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Influence of Differing Nitrogen Nutrition on Polyamines and non-Structural Carbohydrates during Different Developmental Stages of Grapevines (c.v. White Riesling)

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Abstract. Polyamines (PA) are triggered by external inducers (hormones, fertilization, nutrients etc.). In triggered systems, the level of free PAs increase before the onset of cell division. PAs are high during onset of fruit development as compared with other stages of fruit development. During a fertilisation experiment with increasing amounts of N and timing, non-structural carbohydrates and PAs were determined in leaves, flowers and developing berries. Leaves: PAs were lowest in the unfertilized plots; fertilization showed no differentiated effects. PA-concentrations peaked after full bloom and fell to 50% of the starting value at the stage "pea-sized berries". Shoots: belonging to the leaves with the same plastochron-index showed a similar behaviour like PA in leaves. Stems: Putrescine (Put) and Spermidine (Spd) show a clear time course. Put decreases until "flowers fully developed" and afterwards a slight increase until "pea sized berries". Spd behaves nearly inverse: peak at "increasing of the flowers" and a minimum at "fruit set". Berries: all four tested PAs show a clear pattern of their time course. Agmatine (Agm) is highest at 27 May (five to six leaves unfolded), lowest at 5 June and peaking again at "flowers fully developed" (24 June). At "fruit set" concentrations are as low as at 5 June ("flowers are growing"). Put is high at "five to six leaves unfolded", decreases continuously until "flowers fully developed" and a maximum at "fruit set". Spd shows a maximum at "flowers are growing" and reaches a minimum at "fruit set". Spd has nearly constant concentrations through "five to six leaves unfolded" until "flowers are fully developed" in all fertilizer variants. At "fruit set" is a maximum. peaks at "flowers fully developed", decreases to "fruit set" and increases again at "pea sized berries". Treatments show a differentiation; highest values are found with the plot 90/60. A direct connection to the carbohydrate metabolism could not detected during this experiment.

Keywords: Agm, carbohydrates, N-Fertilization, PAs, Spd, Spm, Put

INTRODUCTION

Polyamines (PA) as constituents of plant tissues are known since the beginning of the 20th century. By the late 19th century, Spm had been demonstrated in many animal tissues. In 1925 Dudley and Rosenheim reported that yeast also contains this base.
In this period most of the common diamines and PAs were detected. Put and cadaverine were first found in fungi and later in higher plants. Herbst and Snell (1948) detected Put in orange juice, and proved it as a growth factor for *Hemophilus parainfluenzae*. They also stated „Put and possibly additional compounds of this group play a much more important metabolic role than has been previously indicated."

Richard and Coleman (1952) found with barley that "Put is produced and accumulated under certain conditions of potassium deficiency." Young and Galston (1983) as well as Flores and Galston (1982) reported Put indicates for stress factors (pH, osmotic stress, Cd-toxicity a.s.o.). Concerning the physiological activity of amines Bagni (1967) investigated function and metabolism of PAs in *Helianthus tuberosus* explants. He showed the importance of PAs protein and nucleic acid synthesis. Biosynthesis of PAs is reviewed in Stumpf and Conn (1981).

PAs have a distinct role in cell division. A process, which occurs in developing embryos, fruits, meristimatic tissues in adult plants and proliferating tissues. These systems may be divided into two categories:

1. triggered systems, where cell division is triggered by an external inducer (hormones, fertilization, nutrients etc.), and
2. continuous systems, in which cell division is a non-perturbed state occuring under optimal conditions (meristimatic tissues, cells cultured *in vitro*)

Cell division is correlated with increased PA biosynthesis activity (Heimer *et al.* 1979). In triggered systems, the level of free PAs increase before the onset of cell division. There are several systems, such as developing apple and tomato fruits after fruit-set, in which the PAs seem to accumulate pre-mitotically and decrease sharply upon onset of mitosis which may be a proof for their role in protein, DNA and RNA synthesis.

Fruit development is triggered by pollination. PA levels are high during onset of fruit development, as compared with other stages of fruit development. PA level in tomato fruits at fruit set are close to 104 nmol/g fresh weight (free, conjugated and bound). Also high levels have been reported in apples, *Phaseolus vulgaris*, tobacco, and mandarins. Application of exogenous Put before anthesis or at full anthesis caused an final size increase of fruits and improved set in apples. Pears were found to improve fruit set and delayed senescence of the ovules and enhance pollen germination and fertilization.

Taking into consideration all these findings an experiment was planned with grapevine to get an insight in the dynamics of free PAs and non-structural carbohydrates in different organs under the influence of nitrogen fertilization.

MATERIAL AND METHODS

Field plots: Vineyard planted with White Riesling, clone 239, grafted on 5C. Soil type: loamy sand. Inclination 10^{o}.

Fertilization: Control (no nitrogen),
60 kg N (application after blooming)
90 kg N (application after bud burst)
150 kg N (90 kg after bud burst, 60 kg after blooming).

The experiment started in 1985. Tests were made in 1991 after 6 years of establishment.

Sampling: 1. Leaves from summer shoots were taken according the plastochrone index. Leaves with plastochrone 9-12 (i.e. inserted above the bunches) were sampled at 24 June, 4 July, 6 July, 8 July and 17 July, corresponding to "flowers fully developed", "full bloom", "after blooming", "fruit set", "berries like peas" respectively.
2. Stems: developing flowers were divided in stem and blossoms resp. berries.
3. Berries: flowers resp. developing berries were sampled at 27 May, 5 June, 24 June, 8 July, and 17 July.

Analysis: Plant material was shock frozen (N_2) and freeze dried. After milling free PAs were determined with PCA: 100 mg tissue were extracted with 1 mL 10% PCA for 60 min in a fridge and centrifuged. Supernatant was dansylated and after passing a 0.22 µm filter separation was done with HPLC on a reversed phase C_{18} column. Detection was done fluorimetrically. Carbohydrates were determined enzymatically (Korkas, 1994).

RESULTS AND DISCUSSIONS

1. Leaves

In Fig. 1 the concentrations of Agm, Put, Spd, and Spm in leaves (plastochrone 9-12) are shown during the developmental stages "flowers fully developed" - "berries like peas". For all PAs it can be demonstrated that in the control plot the concentrations are lowest. The fertilized plots do not differentiate according the fertilization scheme but in some cases the after bloom fertilization results in higher concentrations. In the phase from 24 June to 4 July, with exception of Agm, the PA concentrations decreased. At 6 July Spm reached a maximum whereas Put had a minimum. Spm continuously fell until 17 July ("berries like peas"). Agm peaked at 4 July with the control plot and the variant 90/60, whereas 0/60 and 90/0 had a maximum at 6 July. The highest values were reached in the control and 90/60 at 8 July, the other two variants had a further minimum. At the stage "pea sized berries" the final concentration of Spm ranks highest, followed by Agm; Spm and Put fell to 50% of their starting value. Total sum of PAs show a steady decline in concentrations whereas during the bloom phase the control plots show the heaviest drop.

Non-structural carbohydrates (NSC) do not present any reaction following N fertilization nor the developmental stage of the leaves. Only at stage “berry like peas” the sucrose concentration rises markedly.

Stems

In Fig. 2 the concentrations of Agm, Put, Spd from the developmental stage "five to six leaves unfolded" - "pea sized berries" are presented. The Agm concentrations show no regular pattern; it seems that other factors influence the actual concentration at every sampling date.

On the other hand Put and Spd show a clear time course: Put decreases until "flowers fully developed" and afterwards followed by an slight increase until "pea sized berries". Spd behaves nearly inverse, e.g. a peak at "increasing of the flowers" and a minimum at "fruit set".

Spermine shows a similar time course like Put with a distinct peak at “fruit set”. Highest concentrations are measured in all fertilized plots. The sum of PAs is nearly constant throughout this phase of growth.

NSCs show a peak at “full bloom” and then steadily decline towards “berries like peas”. No influence of any N-fertilization regime can be detected.

Berries

Concentrations of Agm, Put, Spd, and Spm are shown in Fig 3. All tested PAs present a clear pattern of their time course. Agm has highest concentrations at 27 May (Five to six leaves unfolded), reaching at 5 June a minimum and peaking at "flowers fully developed" (24 June). At "fruit set" concentrations are as low as at 5 June ("flowers are growing").

Put also has a high concentration at the stage "five to six leaves unfolded" but decreases continuously until "flowers fully developed" and finally reaches a maximum at "fruit set".
Spd shows a maximum at "flowers are growing" and reaches a minimum at "fruit set". Spd has nearly similar concentrations in all test plots through the developmental stages from "five to six leaves unfolded" until "flowers are fully developed". At the stage "fruit set" a maximum is observed. The sum of all PAs show a steady decrease from “flowers are growing” to “fruit set” followed by an increase towards “pea sized berries”.

For all PAs it can be stated that there is a influence of fertilizer treatment on PA concentration. In most cases the treatments 90/0 and 90/60 have higher concentrations. With one exception the control plot has always the lowest concentrations which can be seen best in the plot for the sum of all PAs.

The time course of NSCs illustrates a distinct time dependent pattern. Soluble glucose as well fructose peak at full bloom and are lowest at “fruit set”, indicating that these resources are used for the buildup of new merging tissues. The N-fertilization has no significant influence on the level of NSCs in developing flowers and berries. In contrast fertilization has an effect on the PA contents. The control plots rank almost in the lower part of measured contents in flowers resp. berries.

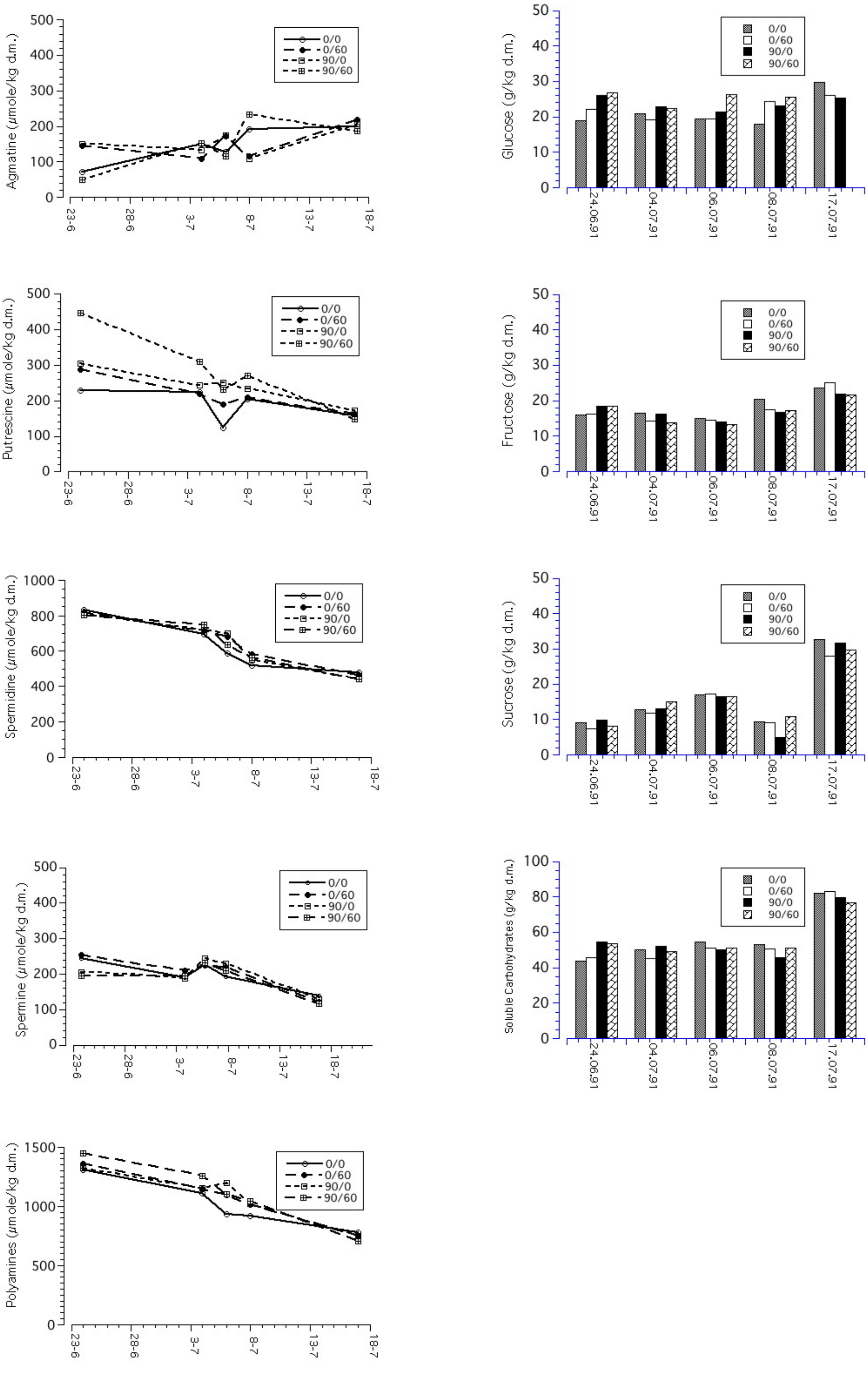

Fig. 1. Polyamine (left) and non-structural carbohydrate (right) concentrations in grapevine leaves (plastochrone index 9-12) during the developmental stages "flowers fully developed" - "pea sized berries" dependant on nitrogen fertilization. (µmol/kg d.m. resp. g/kg d.m.)

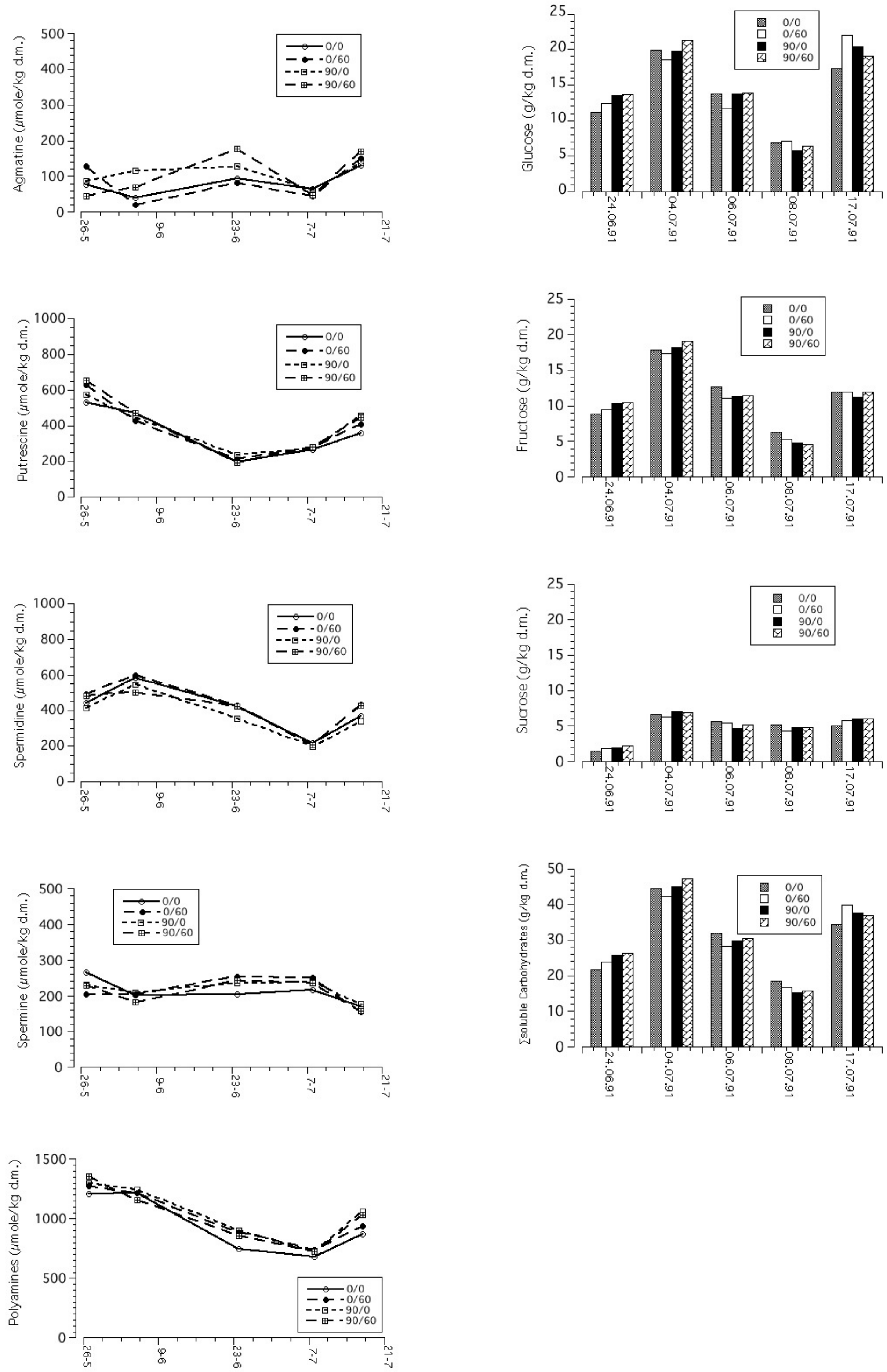

Fig. 2. Polyamine (left) and non-structural carbohydrate (right) concentrations in stems during the developmental stages "flowers fully developed" - "pea sized berries" dependant on nitrogen fertilization. (µmol/kg d.m. resp. g/kg d.m.)

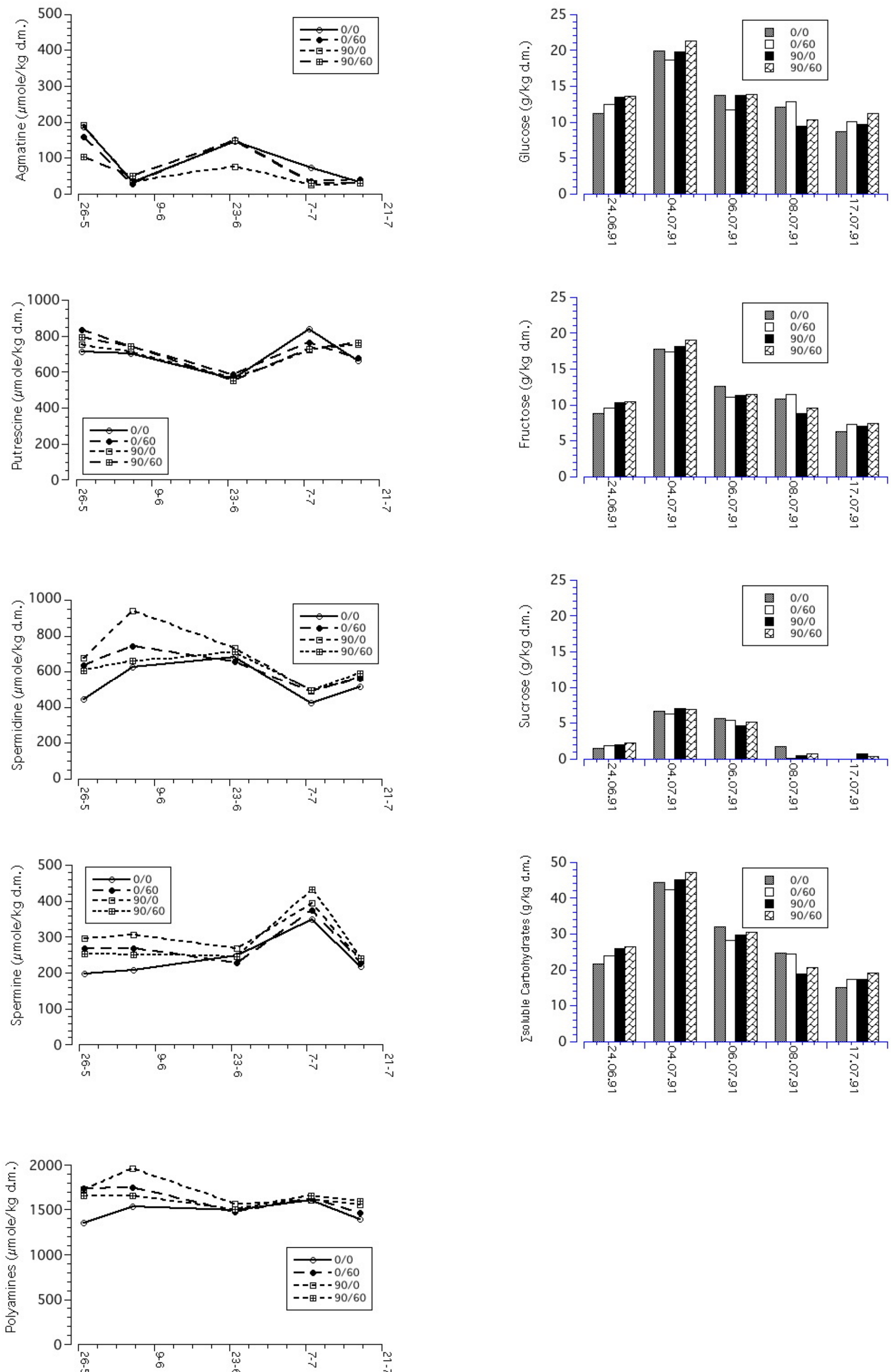

Fig. 3. Polyamine (left) and non-structural carbohydrate (right) concentrations in flowers resp. berries during the developmental stages "flowers fully developed" - "pea sized berries" dependant on nitrogen fertilization. (µmol/kg d.m. resp. g/kg d.m.)

CONCLUSIONS

The aim of the presented investigations was to get an better insight in the process of yielding in grapevines. During our work on the influence of nitrogen fertilization it was found that in such field experiments a long time is needed to get consistent results. This may be caused by the nutrient reserves deposited in trunk and roots, and which can prevent for a long time a clear reaction of the grapevines following a fertilizer application.

On the other hand there must also exist some mechanisms, which are responsible for a successful fruiting. As could be demonstrated PAs show during the flowering and fruiting process a pronounced dynamic. It is also obvious that the particular amines have minimums and maximums at distinct developmental stages of the grapevine. It is also well demonstrated that different nitrogen fertilizer applications influence the PA concentration and content in grapevines. On the other hand no significant influence of a long lasting N-fertilization could be found out.

Finally we have to state that a further and intensive research on PAs is necessary to understand the physiological role during the fruiting and the complete growing process of the grapevine.

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