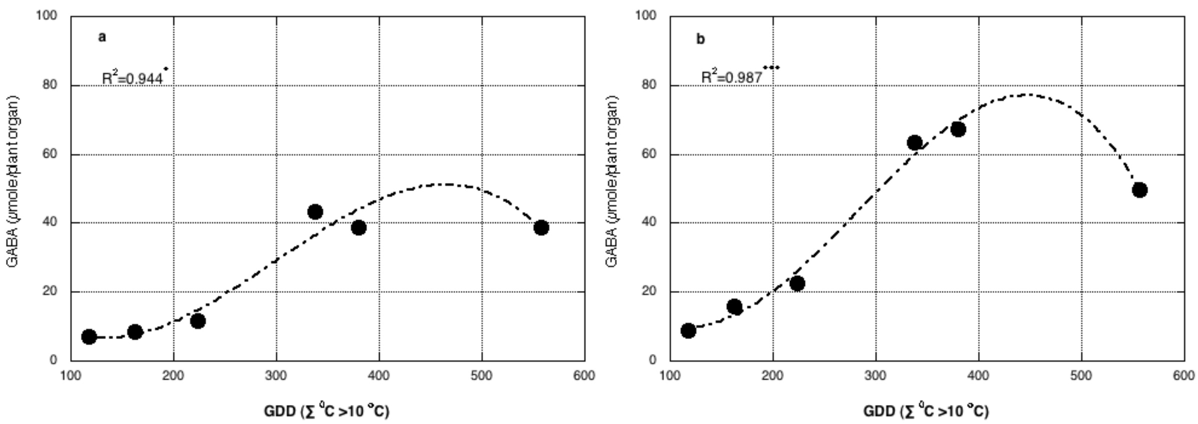

Fig 5: Relationship between GDD (Growing Degree Days >10 °C) and GABA content in leaves (µmole/plant organ) in 1990. 5a: control plot; 5b: fertilized with 30 units N at "5-6 leaves unfolded and 60 units N "after bloom".

It seems that GABA is the driving substance during that time. Its concentration and also total content in leaves is higher than that of Gln and Arg as well as Pro. It can be seen that the nutritional status of the grapevine influences GABA content in leaves. Fig. 5 as well Fig. 6 in addition demonstrates that also GDD has a significant influence on the formation of the GABA content in leaves. The figures indicate also strong interaction with N nutrition. The influence of the year shows Fig. 6 a/b. The level of GABA in 1991 is nearly doubled in comparison to 1990. Also the nutritional status stays constant in subsequent years.

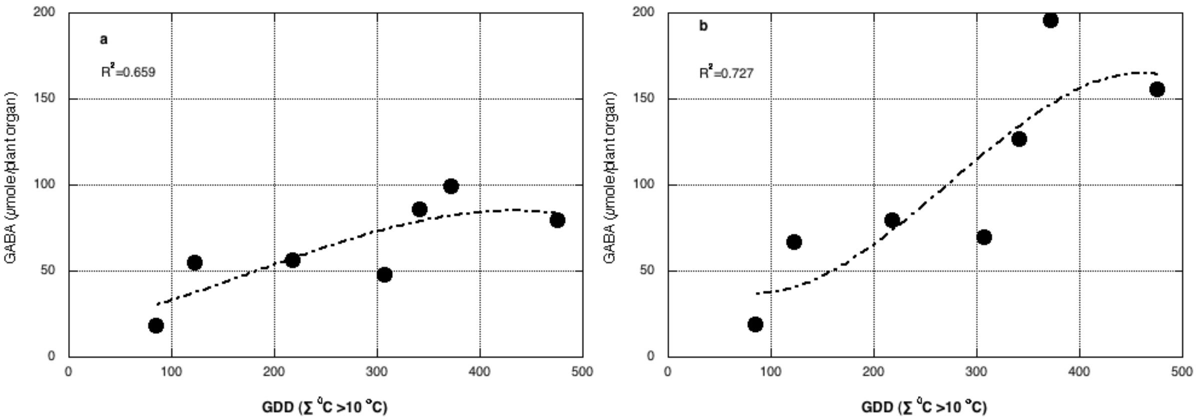

Fig 6: Relationship between GDD (Growing Degree Days >10 °C) and GABA content in leaves (µmole/plant organ) in 1991. 5a: control plot; 5b: fertilized with 30 units N at "5-6 leaves unfolded and 60 units N "after bloom".

Summarizing the findings concerning AAs dynamic in leaves it can be stated that leaves are the real source for the synthesis and distribution of N-compounds.

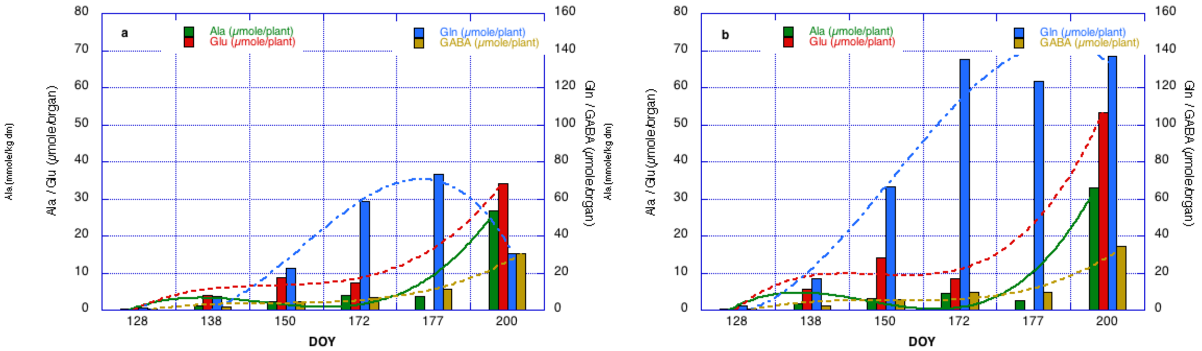

Fig 7: Dynamic of soluble AAs (**Ala, Glu, Gln and GABA**) in flowers and berries from DOY 128 to 200 (µmole/plant organ). 7a: control plot; 7b: fertilized with 30 units N at "5-6 leaves unfolded" and 60 units N "after bloom".

In generative organs Gln is always highest but GABA is present in concentrations higher than Pro (Fig. 7 a and b).

As stated, leaves are the source of AAs and in consequence flowers and berries should be the sink. This is true for Glu and Gln, which accumulate very rapid during flowering (DOY 172) and onset of berries (DOY 177 and 200). It seems that the power of the source is enhanced by N-fertilization (Fig. 4b and 6b). In contrast to wooden parts of the grapevine, which ensures the longevity of the plant and which is a storage place for Arg, fruits are preferred as sink for Gln and Glu. During the ripening phase it may be possible that higher amounts of GABA are formed via the GABA shunt from Glu (Schaller *et al.*, 1990).

A high transfer rate of GABA to musts during wine making can be expected. An influence on wine quality (minerality) is possible (Baron and Fiala, 2012). Fertilization has significant influences.

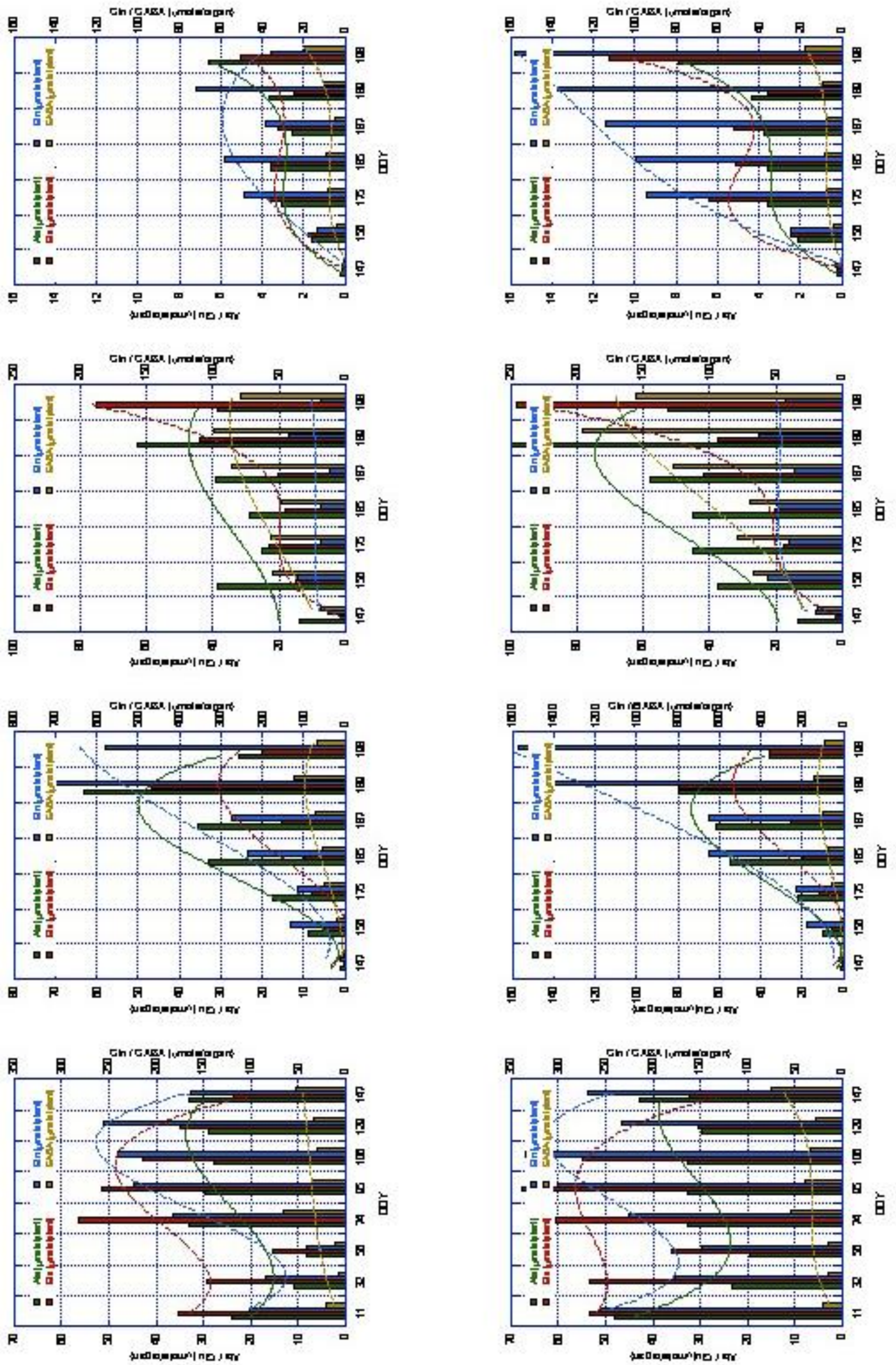

Fig. 8: Soluble AAs in 1 year old wood, shoots, leaves and flowers (berries) in 1991 (µmole/plant part). Upper row control, lower row N fertilization 30/60 units.

In Fig. 8 the results for 1991 are shown. They are in full accordance with those from 1990. The behaviour of all measured compounds (Ala, Glu, Gln, GABA as well as Arg and Pro) is comparable to 1990 with exception of the amount. In the two consecutive years in 1991 concentration as well as amount per plant organ are higher in control plot and fertilized ones.

CONCLUSION

The present work resulted from a fertilizer experiment, which is currently the oldest in Europe. One aim was to apply different amounts of fertilizer N constantly during a complete life cycle of a grapevine stand. In the past most fertilizer experiments with grapevines were conducted only for some years, like field experiments with annuals. Other experiments showed that grapevines have a distinct annual cycle between depleting reserves in woody parts and refilling during autumn and winter rest (Schaller *et al.*, 1989,1990,1991). I.e. such a system has to be brought in new steady state equilibrium before any measurements and further experiments are done. All results, which were elaborated during this two years' experiment show a consistent pattern. There is a clear reaction following fertilization and also a differentiation according the influence of the year. In the wooden parts it was possible to confirm former findings (4). In addition it was possible to show the time course of Gln, Pro and GABA in that organ. There is also an influence of fertilizer N, which increases the amounts of all AAs.

The time course of Ala, Glu, Gln and GABA in shoots, leaves, and flowers resp. berries offers some new insights in the N-metabolism of these organs. Shoots, which act more or less as transport organs have relatively low contents of all AAs. The dominating AA is Gln besides Glu. The influence of N-fertilization is obvious.

The real organs with a character of a N-source are leaves (Fig. 4 a and b). Leaves mirror correctly the N-fertilization. Contents of all AAs are increased. A considerably dynamic exists between Ala, Glu and GABA. The latter peaks during "flower fully developed" until "fruit set". Glu peaks at "berries like peas".

A specific sink for these compounds is, besides the growing shoot tips, the flowers resp. berries. During the process "flower fully developed" until "berries like peas" there is strong import Gln; double amount in the fertilized plots. In the last observed developmental stage also higher amounts of GABA are in these organs. Its amount is higher than Pro, which often is discussed as an stress indicator. The synthesis of GABA seems to follow the GDD; possibly is it an indirect stress indicator? However GABA is especially more important in grapevine than some other bulk amino acids, whose importance was always reported in the past. It seems that GABA plays a key role in grapevine development and also in wine making processes (Baron and Fiala, 2012). It is recommended to do more research on GABA in order to get a clearer picture of its importance in grapevine physiology and wine making.

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<u>Abbreviations</u>:

AA Amino acid
AAs Amino acids
Ala Alanine
Arg Arginine
DOY Day of the year
GABA Gamma-amino butyric acid
GDD Growing Degree Days >10 ^{o}C
Glu Glutamic acid
Gln Glutamine ProProline

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