Vitis **44** (1), 11–16 (2005)

The effect of foliar application of methanol on productivity and fruit quality of grapevine cv. Flame Seedless

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Summary

Field experiments were conducted in 2002 and 2003 on 6-year-old grapevine cv. Flame Seedless. The content of chlorophyll a and b, carotenoids and total carbohydrates increased after methanol application. Foliar application of aqueous methanol was very effective increasing the number of leaves per shoot and leaf area. Furthermore, 30 % methanol increased significantly the number of stomata of developing leaves at the first application time (shoot length: 20-30 cm) while 10, 30, 40 and 50 % methanol solutions were more effective at the second application date (prebloom). Increasing the chlorophyll content, the leaf area and the number of stomata per unit leaf area by methanol application increased net productivity of vines. There was a highly significant positive correlation between total yield, chlorophyll and carbohydrates content. Generally, all methanol treatments significantly increased length and diameter of shoots and internode length at both application dates. Application of methanol increased total soluble solids (TSS), the TSS/acid ratio and total anthocyanins in berry skins but decreased total acidity. Most significant effects were obtained by spraying 30 % methanol at the two application dates.

K e y w o r d s : table grape, fruit quality, yield, foliar application, methanol.

Introduction

Most higher plants produce and emit methanol as a result of pectin demethylation. This volatile organic compound produced especially during the early stages of leaf expansion is released from leaves via stomata (Nemecek-Marshall *et al.* 1995). Plant tissue, however, can also metabolize methanol. Although there is no methanol oxidase in higher plants, they can convert methanol to CO_2 (Cossins 1964). According to Gout *et al.* (2000), assimilation of methanol by plants takes place before its oxidation. The role of methanol as a plant growth regulator (Dwivedi *et al*. 2001) or an agent to enhance fruit quality (colour and composition) and to advance maturity would need to be studied more in detail.

Numerous experiments have shown an increase of yield due to an increase of the CO_2 content in the atmosphere (Devlin *et al.* 1994); moreover, flowering was accelerated (Fisher *et al.* 1996) and plants accumulated more carbohydrates (Abdel-Latif *et al.* 1996). Methanol applied to higher plant cells was readily incorporated into the methyl groups of molecules, such as serine, methionine and phosphatidylcholine (Gout *et al.* 2000). Exogenous application of methanol affected directly metabolic pathways related to plant growth and development (*e.g.* the content of amino acids). In addition, pathways related to plant defence mechanisms such as activation of genes involved in the jasmonic acid biosynthesis were affected.

Methyl alcohol may be an alternate carbon source for plants. According to Nonomura and Benson (1992 a, b), methanol-treated C3-plants increased turgor, had higher growth rates and consequently higher yields. Hemming *et al.* (1995) found that brief exposure to aqueous methanol solutions increased the metabolic heat rate resulting in an increased carbon conversion efficiency. Furthermore, Zbieć *et al.* (1999) found that plants grown in CO_2-enriched atmosphere were less susceptible to drought due to a decrease of stomatal conductance and transpiration and an increase of net photosynthesis.

This study investigates the effect of foliar application of methanol on the contents of chlorophyll and carotenoides in leaves, carbohydrates in canes, anthocyanins in berry skins and growth parameters reflecting total yield of grapevine.

Material and Methods

The study was carried out in 2002 and 2003 using 6-year-old Flame Seedless grapevines grown in the vineyard of the Faculty of Agriculture, Assiut University, Assiut, Egypt. Experimental vines were planted at 2m x 3m in loamy-clay soil and were watered by drip irrigation. A double cordon was used as training system. Healthy vines were selected for uniform vigor; they were pruned in the first week of January in each season to leave about 60 buds per vine (20 fruiting spurs, 3 buds per spur). All vines were regularly fertilized. Sixty vines were chosen and divided into 12 groups. In the second season another set of vines was chosen to avoid carry over effects of treatments from the first season.

Thirty vines (5 as replicates for each treatment) were sprayed with 0 (control), 10, 20, 30, 40 and 50 % aqueous methanol when shoot length was 20-30 cm (first application date). The other 30 vines were sprayed two weeks before

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bloom (second application date) with the same methanol concentrations. One liter of solution was sprayed on each vine.

Photosynthetic pigments: Ten leaves per replicate were collected from the middle part of the shoots for determination of chlorophyll a, chlorophyll b, and total carotenoids (mg·g^{-1} fresh weight) according to WELLBURN (1994).

Number of stomata: Leaf prints were prepared from both the adaxial and abaxial surface of intact leaves according to the method described by HILU and RANDALL (1984). Leaf prints were taken in triplicate from three different lobes of the lamina. Stomata were counted using a light microscope in three areas between the leaf midrib and the blade margin. The average of these three areas was considered as one replicate for each leaf.

Vegetative growth: The number of young and fully expanded leaves per shoot was counted and leaf area was calculated from 20 leaves per vine positioned opposite to the basal clusters according to the equation reported by SOURIAL *et al.* (1985): Leaf area (cm^2) = 0.785(diameter)^2.

Final shoot length, shoot diameter and internode length of 5 shoots per vine were estimated; the mean was considered as one replicate. The weight of one-year-old pruning wood, including laterals, was determined in the first week of January.

Yield and its components: Yield per shoot (g) and total yield per vine (kg) were determined at the time of harvest (June 14, 2002 and June 18, 2003 for treated vines, the control was delayed by about 10 d). Samples of 15 bunches per treatment (the mean of three bunches was considered as a replicate) were picked at harvest and the following parameters were determined: (1) Diameter, length and weight of each bunch, weight of 100 berries and number of bunch ramifications. (2) Fruit composition: 100 berries were taken from each replicate and crushed to determine total soluble solids (TSS) by using a hand refractometer. Total acidity (as g tartaric acid per 100 ml juice) was determined according to the A.O.A.C (1975), and the ratio of TSS/acid was calculated.

In addition, the content of total anthocyanins in berry skins was determined according to RABINO *et al.* (1977). Total carbohydrates in the basal parts of canes were determined according to DUBOIS *et al*. (1956).

Statistical analysis: Data were statistically analysed using SPSS (SPSS Inc.). Analysis of variance was carried out using a general one-way model, and Student-Newman-Keuls (S-N-K) was used for comparison between particular means. Simple correlations or linear regression were carried out between different parameters.

Results and Discussion

Effect of methanol on photosynthetic pigments and leaf area: Fig. 1 shows the effect of spraying various concentrations of methanol on the content of chlorophyll a (chl.a), chlorophyll b (chl.b) and total carotenoids in leaves in two seasons. There was no significant difference between seasons. It is evident that foliar application of methanol induced significant increments of chl.b at the first date, chl.a and chl.b at the second date and total chlorophyll at both dates. Considerable increases were obtained by all concentrations especially by 30 % and 40 % of methanol at the second application date. Spraying leaves with methanol resulted in a significant decrease of the chl.a/b ratio due to an increase in chl.b relative to chl.a (Table). The decreased ratio of chl. a/b in the leaves means an extension of the absorption band of mixed pigments towards the green part of the system (THIMANN 1980, 1987; VEIERSKOV and THIMANN 1988). In contrast, the ratio of chl.a+b/carotenoids increased significantly in methanol-sprayed leaves (Table), despite there was no significant change in the content of carotenoids between different treatments. Chlorophylls are particularly sensitive to oxidation and photodamage, while carotenoids function as anti-oxidants

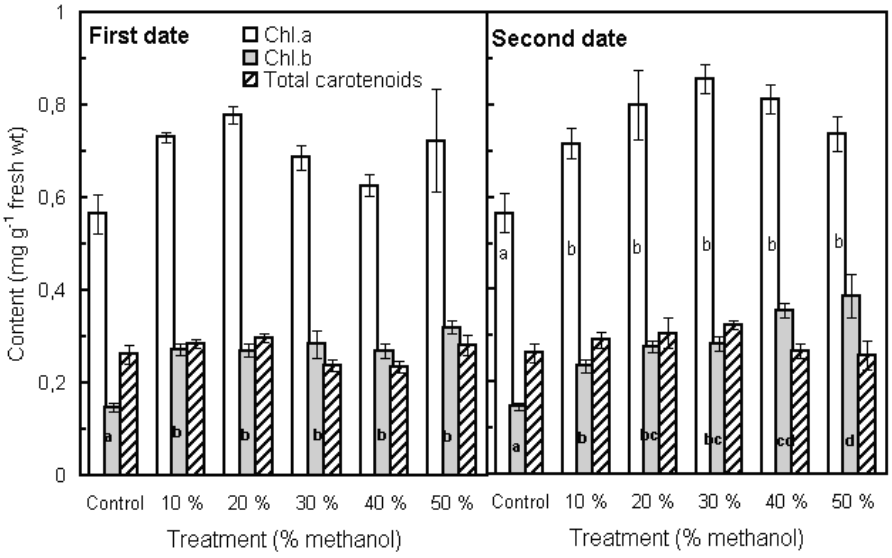

Fig. 1: Content of chlorophyll a, b and total carotenoids in leaves of vines treated with various concentrations of methanol. Values are means ±SE, n= 3. At the first or second date of methanol application, means of each component with different letters are significantly different at $p< 0.05$ according to the S-N-K test.

Table 1

Effect of foliar application of methanol (10-50 %) on the ratio of chl. a/b and chl. a+b/carotenoids, and on the number of stomata per mm^2 leaf area of grapevines. Treatments at 20-30 cm shoot length (I) or at pre-bloom (II). Values are means ±SE, n= 10. Values for each date of application with different superscript letters are significantly different at $p< 0.05$ according to the S-N-K test.

		Methanol treatments					
		Control	10 %	20 %	30 %	40 %	50 %
Chl.a/b	I	$3.85^{b}\pm0.09$	$2.70^{a}\pm0.09$	$2.86^{a}\pm0.07$	$2.46^{a}\pm0.18$	$2.38^{a}\pm0.21$	$2.24^{a}\pm0.26$
	II	$3.85^{c}\pm0.09$	$3.07^{b}\pm0.08$	$2.90^{b}\pm0.22$	$3.08^{b}\pm0.27$	$2.31^{a}\pm0.17$	$1.95^{a}\pm0.15$
Chl.a+b/	I	$2.72^{a}\pm0.04$	$3.54^{b}\pm0.16$	$3.54^{b}\pm0.03$	$4.07^{b}\pm0.25$	$3.81^{b}\pm0.12$	$3.71^{b}\pm0.21$
carotenoids	II	$2.72^{a}\pm0.04$	$3.27^{ab}\pm0.03$	$3.55^{ab}\pm0.15$	$3.52^{ab}\pm0.04$	$4.38^{b}\pm0.18$	$4.48^{b}\pm0.68$
No. of stomata	I	$218.23^{ab}\pm6.67$	$240.05^{b}\pm7.11$	$205.14^{a}\pm7.18$	$208.96^{a}\pm7.97$	$228.60^{ab}\pm5.01$	$220.96^{ab}\pm7.29$
per mm^2	II	$218.23^{a}\pm6.67$	$233.51^{ab}\pm6.57$	$214.96^{a}\pm6.54$	$237.33^{ab}\pm7.16$	$249.33^{b}\pm9.79$	$241.69^{ab}\pm8.22$

and in quenching photo-induced excitation. Changes in the chlorophyll:carotenoid ratio are potentially sensitive indicators of oxidative damage. According to THIMANN (1980), carotenoids are more stable than chlorophylls. ROBERTSON *et al*. (1966) reported that the amount of chlorophyll will not increase above that which can be protected by carotenoids. The concept of many authors (KRINSKY 1968; GOLBECK *et al*. 1977; THOMAS 1978; DÜRING 1999) towards the protective role of some carotenoids under conditions of excessive light led us to conclude that foliar spraying with methanol did not cause any stress for the plants.

Data in Fig. 2 A show that spraying vines with methanol increased significantly the number of leaves per shoot. The maximum increase was observed when vines were sprayed with 30 % methanol at both application dates. Application of methanol at the two dates very effectively increased leaf area. Application of 30 % methanol at 20-30 cm shoot length (first date), increased leaf area by about 26 % (Fig. 2 A). A similar but smaller effect was observed by applying 20 % methanol. At 20-30 cm shoot length, 20-40 % methanol treatments increased significantly leaf area as compared to applications at the second date. The positive effect of methanol application on leaf area may be due to abundant CO_2 supply from methanol as suggested by HEMMING *et al.* (1995). This may have reduced photorespiration in favour of photosynthesis. Moreover, application of methanol was found to play an important role in balancing the nutritional status of leaves by acting as a carbon source (BENSON and NONOMURA 1992; MAUNEY and GERIK 1994) or by enhancing the engendered root activity (MAKHDUM *et al.* 2002).

Moreover, it is clear from the data shown in the Table that the number of stomata per mm^2 at the abaxial leaf surface increased significantly after application of 40 % methanol only; according to DÜRING (1980) vine leaves are hypostomatal. When estimated per leaf, the number of stomata increased significantly by spraying methanol at any concentration (Fig. 2 B). The increase was also significant with 20 and 30 % methanol at the first date and with 50 % at the second date (Fig. 2 B).

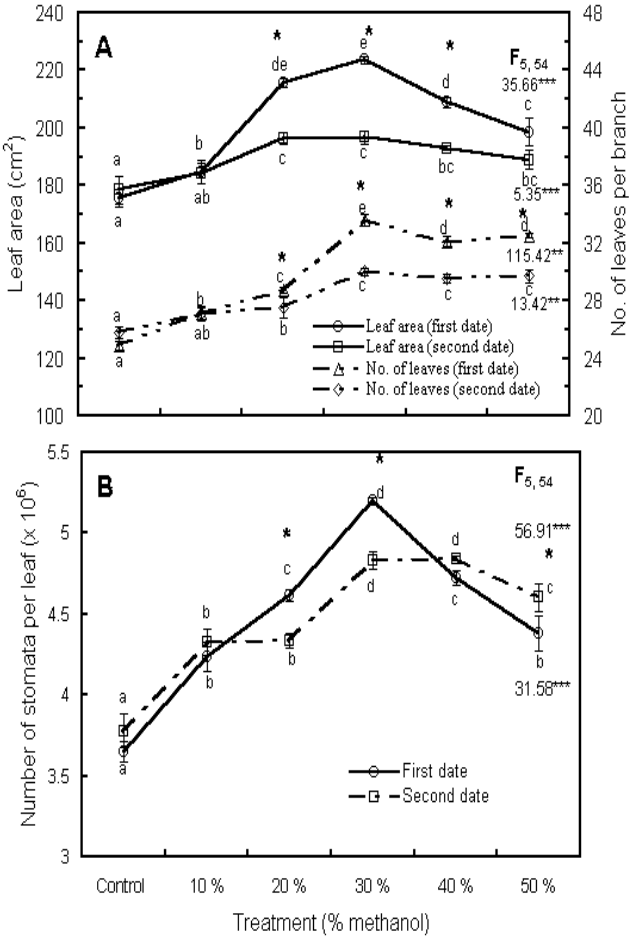

Fig. 2: Effect of foliar sprays with methanol on leaf area (cm^2) and the number of leaves per shoot at two different dates of application (A), and on the number of stomata per leaf (B). Values are averages of the two seasons ±SE, n = 10. For each line, values with different letters are significantly different. For each panel, values of the first and second dates of methanol application with statistically significant differences are marked with stars ($P<0.05$) according to Duncan test. The figures also display the F-values for one-way ANOVAs.

Vegetative growth: Fig. 3 A shows that methanol significantly increased length and diameter of shoots at the two dates of application. The highest length of shoots was observed after using 30 % methanol followed by 20 %, regardless of the time of application. Shoot length increased by about 40 % and 20 % after spraying 30 % and 20 %

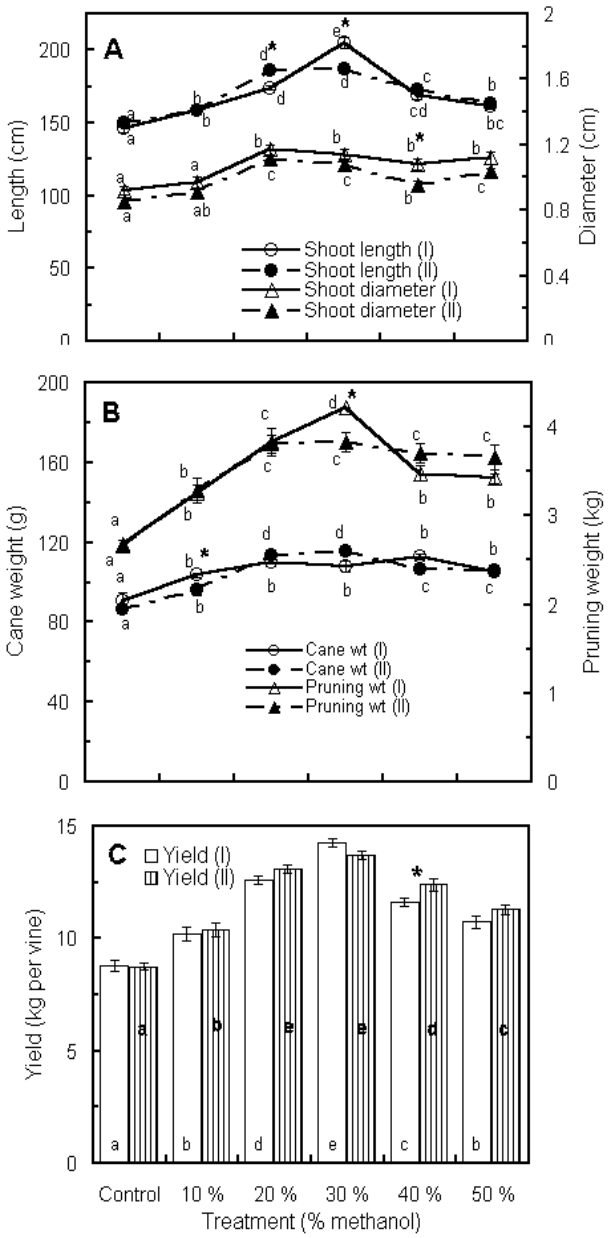

Fig. 3: Effect of various concentrations of methanol on shoot length and diameter (A), cane and pruning weight (B) and on total yield per vine (C) for two different application dates. Values are averages of the two seasons ±SE, n=10. For each line or column, values with different letters are significantly different. For each panel, values of the first and second dates of methanol application with statistically significant differences are marked with stars ($P < 0.05$) according to the S-N-K test.

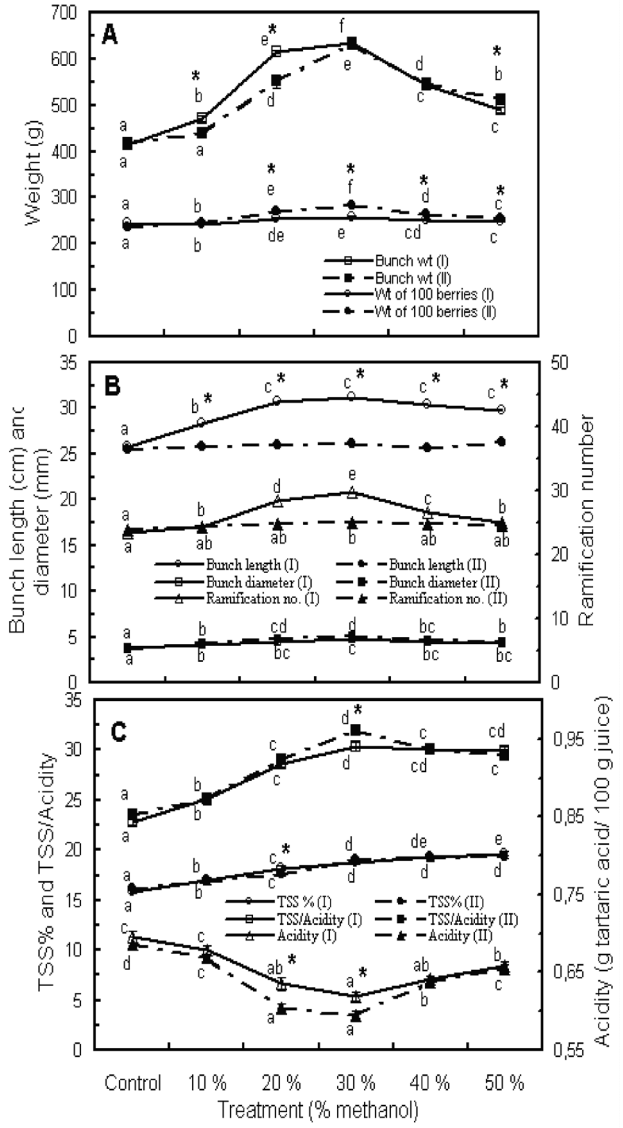

Fig. 4: Influence of foliar application of methanol on bunch weight and 100-berry-weight (A), bunch length and diameter and ramification number (B); total soluble solids (TSS%), total acidity and TSS/acidity (C). For details see Fig. 3.

methanol, respectively. These results were confirmed in two seasons. Furthermore, all levels of methanol caused an increase in internode length: while the internode length in control vines was 4.7 cm it ranged between 5.2 and 5.5 cm in treated vines (without significant differences between treatments). The increase in shoot length, diameter and internode of shoots could be due to the important role of methanol in facilitating the availability of mineral or organic nutrients to vines and the utilization of methanol as a carbon source (NONOMURA and BENSON 1992 a). As CO_2 increased in the ambient air as a result of methanol oxidation (GOUT *et al.* 2000), the photosynthetic efficiency of leaves will increase and hence more carbohydrates, amino acids and proteins may be transported to the shoots.These results are supported by finding of ZBIEĆ *et al.* (2003) who reported that application of methanol solutions (10-40 %) on winter-rape significantly increased shoot length and growth. Also, pruning weight increased significantly by methanol application (Fig. 3 B), reaching highest values (4.22 and 3.83 kg vine^{-1} for the two dates of application) after spraying with 30 % methanol.

Yield and fruit quality: Total yield per vine was increased significantly by all treatments at each date of application (Fig. 3 C). Maximum yield was obtained by spraying with 30 %, followed by 20 % methanol. Highly significant positive correlations were found between total yield and chlorophyll ($R^2 = 0.823$ and 0.955, for the two dates of application), and between total yield and the content of carbohydrates ($R^2 = 0.952$ and 0.974). These results are in agreement with those obtained by NONOMURA and BENSON (1992 b) who reported that methanol-treated C_3-plants showed high

growth rates and consequently had higher yield. In contrast, WILSON *et al.* (1996) applied aqueous methanol (6 concentrations from 0 to 50 %) on barley and found that none of the treatments significantly affected crop performance.

The linear regression between total yield and pruning weight indicated that total yield was 3.3-fold the pruning weight ($R^2 = 0.92$), while bunch weight was about 5-fold the cane weight ($R^2 = 0.67$). These relationships were confirmed in two seasons.

Bunch weight increased with methanol spraying, the maximum weight was obtained from vines sprayed with 20 % or 30 % methanol at both application dates (Fig. 4 A). Furthermore, all treatments significantly increased berry weight, irrespective of the application date. In two seasons, the highest berry weight was obtained by spraying 2 weeks before bloom with 30 % methanol (278.8 and 283.5 g 100 berries^{-1}, respectively).

Data presented in Fig. 4 B reveal that bunch length was increased significantly by methanol treatments only at the first date of application. At both application dates, the diameter of bunches was increased significantly by all treatments. In addition, at the first application date all treatments significantly increased bunch ramification. At the second application date, only 20 % and 30 % methanol significantly increased the rate of ramification.

Foliar application of methanol (10-50 %) caused a significant increase in total soluble solids (TSS) of berries at the two dates of application and in both seasons (Fig. 4 C). The increase in TSS was proportional to the concentration of methanol. The results indicate that all methanol treatments significantly decreased total acidity, the lowest acidity percentage was obtained after spraying 30 % followed by 20 % methanol. Methanol significantly increased the ratio of TSS/acidity, 30 % methanol exerting the highest ratio (Fig. 4 C).

All treatments significantly increased the anthocyanin content in the skins of berries, especially at 20 % and 30 % methanol at both application dates (Fig. 5). At the second date, the regression slope of contents of anthocyanins accumulated in the skins of berries against the concentrations of applied methanol was 11.5 µg anthocyanins g^{-1} fruit per 1 % methanol with an intercept of 0.93 that equals to the content in control fruits ($R^2 = 0.94$). At the first time, the slope was 9.0 and R^2 was 0.86. According to CHERVIN *et al.* (2001), spraying bunches of vines with 5 % aqueous ethanol increased the internal ethylene concentration in treated berries and substantially increased berry colour. In agreement with CHERVIN's result, NIKOLAOS *et al.* (2003) found that spraying vines but with methanol advanced and increased the anthocyanins in skins of berries via induction of ethylene synthesis. In addition, methanol caused a significant increase in the content of total carbohydrates in canes, especially by 30 % and 20 % (Fig. 5). There was a highly significant positive correlation between the chlorophyll concentration in the leaves and the content of carbohydrates at both application dates ($R^2 = 0.910$ and 0.947).

The flow of carbon in leaves is determined by the balance between two mutually opposing cycles: the Calvin cycle resulting in a consumption of CO_2 (carbon gain) and the photorespiratory carbon oxidation resulting in a release of CO_2 (carbon loss). In normal air the two cycles operate simultaneously, carboxylating and oxygenating ribulose bisphosphate in a ratio of about 3:1 (TAIZ and ZEIGER 1998). GOUT *et al.* (2000) documented, using [^{13}C]methanol, that the plant cells metabolize methanol slowly through readily incorporation into the methyl group of some compounds such as methylenetetrahydrofolate, methyltetrahydrofolate and *S*-adenosyl-methionin. The subsequent utilization of these compounds will yield serine, methionine and phosphatidylcholine. Increasing accumulation of carbohy-

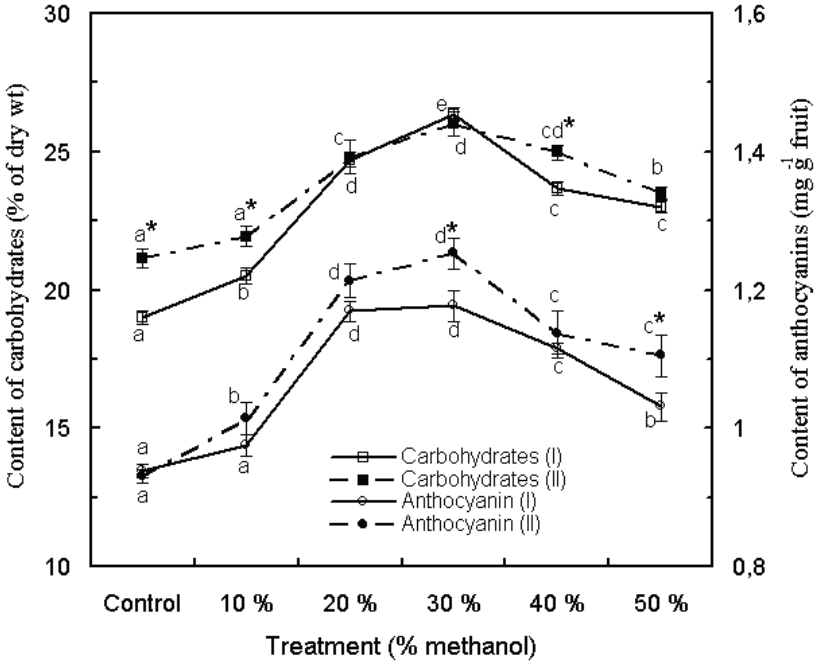

Fig. 5: The content of total carbohydrates in canes (% of dry wt) and anthocyanins in the berry skin (mg g^{-1} fresh weight) as influnced by foliar application of methanol at two different dates. For details see Fig. 3.

drates after methanol application was explained by ROWE *et al.* (1994) by the production of sucrose through the serine intermediate. In addition, the amino acid methionine is the precursor of ethylene (LIBERMAN and MAPSON 1964; ADAMS and YANG 1979; MCKEON *et al.* 1995) which can be completely oxidized to CO_2 (BEYER 1979). However, the decrease of photorespiration reported by HEMMING *et al.* (1995) may be due to blocking the cycle by increasing the concentration of methylenetetrahydrofolate in the mitochondria by foliar application of methanol instead of decarboxylation of glycine.

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Received July 10, 2004