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Biochemical Changes in the Germinating Seeds of *Trigonella foenum graecum* L. in Relation to *s*-Triazine Herbicides

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Abstract: The *s*-triazine herbicides studied (atrazine and simazine) inhibited the growth of the seedlings of *Trigonella foenum graecum* L. (*Viciaceae*). These herbicides caused a decrease in the contents of total water soluble carbohydrates and reducing sugars. Pentose sugars increased in the cotyledons. The total protein and free amino acid contents were enhanced. The activity of peroxidase was inhibited in the cotyledons, but was enhanced in the seedling axes.

The *s*-triazine herbicides have been shown to inhibit the growth of all organs of intact plants (ASHTON *et al.* 1966). However, subtoxic concentrations of certain triazines have been found to stimulate growth and seed germination (LORENZANI 1962, JORDON and DAY 1968). The effect of triazines on chlorophyll content (FREEMAN *et al.* 1966); Hill reaction (MORELAND *et al.* 1958, 1959); respiration (FUNDERBURK and DAVIS 1963, FOY and PENNER 1965, PALMER and ALLEN 1962); contents of sugars and free amino acids (PLOZYŃSKI and ŻURAWSKI 1967, TIMOFEEVA 1967); and enzymes of carbohydrate and nitrogen metabolism have been studied (WU *et al.* 1971). Various workers have reported an increase in total nitrogen, protein and nitrate reductase activity by triazines in plants (DE VRIES 1963, TWEEDY and RIES 1967, RIES *et al.* 1967, SCHWEIZER and RIES 1969, ASLAM and HUFFAKER 1973).

The present communication deals with the changes in the contents of carbohydrates, protein, free amino acids and the activity of peroxidase (EC. 1.11.1.7) in germinating seeds of *Trigonella foenum graecum* L. treated with 2-chloro-4-(ethyl amino)-6-(isopropyl amino)-*s*-triazine (atrazine), and 2-chloro-4,6-bis(ethyl amino)-*s*-triazine (simazine).

Material and Methods

Surface sterilized seeds of *Trigonella* were soaked in aqueous suspensions of different concentrations (5, 50, 500 and 5000 mg l^{-1}) of herbicides for a period of 16 h at room temperature. The control seeds were soaked in glass

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distilled water for the same period of time. The imbibed seeds were placed on moist filter papers in sterilized Petri dishes and were allowed to germinate at 26 ± 2 °C and 60 per cent relative humidity in the dark. The zero time of germination period in all the experiment was 16 h after the initiation of seed imbibition. The growth of seedlings was measured after 5 days of germination.

Amino acids were separated on two-dimensional paper chromatography using Whatman No. 1 filter paper. The solvent system consisted of butanol: acetic acid : water (4 : 1 : 1) and phenol : water (3 : 1). The colour was developed by spraying ninhydrin reagent (0.25% in acetone) and drying at 80 °C for 15 min. The spots were identified, eluted in 5 ml 71% ethanol and read at 570 nm in a Beckman DU-2 spectrophotometer. The amount of amino acids was expressed in terms of α-alanine.

Total carbohydrates were estimated using anthrone reagent, pentose sugars by the orcinol method and reducing sugars by the method of Somogyi (Ashwell 1957). Peroxidase activity was estimated by Worthington procedures (1963) using *o*-dianisidine as hydrogen donor. Enzyme activity is expressed as change in absorbance $min^{-1}mg^{-1}$ protein. The protein content was estimated using phenol reagent (Lowry *et al.* 1951).

All the experiments were carried out in duplicate and repeated six times.

Results

Atrazine and simazine inhibited the growth of seedlings of *Trigonella* (Fig. 1). With increasing concentrations of atrazine the inhibition became pronounced. Simazine, however, stimulated hypocotyl elongation at a concentration of 500 and 5000 mg l^{-1}.

In the seedling axes both the herbicides decreased the contents of total carbohydrates and pentoses. In the cotyledon, however, both the chemicals

Table 1

Free amino acid composition as influenced by atrazine and simazine (5000 mg l^{-1}) after 5 days of germination

Amino acid	Cotyledon [mg g^{-1} fr. w.]			Seedling axes [mg g^{-1} fr. w.]		
	Control	Atrazine	Simazine	Control	Atrazine	Simazine
Alanine	1.64	1.79	0.57	0.15	0.16	0.07
Aspartic acid	0.91	0.77	0.71	0.18	0.08	0.01
Arginine	1.74	1.40	2.61	0.27	0.69	0.62
Aminobutyric acid	—	1.40	—	—	—	—
Glycine	1.17	—	—	1.27	0.03	0.16
Histidine	—	—	—	—	—	0.02
Hydroxyproline	2.42	1.96	0.14	—	—	0.07
Leucine/isoleucine	2.16	1.40	1.57	0.21	0.19	0.27
Methionine	—	0.77	—	—	—	—
Ornithine	—	0.84	0.71	—	—	—
Proline	—	—	—	—	0.02	—
Serine	0.78	0.89	0.76	0.24	0.02	0.01
Threonine	—	0.77	0.76	—	—	—
Tyrosine	—	0.77	—	0.27	0.27	0.08

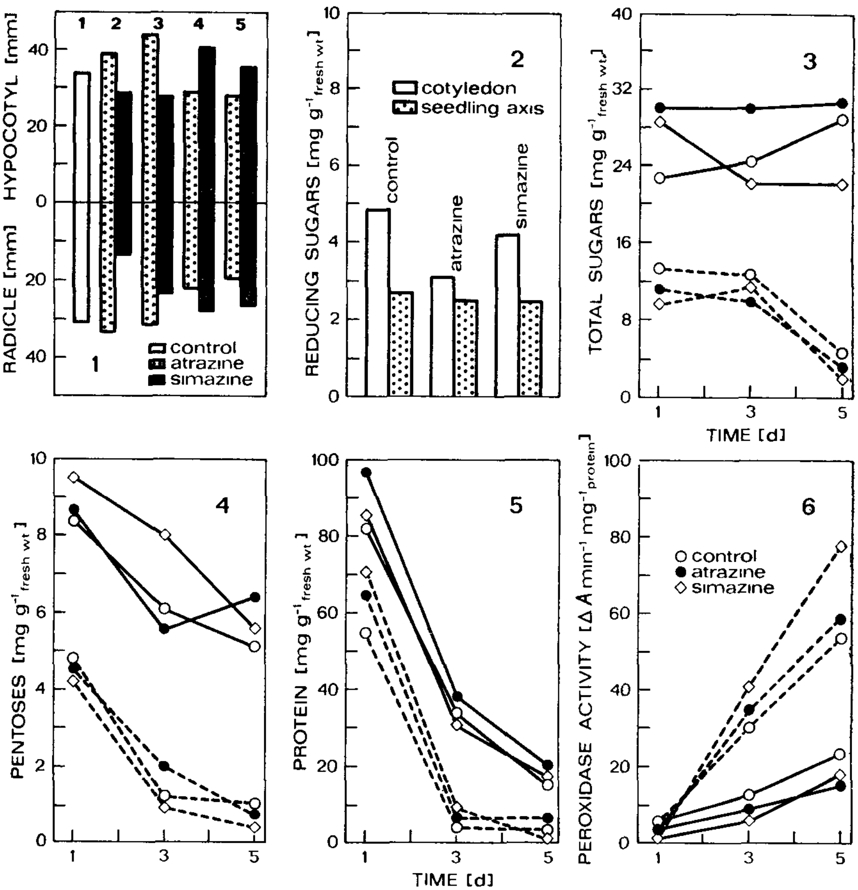

Fig. 1. Effect of different concentrations of atrazine and simazine on growth of *Trigonella* seedlings after 5 days of germination. (Nos. 1, 2, 3, 4 and 5 indicate 0, 5, 50, 500 and 5000 mg l^{-1} concentrations).
Fig. 2. Effect of atrazine and simazine (5000 mg l^{-1}) on reducing sugar content after 5 days of germination.
Fig. 3. Effect of atrazine and simazine at a concentration of 5000 mg l^{-1} on total sugars in cotyledon (full line) and seedling axis (dashed line) during germination.
Fig. 4. Effect of atrazine and simazine (5000 mg l^{-1}) on pentoses during germination (symbols as in Fig. 3).
Fig. 5. Changes in protein content in relation to atrazine and simazine (5000 mg l^{-1}) treatments (symbols as in Fig. 3).
Fig. 6. Changes in peroxidase activity in relation to atrazine and simazine (5000 mg l^{-1}) treatments (symbols as in Fig. 3).

increased the contents of pentoses. Atrazine increased the total water soluble carbohydrates in cotyledons, whereas simazine decreased the content. Both the chemicals decreased the levels of reducing sugars (Figs. 2, 3 and 4).

In the control seedlings alanine, arginine, aspartic acid, glycine, hydroxyproline, leucine/isoleucine and serine were detected. The changes brought about by atrazine and simazine on the 5th day of germination are presented in Table 1. Atrazine treated seedlings showed the presence of amino-butyric acid, methionine, ornithine, threonine, and tyrosine in the cotyledons and proline in the seedling axes while all these amino acids were found to be absent in water control. The amino acids ornithine, threonine appeared in the cotyledons and histidine, hydroxyproline were found in simazine treated seedlings.

Both chemicals increased the protein content in the cotyledons. Atrazine increased the content in the seedling axes also, whereas simazine caused a decrease (Fig. 5). The activity of peroxidase was inhibited by both the herbicides in the cotyledons but an enhanced activity in the seedling axes was observed in treated seedlings (Fig. 6).

Discussion

The growth inhibition by triazine herbicides is generally attributed to a deficiency of photosynthesis. At low concentrations the herbicides showed little or no effect. Hence, suspensions of higher concentrations of the herbicides were used since they have low solubilities in water (Martin 1972). With increasing concentrations of these herbicides the inhibition of hypocotyl and radicle became more pronounced, but simazine at 500 and 5000 mg l^{-1} stimulated hypocotyl elongation. This is contrary to the earlier reports where sublethal concentrations are known to stimulate growth (Lorenzani 1962, Jordon and Day 1968). A decrease in the sugar content has been attributed to photosynthesis inhibition (Ploszyński and Żurawski 1967, Timofeeva 1967). But the present investigations were conducted in the dark, and the inhibition of carbohydrate metabolism and peroxidase activity seem to show that these chemicals invariably interfere also with other metabolic processes. Subtoxic levels of triazines increased growth and the amount of nitrogen and/or protein in the plants (De Vries 1963, Tweedy and Ries 1967, Schweizer and Ries 1969). In the present study, however, it was found that even at higher concentrations where growth was inhibited, protein content and the number of free amino acids increased as a result of treatment with atrazine and simazine in the seedlings of *Trigonella*. The appearance of amino butyric acid, methionine, ornithine, threonine, tyrosine, histidine and hydroxyproline in the free amino acid pool of treated seedlings might be due to their non-utilization for protein biosynthesis.

The activities of various enzymes have been reported to be stimulated by triazines. The activities of phosphorylase, pyruvate kinase, cytochrome oxidase, glutamate dehydrogenase (Wu *et al.* 1971) and nitrate reductase (Aslam and Huffaker 1973) were enhanced by triazines in pea and corn. The increase in nitrate reductase activity might be responsible for the increase in nitrogen and protein content. The peroxidase activity was inhibited in the cotyledons, but it was stimulated in seedling axes by these chemicals. Thus there was an inverse correlation between growth and peroxidase activity in the seedling axes. This might possibly be explained in the light of correlation between peroxidase and IAA oxidase activities. The enhanced peroxidase activity apparently results in IAA destruction which is essential in growth and elongation of the growing region of the seedling. Most peroxidase isozymes of Japanese morning glory, *Pharbitis nil* has been shown to possess IAA oxidase activity (Yoneda and Endo 1970).

Recently Gove and Hoyle (1974) have shown a similar correlation between peroxidase isozymes and IAA oxidase activity in horseradish and yellow birch.

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