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Effect of the herbicide atrazine on the bean leaf lipids

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Abstract

Eight-day-old bean plants, grown in a nutrient solution, were sprayed with 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M atrazine. The lipid changes in primary and trifoliate leaves were studied 6 d after the herbicide application. The atrazine treatment inhibited the growth of the trifoliate leaves, and decreased photosynthetic and transpiration rates, the stomatal conductance, and the total lipid content. Atrazine treatment increased 16:0 and 18:3 acids and decreased trans-3-hexadecenoic and 18:2 acids in the phospholipids. The herbicide also increased 16:0 and 18:1 acids in glycolipids and decreased 18:3 acid in monogalactosyl diacylglycerols and digalactosyl diacylglycerols. In most cases the marked changes in fatty acid composition of the main lipid classes were observed at 10^{-4} and 10^{-3} M atrazine.

Additional key words: lipid composition, *Phaseolus vulgaris*, triazine herbicide.

Introduction

Atrazine is a triazine herbicide, wide-spread because of its superior tolerance in maize. All triazine herbicides have been noted to inhibit the growth of all organs in intact plants. When the roots of maize were treated with atrazine, chloroplasts were destroyed and photosynthesis in the leaves decreased (Ashton *et al.* 1963, Ashton and Crafts 1981). However, certain triazine herbicides have been shown to stimulate growth at low concentrations (Ashton and Crafts 1981). As a rule, the lipid synthesis in leaves is stimulated at low herbicide concentrations and inhibited at high concentrations.

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Abbreviations: DGDG - digalactosyl diacylglycerols; d.m. - dry mass; FA - fatty acids; FAME - fatty acids methyl esters; 16:1 - hexadecenoic acid; 18:2 - linoleic acid; 18:3 - linolenic acid; MGDG - monogalactosyl diacylglycerols; 18:1 - oleic acid; 16:0 - palmitic acid; PL - phospholipids; 18:0 - stearic acid; TAG - triacylglycerols.

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Although the effect of atrazine on the photosynthetic apparatus in plant leaves and algae has been well studied (*e.g.* Holt 1993, Zheleva *et al.* 1993, Fournadzhieva *et al.* 1995, Iliev 1991), the data on its effect on lipid metabolism are limited (Ashton and Crafts 1981, Fedtke 1982, Harwood 1991). The aim of this work was to study the lipid changes in bean leaves after treatment with different concentrations of atrazine.

Materials and methods

Bean plants (*Phaseolus vulgaris* L., cv. Cheren Starozagorsky) were grown as a hydroponic culture in growth chambers (irradiance of 100 μmol m^{-2} s^{-1}, 12-h photoperiod, day/night temperature of 26/24 °C). A series of preliminary experiments with untreated plants, grown for the same period in the growth chamber did not show statistically significant differences in the net photosynthetic and oxygen evolution rates induced by plant ontogeny. Eight-day-old plants were divided into five groups (for each group at least 25 plants were taken): control plants, and plants sprayed with 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M water solution of atrazine, containing 0.5 % (v/v) *Tween 80* as a surfactant. Each plant was sprayed with approximately 0.3 cm^3 of the herbicide solution. All plant groups were grown further under the same conditions (temperature and irradiance). A nutrient Knop's solution was added when required. After 6 d (14-d-old plants) leaf samples were taken for lipid analysis and determination of photosynthetic rate, stomatal conductance and transpiration rate.

The total lipids were extracted according to Bligh and Dyer (1959). The main lipid classes were separated by preparative thin-layer chromatography (*Silicagel G, Merck*, Germany, layer thickness 0.5 mm). The lipid spots were vizualized under UV-light after spraying with fluorescent indicator, scrapped off into small glass containers with teflons crewcaps. After addition of internal standard (heptadecanoic acid) all lipid classes were transesterified with 15 % acetylchloride in methanol. The analysis of the obtained fatty acid methylesters (FAME) was carried out by flame-ionization detector of gas-liquid chromatography (*FID-GLC*) on a glass capillary column (30 m, 0.2 mm i.d. coated with *SILAR10C*). The column temperature was increased from 165 to 220 °C (2 °C min^{-1}) with nitrogen as a carrier gas at a flow rate 14 cm^3 s^{-1}. The amount of each lipid class was determined on the basis of the FAME mass using converting factors as follows: 1.0 for TAG; 1.4 for MGDG and PL, and 1.8 for DGDG (Elenkov *et al.* 1993).

The net photosynthetic rate, stomatal conductance, and transpiration rate in bean leaves were measured by a Portable System for Photosynthesis Measurements *LI-6000* (*Li-Cor*, Lincoln, USA).

Results and discussion

The plant responses to herbicide treatment were more expressed in trifoliate than in primary leaves. The action of atrazine was observed already on the third day after treatment, but the plant injuries continued until the end of the experiment. This could

be due to the relatively slow penetration of the herbicide through the inner cell membranes. The herbicide treatment led to growth inhibition of the trifoliate leaves. Surprisingly, in the primary leaves the 10^{-4} M atrazine increased their dry matter. This could be due to the possibility that triazines at low concentrations were able to stimulate the plant growth (Ebert and Dumford 1976). A partial necrosis of the roots was observed with the increase in atrazine concentration.

Table 1. Effect of atrazine on the net photosynthetic rate, transpiration rate and stomatal conductance in bean leaves (means ± SE, $n = 3$).

	Atrazine [M]	Photosynthetic rate [mg(CO_2) m^{-2} s^{-1}]	Transpiration rate [mg(H_2O) m^{-2} s^{-1}]	Stomatal conductance [cm s^{-1}]
Primary leaves	control	0.257 ± 0.042	27.0 ± 1.1	0.162 ± 0.008
	10^{-5}	0.164 ± 0.050	22.5 ± 1.5	0.108 ± 0.006
	10^{-4}	0.111 ± 0.012	19.3 ± 0.5	0.081 ± 0.005
	10^{-3}	0.019 ± 0.002	17.9 ± 0.8	0.090 ± 0.005
	10^{-2}	0.022 ± 0.011	16.1 ± 0.9	0.075 ± 0.004
Trifoliate leaves	control	0.234 ± 0.030	25.7 ± 1.9	0.149 ± 0.007
	10^{-5}	0.198 ± 0.051	23.2 ± 0.7	0.115 ± 0.006
	10^{-4}	0.141 ± 0.040	21.9 ± 0.4	0.094 ± 0.005

The treatment with atrazine caused a decrease in the net photosynthetic and transpiration rates, and in the stomatal conductance in both primary and trifoliate leaves (Table 1). The effect was observed even at concentration of 10^{-5} M. These results are in agreement with the changes in pea leaves after atrazine treatment (Zheleva *et al.* 1994).

The herbicide treatment decreased the relative amounts of all lipid classes, as well as in the total lipid content (Table 2). The MGDG/DGDG ratio increased in the course of atrazine treatment. This corresponded with the data of Dominguez *et al.* (1994), Goasdoue *et al.* (1993), and Mishra *et al.* (1992).

The amount of the total lipids, as well as of the MGDG and DGDG decreased after atrazine treatment (Table 2). We suppose that the changes in the amount of glycolipids and phospholipids can be involved in a protective reaction of the plant membranes against harmful treatment.

A detailed investigation on the content of 16:1 acids in PL showed that atrazine treatment decreased more content of trans-9-hexadecenoic than cis-3-hexadecenoic acid (Ivanova *et al.* 1997). Because trans-3-hexadecenoic acid is concentrated mainly in phosphatidyl glycerols, it can be supposed that the relative amount of these PL decreased also.

The amount of DGDG in the survived trifoliate leaves after 10^{-2} M atrazine treatment was even larger than the amount of MGDG. This led to better control of the membrane permeability and could play an adaptive role against herbicide stress (Ivanova *et al.* 1995).

Table 2. Effect of the concentration of atrazine on the main lipid classes in primary and trifoliate bean leaves (means ± SE, $n = 3$).

Atrazine [M]	Lipid class	Primary leaves lipid content [mg g^{-1}(d.m.)]	[% of total]	Trifoliate leaves lipid content [mg g^{-1}(d.m.)]	[% of total]
Control	TAG	3.4 ± 0.3	4.0	1.7 ± 0.2	2.2
	MGDG	36.2 ± 3.2	42.4	33.0 ± 3.0	42.8
	DGDG	27.4 ± 2.5	32.1	27.4 ± 2.5	35.5
	PL	18.4 ± 1.7	21.5	15.0 ± 1.4	19.5
	total	85.4 ±7.7	100.0	77.1 ± 6.9	100.0
	MGDG/DGDG	1.3		1.2	
10^{-5}	TAG	3.8 ± 0.3	6.2	3.0 ± 0.3	4.9
	MGDG	20.4 ± 1.8	33.4	23.6 ± 2.1	38.6
	DGDG	10.1 ± 0.9	16.6	16.6 ± 1.6	27.2
	PL	26.7 ± 2.4	43.8	17.9 ± 1.6	29.3
	total	61.0 ± 5.5	100.0	61.1 ± 4.6	100.0
	MGDG/DGDG	2.0		1.4	
10^{-4}	TAG	2.7 ± 0.2	6.1	4.7 ± 0.4	10.0
	MGDG	17.7 ± 1.6	40.2	14.3 ± 1.3	30.3
	DGDG	6.1 ± 0.5	13.8	8.8 ± 0.8	18.7
	PL	17.6 ± 1.6	39.9	19.3 ± 1.7	41.0
	total	44.1 ± 4.0	100.0	47.1 ± 4.2	100.0
	MGDG/DGDG	2.9		1.6	
10^{-3}	TAG	2.0 ± 0.2	6.4	3.0 ± 0.3	6.4
	MGDG	13.6 ± 1.2	43.7	18.0 ± 1.6	38.5
	DGDG	4.6 + 0.4	14.7	14.8 + 1.3	31.6
	PL	11.0 ± 1.0	35.2	11.0 ± 1.0	23.5
	total	31.2 ± 2.8	100.0	46.8 ± 4.2	100.0
	MGDG/DGDG	3.0		1.2	
10^{-2}	TAG	2.7 ± 0.2	5.8	4.0 ± 0.4	6.3
	MGDG	15.0 ± 1.4	32.4	23.4 ± 2.1	36.8
	DGDG	9.7 ± 0.9	20.9	26.2 ± 2.4	41.2
	PL	19.0 ± 1.7	40.9	10.0 ± 0.9	15.7
	total	46.4 + 4.2	100.0	63.6 + 5.8	100.0
	MGDG/DGDG	1.5		0.9	

In MDGD 10^{-5} and 10^{-4} M atrazine had insignificant effect on the FA composition in the primary and trifoliate leaves (Table 3). The higher atrazine concentrations increased palmitic and oleic acid contents and decreased linoleic and linolenic acid contents. This is in accordance with the literature data on the inhibition of the desaturation of 18:1 and 18:2 acids caused by some herbicides (Cohen *et al.* 1993).

In DGDG the effect of different concentrations of atrazine on the DGDG content in primary bean leaves (Table 3) was similar to those of MGDG, but less expressed. There was even a decrease in 18:3 acid in trifoliate leaves (Table 3). The FA composition of trifoliate leaves was affected more than in primary leaves, which can be due to more severe injuries in trifoliate leaves.

Table 3. Effect of atrazine concentration on the fatty acid composition in the main lipid classes of primary and trifoliate bean leaves [triplicate values for methyl esters in standard mixture by gas chromatographic analysis varied within 12 % for minor components (< 5 % content) and within 5 % for the others].

Atrazine [M]	Lipid class	Fatty acids [% of total] 16:0	16:1	18:0	18:1	18:2	18:3	unsat/sat
Primary leaves								
Control	MGDG	2.8	0.8	0.9	0.3	3.2	91.9	26.0
	DGDG	13.9	8.7	6.0	1.6	13.9	55.8	4.2
	PL	31.7	9.9	7.0	1.0	16.5	33.9	1.6
10^{-5}	MGDG	3.2	1.1	1.2	0.7	3.5	90.3	21.7
	DGDG	14.8	4.0	7.2	2.0	17.7	54.3	3.6
	PL	31.4	6.6	4.8	1.4	5.6	50.2	1.8
10^{-4}	MGDG	3.7	0.4	0.7	0.2	2.6	92.4	21.9
	DGDG	21.2	0.5	2.6	4.4	14.3	57.0	3.2
	PL	35.6	5.8	3.9	1.4	5.4	47.9	1.5
10^{-3}	MGDG	5.0	2.3	0.9	1.0	2.4	88.4	15.9
	DGDG	18.0	6.5	4.1	0.9	10.3	60.1	3.5
	PL	43.5	5.6	3.5	1.2	3.8	42.4	1.1
10^{-2}	MGDG	7.1	4.8	1.4	1.8	3.2	81.6	10.7
	DGDG	19.4	5.7	3.4	1.7	9.8	60.0	3.4
	PL	47.6	4.7	4.3	1.9	3.4	38.1	0.9
Trifoliate leaves								
Control	MGDG	4.5	1.3	1.4	1.1	4.9	86.7	15.9
	DGDG	17.1	5.5	8.3	3.6	15.0	50.5	2.9
	PL	28.8	15.6	5.0	1.4	18.3	30.9	2.0
10^{-5}	MGDG	3.5	0.3	0.6	0.3	4.4	90.8	23.3
	DGDG	21.4	1.9	7.7	3.5	28.2	37.3	2.4
	PL	28.9	15.0	4.6	1.2	10.7	39.6	2.0
10^{-4}	MGDG	3.7	0.4	0.9	0.8	3.6	90.6	20.7
	DGDG	23.9	3.0	7.8	3.3	23.5	38.2	2.2
	PL	30.5	15.4	4.4	1.7	10.1	37.9	1.9
10^{-3}	MGDG	5.5	4.2	0.9	0.4	4.0	85.0	14.6
	DGDG	22.3	15.7	6.3	8.3	6.0	41.4	2.5
	PL	30.3	12.7	4.4	2.2	9.5	31.9	1.3
10^{-2}	MGDG	24.9	10.3	6.9	3.4	3.4	51.0	2.1
	DGDG	21.3	10.3	5.2	1.3	19.0	42.8	2.8
	PL	41.4	9.2	4.7	4.5	3.3	36.9	1.2

In PL the effect of atrazine on the FA composition decreased the amounts of 18:2 and 16:1 acids and increased the amount of 16:0 and 18:3 acids. The same was observed with other triazine herbicides (Dominguez *et al.* 1994). Surprisingly, the lowest atrazine concentration induced most expressed effect on the 18:3 acid in both primary and trifoliate leaves.

Generally, it was found that even in the concentrations range of 10^{-5} - 10^{-4} M, atrazine has an expressed effect on the lipids composition in bean leaves. Although at this concentrations the leaves have not visible injuries, their photosynthetic apparatus

and lipid membrane composition are affected. This results can be helpful in practice forage estimation of the field doses of atrazine.

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