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The phytotoxic action of triazine herbicides on flax, beets and buckwheat seedlings, and some physiological changes connected with it

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Abstract:

It was found that triazine herbicides exerted, when applied for a longer time, an increasing inhibitory effect on dry and fresh weight yields, transpiration and growth of the seedlings of flax, beet and buckwheat. At the same time enhanced accumulation of free amino acids and decrease of simple sugar values was noted in the aboveground parts of the test plants.

The results are interpreted as the secondary effects of the inhibitory action of triazines on plant photosynthesis. The toxicity of the tested chemicals to flax, beet and buckwheat decreased in the following order: atrazine, simazine, propazine, atratone, prometone and prometryne. Flax was more resistant to triazines than beets and buckwheat.

INTRODUCTION

Triazine herbicides (Overbeek 1964) are widely used in many cultivated crops (Crafts 1961; Detroux 1957; Holstun 1963; Kraśnikow et al. 1962) and in agricultural practice.

The action of these preparations on plants is probably connected with their inhibitive influence upon the process of photosynthesis (Ashton et al. 1960; Crafts 1961; Couch et al. 1966; Oorschot et al. 1961; Overbeek 1962; Roth 1958), and particularly upon Hill's reaction (Crafts 1961; Exter 1961; Moreland et al. 1959; Overbeek 1962); nevertheless they can exert various effects on the metabolism of plants (Baranowski et al. 1967; Crafts 1961; Funderburk et. al. 1963; Płoszyński 1966). Investigations on the activity of triazine preparations in plants and their influence upon vegetable metabolism are now one of the main problems which various scientific centres are concerned with.

In this paper the phytotoxic action of basic triazine preparations is compared in the seedlings of flax (*Linum usitatissimum*), sugar beets (*Beta vulgaris*) and buckwheat (*Fagopyrum esculentum*), and their influence on the levels of some metabolic components was determined in the tested plants.

The investigations involved the dynamics of dry and fresh matter yields, the growth and transpiration of plants, determination of the contents of free amino acids and saccharides in the aboveground parts in both control and herbicide-treated seedlings. The experiments were carried out under controlled conditions.

EXPERIMENTAL METHODS

The investigations of the influence of triazines on flax, beets and buckwheat were based on three vegetation experiments carried out according to the method described by Świętochowski et al. (1962 a, 1962 b), applying the technique of paraffin cups. A light soil (coarse sandy soil) with slightly acid reaction (pH in KCl = 5,8), containing 0,5% of organic carbon and 8,0 mg of assimilable P_2O_5 nad 6,0 mg of assimilable K_2O (acc. to Egner) per 100 g of soil was used as substrate.

Table 1

Scheme of experiments concerning the influence of triazine herbicides on flax, sugar beet and buckwheat seedlings

Treatments in each experiment	Number of replications	Doses of preparations in mg/cup used in experiments with			Number of seeds sowed per one cup	Number of plants in cup after thinning		
		flax	sugar beets	buckwheat		flax	sugar beets	buckwheat
Control	15	0	0	0	25	15	10	10
Atrazine	15	0,8	0,4	0,8	25	15	10	10
Simazine	15	0,8	0,4	0,8	25	15	10	10
Propazine	15	0,8	0,4	0,8	25	15	10	10
Prometone	15	0,8	0,4	0,8	25	15	10	10
Atratone	15	0,8	0,4	0,8	25	15	10	10
Prometryne	15	0,8	0,4	0,8	25	15	10	10

For the experiments triazine herbicides produced by "J. R. Geigy A.G.", Bale, Switzerland were used, each containing 50% of active component. They were: simazine, atrazine, propazine (chloro-triazines), atratone, prometone (methoxy-triazines) and prometryne (methyltio-triazine). Doses of applied herbicides are presented in this paper in mg of total preparation used per cup. Each of these preparations in the form of aqueous suspension together with NPK fertilizers (aqueous solutions of NH_4NO_3, KH_2PO_4 and K_2SO_4 in doses of 25 mg of pure component per cup), was mixed before sowing with the whole quantity of air-dry soil (400 g) prepared for each a cup. Substrate prepared in such a way was placed in paraffin cups and seeds of the test plants were sown in them. Three experiments were carried out altogether, each plant being tested in à separáte one. The scheme of the experiments is shown in Table 1.

All the experiments were carried out in glasshouse conditions under natural light. In the course of the experiment temperature varied from 20 to 23°C in the daytime, and from 15 to 17°C at night. Air humidity varied from 60 to 85% of relative humidity. After the test plants had sprouted they were watered every day through a drain to a constant weight, keeping the soil humidity at 50% of maximum water capacity. During the experiments daily measurements of plant growth were carried out. Transpiration was determined by daily comparison of water loss in the cups with plants against that in cups without plants. In each experiment and in all treatments, investigations were carried out on the dynamics of fresh and dry matter yields as well as on the dynamics of the contents of free amino acids and saccharides in the aboveground parts of the test plants.

For this purpose plants in certain replications were cut at soil level at established periods to determine the contents of free amino acids and sugars in them as well as the yields of fresh and dry matter. The plant material was subjected to analysis by ascending paper chromatography in n-butyl alcohol:acetic acid:water (4:1:1). Sugars and amino acids were developed three times.

Amino acids were developed with ninhydrin, aldoses with aniline phthalate and ketoses with anthrone (Opieńska-Blaut et al. 1957). Quantitative interpretation of the amino acid spots was achieved by means of a densitometer (Glabiszewski et al. 1966), and that of sugars by comparing the sizes and intensity of the spots with adequate standart spots (Opieńska-Blaut et al. 1957).

RESULTS AND DISCUSSION

The investigations results, showing the influence of triazine herbicidies upon the dynamics of dry matter yields of flax, are given in Fig. 1.

As seen from the shapes of the curves in Fig. 1, relative inhibition of the growth dynamics of the crops in relation to the control occurred

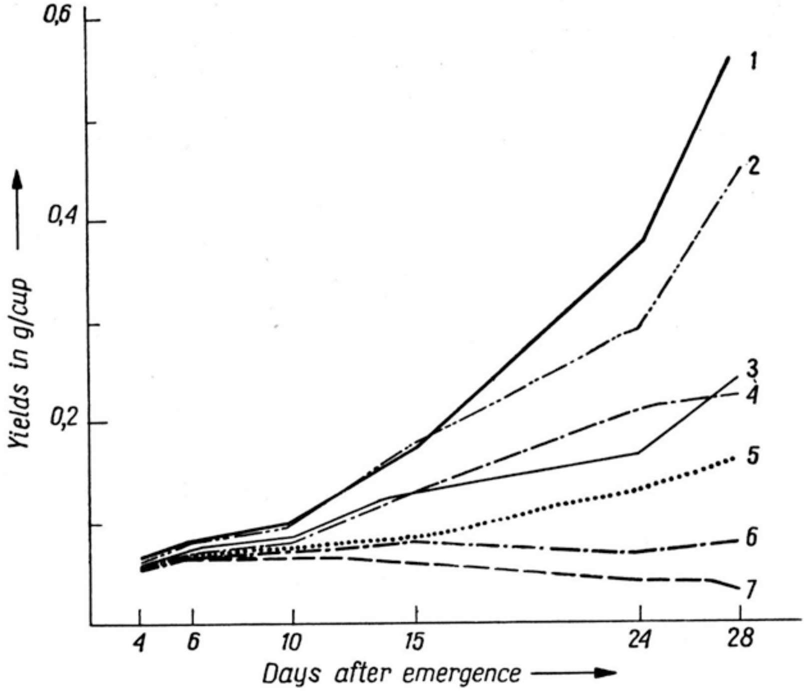

Fig. 1. Influence of triazine herbicides on the dynamics of dry weight yields of flax. Dose of herbicides 0.8 mg/cup

1 — Control; 2 — Prometryne; 3 — Prometone; 4 — Atratone; 5 — Propazine; 6 — Simazine; 7 — Atrazine.

rapidly and was considerable, particularly in treatments with simazine and atrazine. Withering and death of plants was observed in these treatments as late as 28 days after sprouting. The action of herbicides on beets and buckwheat was equally rapid.

Figures 2 and 3 show that triazine herbicides, particularly atrazine and simazine, rapidly inhibit the dry matter yields of beets and buckwheat. However, contrary as the flax, withering and death of seedlings of both these plants occurred very early (9 days afters prouting). The dynamics of fresh matter yields, growth and transpiration of all the tested seedlings was similar to that of dry matter yields, the only difference being that the influence of herbicides upon the dynamics of fresh matter yields was somewhat greater, and transpiration in the final stages of the experiments in treatments with atrazine and simazine decreased almost to zero.

In parallel to the investigations on the dynamics of yields, the influence of triazine preparations upon the dynamics of the free amino acids and saccharides content was analysed in the aerial parts of the tested

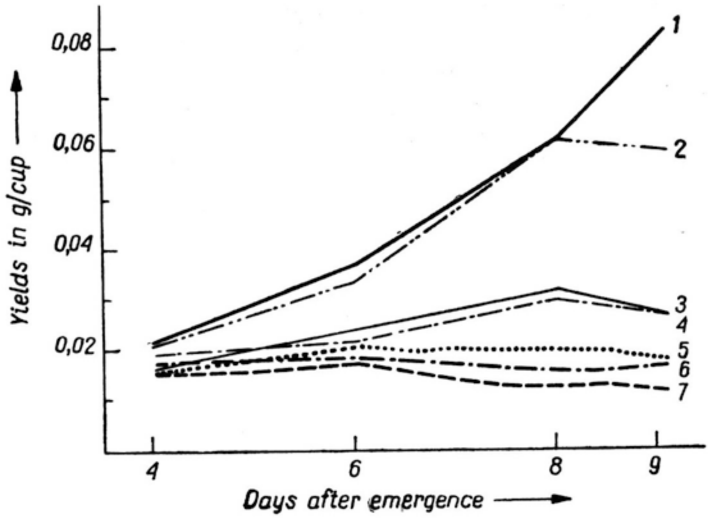

Fig. 2. Influence of triazine herbicides on the dynamics of dry weight yields of sugar beets. Dose of herbicides 0.4 mg/cup

1 — Control; 2 — Prometryne; 3 — Prometone; 4 — Atratone; 5 — Propazine; 6 — Simazine; 7 — Atrazine.

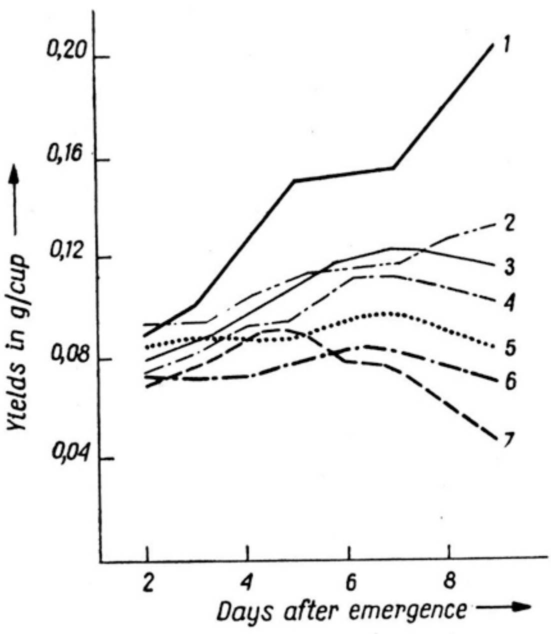

Fig. 3. Influence of triazine herbicides on the dynamics of dry weight yields of buckwheat. Dose of herbicides 0.8 mg/cup

1 — Control; 2 — Prometryne; 3 — Prometone; 4 — Atratone; 5 — Propazine; 6 — Simazine; 7 — Atrazine.

plants. However, demonstration of the dynamics of the content of these components during the experiment was technically complicated.

Therefore in this paper we restricted ourselves to discussing the results obtained in the final stages of the experiments, particularly as they give the most characteristic picture of the physiological changes, which became more intensive with time.

In Table 2 the influence of triazines upon the free amino acids and saccharides content in flax is shown.

Table 2

Influence of triazine herbicides on the contents of free amino acids and saccharides in the aboveground parts of flax seedlings

Dose of herbicides 0,8 mg/cup. Term of plant harvest — 28 days after sprouting. Results in mg/100 g of dry weight

Saccharides Amino acids	Treatments						
	control	atrazine	simazine	propazine	prometone	atratone	prometryne
Saccharose	891	—	+	50	200	+	462
Fructose	231	50	59	124	108	86	205
Glucose	294	150	164	172	+	140	260
Mannose	162	—	40	85	78	118	140
Leucine	12	148	92	54	38	44	25
Phenylalanine	—	216	117	126	38	64	+
Valine	28	165	124	70	35	52	36
Methionine	—	30	24	—	—	—	—
Tryptophane	—	—	—	+	—	—	+
γ-Aminobutyric acid	24	118	95	60	48	48	35
Tyrosine	34	96	80	45	43	42	38
Alanine	74	155	124	88	70	78	75
Threonine	30	63	58	26	24	32	35
Glutamic acid	176	145	135	184	172	158	195
Glycine	78	262	128	75	38	40	40
Serine	60	185	105	75	55	82	75
Aspartic acid	162	135	122	134	126	130	134
Glutamine	208	462	428	358	242	358	225
Asparagine	—	1352	1218	792	486	520	253
Arginine	+	688	534	308	136	188	120
Histidine	+	296	182	76	+	+	—
Lysine	—	258	195	122	42	88	54
Cystine and cysteine	—	352	384	235	158	184	50

According to the data in Table 2, in the treatments with herbicides, and particularly atrazine, a definite drop in the content of saccharose could be observed, while the content of glucose, mannose and fructose also decreased but did not differ so much as compared with the control.

In comparison with the control an apparent accumulation of free amino acids was observed particularly asparagine, arginine, histidine, glutamine, lysine, cystine and cysteine. There was less increase in the contents of other amino acids. Similar data concerning beets are presented in Table 3.

Table 3

Influence of triazine herbicides on the contents of free amino acids and saccharides in the aboveground parts of sugar beet seedlings

Dose of herbicides 0,4 mg/cup. Term of plant harvest — 9 days after sprouting. Results in mg/100 g of dry weight

Saccharides Amino acids	Treatments						
	control	atrazine	simazine	propazine	prometone	atratone	prometryne
Saccharose	548	—	—	—	80	—	326
Fructose	70	—	+	—	+	—	+
Glucose	284	63	168	163	88	156	336
Mannose	150	+	+	104	116	124	136
Leucine	—	133	118	118	42	55	—
Phenylalanine	—	175	146	104	28	46	—
Valine	—	108	92	90	80	37	—
γ-Aminobutyric acid	+	75	60	54	+	25	+
Tyrosine	5	94	65	60	10	18	10
Alanine	16	92	65	60	33	40	27
Threonine	—	15	+	+	+	+	—
Glutamic acid	178	133	124	120	145	118	167
Serine + glycine	18	112	74	90	38	49	22
Aspartic acid	105	192	134	124	120	92	102
Glutamine	78	403	305	275	203	234	140
Asparagine	55	1368	1204	886	389	485	70
Histidine	—	270	256	291	72	100	—
Lysine	30	317	240	224	155	154	46
Cystine + cysteine	20	614	502	463	286	335	35

In beets, a considerable reduction of the saccharose and hexoses contents were accompanied by considerable increase of free amino acids, mainly asparagine, glutamine, histidine, cysteine, cystine, leucine and phenylalanine. Only glutamic acid showed some tendency to decrease.

The data concerning buckwheat are presented in Table 4.

In buckwheat, as in flax and beets, a considerable decrease of the contents of sugars under the influence of herbicides was observed. As regards free amino acids, the most significant increase of the contents of asparagine, glutamine, histidine, phenylalanine, cystyne and cysteine was noted under the influence of herbicides.

Table 4

Influence of triazine herbicides on the contents of free amino acids and saccharides in the aboveground parts of buckwheat seedlings
Dose of herbicides 0.8 mg/cup. Term of plant harvest — 9 days after sprouting.
Results in mg/100 g of dry weigth

Saccharides Amino acids	Treatments						
	control	atrazine	simazine	propazine	prometone	atratone	prometryne
Saccharose	786	+	+	+	165	40	194
Fructose	178	—	82	95	108	102	122
Glucose	112	86	36	105	56	72	95
Leucine	15	115	108	74	48	77	58
Phenylalanine	+	205	192	186	97	127	108
Valine	32	146	146	137	65	74	60
γ-Aminobutyric acid	34	186	204	155	74	136	80
Tyrosine	18	123	95	104	35	50	40
Alanine	45	95	92	88	64	74	60
Glutamic acid	143	83	79	76	122	164	175
Threonine	—	27	35	16	5	10	15
Serine + glycine	20	102	95	79	35	58	60
Aspartic acid	165	96	113	108	164	160	152
Glutamine	180	440	395	362	286	328	305
Asparagine	158	1386	1315	1212	685	812	632
Histidine	130	435	420	386	205	293	204
Lysine	—	140	136	108	124	112	105
Cystine + cysteine	135	471	422	360	285	355	228

Analysis of the data contained in the figures and tables leads to the conclusion that all the herbicides used brought about similar disturbances in the tested plants, differing only in intensity of symptoms; it is suggested that these triazine preparations act in a similar way on the metabolism of various species of plants. The negative influence of these preparations upon the yields of dry matter and the contents of sugars in the aboveground parts of the tested plants is well explained by the fact known from literature (Ashton et al. 1960; Couch et al. 1966; Oorschot et al. 1961; Overbeek 1962) that triazines inhibit the photosynthetic activity of plants.

Accumulation of free amino acids in the aboveground parts of the tested plants was probably connected with a disturbance of the dynamic equilibrium of proteins, caused by triazines (velocity equilibria of anabolic and catabolic processes involving protein syntheses).

This can be manifested by the results of our earlier investigations in which triazines were found to decrease the contents of proteins in oats (Glabiszewski et al. 1966), maize and wheat (Płoszyński

1966). Nothwithstanding the possibility of a direct influence of triazine herbicides upon the synthesis of proteins, the accumulation of free amino acids can be explained as a secondary effect of the action of triazines on photosynthesis, the more so as some authors point to the existence of certain links between photosynthesis and protein synthesis (Calvin and Benson 1948; Stepka et al. 1948; Stephenson et al. 1956).

The final level of several amino acids probably resulted from one or another of the dynamic equilibria of proteins, and also from relation between synthesis and metabolism of amino acids with other metabolic pathways (together with the equilibrium of various nutrients uptake from soil and CO_2 from air). Since these connections can be very complex, we limit ourselves to an interpretation of the more basic dependences.

The accumulation of amides (asparagine and glutamine) and basic amino acids is supposed to be provoked by the action of the regulation systems of plants, which with the inhibition of photosynthesis and lack of carbon chains (sugars) lead to an advantageous fixation of excess NH_2 groups. The lack of increase of glutamic and aspartic acids contents in plants treated with herbicides, as compared to the control, may be explained by their transformation into amides after combining with the NH_2 groups or after deamination by consumption in the Krebs cycle.

The phytotoxic action of the tested preparations on the experimental plants (the rate and magnitude of accumulation of definite effects studied in this paper) may be considered as an aspect of the structure of a herbicide or of the plant species. The dependence of phytotoxicity on the chemical structure of a preparation is quite apparent. The highest activity was that of chloro-triazines in the order: atrazine- simazine-propazine. The methoxy-triazines prometone and atratone were less effective, while prometryne the only methyltio-triazine used in the experiments — showed but slight phytotoxicity. The phytotoxic effect of triazine herbicides, although apparently connected with the chemical structure of the preparation, appeared with different intensity in the test plants. Flax was much more resistant to the action of triazines than buckwheat and beets, the latter plants were very sensitive under the conditions of our experiments. Most probably the rate of herbicide uptake, their detoxification in the plant and the velocity at which they reached the site of action (chloroplasts) must have been different in flax than in buckwheat and beets.

CONCLUSIONS

1) Triazine herbicides inhibited plant growth, dry and fresh matter yields and transpiration in flax, buckwheat and beets.

2) The preparations caused a marked decrease in the contents of saccharose and hexoses, and a considerable increase in the contents of free amino acids in the aboveground parts of the tested plants.

3) The phytotoxicity of triazine herbicides to all the three tested plants decreased in the following order: atrazine – simazine – propazine-prometone – atratone – prometryne.

4) Flax was more resistant to the examined triazines than were buckwheat and beets.

5) The effects of triazines on plants observed in our experiments can be recognized as secondary effects of the reported in literature ability of these preparations to inhibit the photosynthetic activity of plants.

REFERENCES

Ashton F. M., Zweig G., Mason G. W., 1960, Weeds 8, 448 - 451.

Baranowski R., Bors J., Płoszyński M., Żurawski H., 1967, Pam. Puł. 28, 63 - 77.

Calvin M., Benson A. A., 1948, Science 107, 476 - 480.

Crafts A. S., 1961, The Chemistry and Mode of Action of Herbicides, pp. 269, Intersc. Publ. New York.

Couch R. W., Davis D. E., 1966, Weeds 14, 251 - 255.

Detroux L., 1957, Parastica 13, 144 - 154.

Exter B., 1961, Weed Res. 1, 233 - 244.

Funderburg H. H., Davis D. E., 1963, Weeds 11, 101 - 104.

Glabiszewski J., Płoszyński M., Szumilak G., Żurawski H., 1966, Pam. Puł. 21, 233 - 257.

Holstun T. J., 1963, J. Agric. Food Chem. 11, 442 - 443.

Kraśnikow W., Chikuślew W., Kukuruża 54, 51 - 55, Chem. Abstr. 1964, 2411 h.

Moreland D. E., Gentner W. A., Hilton J. L., Hill K. L., 1959, Plant Physiol. 34, 432 - 445.

Oorschot van P. J. L., Belksma M., 1961, Weed Res. 1, 245 - 257.

Opieńska-Blaut J., Waksmundzki A., Kański M., 1957, Chromatografia, pp. 987, PWN, Warszawa.

Overbeek van J., 1962, Weeds 10, 170 - 173.

Overbeek van J., 1964, The Physiology and Biochemistry of Herbicides, pp. 555, (L. J. Audus) Academic Press, London.

Płoszyński M., 1966, Doctor Dissertation, Technical University, Wrocław.

Roth W., 1958, Experientia 14, 137 - 138.

Stephenson M. L., Thiman K. V., Zamecnik F. C., 1956, Arch. Biochem. et Biophys. 65, 195 - 209.

Stepka W., Benson A. A., Calvin M., 1948, Science 108, 304 - 308.

Świętochowski E., Żurawski H., Piss J., 1962 a, Zesz. Nauk. WSR, Wrocław 46, 74 - 81.

Świętochowski B., Skowrońska M., Żurawski H., 1962 b, Zesz. Nauk. WSR Wrocław 46, 84 - 89.

Fitotoksyczne działanie herbicydów triazynowych na siewki lnu, buraków i gryki oraz związane z nim niektóre zmiany fizjologiczne

Streszczenie

Stwierdzono, że preparaty triazynowe wykazywały pogłębiający się w miarę czasu ich działania, hamujący wpływ na plony suchej i świeżej masy, transpirację i wzrost siewek lnu, buraków i gryki. Działaniu preparatów towarzyszyła również wzrastająca akumulacja wolnych aminokwasów oraz spadek zawartości cukrów prostych w częściach nadziemnych badanych roślin.

Uzyskane rezultaty interpretowano jako efekty wtórne hamującego działania triazyn na aktywność fotosyntetyczną roślin. Toksyczność badanych preparatów na len, buraki i grykę malała w następującej kolejności: atrazin, simazin, propazin, atraton, prometon i prometryn. Len był bardziej odporny na działanie triazyn niż buraki i gryka.