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EFFECT OF TEMPERATURE ON LIPID, STARCH AND ENZYMES OF STARCH METABOLISM IN GRAPE, TOMATO AND BROAD BEAN LEAVES

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Key Word Index—*Vitis vinifera*; Vitaceae; *Lycopersicon esculentum*; Solanaceae; *Vicia faba*; Leguminosae; leaves; starch; lipid; starch synthesis; ADPglucose pyrophosphorylase; ADPglucose starch synthase; fatty acids; temperature dependence.

Abstract—Grapevine (*Vitis vinifera* cv Cabernet Sauvignon) leaves have previously been shown to accumulate starch at temperatures of *ca* 18/13° (day/night) and 25/20° whereas at 35/30° starch is largely replaced by lipid as a storage product. There was no indication that leaves at 35/30° were undergoing senescence. The effect of temperature on the accumulation of lipid and starch in tomato (*Lycopersicon esculentum*) leaves and broad bean (*Vicia faba*) leaves has now been studied in addition to grapevine leaves. The change from starch to lipid storage does not occur in tomato or broad bean leaves and in grapevine leaves it only occurs under specialized conditions, one of which is probably the level of reserves in the parent plants. The activities of ADPglucose pyrophosphorylase and ADPglucose starch synthase were assayed in the leaves from the plants grown at different temperatures to determine whether the levels of these enzymes correlated with the concentrations of starch present. Decreases in enzyme activity at the higher temperatures in grapevine were not sufficient to account for the reduction in starch synthesis.

INTRODUCTION

Higher plants partition photosynthate into storage products such as sugar, starch or lipid or into secondary products such as phenolic compounds or hydrocarbons. Temperature has an effect on the type of compound stored in the grapevine [1]. It has been shown that grapevine (*Vitis vinifera* cv Cabernet Sauvignon) leaves synthesize and accumulate starch in the light at temperatures of *ca* 18/13° (day/night) and 25/20°, whereas at 35/30° starch is largely replaced by lipid as a storage product [1]. The total energy stored in the leaves was hardly affected by temperature [1] and dry wt production of the whole plants would not be lower at the lower temperatures [2]. Under the conditions used, photosynthesis rates would not have been greatly different at the different temperatures [3], and there was no evidence that leaves were undergoing senescence at the higher temperatures [1]. No biochemical investigations were carried out by the authors to explain the change-over from starch to lipid accumulation.

Two important enzymes in starch synthesis in leaves are ADPglucose pyrophosphorylase and ADPglucose starch synthase [4]. From evidence obtained on enzyme activities in developing leaves and in leaf discs cultured under different light conditions it was postulated that the synthesis as well as the activity of ADPglucose pyrophosphorylase is subject to metabolite control in leaves [5]. ADPglucose starch synthase activity on the other hand, does not vary in leaf tissue to the same extent [5]. It was considered possible that with increasing temperature the level of these enzymes might decrease in grapevine leaves and thus result in a change of storage product at higher temperatures. Consequently, enzyme activities of grapevine leaves from plants grown at different temperatures were determined and for comparison leaves of broad bean and tomato plants were also used.

RESULTS AND DISCUSSION

The starch and lipid content of leaf laminae of grapevine, tomato and broad bean from plants grown at different temperatures are shown in Tables 1 and 2.

After 16 days tomato leaves contained *ca* 6 times more starch at 25/20° than at 32/27° but at both temperatures lipid levels were the same. There was a 10-fold difference in starch after 4 weeks at either 22/17° or 35/30° but lipid again did not change. Possibly changes in photosynthetic, respiration, or growth rates resulted in changes in starch content without the storage of an alternate reserve at higher temperatures [6]. Broad bean leaves did not have different concentrations of either starch or lipid at 22/17° compared with 35/30° and in some cases the bean leaves became chlorotic at the higher temperature regime.

The amount of starch and lipid was not different after 1 week in leaves of grapevines transferred from a glasshouse at *ca* 30/25° to growth cabinets at 22/17° or 35/30°. After 3 weeks at the different temperature regimes, mature leaves on both primary and secondary shoots had higher starch and lower lipid concentrations at 22/17° than at 35/30° and the effect was still evident at the fourth week. The protein content of tomato leaves (not shown) was *ca* 2% fr. wt and that of the grapevine leaves between 3.5 and 4%. The relationship between temperature and starch and lipid content was not altered by expressing the results on a protein basis. The highest starch content in grapevine leaves at 22° (0.60% fr. wt which is equivalent to 2.5% dry

Table 1. Starch and lipid content of tomato and broad bean leaves grown at different temperatures for different times

Environment Temperature/time		Starch (g/100 g fr. wt)	Lipid
Tomato leaves			
25/20°	16 days	0.97	1.31
35/27°	16 days	0.17	1.27
	L.S.D.	0.60	n.s.
22/17°	4 weeks	5.91	1.41
35/30°	4 weeks	0.60	1.47
	L.S.D.	0.37	n.s.
Broad bean leaves			
Basal leaves			
22/17°	4 weeks	0.38	0.89
35/30°	4 weeks	0.36	0.86
		n.s.	n.s.
Apex leaves			
22/17°	4 weeks	0.49	0.96
35/30°	4 weeks	0.42	0.67
		n.s.	n.s.

L.S.D. values are for $P = 0.05$, n.s. = no significant difference.

Each value is the mean of 4 assays on 4 different plants from the same batch ($n = 4$).

Table 2. Starch and lipid content of grapevine leaves grown at different temperatures for different times

Environment Temperature, time		Starch (g/100 g fr. wt)	Lipid
Grapevine leaves			
22/17°	1 week	0.30	2.38
35/30°	1 week	0.21	2.59
	L.S.D.	n.s.	n.s.
Secondary shoot			
22/17°	3 weeks	0.60	1.68
35/30°	3 weeks	0.37	2.41
	L.S.D.	0.14	0.14
Main shoot			
22/17°	3 weeks	0.53	2.25
35/30°	3 weeks	0.23	3.21
	L.S.D.	0.30	0.42
22/17°	4 weeks	0.41	1.88
35/30°	4 weeks	0.23	2.48
	L.S.D.	0.13	0.39
Main shoot (different plants)			
25/20°	1 week	1.74	2.64
32/27°	1 week	0.88	4.39
	L.S.D.	0.56	0.37

L.S.D., n.s. and n as in Table 1.

wt) is low compared to the 11 and 23 % of dry wt at 25 and 18° reported previously [1]. The low starch values were not due to an error in the current work because values as high as 17 % dry wt were found during tests on other grapevine leaves, and tomato leaves at 22° for 4 weeks gave high values for starch (Table 1).

In the current experiments (not all of which are reported) it was found generally that much smaller differences in starch content occurred at the different temperatures and then only after a few weeks. However, with another set of grapevines, differences occurred sooner (after only 1 week) and the starch levels were higher than in the former set of plants (Table 2, last data), but still the differences were not as great as those observed previously [1]. The age and nutritional status of the grapevine cuttings used to establish the plants and the age of the plants when transferred to the growth cabinets probably was different from those in the earlier experiments. The lipid content of the grapevine leaves in the present work was between 7 and 18 % on a dry wt basis compared with between 6 and 16 % in the previous work.

The activities of ADPglucose pyrophosphorylase and ADPglucose starch synthase were assayed in the leaves from the plants grown at different temperatures to determine whether the levels of these enzymes correlated with the concentrations of starch present (Table 3). In tomato leaves the activity of the former enzyme was not affected by the growth temperature. The total activity of ADPglucose starch synthase also was not different but a greater proportion of the enzyme was insoluble (presumably adsorbed on starch grains) at the lower temperature than at the higher temperature after 16 days. After 4 weeks more starch had accumulated in tomato leaves and the activity of ADPglucose starch synthase adsorbed on starch grains was similar at both temperatures, but inexplicably there was more soluble enzyme at 35/30°.

Broad bean leaves at the higher temperatures had lower activities of both enzymes but this was probably due to senescence of the leaves under these conditions and the values are now shown in Table 3.

After 1 week and 3 weeks in the growth cabinets, grapevine leaves contained less ADPglucose pyrophosporylase and less insoluble starch synthase at the higher temperature regime than at the lower. However, the decrease in these enzyme activities was not sufficient to explain the reduction in the concentration of starch accumulated at the higher temperature, because *in vivo* the activity of the enzymes would be *ca* the same in the leaves at 22° and 35° due to increasing activity with increasing temperature [7]. The values shown in Table 3 are the results of assays carried out at 30°. The relationships were not changed by expressing the activities on a protein basis. Possibly enzymes of lipid metabolism may be either activated or synthesized at higher temperatures, but the multitude of possible enzymes makes choice of likely ones difficult.

The fatty acid composition of the lipids of tomato leaves was different after 4 weeks at the two different temperatures (Table 4). At 35° there were lower percentages of palmitic and linoleic acids and a higher percentage of linolenic acid than at 22°. In the grapevine leaves of the secondary shoot containing lower amounts of lipid (Table 2) there was virtually no difference in the fatty acid composition of the lipid at the two temperatures (Table 4). However, in the leaves of the main shoot grown at 35° containing higher amounts of lipid there was a higher percentage of palmitic and a lower percentage of linolenic acid than at 22°. This was the reverse to that which occurred in tomato leaves.

Table 3. Activity measured at 30° of enzymes in tomato and grapevine leaves grown at different temperatures for different times

Environment Temperature/time		ADPG pyrophosphorylase (nmol/min g fr. wt)	ADPG starch synthase Sol.	Insol.	Total
		Tomato leaves			
25/20°	16 days	260	31	45	76
32/27°	16 days	320	36	28	64
	L.S.D.	n.s.	n.s.	10	n.s.
22/17°	4 weeks	150	11	48	59
35/30°	4 weeks	200	26	46	71
	L.S.D.	n.s.	5	n.s.	n.s.
		Grapevine leaves			
22/17°	1 week	430	30	82	112
35/30°	1 week	340	25	28	53
	L.S.D.	80	n.s.	41	n.s.
Secondary shoot					
22/17°	3 weeks	200	9	38	47
35/30°	3 weeks	115	11	14	24
	L.S.D.	50	n.s.	7	8
Main shoot					
22/17°	3 weeks	340	11	41	51
35/30°	3 weeks	240	9	21	30
	L.S.D.	n.s.	n.s.	8	7

L.S.D., n.s. and *n* as in Table 1.

Quinn and Williams [8] in a recent comprehensive review on plant membrane lipids have discussed lipid composition as related to temperature of growth of plants. Although in some plants an increase in the degree of unsaturation of the fatty acids of the lipids is observed when the plants are grown at lower temperatures, there does not seem to be general acceptance that a causal relationship exists between cold-hardiness or chilling resistance and the degree of unsaturation of the lipids. The osmiophilic globules of *Beta vulgaris* leaves contain many lipids including galactolipids and sulpholipids [9]. The authors consider that the globules are lipid depots which serve as a source of energy in addition to that of starch. In grapevine leaves the lipid droplets were much larger [1] than the osmiophilic globules found in other leaves [9]. The decrease in the percentage of linolenic acid observed at the higher temperature (Table 4) may be due to a different composition of the storage lipid compared to the other lipids of the leaves. Possibly sulpholipids, known to contain less linolenic acid and more palmitic acid [8], may be present in larger amounts in the lipid droplets. The change in overall fatty acid composition could be reflected in a change from a low to a high content of storage lipid.

The current work has confirmed that grapevine leaves under some circumstances change from starch storage at low temperatures to lipid storage at higher temperatures although the differences were never as great as those found previously [1]. At the present time no explanation can be offered for the effect which is sometimes observed in grapevine, a woody perennial, but not in tomato or broad bean, both of which are herbaceous annuals. The presence of woody stem tissues containing large rays as sites of storage of starch in grapevine would not seem to be an explanation for the difference between the perennial and annual species. However, the amount of starch stored in the rays of the cuttings from which the plants were grown may have influenced the subsequent size of the sink and hence the levels of starch stored in the leaves. Possibly Buttrose and Hale [1] used cuttings containing very high concentrations of starch which could explain their subsequent high starch values in leaves at the lower temperatures.

EXPERIMENTAL

Vines of *Vitis vinifera* L. cv Cabernet Sauvignon, were established from cuttings in a glasshouse at *ca* 30°/25° [10] and after 3 months transferred to growth cabinets under conditions described previously [1]. In initial expts plants were tipped and laterals were removed as described in ref. [1]. When these plants did not show the switch from starch to lipid storage at high temps,

Table 4. Fatty acid composition of lipids extracted from leaves of tomato plants and grapevines grown at different temperatures for different times

Environment Temperature/time		Fatty acid composition (% total) Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
			Tomato leaves			
22/17°	4 weeks	30	1–2	1–2	17	50
35/30°	4 weeks	23	1–2	1–2	10	64
	L.S.D.	4			3	5
			Grapevine leaves			
Secondary shoot						
22/17°	3 weeks	20	1–2	6–7	14	58
35/30°	3 weeks	18	1–2	5–6	16.5	59
	L.S.D.	n.s.			1.7	n.s.
Main shoot						
22/17°	3 weeks	17	1–2	1–2	7	74
35/30°	3 weeks	22	1–2	1–2	9	66
	L.S.D.	2			n.s.	2

L.S.D., n.s. and *n* as in Table 1.

subsequent expts were carried out using plants which had their leaves removed from the lower half of the stems. These partially shaded leaves were removed to avoid them acting as sinks and receiving photosynthate from the upper leaves. Data for the latter plants only are reported in this paper.

Broad bean seeds (*Vicia faba* L.) and tomato seeds (*Lycopersicon esculentum* Mill.) were sown in a glasshouse and plants were transferred to growth cabinets when they were *ca* 60 cm high. During the 16 hr days the temp was maintained at 5° higher than that during the night [1].

After various numbers of days in the growth cabinets as described in the text, leaves were sampled at the end of the night period. Starch and protein were determined as described previously [11].

Lipid was determined gravimetrically [12]. Lipid was also extracted from 0.5 g leaf lamina as above and the solvent removed *in vacuo*. The lipid was dissolved in 0.25 ml C_6H_6 containing 0.25 mg heptadecanoic acid, 1 ml BCl_3 in MeOH added, and the mixture heated at 100° for 3 min. After evapn to near dryness in a desiccator, the Me esters were extrd with 1 ml hexane, dried (Na_2SO_4) and separated by GC at 200° on a 25-m SCOT Column coated with SP 1000.

Extraction of enzymes in a medium containing DIECA and Carbowax was carried out as before [11]. The activities of ADPglucose starch synthase (EC 2.4.1.21) and ADPglucose pyrophosphorylase (EC 2.7.7.27) were determined at 30° using ^{14}C-labelled substrates [11].

Extraction media and assay conditions were used which gave maximum activities of enzymes in the tissues studied [11, 13–15]. Inhibition and/or pption of enzymes by tannins was avoided by the use of DIECA and Carbowax [11, 13, 14]. Both supernatants and ppts (which contained all the precipitable material of the homogenates) were assayed for enzyme activity in the linear part of the reactions at optimum pH values with optimum concns of substrates, metal ions and activators. No evidence for inhibitors (apart from tannins) of the two enzymes has been found in the tissues studied.

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