Effects of Foliar Application of *s*-Triazines on Protein, Amino Acids, Carbohydrates, and Mineral Composition of Pea and Sweet Corn Seeds, Bush Bean Pods, and Spinach Leaves

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Foliar applications of 2 or 5 mg/l. of simazine, prometone, and terbutryn increased the protein content of seeds of peas and sweet corn growing under field conditions. A similar application of 0.5 or 1.0 mg/l. of simazine, atrazine, and terbutryn increased the protein content in pods of bush beans and leaves of spinach under field and/or growth room conditions. None of the compounds had any significant effect on dry weight of the plant parts. Analyses of amino acid pattern indicated quantitative changes in the treated sweet corn and pea seeds. Soluble amino acids were higher in the treated bush bean seeds and spinach leaves. Fe content in bush bean seeds and Fe, Mg, P, and K contents in spinach leaves were higher in the treated plants than in the controls.

Recent studies have shown that protein in various crops is increased considerably when sublethal concentrations of *s*-triazine compounds are applied. For example, simazine increased the protein content in corn (Ries and Gast, 1965), rye (Ries *et al.*, 1967), peas, beans, rice, alfalfa, oats (Ries *et al.*, 1968), and a number of forage crops (Allinson and Peters, 1970). Vergara *et al.* (1970) found that the application of simazine would increase the protein content of rice, but at the cost of a reduction in total grain and protein yields. Fink and Fletchall (1967) reported significant increases in nitrate and total nitrogen, but a decrease in dry matter after simazine was applied to corn.

Earlier, we have reported that foliar application of sublethal concentrations of several *s*-triazines caused significant increases of protein and amino acids in leaves of beans, peas, and corn under growth room and greenhouse conditions (Singh and Salunkhe, 1970b; Wu, 1971). Soil application of several *s*-triazines also caused significant increases of total nitrogen, soluble protein, and total amino acids in sweet corn and pea seeds (Salunkhe *et al.*, 1971). In the present paper, the effects of foliar application of sublethal concentrations of *s*-triazine compounds on protein, amino acids, carbohydrates, and mineral composition of pea and sweet corn seeds, bean pods, and spinach leaves are described.

MATERIAL AND METHODS

Bush beans (*Phaseolus vulgaris* L. cv. Tendergreen), peas (*Pisum sativum* L. cv. Perfected Freezer), and sweet corn (*Zea mays* L. cv. Iochief) were grown at the Utah State University farm, North Logan, on a Millville silt loam soil. Each treatment was replicated three or six times in a randomized block design. Each plot consisted of two rows 30 in. apart for beans and peas and 36 in. apart for corn. The length of each row was 43 ft. Plots were separated by a border row and were maintained weed-free without use of chemicals. The crops were irrigated as needed. In the case of bush beans, an aqueous solution of 0.5 and 1.0 mg/l. of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine), terbutryn (2-methylmercapto-4-ethylamino-6-isobutylamino-*s*-triazine), or GS-14254 (2-methoxy-4-isopropylamino-6-butylamino-*s*-triazine), each containing 0.2% triton B-1956, was sprayed until run off on leaves of 42-day-old plants. An aqueous solution containing 2 and 5 mg/l. of atrazine, simazine, terbutryn, GS-14254, propazine (2-chloro-4,6-bis(isopropylamino)-*s*-triazine), prometryne (2-methylmercapto-4,6-bis(isopropylamino-*s*-triazine), prometone (2-methoxy-4,6-bis(isopropylamino)-*s*-triazine), or ametryne (2-methylmercapto-4-ethylamino-6-isopropylamino-*s*-triazine), each with 0.2% triton B-1956, was applied to the leaves of pea plants 45 days after planting and to sweet corn 60 days after planting. In every case, control plants were sprayed with a water solution of the surfactant only. The bean pods were harvested after 28 days and peas and sweet corn seeds were harvested 30 days after application.

Experiments were also conducted with bush beans and spinach (*Spinacia oleraceae* cv. Bloomsdale) growing in a growth room on vermiculite in 1.5-l. plastic pots. Plants were watered daily with a modified Hoagland's solution. The newly emerged bean seedlings were thinned for uniformity to three plants per pot. The spinach plants were thinned to two plants per pot. The pots were arranged in a randomized complete block design with six pots per treatment under 16-hr photoperiod at 2000 ft-candles. Day and night temperatures were 28° and 21° and the relative humidity was maintained at 70–75%. The solutions containing 0.5 or 1.0 mg/l. of simazine, atrazine, terbutryn, or GS-14254 with 0.2% triton B-1956 were sprayed on foliage of 35-day-old plants. The bean pods or spinach leaves were harvested 18 days after application.

Each of the three or six replications were sampled and analyzed separately. Other than amino acid analyses, a minimum of three determinations were made on each replicate. For amino acids only one sample from each replicate was analyzed. An analysis of variance was performed on the data and the means were compared according to Tukey's *w* procedure (Steel and Torrie, 1960).

For dry-weight determinations, the freshly harvested samples were dried in an oven at 85° to a constant weight. For chemical analyses, samples were cooled immediately to 0° and then freeze dried and ground to pass a 40-mesh sieve, bottled, and dried again before analyses.

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Table I. Effects of *s*-Triazines on Dry Weight, Protein, Total N, Starch and Sugars in the Seeds of Peas and Sweet Corn 30 Days after Application under Field Conditions[a]

Crop	Treatment	Concn, mg/l.	Dry wt, %	Total N, %	Soluble protein, %	Starch, %	Total sugars, %	Crude fat, %
Peas	Control	0	23.3	4.4	12.9	48.4	6.6	2.7
	Simazine	5	22.9	5.2*	15.6*	44.2*	5.7*	2.9
	Terbutryn	5	22.4	5.1*	14.9*	45.2*	5.7*	. . .
	Prometone	2	22.7	5.1*	14.6*	45.0*	5.5*	2.9
Sweet corn	Control	0	28.3	1.2	4.6	59.9	14.3	5.9
	Simazine	5	28.5	1.4*	5.3*	49.0*	12.4*	5.9
	Terbutryn	5	27.9	1.3*	5.2*	49.3*	12.6*	. . .
	Prometone	5	28.4	1.4*	5.3*	49.5*	12.2*	5.9

[a] All data expressed on dry weight basis. * = significantly different at 0.05 level.

Table II. Effects of *s*-Triazines on Total N, Soluble Amino Acids, Starch, and Sugars in the Pods of Bush Beans under Field and Growth Room Conditions[a]

Treatment	Concn, mg/l.	Dry wt, %	Total N, %	Nitrate, μg/g	Soluble amino acids, mg/100 g	Starch, %	Total sugars, %
			Field experiments[b]				
Control	0	8.8	1.8	38	33.3	69.3	5.9
Atrazine	0.5	9.5	2.4*	40	38.7*	68.5	5.9
Simazine	0.5	9.0	2.0*	36	39.0*	68.9	5.2
Terbutryn	0.5	8.5	2.2*	37	39.2*	69.1	5.9
			Growth room experiments[c]				
Control	0	9.1	2.8	32	25.0	63.3	4.5
Atrazine	0.5	8.8	3.2*	29	39.1*	62.8	4.6
Simazine	0.5	9.5	3.5*	30	36.2*	67.6	4.7
Terbutyrn	0.5	9.0	3.7*	32	31.2*	64.2	4.3

[a] All data expressed on dry-weight basis. [b] Bean pods were harvested 28 days after application. [c] Bean pods were harvested 18 days after application. * = significantly different at 0.05 level.

Table III. Effects of *s*-Triazines on Total N, Soluble Amino Acids, and Sugars in the Leaves of Spinach 18 Days after Application under Growth Room Conditions[a]

Treatment	Concn, mg/l.	Dry wt, %	Total N, %	Soluble protein,[b] mg/g	Starch, %	Nitrate, μg/g	Soluble amino acids, mg/g	Total sugars, %
Control	0	9.4	4.1	12.9	28.1	31	4.3	7.6
Atrazine	0.5	9.7	5.0*	14.1*	27.9	29	5.1*	6.8*
Simazine	0.5	9.2	5.2*	13.7*	27.5	28	5.6*	6.3*
Terbutyrn	0.5	9.2	5.5*	13.9*	28.5	32	4.9*	6.7*

[a] All data other than soluble protein are expressed on dry weight basis. Expressed on fresh weight basis. * = significantly different at 0.05 level.

Sugars and starch were extracted according to the procedure of McCready *et al.* (1950). The ethanolic extract was cleared and deleaded (Loomis and Shull, 1937). The total sugars were determined by using the method of McCready *et al.* (1950). The sugar-free residue was solubilized by perchloric acid for starch determination (McCready *et al.*, 1950). Crude fats were extracted and determined by AOAC methods (1960).

Total nitrogen was determined by the standard Kjeldahl method. Soluble protein was extracted with water and precipitated with trichloroacetic acid. The protein content was determined by the method of Lowry *et al.* (1951). The nitrate content was determined by the method of Wooley *et al.* (1960). The soluble amino acids were determined colorimetrically (Rosen, 1957). The amino acid analyses were done at the Analytical Biochemistry Laboratories, Inc., Columbia, Mo. The samples were hydrolyzed in 6 *N* HCl, at 145° for 4 hr in an inert atmosphere. After hydrolysis, the amino acids were measured by the gas–liquid chromatographic method as described by Roach and Gehrke (1969). The determination of tryptophan was made in the same manner as that of the other amino acids, but the samples were hydrolyzed by pronase (Spies, 1968).

For determination of Ca, K, S, Fe, P, and Mg, 1 g of dried samples was digested with 20 ml of concentrated nitric acid and 4 ml of 72% perchloric acid and then diluted to a known volume with distilled and deionized water. Phosphorus was determined cholorimetrically using ammonium–metavanadate–molybdate reagent (Quinlan and DeSesa, 1955). Sulfur content was estimated by the turbidimetric method described by Chesnin and Yiens (1950). Determinations of Ca, K, Fe, and Mg were made with a Perkin-Elmer model 303 Atomic Absorption Spectrophotometer.

RESULTS

The effects of different *s*-triazines on the seeds of peas, corn, and beans and the leaves of spinach depended on the compound and its concentrations under field and growth room conditions. Only the effects of those compounds and the levels of application that significantly increased total N are presented in the tables. None of the compounds produced any significant effect on the dry weight of the seed of peas and sweet corn, pods of beans, or the leaves of spinach (Tables I–III).

Simazine or terbutryn at the rates of 5 mg/l. and prometone at 2 mg/l. caused significant increases in the amounts of total

Table IV. Effects of Foliar Applications of Simazine and Prometone on Amino Acids in the Seeds of Peas and Sweet Corn[a]

Amino acid	Peas[b] Control	Peas[b] Simazine, 5 mg/l.	Peas[b] Prometone, 2 mg/l.	Sweet corn[c] Control	Sweet corn[c] Simazine, 5 mg/l.	Sweet corn[c] Prometone, 5 mg/l.
Alanine	1.10	1.48*	1.45*	0.55	0.65*	0.65*
Valine	1.09	1.18*	1.15*	0.36	0.41*	0.36
Glycine	0.99	1.21*	1.22*	0.28	0.36*	0.31*
Isoleucine	0.96	1.00	0.84*	0.22	0.25*	0.24*
Leucine	1.78	2.10*	2.01*	0.78	0.93*	0.82*
Proline	0.84	1.14*	1.06*	0.64	0.73*	0.70*
Threonine	0.86	1.03*	1.00*	0.26	0.31*	0.33*
Serine	1.13	1.42*	1.38*	0.34	0.40*	0.38*
Methionine	0.20	0.23*	0.19*	0.17	0.23*	0.17
Hydroxyproline	0.01	0.03*	0.04*	0.02	0.02	0.02
Phenylalanine	1.08	1.38*	1.25*	0.39	0.43*	0.46*
Aspartic acid	2.44	2.90*	2.87*	0.56	0.62*	0.62*
Glutamic acid	3.69	4.34*	4.20*	1.43	1.43	1.48*
Tyrosine	0.54	0.89*	0.81*	0.22	0.27*	0.28*
Lysine	1.66	2.05*	2.03*	0.24	0.34*	0.32*
Histidine	0.83	0.60*	0.68*	0.26	0.33*	0.32*
Arginine	1.47	1.68*	1.77*	0.23	0.26*	0.25*
Cystine/2	0.13	0.11*	0.10*	0.03	0.04*	0.04*
Tryptophan	0.11	0.17*	0.18*	0.03	0.05*	0.04*
Total	20.91	24.94*	24.23*	7.06	8.06*	7.78*

[a] Data are expressed as percent of dry weight. [b] Treatments at 45 days after planting. [c] Treatments at 60 days after planting. * = significantly different at 0.05 level.

nitrogen and soluble protein in pea seeds when applied to the leaves of 45-day-old plants (Table I). The starch and total sugar contents were decreased.

Foliar applications of 5 mg/l. of simazine, terbutryn, and prometone to 60-day-old sweet corn plants significantly increased total N and soluble protein but decreased starch and total sugars (Table I).

The total N and soluble amino acids were significantly increased by the application of 0.5 mg/l. of atrazine, simazine, and terbutryn to 35–42-day-old bean plants under both field and growth room conditions. These same treatments did not have any significant effect on nitrate-N total sugars or starch (Table II).

The effects of 1.0 ppm of simazine, atrazine, and terbutryn on spinach were determined only under growth room conditions. As evident from Tab'e III, both total N and amino acids were increased in the leaves. Treatments did not have significant effects on total starch, but total soluble sugars were lower than the control plants. The nitrate content of spinach leaves was not significantly affected by any of the three treatments.

The effects of simazine and prometone on amino acid patterns of peas and sweet corn seeds are presented in Table IV. In every case, total amino acids content was higher in the simazine- or prometone-treated plants than the untreated controls. Compared to the controls, simazine and prometone increased the concentrations of most of the amino acids in pea seeds, except that histidine and cystine in simazine-treated plants and isoleucine, methionine, and histidine in prometone-treated plants were lower. In sweet corn, the amounts of each individual amino acid were higher, except for hydroxyproline and glutamic acid in simazine-treated plants and valine, methionine, and hydroxyproline in prometone-treated plants.

Atrazine, simazine, or terbutryn did not have any significant effect on the Ca, K, S, P, or Mg of bean pods growing under field or growth room conditions. However, the Fe content was significantly higher than controls in every case (Table V). In the case of spinach, atrazine, simazine, and terbutryn increased the amounts of K, Fe, P, and Mg (Table V). None of the compounds significantly influenced the Ca or S contents of spinach leaves.

The total fat content in the seeds of peas and sweet corn was not significantly affected by *s*-triazine compounds (Table I).

DISCUSSION

The data indicated that the most effective chemicals to cause significant increases in protein content of corn and peas were simazine, terbutryn, and prometone; and for beans and spinach, simazine, terbutryn, and atrazine were the most effective. It was also evident that for peas and corn the concentrations needed to produce a significant effect were much higher than for beans and spinach. The differences in response to these compounds by different species may be due to the ability of the plants to metabolize *s*-triazines. Shimabukuro (1967) demonstrated that all species studied were able to metabolize atrazine to a nonphytotoxic compound; however, the most susceptible species, soybeans and wheat, were much slower in metabolizing atrazine than the intermediately susceptible peas or the resistant corn. Apparently, beans and spinach would fall under the category of most susceptible species, because a concentration higher than 1 mg/l. caused injury to the treated plants. Corn and peas, however, did not show any injury symptoms, even at a level of 5 mg/l. of the application.

Table V. Effects of *s*-Triazines on Mineral Composition of Bean Pods and Spinach Leaves[a]

Crop	Treatment	Concn, mg/l.	Ca, %	K, %	S, %	Fe, mg/100 g	P, %	Mg, %
Beans under field conditions	Control	0	0.61	2.33	0.11	8.2	0.36	0.24
	Atrazine	0.5	0.58	2.45	0.12	9.8*	0.35	0.26
	Simazine	0.5	0.66	2.46	0.12	9.2*	0.39	0.26
	Terbutryn	0.5	0.68	2.37	0.13	9.2*	0.40	0.26
Beans under growth room conditions	Control	0	0.65	2.91	0.12	7.8	0.36	0.17
	Atrazine	0.5	0.67	2.87	0.13	9.2*	0.38	0.20
	Simazine	0.5	0.68	2.83	2.12	9.5*	0.35	0.18
	Terbutryn	0.5	0.67	2.79	0.12	9.8*	0.36	0.17
Spinach under growth room conditions	Control	0	1.94	0.49	0.39	15.2	0.37	0.80
	Atrazine	1.0	1.83	0.61*	0.37	23.6*	0.41*	0.84*
	Simazine	1.0	1.77	0.60*	0.38	19.8*	0.46*	0.85*
	Terbutryn	1.0	1.69	0.55*	0.42	24.1*	0.44*	0.97*

[a] Data expressed on dry weight basis. * = significantly different at 0.05 level.

The data on dry weight, protein, minerals, and general appearance of the seeds and pods, together with the visual appearance of the plants growing under field and growth room conditions, indicate that sublethal concentrations of *s*-triazine compounds act as a growth hormone rather than an herbicide on peas, sweet corn, beans, and spinach. Further support is given to this contention by our earlier work on protein-stimulative properties of these compounds in the leaves of peas, sweet corn, and beans (Singh and Salunkhe, 1970b; Wu, 1971; Wu *et al.*, 1971a,b).

That these compounds stimulate protein production and cause quantitative differences in amino acid patterns in seeds of peas and sweet corn when applied through the soil has been demonstrated earlier from this laboratory (Salunkhe *et al.*, 1971). The foliar application data indicate that *s*-triazine induces similar responses in corn and peas and also in spinach and beans. This could have certain advantages of rapid and uniform response. It has been pointed out by Kozlowski and Kuntz (1963) that the slow response to the soil-applied atrazine probably reflects a slow movement of the herbicide to the zone of absorbing roots and hence limited uptake.

The increased amount of soluble amino acids may be the results of an increased rate of synthesis of amino acids or of an increased rate of degradation of proteins or both. The level of free amino acids and the capacity of the leaves of treated *s*-triazines to reduce nitrate (through nitrate reductase) and synthesize amino acids (through glutamic acid dehydrogenase, transaminase, respiratory enzymes, pyruvate kinase, and cytochrome oxidase) (Singh and Salunkhe, 1970a; Wu *et al.*, 1971) apparently led us to believe that *s*-triazines induce efficient synthesis of amino acids. Amino acids are the precursors of storage proteins, hence a higher level of these amino acids will contribute to a faster and greater accumulation of protein in the so-called protein bodies (Singh *et al.*, 1972; Wu, 1971). The greater capacity for amino acid incorporation in the leaf disks of peas treated with *s*-triazine (Wu, 1971), together with an increased number of endoplasmic reticulum in bean cotyledons (Singh *et al.*, 1972), provide interesting biochemical and ultrastructural explanations for increased protein contents in plants.

The data on the mineral composition of the treated bean pods and spinach leaves indicate that these compounds do not have any deleterious effects on the nonprotein nutrients of the plants. In bean pods, *s*-triazines did not have significant effect on the concentrations of Ca, K, S, P, or Mg; however, Fe content was significantly higher than the untreated controls. In spinach leaves, we noted increases in K, P, Fe, and Mg. This difference in response may be due to more absorption of these nutrients by spinach. It is also possible that K, P, and Mg did not translocate from leaves to the pods in the case of beans. It has been demonstrated by Ries *et al.* (1967) that simazine affects nitrate absorption in plants. In the analyses of bean pods and spinach leaves, however, we did not notice significant differences in the concentrations of nitrate in treated and untreated plants. The total fat content in the seeds of peas and sweet corn also was not affected by *s*-triazine compounds.

The percent dry weight of the seeds or plant parts was not affected by any of the treatments. Apparently, this indicates that the protein content of peas and sweet corn seeds, bean pods, and spinach leaves was increased without any loss in total dry matter. Ries *et al.* (1968) also confirmed that simazine increases protein in several crops without a concomitant loss in total yield.

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