IV- Results And Discussions

Photoplate I showing

A. Effect of glyphosate on growth of monocotyledonous plants on the II day after treatment in

a) <u>Cyperus rotundus</u> - Control

b) <u>Cyperus rotundus</u> - Sprayed

c) <u>Triticum aestivum</u> - Control

d) <u>Triticum aestivum</u> - Sprayed

B. Effect of glyphosate on growth of monocotyledonous Plants on the X day after treatment in

a) <u>Cyperus rotundus</u> - Control

b) <u>Cuperus rotundus</u> - Sprayed

c) <u>Triticum aestivum</u> - Control

d) <u>Triticum aestivum</u> - Sprayed

Photoplate II showing

A. Effect of glyphosate on growth of dicolyledonous plants on the II day after treatment in

a) <u>Celosia argentea</u> - Control

b) <u>Celosia argentea</u> - Sprayed

c) <u>Glycine max</u> - Control

d) <u>Glycine max</u> - Sprayed

B. Effect of glyphosate on growth of dicotyledonous plants on the X day after treatment in

a) <u>Celosia argentea</u> - Control

b) <u>Celosia argentea</u> - Sprayed

c) <u>Glycine max</u> - Control

d) <u>Glycine max</u> - Sprayed

PLATE - II

A

B

PLATE - I

A

B

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The crops wheat and soybean and weeds Cyperus and Celosia were treated with 300 ppm foliar sprays of glyphosate and the leaf material was harvested on the II and X day after treatment for analysis. The growth and appearance of these plants after the treatment of glyphosate has been well demonstrated in plates I and II. Glyphasate affected the growth of both the crops and weeds from the initial phase of treatment and the severity increased with the time. Both the crops and weeds exhibited a conspicuous reduction in the biomass production as compared to the control plants (b and d from plates I and II). A reduction in the height and yellowing of the leaves were specific features of the glyphosate treated plants which were clearly evident on the X day after treatment. The results obtained with respect to various physiological and biochemical analyses of the control and glyphasate treated plants have been discussed in the following pages.

A. WATER RELATIONS

1. STOMATAL BEHAVIOUR

Effect of foliar spray of glyphosate on stomatal behaviour in the crops wheat and soybean and weed Celosia argentea has been depicted in Table 2. In soybean and wheat diffusive resistance for H_2O increased on the II day after treatment with a decrease in the transpiration rate

Table 2. Stomatal response to glyphosate treatment in *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species			Diffusive resistance for H_2O ($Sec\ cm^{-1}$)		Diffusive conductance for H_2O ($cm\ Sec^{-1}$)		Transpiration rate ($\mu g\ cm^{-2}\ Sec^{-1}$)		Diffusive conductance for CO_2 ($cm\ Sec^{-1}$)		Diffusive resistance for CO_2 ($Sec\ cm^{-1}$)	
			II day	X day	II day	X day	II day	X day	II day	X day	II day	X day
Triticum aestivum	Control	L	5.59	6.78	0.178	0.147	0.519	0.521	1.362	0.940	0.734	1.063
		U	1.49	2.30	0.671	0.439	1.479	1.823				
	Sprayed	L	12.30	13.73	0.081	0.072	0.214	1.760	0.897	1.144	1.114	0.874
		U	2.09	1.56	0.478	0.641	1.165	1.916				
Celosia argentea	Control	L	5.06	7.37	0.197	0.135	4.370	0.779	0.816	0.725	1.225	1.379
		U	3.20	3.15	0.312	0.317	7.720	1.156				
	Sprayed	L	0.95	7.46	0.202	0.134	3.920	1.294	0.685	0.627	1.451	1.609
		U	4.44	3.89	0.225	0.257	5.000	2.443				
Glycine max	Control	L	2.06	4.17	0.485	0.239	10.210	11.890	0.974	0.497	1.026	2.012
		U	8.19	14.00	0.122	0.071	2.500	4.090				
	Sprayed	L	2.61	2.81	0.383	0.355	7.020	9.440	0.730	0.666	1.369	1.501
		U	13.70	16.55	0.072	0.060	1.300	1.230				

Measured at humidity - II day 33.40%
X day 40.80%

Values are mean of three determinations

as compared to the untreated plants. A further increase in the diffusive resistance was observed in both the plants on the X day of analysis. The rate of transpiration was higher as compared to the control plants. In both these crops, glyphosate induced an increase in diffusive resistance for CO_2 initially, which was minimised after the X day of treatment.

In the weed C. argentea a slight rise in the diffusive resistance for H_2O was observed on both the days of investigation. Transpiration rate decreased a little on the II day but increased on the X day after treatment. Diefusion resistance for CO_2 remained more or less the same on II and X day after treatment in the sprayed plants.

Stomata regulate the fluxes of CO_2 and water vapours, entering and leaving leaf surfaces. The supression of stomatal opening reduces the water needs of the plant, while enhanced stomatal opening increases transpiration, leading to the dessication of the plant. Herbicides such as paraquat and 2, 4, 5-T are known to kill the plants by enhancing transpirational water loss (Rao et. al. 1977).

In the present study the herbicidal treatment decreased transpiration rate in soybean but it was enhanced in the C_3 weed C. argentea. Das and Santakumari (1975) have reported that thiocarbamates inhibit transpiration in

C_4 crops and enhance in C_3 weeds. They have also reported that several herbicides such as alachlor and butachlor reduce transpiration by restricted stomatal openings. Decreased rate of transpiration helps in better management of water in plant whereas higher transpiration leads to the dessication and death of the plant.

Glyphosate treatment enhanced transpiration rate in the dicot weed (C. argentea) and inhibited it in dicot crop soybean. In the monocot crop i.e. Wheat, also an increase in transpiration rate was observed. Information regarding the stomatal behaviour of the monocot weed (C. rotundus) taken for investigation was not available due to certain difficulties in recording the data.

STOMATAL INDEX

The values of stomatal indices as observed on the II and X day of glyphosate spray, have been presented in Table. 3. Stomatal index decreased in the glyphosate treated plants and the decrease was more prominent on the X day after treatment. Further the values were lower in the upper epidermis of the treated plants as compared to the lower epidermis. In both the weeds the values of stomatal indices decreased by 11 and 19 for lower and upper epidermis respectively. In case of the crops the reduction was in the range of 5 to 7 and 15 to 17 for the lower and upper epidermis respectively. These results

Table 3. Effect of glyphosate on Stomatal Index in the leaves of *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species			Stomatal Index (no. of stomata mm^{-2})	
			II day	X day
	Control	L	39.88	42.00
Cyperus rotundus		U	38.35	48.19
	Sprayed	L	34.88	31.00
		U	31.57	29.90
	Control	L	24.80	32.00
Tritium aestivum		U	24.68	37.50
	Sprayed	L	21.12	27.30
		U	17.68	22.60
	Control	L	30.12	31.42
Celosia argentea		U	29.70	38.88
	Sprayed	L	25.00	22.40
		U	23.85	19.00
	Control	L	24.66	29.08
Glycine max		U	27.37	37.50
	Sprayed	L	24.00	22.90
		U	20.30	20.00

Values are mean of three determinations

suggested that the stomatal index is affected more in the weeds than in the crops by the glyphosate treatment. Umamahesh *et. al.* (1984) have recorded a decrease in stomatal index in *C. argentea* subjected to foliar sprays of DNP and SMA.

2. RELATIVE WATER CONTENT

Table 4 describes the effect of glyphosate on relative water content in the plants under investigation. RWC decreased more in the sprayed plants as compared to the untreated plants on the X day after treatment. In *C. rotundus* the values of RWC decreased more than in the remaining plants. The decrease was 14% in *Cyperus* and 6 to 8% in the remaining plants on II day after treatment. On the X day, RWC decreased by 20% in *Cyperus* whereas in others the range of decrease was 10-16%. Here again the weeds seem to be more affected by the glyphosate treatment. Increased transpiration rate in *Celosia* might have resulted in more loss of water on the X day after treatment. In wheat also the increased transpiration rate correlates with the decreased value of R W C

3. OSMOTIC POTENTIAL

In general the values of osmotic potential (Table 4) increased in both the control as well as treated plants on the X day after treatment. Decrease in RWC and increased rate of metabolism during the development of

Table 4. Effect of glyphosate on relative water content and osomotic potential in the leaves of *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species		Relative water content (%)		Osmotic potential (-bars)	
		II day	X day	II day	X day
Cyperus rotundus	Control	80.50	56.70	6.90	11.50
	Sprayed	66.77	36.40	7.02	12.03
Triticum aestivum	Control	81.30	78.63	5.96	9.48
	Sprayed	72.72	66.66	6.14	9.72
Celosia argentia	Control	80.15	75.20	3.65	7.25
	Sprayed	73.70	59.67	3.93	7.30
Glycine max	Control	81.40	64.60	5.02	7.00
	Sprayed	73.00	58.62	5.46	7.76

Values are mean of three determinations

leaves, from II and X day might have increased the cellular content resulting in an increase in the electrical conductivity of the cell sap. However osmotic potential in the leaves of control and treated plants did not change much due to glyphosate. Only a marginal increase in the osmotic potential was observed in all the plants studied on II as well as X day after treatment.

Maintenance of turgor depends upon the water status of leaf tissue and a change in water status brings about a change in the osmotic potential. Increased concentration of certain solutes, particularly sugars, free amino acids helps to decrease the osmotic potential and to maintain water balance in the plants growing under water deficits. (Barnett and Naylor, 1966, Singh et. al. 1973, Munns et. al. 1979). In the present study a failure in the osmotic adjustment has been observed eventhough the water contents decrease by the herbicidal treatment. This might be due to a negative influence of the herbicide on the plant metabolism, decreasing there the content of solutes in the tissues.

B. ORGANIC CONSTITUENTS

Influenced of glyphosate on various organic constituents in the plants selected for study was analysed on the II and the X day after treatment. Results are discussed in the following pages.

1. PHOTOSYNTHETIC PIGMENTS

Total chlorophylls and carotenoids

Effect of glyphosate on photosynthetic pigments in the weeds and crops under study has been presented in Table 5 and 6 and figs. 1 and 2. In C. rotundus and T. aestivum total chlorophyll content decreased by about 8% on the II day after treatment. The loss in chlorophylls was doubled (16%) on the X day in Cyperus while in wheat there was about 44% decrease in the total chlorophylls on X day after treatment. In the dicotyledonous species taken for investigations the decline in the total chlorophylls was about 19% in both the weed and the crop on the II day after treatment (Table 6). In the weed C. argentea there was no further decrease in total chlorophyll content on X day after treatment. However in G. max the loss in chlorophyll was doubled (39%).

Carotenoid content in Cyperus decreased by 7 and 14% respectively on the II and X day after treatment. In case of Celosia the % decrease was somewhat more (9 and 28%) than in Cyperus on both the days of anlysis (Tables 5 and 6 and fig 1 and 2). In both the crops the loss in carotenoid content was much higher (more than 50%) as compared to the weeds.

A reduction in photosynthetic pigments has been reported by Munoz-Rueda and Gonzalez-Diaz. (1986) in the

Table 5. Effect of glyphosate on chlorophyll and carotenoid content in the leaves of *Cyperus rotundus* and *Triticum aestivum*

Parameters	Day after treatment	*Cyperus rotundus*		*Triticum aestivum*	
		Control	Sprayed	Control	Sprayed
Chlorophyll a	II	059.00	053.45	083.15	071.95
(mg 100^{-1} g fr.wt.)	X	068.15	054.84	068.25	034.10
Chlorophyll b	II	067.00	062.45	067.95	066.75
(mg 100_g^{-1} fr.wt.)	X	058.00	050.65	052.75	033.85
Total Chlorophylls	II	126.00	115.85 (8.05)	151.10	138.70 (7.94)
(mg 100_g^{-1} fr.wt.)	X	126.15	105.75 (16.19)	121.00	067.90 (43.80)
Chlorophyll a/b	II	00.88	00.85	1.22	1.07
	X	1.17	1.08	1.24	1.01
Carotenoids	II	1.70	1.58 (7.05)	1.50	1.00 (33.33)
(g 100_g^{-1} fr.wt.)	X	1.72	1.50 (14.66)	1.40	0.70 (50.00)

Values in parantheses indicate percent decrease

Values are mean of three determinations

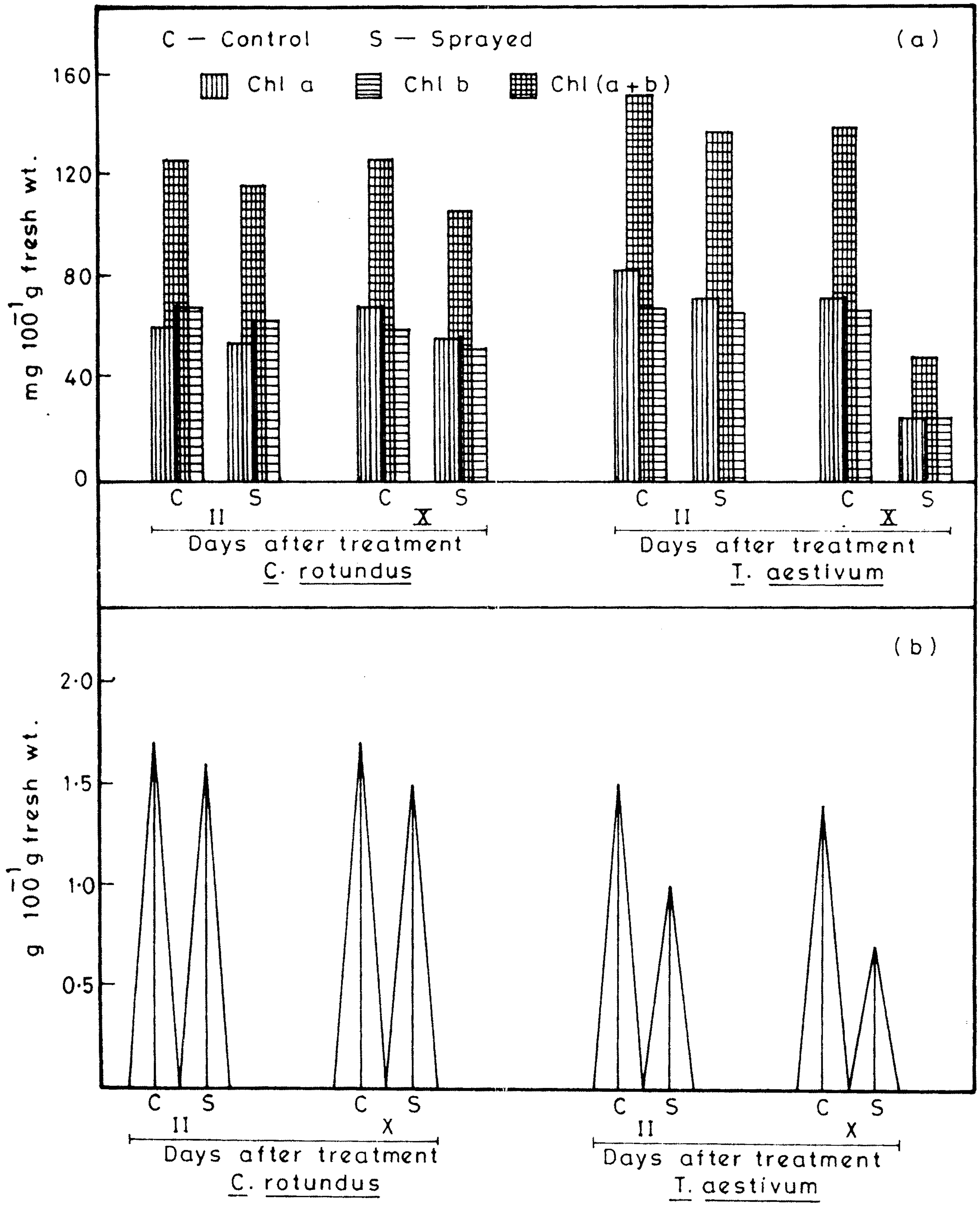

FIG. 1 (a&b) — EFFECT OF GLYPHOSATE ON CHLOROPHYLL AND CAROTENOID CONTENT IN *C. rotundus* and *T. aestivum*.

Table 6. Effect of glyphosate on chlorophyll and carotenoid content in the leaves of Celosia argentea and Glycine max

Parameters	Day after treatment	Celosia argentea		Glycine max	
		Control	Sprayed	Control	Sprayed
Chlorophyll a ($mg\ 100_g^{-1}$ fr.wt.)	II	36.55	33.40	54.90	46.70
	X	41.00	34.09	35.10	21.25
Chlorophyll b ($mg\ 100_g^{-1}$ fr.wt.)	II	30.50	21.00	49.85	37.65
	X	23.25	17.80	29.25	17.90
Total Chlorophylls ($mg\ 100_g^{-1}$ fr.wt.)	II	67.05	54.40 (18.86)	104.75	84.35 (19.23)
	X	64.25	51.89 (14.23)	64.35	34.15 (39.16)
Chlorophyll a/b	II	1.19	1.59	1.10	1.24
	X	1.76	1.91	1.20	1.28
Carotenoids ($g\ 100_g^{-1}$ fr.wt.)	II	1.06	0.96 (9.43)	1.56	0.96 (36.46)
	X	1.12	0.80 (28.57)	0.72	0.34 (52.77)

Values in parantheses indicate percent decrease

Values are mean of three determinations

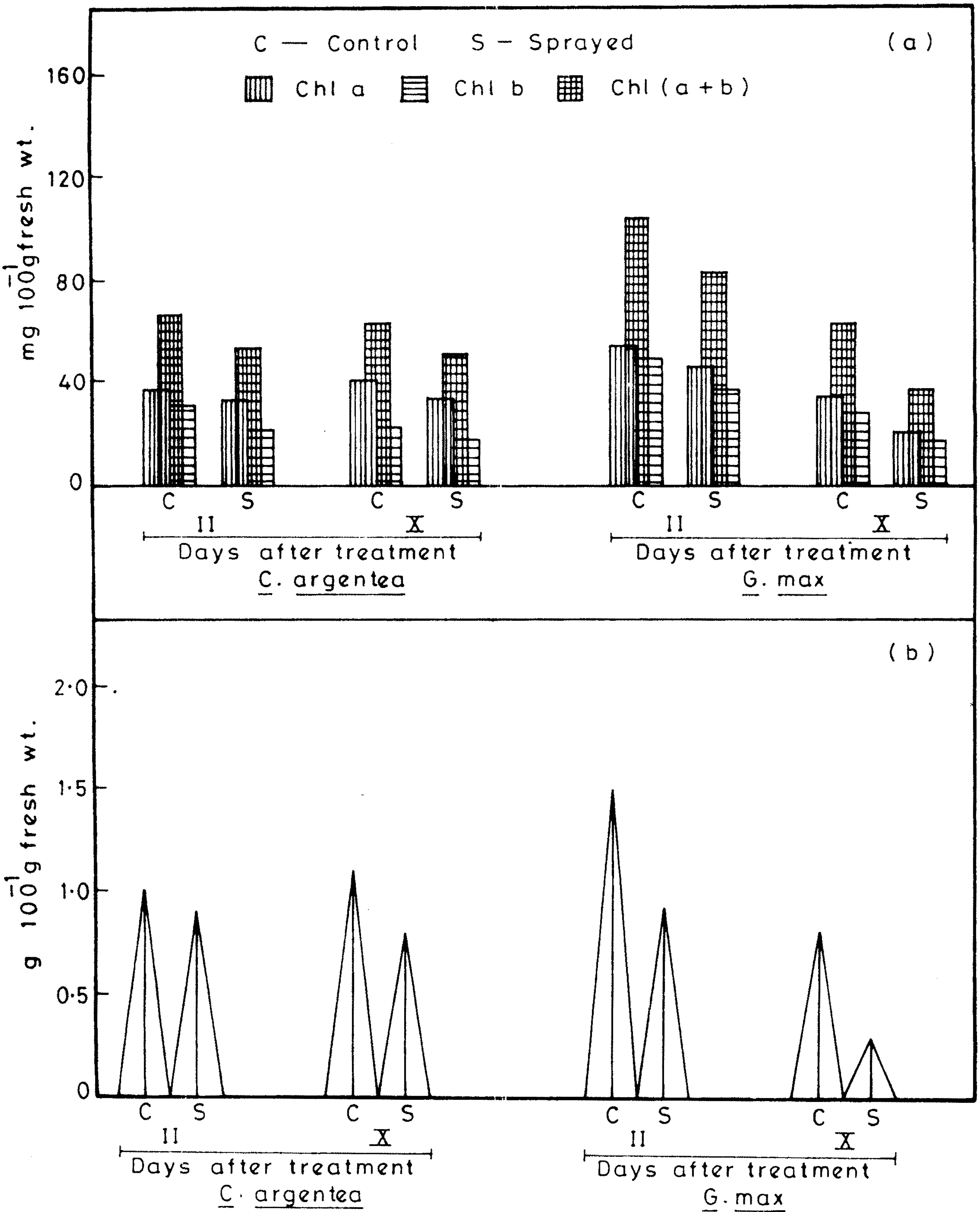

FIG. 2 (a&b) — EFFECT OF GLYPHOSATE ON CHLOROPHYLL AND CAROTENOID CONTENT IN *C. argentea* and *G. max*.

plants exposed to various glyphosate concentrations. They also observed a greater effect on chlorophyll pigments than on the carotenoids. In *Cyperus esculentus* treated with foliar sprays of glyphosate, more than 50% decrease in the photosynthetic pigments has been reported (Villanueva *et*. *al*. 1985). Inhibition of chlorophyll accumulation by glyphosate (1mm) in various crops was studied by Kitchen *et*. *al*. (1981). They observed that in soybean the rate of chlorophyll accumulation was reduced by 82% whereas in corn shoots it was reduced by 64%. They further suggested that glyphosate interferes with the growth of the plant by inhibiting the synthesis of chlorophylls. In callus cultures of tobacco and soybean also the presence of glyphosate in the medium inhibited chlorophyll accumulation. (Lee 1981).

In the present investigation about 40% decrease in the total chlorophyll content has been observed in the crops soybean and wheat treated with 300ppm glyphosate. A greater loss in photosynthetic pigments suggested that crops are more sensitive to the herbicide than the weeds. Chlorophyll a/b ratio was found decreased in *Cyperus* and wheat as a rsult of decrease in chlorophyll a, as compared to chlorophyll b due to the glyphosate treatment. On the contrary in *C*. *argentea* and soybean chlorophyll a/b ratio increased in the treated plants due to decreased synthesis of chlorophyll b. Munoz-Rueda and

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Gonzolez-Digg (1986) have also reported an increase in chlorophyll a/b ratio as a consequence of major effects on chlorophyll b in _Trifolium_ and _medicago species_ exposed to glyphosate.

2. TOTAL POLYPHENOLS

Polyphenol content in different plants exposed to the glyphosate treatment revealed an increase in this component in all the plants studied (Table 7). Further the polyphenol accumulation was more on the X day as compared to the II day after treatment, the maximum increase being observed in _Cyperus_ (57%) followed by soybean (50%). In wheat and _Celosia_ percent increase was less as compared to the other plants.

Secondary products of many chemical classes exist in plants and are thought to function in interaction of plants with pathogentes herbivores and other plants and also as structural components of cell walls such as lignins. Any type of stress can exert a profcund effect on the biosynthesis of these compounds. (Dicosmo and Towers 1984). Herbicides may cause a decrease or increase in the content of secondary compounds, for example, 2,4,5-T increase alkaloid contents of some plants, which is poisonous (Williams and Cronin 1963), and important in pasture or rangeland (williams and James 1983). Maleic hydrazide has been reported to cause a significant decrease

Table 7. Effect of glyphosate on polyphenol content in the leaves of *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species		Polyphenols ($g\ 100_g^{-1}$ fr. wt.)		Percent increase	
		II day	X day	II day	X day
Cyperus rotundus	Control	0.40	0.47	—	—
	Sprayed	0.47	0.74	17.50	57.44
Triticum aestivum	Control	1.25	1.12	—	—
	Sprayed	1.50	1.40	20.00	25.00
Celosia argentea	Control	1.07	1.12	—	—
	Sprayed	1.27	1.55	18.69	29.48
Glycine max	Control	0.57	0.60	—	—
	Sprayed	0.75	0.90	31.57	50.00

Values are mean of three determinations

in nicotine content of tabacco which is undesirable (Birch and Vickery 1961). PAL is the first enzyme in the production of secondary phenolic compounds whose activity is roughally correlated with the anthocyanin content. Hoagland and Duke (1983) observed a significant reduction in anthocyanin content in soybean hypocotyls exposed to various herbicides such as atrazine, fenuran, paraquat propanil, profan, TCA etc.

Glyphosate has been reported to block synthesis of aromatic amino acids and all phenolic compounds derived from them such as flavanoids, lignins, cinnamic acids, flavans. (Hollander and Amrhein, 1980; Saltveit 1988; Canal et. al. 1987 and Ishikura et. al. 1986). However Duke and Hoagland (1978) and Duke et. al. (1979) while studying the effects of glyphosate on metabolism of phenolic compounds have found that increased levels of PAL activity can inhibit biosynthesis of aromatic amino acids with increased synthesis of phenolic compounds. They observed stimulation in PAL activity in both light and dark-grown soybean seedings during glyphosate treatment. Deregulation of shikimate pathway by glyphosate may result in an increase in tannins, or protocatechuic acid in certain plants. (Kakhniashvili et. al. 1989, and Lydon and Duke 1988).

In the present study, observed higher levels of secondary phenolic compounds on the X day after glyphosate treatment may be attributed to the enhanced protein degradation induced by glyphosate which results in an increased pool of aromatic amino acids. However a further study regarding the metabolism of phenolic compounds in this respect is essential.

3. CARBOHYDRATES

Soluble sugars and starch content in the plants under investigation was greately influenced by the sprays of glyphosate as presented in Tables 8 and 9. In general a decline in the total sugars and starch was observed in all the plants on the II as well as X day after treatment (fig. 3 and 4). As a consequence the content of reducing sugars was found increased in all the treated plants as compared to the control. Soybean and Cypetus exhibited a marked increase in the reducing sugars on X day as compared to the II day after treatment of glyphosate. In wheat and Celosia only a marginal increase in reducing sugars was observed. Das et. al. (1977), have reported an increase in reducing and nonreducing sugars with both alachlor and PMA treatment in corn plants.

Glyphosate is mainly translocated via phloem in plants, with a small amount transferred from phloem to xylem. Phloem translocation of glyphosate is also limited due to indirect effect on carbon metabolism as Glyphosate affects carbon fixation through an effect on RuBP levels and on starch production. (Geiger et. al. 1986; Servaites et. al. 1987 and Geiger and Bestman 1990).

Table 8. Effect of glyphosate on soluble sugars and starch in the leaves of *Cyperus rotundus* and *Triticum aestivum*

Parameters	Day after treatment	*Cyperus rotundus*		*Triticum aestivum*	
		Control	Sprayed	Control	Sprayed
Reducing Sugars	II	0.40	0.50	0.18	0.19
($g\ 100_g^{-1}$ fr.wt.)	X	0.75	1.00	0.12	0.21
Total Sugars	II	0.82	0.57	0.30	0.23
($g\ 100_g^{-1}$ fr.wt.)	X	1.35	1.10	0.35	0.22
Starch	II	1.56	0.68	0.60	0.41
($g\ 100_g^{-1}$ fr.wt.)	X	0.79	0.52	0.57	0.30
Total Carbohydrates	II	2.38	1.25 (47.47)	0.90	0.64 (28.88)
($g\ 100_g^{-1}$ fr.wt.)	X	2.14	1.62 (24.29)	0.92	0.52 (43.47)

Values in parantheses indicate percent decrease

Values are mean of three determinations

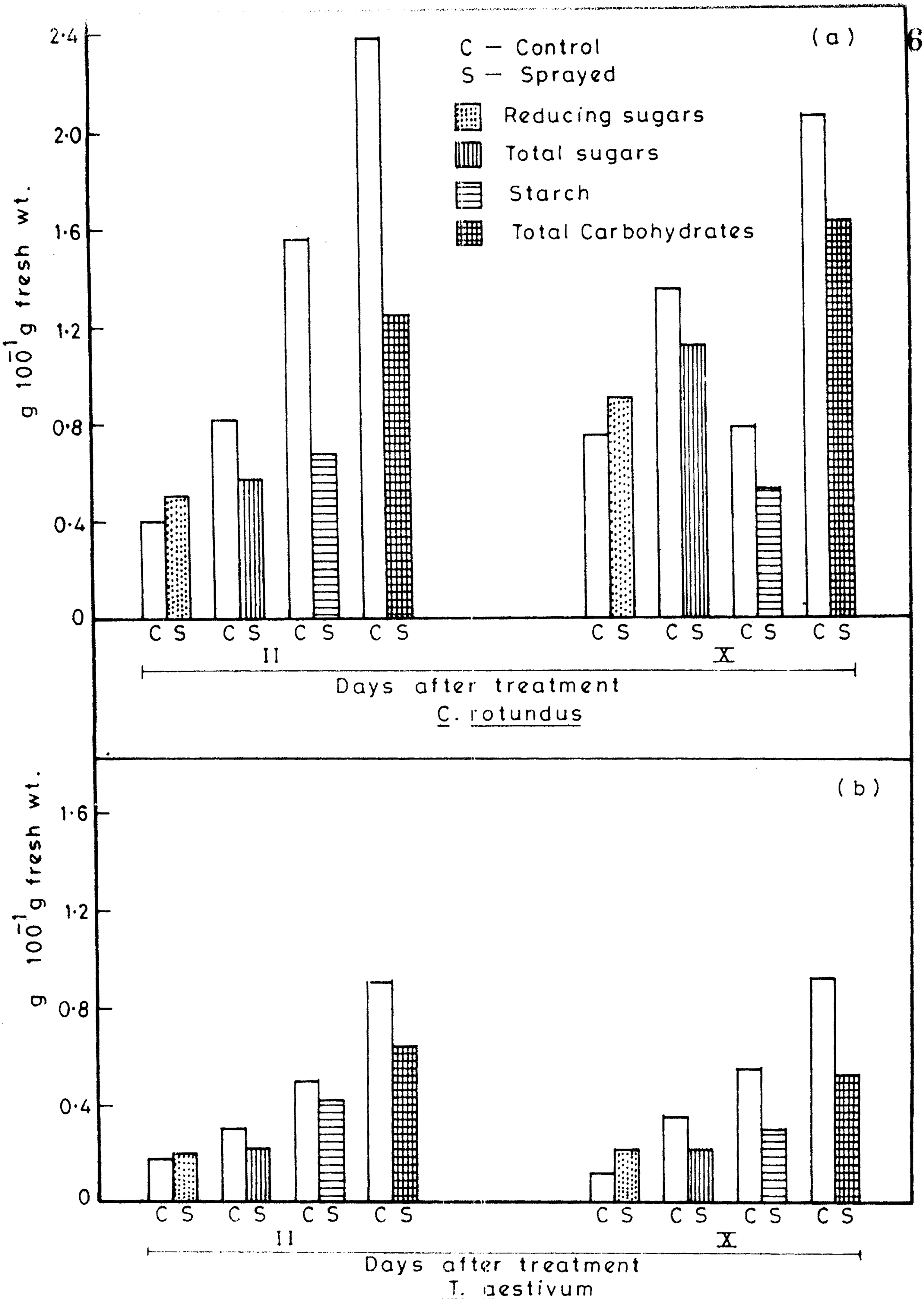

FIG. 3(a & b) — EFFECT OF GLYPHOSATE ON CARBOHYDRATES IN *C. rotundus* AND *T. aestivum*.

Table 9 Effect of glyphosate on soluble sugars and starch content in the leaves of Celosia argentea and Glycine max

Parameters	Day after treat-ment	Celosia argentea		Glycine max	
		Control	Sprayed	Control	Sprayed
Reducing sugars	II	0.27	0.30	0.52	0.60
($g\ 100_g^{-1}$ fr. wt.)	X	0.27	0.34	0.57	0.97
Total sugars	II	0.42	0.34	1.00	0.70
($g\ 100_g^{-1}$ fr. wt)	X	0.43	0.35	1.87	1.15
Starch	II	1.67	1.40	1.92	0.63
($g\ 100_g^{-1}$ fr. wt)	X	1.65	1.05	1.21	0.96
Total carbo-hydrates	II	2.09	1.74 (16.74)	2.92	1.33 (54.45)
($g\ 100_g^{-1}$ fr. wt)	X	2.08	1.40 (32.69)	3.08	2.11 (31.49)

Values in parantheses indicate percent decrease

Values are mean of three determinations

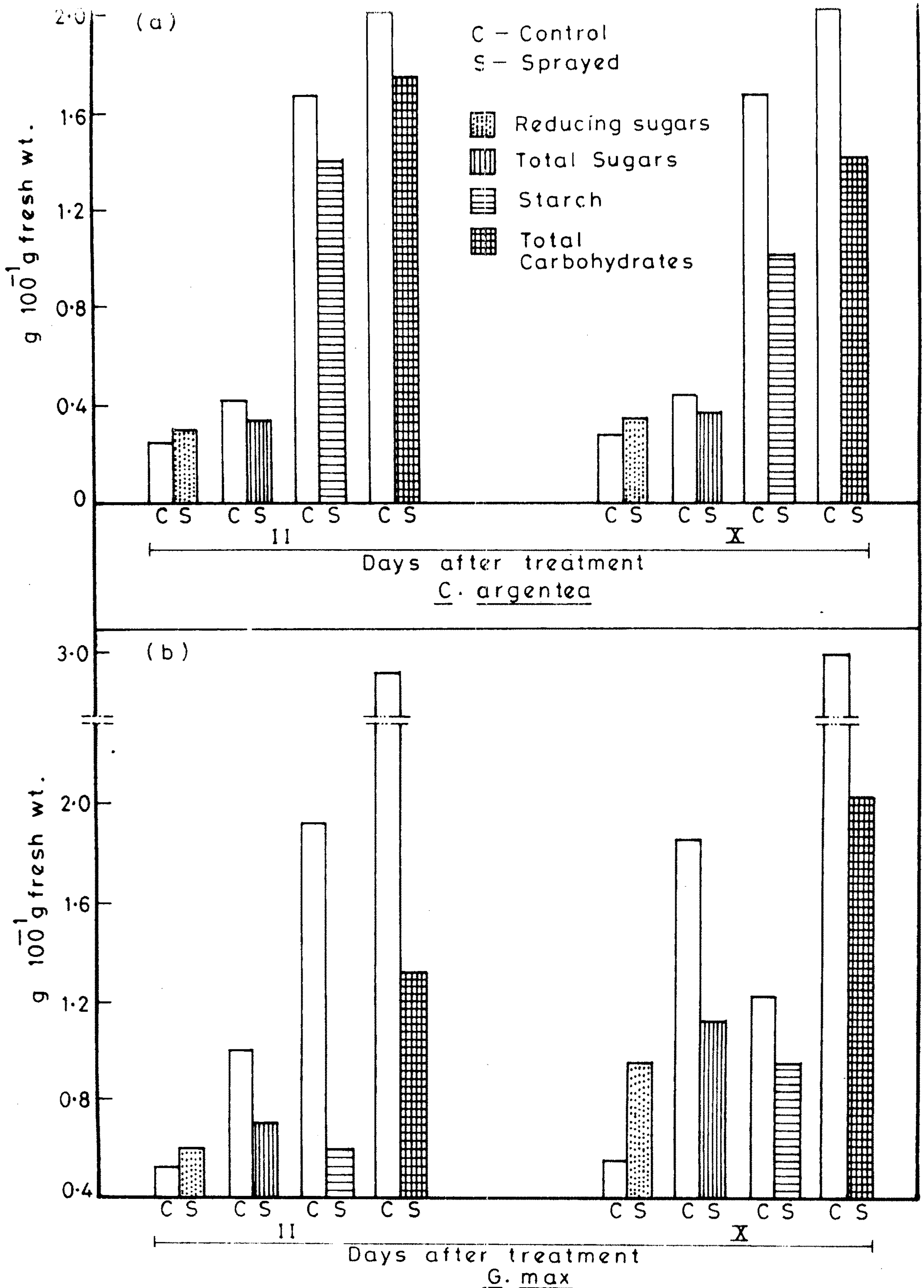

FIG. 4 (a & b) – EFFECT OF GLYPHOSATE ON CARBOHYDRATES IN *C. argentea* AND *G. max*.

Initially sugars derived from starch, maintain supply of assimilate for translocation but starch also becomes soon depleted. Hence the effect of glyphosate may not be detectable immediately after application.

In the present study a prominent reduction in the starch content has been observed in all the plants on the X day after treatment. Santakumari and Das (1979) have reported an overall decrease in total carbohydrate content of four plant species treated with herbicides diuron and atrazine. They have also correlated increased starch content in C. rotundus in their study, with the resistance of the plant towards the herbicides. In our study total carbohydrates in Cyperus decreased by 47% initially and the content increased with a further recovery on the X day after treatment. In soybean also total carbohydrate content increased on the X day as compared to the II day after treatment. In wheat and Celosia a continuous decline in the total carbohydrate content was observed due to glyphosate treament. This suggested that Cyperus and soybean had some mechanism by which they were able to maintain their sugar levels during the herbicidal treatment. Wheat and Celosia seem to be susceptible to the action of glyphosate as far as carbohydrate metabolism is concerned.

4. SOLUBLE PROTEINS

Protein content in the glyphosate treated plants

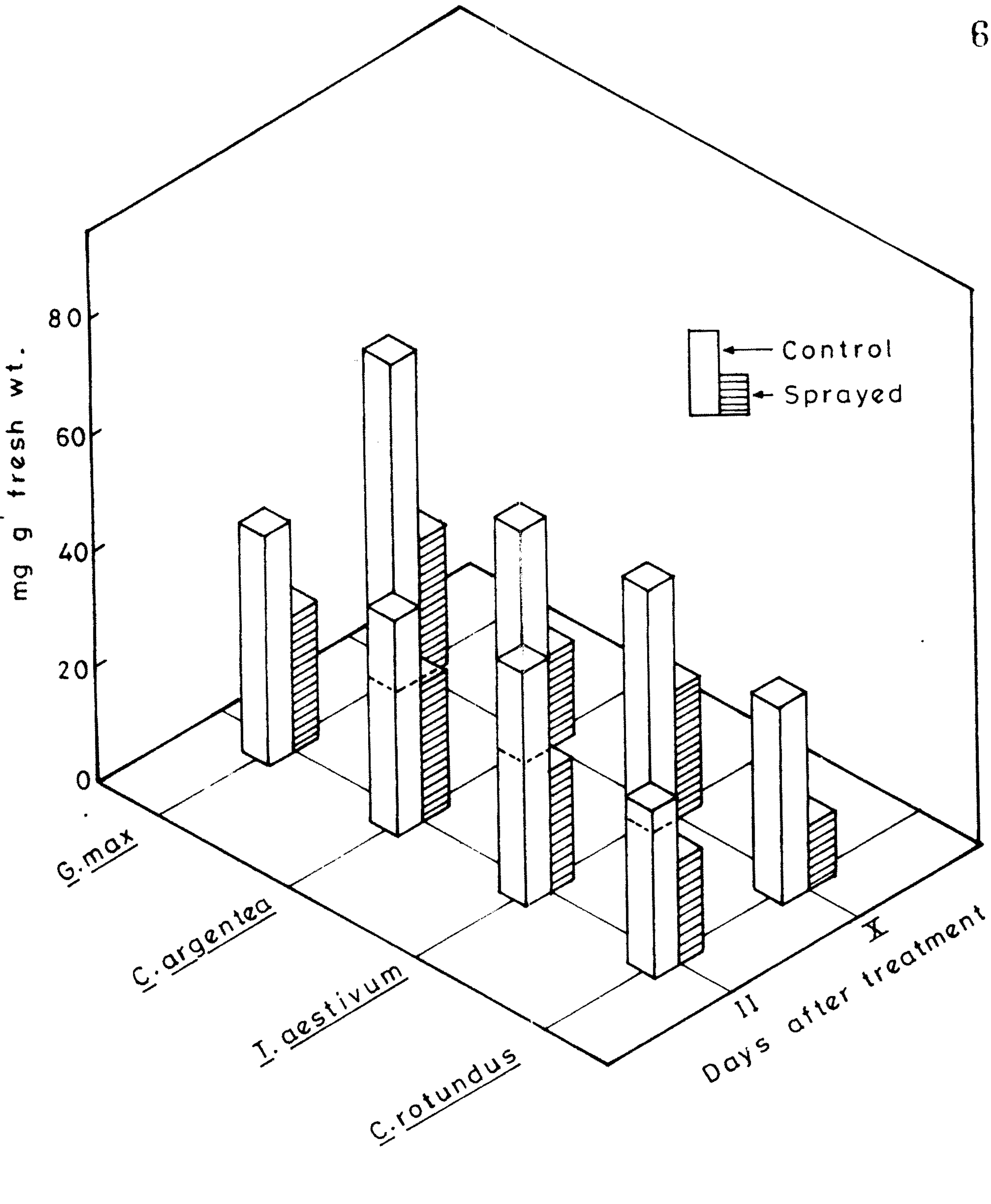

FIG. 5 — EFFECT OF GLYPHOSATE ON PROTEIN CONTENT IN *C. rotundus*, *T. aestivum*, *C. argentea*, *G max*.

was found greately affected as evident from Table 10. The effect was more pronounced on the X day after treatment as compared to the II day, in most of the plants (Fig.5). C. argentea exhibited a maximum decrease (85%) in protein content followed by C. rotundus (66.6%). In both the crops, values of percent decrease in proteins were less as compared to the weeds.

No commercially available herbicides are known to affect protein synthesis directly, but there are many reports of apparent indirect effect of herbicide on these processes (Mann et al. 1965, Moreland et al. 1969; Ashton et al 1977; Bellinder et al. 1985) Simazine and atrazine (Wu et al. 1971) and alachlor (Das et. al. 1977) are known to increase protein content in certain crops and the increase may be associated with an increased rate of amino acid formation (Singh and Salunke 1970). Most of the herbicides affect the major metabolic processes such as nucleic acid and protein synthesis. However their relationship with primary mechanism of action has not yet been discovered. Generally protein synthesis is inhibited more than RNA synthesis and herbicides that disrupt amino acid metabolism can affect the protein synthesis. (Devine et al. 1993). Further the effect of herbicide varies considerably with the plant species, plant tissue, environmental conditions and also depends on the concentration used.

Table 10 Effect of glyphosate on protein and proline content in *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species		Proteins ($mg\ g^{-1}$ of fr. wt)		Proline ($g\ 100g^{-1}$ dry wt)	
		II day	X day	II day	X day
Cyperus rotundus	Control	28.00	33.60	0.702	0.772
	Sprayed	17.60 (38.88)	11.20 (66.66)	1.053	1.540
Triticum aestivum	Control	40.00	42.40	0.351	0.421
	Sprayed	21.60 (47.76)	22.40 (47.16)	0.491	0.842
Celosia argentea	Control	37.60	40.00	0.315	0.386
	Sprayed	24.00 (36.17)	16.00 (85.00)	0.561	0.877
Glycine max	Control	40.00	56.80	0.631	0.772
	Sprayed	24.00 (40.00)	24.00 (57.74)	1.260	1.750

Values in parantheses indicate percent decrease

Values are mean of three determinations

Haderlie (1975), has reported a decrease in protein level in glyphosate treated soybean plants which was observed after 30 h and continued upto 90 h. He also observed decreased protein content in callus cell suspenssion after 48 h, treated with glyphosate.

Isolated cell studies of Tymonko and Foy (1978) have indicated that glyphosate inhibits protien synthesis, RNA synthesis, photosynthesis and respiration. In dark grown soybean seedlings higher concentrations of glyphosate (.1M) caused a slight decrease in soluble proteins while lower concentrations did not cause any significant change in protein level (Hoagland et al. 1979), In the light treated soybean seedlings glyphosate reduced the amount of protein per axis after 72 h. (Duke et al. 1979).

Thus there is no clear evidence for the inhibition of protein synthesis by the glyphosate, however a decrease in the content of proteins has been reported by few workers as discussed above. In our study also a profound decrease in protein level has been observed especially on the X day after treatment. Herbicides such as diquat and paraquat have been shown to act readily on proteins modifying their structure and function (Szogyi et al. 1989). Many herbicides are known to form conjugates with different cell constituents such as sugars, amino acids, peptides, lignin by chemical bonding. Much of the fraction of such conjugates is insoluble and can not be extracted by standard extraction procedures. (Devine et al. 1993). This is quite possible in the present study as the analysis has been carried out a longer period after the treatment. Increased degradation of proteins and decreased amino acid synthesis during herbicidal treatment may also account for the decline in protein content.

5. FREE PROLINE

Effect of glyphosate on free proline content has been presented in Table 10. A tremendous increase in proline level was observed in glyphosate treated plants as compared to the control plants. The increase was about 100% or more in all the plants studied on the X day after treatment. As evident from fig.6, in all the control plants the proline content has increased marginally from the II to the X day after treatment. Whereas in the glyphosate treated plants the increase in proline content is distinct as compared to the control plants.

Accumulation of free proline in plants under any physiological stress is very common. Proline accumulates readily in the leaves of many crop plants exposed to water stress conditions as observed in wheat (Singh et al. 1973), Sorghum (Blum and Ebercorn 1976), soybean (Waldren and Teare 1974), etc. Halophytes growing in saline conditions also have the proline as the major component of the amino acid pool (Stewart and Lee 1974). Capacity to accumulate proline is often correlated with the salt tolerance of the halophytes.

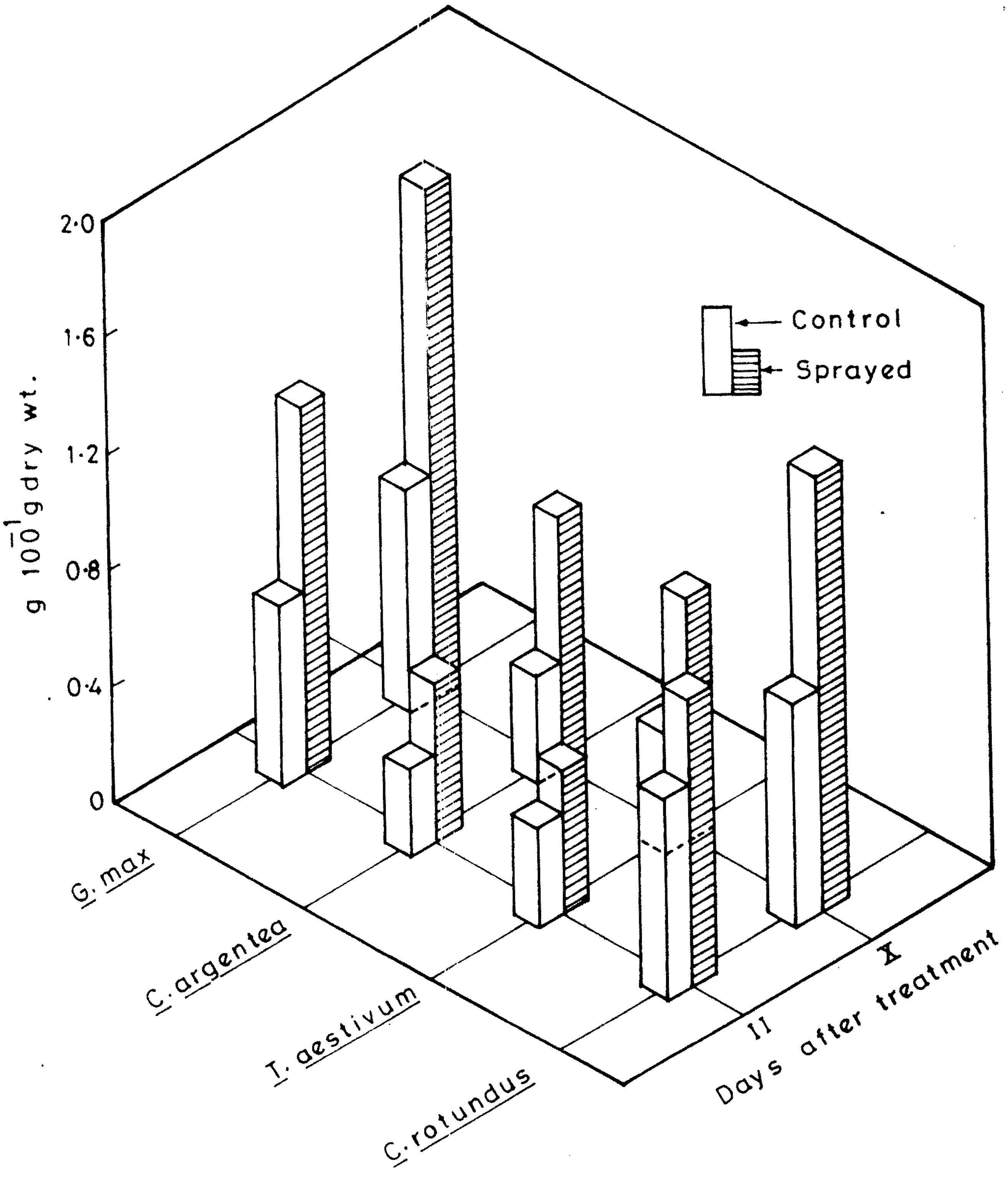

FIG. 6 – EFFECT OF GLYPHOSATE ON PROLINE CONTENT IN C. rotundus, T. aestivum, C. argentea, G max.

Causes of proline accumulation in its response to water stress have been discussed by Stewart and Hanson (1980). According to them proline accumulation may be a combined or individual effect on the stimulation of its synthesis, inhibition of its oxidation and impaired protein synthesis. In wilted plant leaves, synthesis of proline gets enhanced than in the turgid leaves (Boggess et al. 1976). Membrane damage in the stressed tissue may also lower down the oxidation of proline taking place in mitochondria (Nir & Klein 1970 Bell et al. 1971). When protein synthesis gets affected, the incorporation of all amino acids into proteins may also be inhibited by water stress. This may lead to an increase in the amino acids like proline.

In the present study a loss in turgidity of the leaves was observed in the glyphosate treated plants. The wilting observed may be possibly, due to reduced uptake of water by these plants under the influence of glyphosate. This was also evident from reduced water content in the treated plant leaves (Table 4). Thus the conditions resembling to those that occur in the plants under water stress may be created in the plants exposed to glyphosate treatment, resulting in an elevation in the free proline content. Enhanced protin degradation observed in all these plants may also be associated with the rise in amino acid proline. Duke et al. (1979) have reported a five-fold increase in proline content in light due to glyphosate.

According to them proline increases due to general stress conditions.

6. FREE AMINO ACIDS

Distribution of various protein amino acids in the plants under study has been represented in Tables 11 and 12. The no. of total amino acids detected was found increased in glyphosate treated plants. Specifically the number was higher for X day after treatment. Plates III, IV, V, and VI reveal chromatographic representation of various amino acids in control and glyphosate treated plants. Chromatographic analysis clearly indicates that the turnover of amino acids has increased on the X day after treatment in both the weeds and the crops. This is particularly true in case of soybean and Cyperus, as indicated by the number of amino acids detected. Amino acids tryptophan, phenylalanine, leucine, valine, alanine, lysine, arginine, etc. were not detected in control plants but appeared in the treated plants.

Glyphosate interrupts the biosynthesis of aromatic amino acids by blocking an enzymatic step of shikimate pathway in plants (Jaworsky 1972). Its mechanism of action has been reviewed by many workers (Kishore and Shah 1988, Duke 1988, and Cole 1985). Phenylalanine, tyrosine and tryptophan are the three aromatic amino acids produced by shikimate pathway. Inhibition of specific site of amino

Table 11. Distribution of free amino acids in the leaves of *Cyperus rotundus* and *Triticum aestivum* exposed to glyphosate

Amino acids	*Cyperus rotundus*				*Triticum aestivum*			
	II day		X day		II day		X day	
	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
Alanine	+	+	–	+	–	–	–	+
Dl-Alanine	+	+	+	+	+	+	+	–
Dl-Valine	+	–	+	+	+	–	–	–
Glycine	+	+	+	+	+	–	+	+
L-Arginine	–	–	–	+	+	+	+	+
L-Cystine	+	+	+	+	–	+	+	+
L-Histidine	–	+	+	+	+	+	+	–
L-Leucine	–	–	–	–	+	+	–	–
L-Lycine	–	–	+	+	–	+	–	+
Phenyl alanine	–	–	–	–	–	–	–	–
Proline	+	+	+	+	+	+	+	+
Trypto phan	–	+	–	–	–	–	–	+
No. of amino acids detected	6	7	7	9	7	7	6	7

\+ indicates presence
– indicates absence

Table 12. Distribution of free amino acids in the leaves of <u>Celosia argentea</u> and <u>Glycine max</u> exposed to glyphosate

Amino acids	*Celosia argentea*				*Glycine max*			
	II day		X day		II day		X day	
	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
Alanine	-	-	-	+	-	+	+	+
DL-Alanine	+	+	-	-	+	+	+	+
DL-Valine	-	-	+	+	-	-	-	+
Glycine	+	+	+	+	+	+	+	+
L-Argenine	+	+	+	+	-	-	-	+
L-Cystine	-	-	+	+	+	+	+	-
L-Histidine	+	+	+	+	+	+	+	+
L-Leucine	-	-	+	+	-	+	-	+
L-Lycine	-	+	-	-	+	+	+	+
Phenyl-alanine	-	-	-	-	-	+	-	+
Proline	+	+	+	+	+	+	+	+
Tryptophan	+	+	+	+	-	-	-	+
No. of amino acids detected	6	7	8	9	6	9	7	11

\+ indicates presence
\- indicates absence

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Photoplate III showing

Effect of glyphosate on amino acid distribution in the leves of Cyperus rotundus on

A. II day after treatment

B. X day after treatment

Photoplate IV showing

Effect of glyphosate on amino acid distribution in the leaves of *Triticum aestivum* on

A. II day after treatment

B. X day after treatment

Photoplate V showing

Effect of glyphosate on amino acid distribution in the Leaves of *Celosia argentea* on

A. II day after treatment

B. X day after treatment

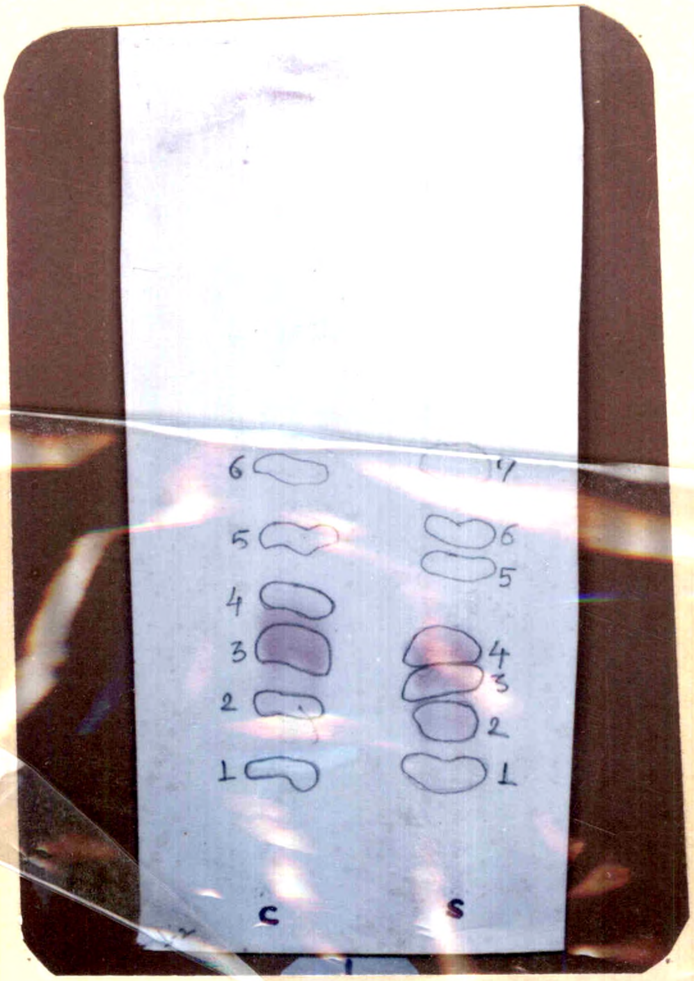
Photoplate VI showing

Effect of glyphosate on amino acid distribution in the leaves of *Glycine max* on

A. II day after treatment

B. X day after treatment

PLATE - III



A

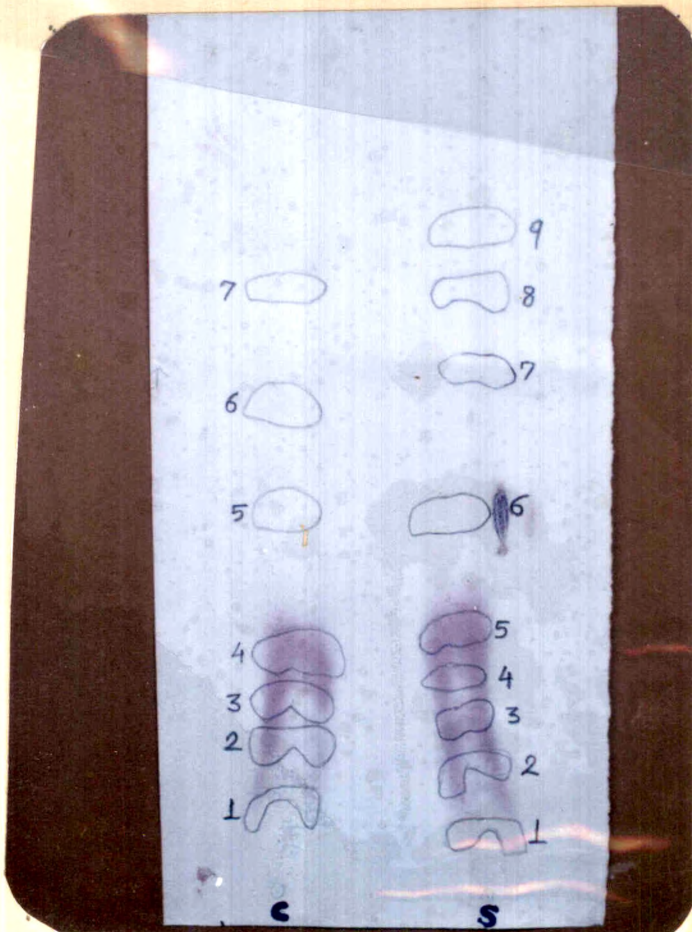


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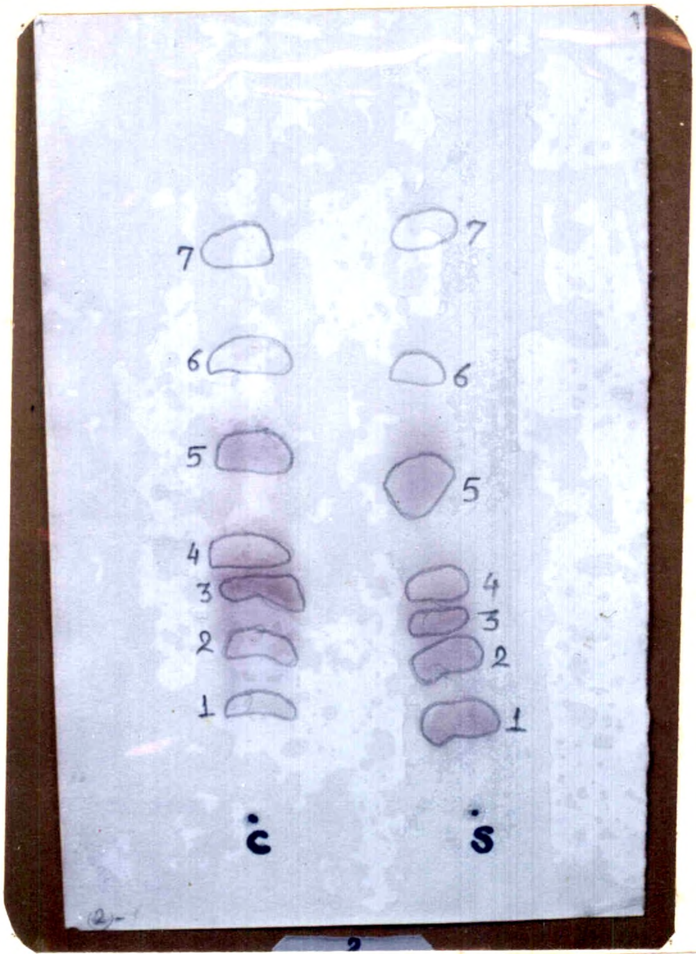
PLATE - IV



A

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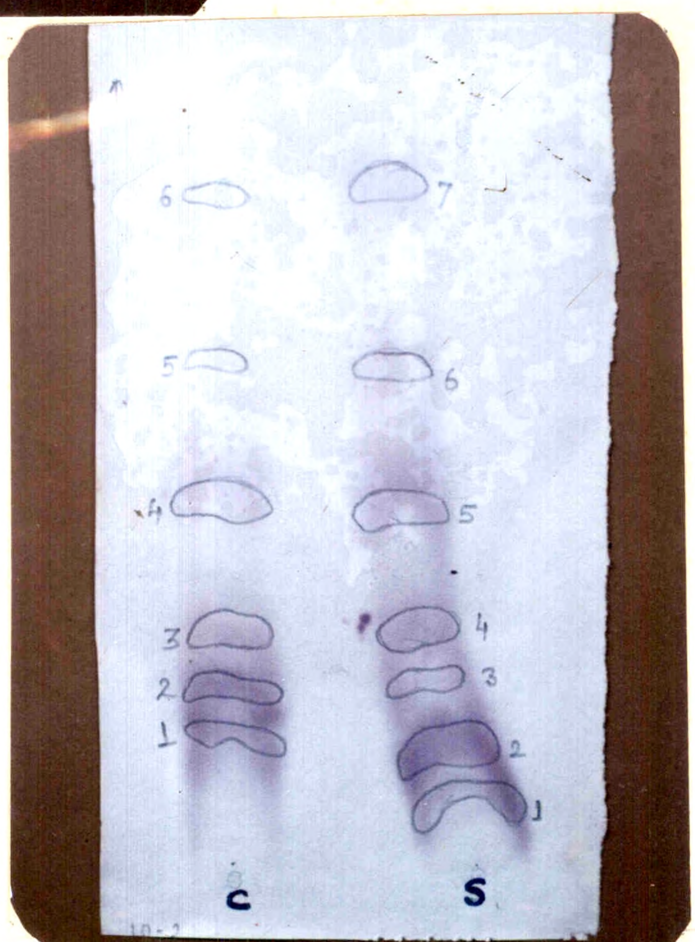
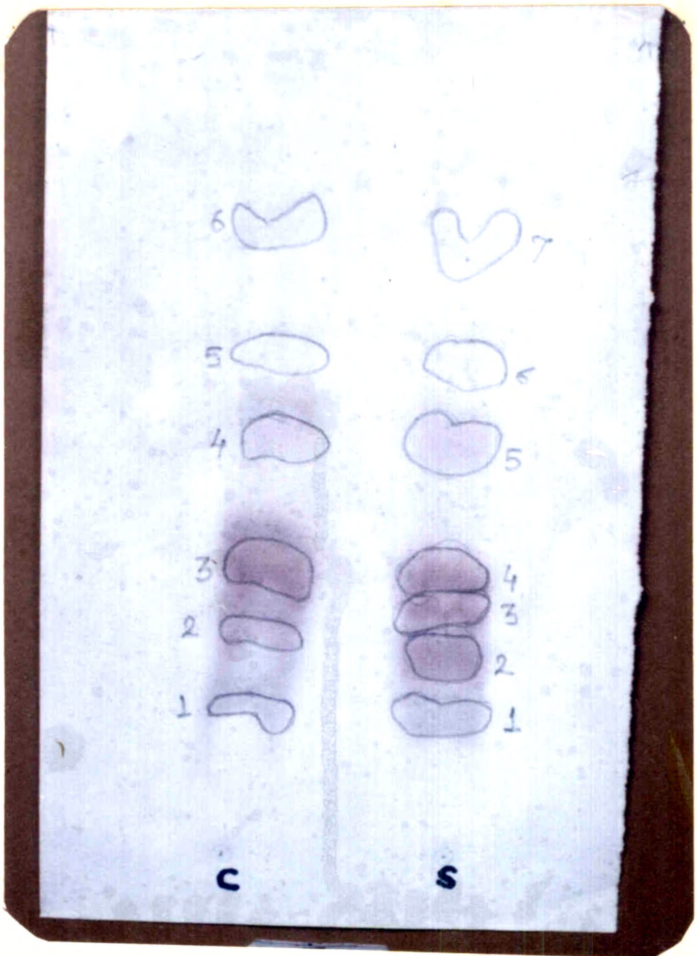
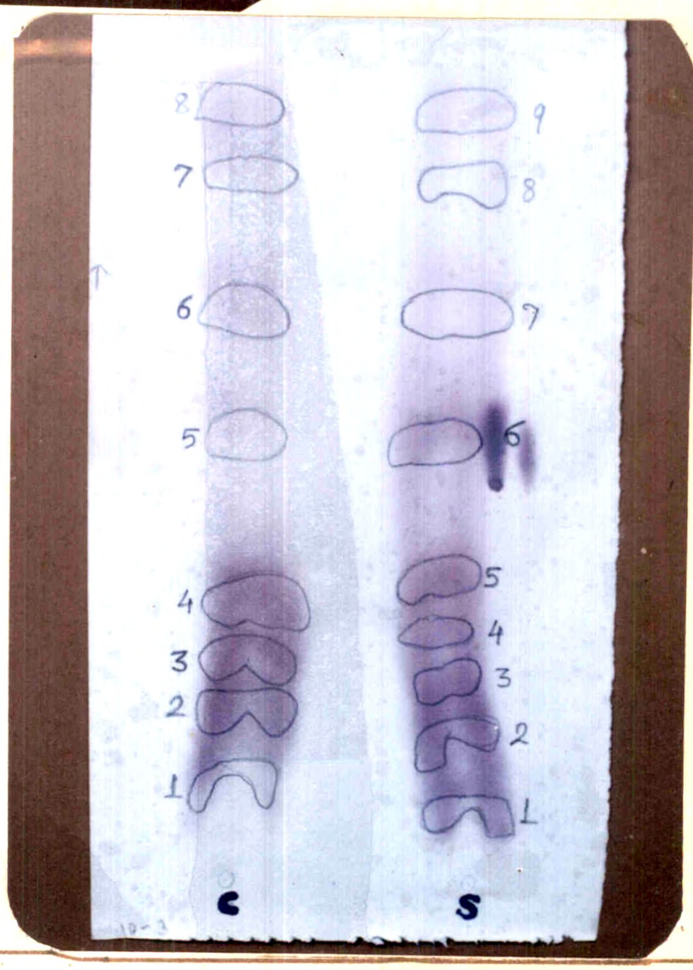


PLATE - V

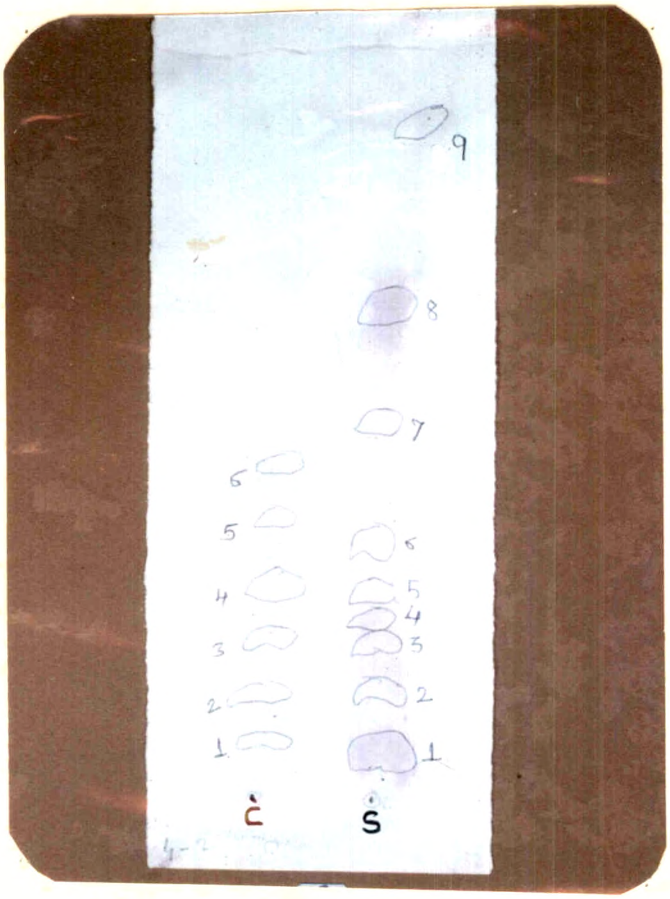


A



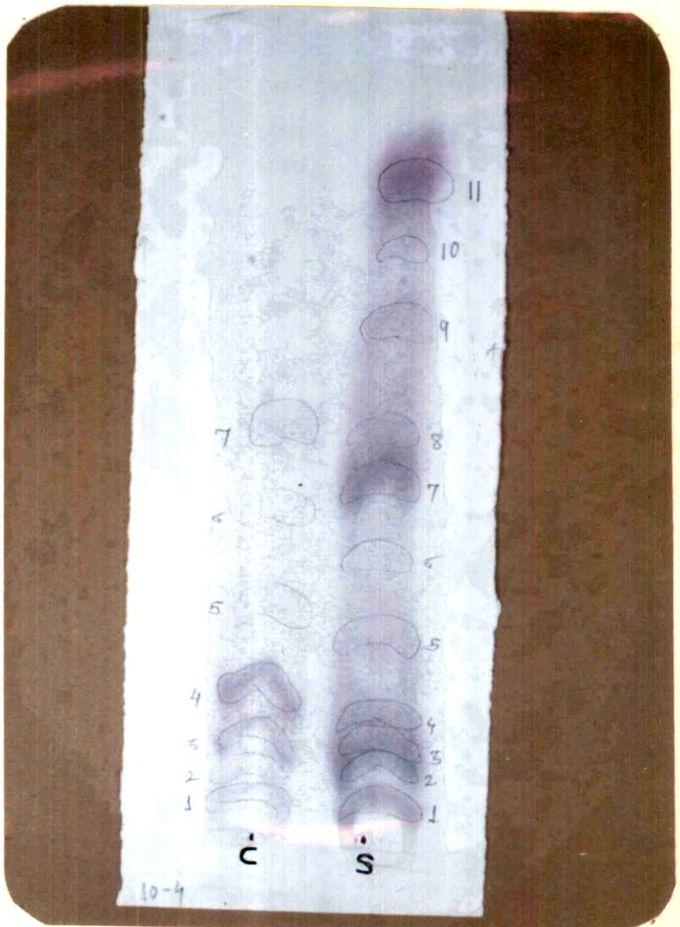
B

PLATE - VI



A

B



acid synthesis is expected to result in depleted metabolic pools of certain amino acids, leading to a decrease or cessation of all processes dependent on amino acids. Metabolic synthesis of amino acids and the amino acids stored in the vacuoles of cell,both account for the total free amino acid pools in the cell. Inhibition of synthesis of a particular amino acid can result in an increased protein turnover which can increase the free amino acid content. Even the total free pools of amino acids whose synthesis has been blocked, may also increase due to metabolic stress (Devine et al. 1993).

Nilsson (1977) observed an increase in tyrosine and phenylalanine percentage in young *T. aestivum* plants exposed to foliar sprays of glyphosate. He also observed increased percentage of glutamine. Haderlie et al. (1977) found increased levels of histidine, arginine and glutamic acid in carrot suspension culture, due to glyphosate treatment. No significant effect on aromatic amino acid level was observed by them. In light grown soybean also a severe reduction in phenylalanine and tyrosine was observed due to glyphosate by Duke et al. (1979) along with an increase in glutamine, alanine, valine and other amino acids.

From the above discussion it is clear that glyphosate causes a reduction in aromatic amino acid levels. In our study the total content of free amino acids is not analysed. However absence of phenylalamine in *Cyperus*, *Celosia* and wheat supports the above discussion. Though tryptohan was detected in glyphosate treated plants its level may be increased due to enhanced protein turn-over as discussed earlier.

C. **Mineral Constituents**

Healthy growth and development of any plant depends upon the availability of various organic and inorganic constituents absorbed from the soil, water and air. About 16 elements are required for the normal development of the plant and they are known as essential elements (Epstein 1972). Out of 16 elements, carbon, oxygen and hydrogen constitute major weight of the plant. Remaining 13 elements include the major or macroelements and the micronutrients or trace elements. The essetial elements function varicusly as the structural elements or acts as regulators and carriers in various metabolic processes in the cell. Some elements also function as catalysts,cofactors, and activators. All the mineral elements required by the plants are absorbed mostly from the soil through the roots. A change in the physical and biochemical environment of the plant can affect the uptake of various essential nutrients from the soil. The mineral content in glyphosate treated plants was analysed with respect to major nutrients, such as nitrogen, phosphorus, potossium, calcium, magneium, and iron.

1. NITROGEN

Nitrogen content in the crops and the weeds under study has been presented in Table 13. A general trend of decrease in nitrogen percentage was observed in the plants sprayed with glyphosate on both the days of analysis. In both weeds, the nitrogen content was reduced more than half as compared to the control on II day after treatment. A further decrease which was more evident in Cyperus than in Celosia was observed on the X day after treatment. Glyphosate did not reduce significantaly the amount of nitrogen in wheat on the II day, however it caused more than 50% decrease in the X day after treatment. Nitrogen content in soybean was reduced to less than half, on both the days after treatment.

Nitrogen is the fourth most abundant element in plants following, carbon, hydrogen,, oxygen. It is a constituent of proteins, amino acids, nucleic acids, chlorophylls and various co-enzymes. Proteins contain about 18% Nitrogen. Nitrogen is absorbed by the higher plants in oxidised form such as nitrate. Nitrate is reduced to ammonia and then assimilated further to form amino acids and other cell constituents.

There are very few reports regarding the effect of herbicides on the nitrogen contents in the plants. Ravel and Leela (1976) have reported an increase in nitrogen level in pineapple leaves under the influence of bromacil

Table 13 Effect of glyphosate on nitrogen, phosphorus and potassium content in the leaves of *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species		Nitrogen		Phosphorus		Potassium	
		II day	X day	II day	X day	II day	X day
Cyperus rotundus	Control	2.60	3.10	1.10	1.11	0.18	0.12
	Sprayed	1.02	1.18	0.60	0.59	0.12	0.06
Triticum aestivum	Control	1.14	2.50	1.60	1.72	0.14	0.14
	Sprayed	1.10	1.18	0.81	0.73	0.12	0.10
Celosia argentea	Control	2.40	3.40	1.80	1.92	0.16	0.14
	Sprayed	1.16	2.51	1.52	1.45	0.80	0.04
Glycine max	Control	2.00	3.80	3.10	3.40	0.18	0.24
	Sprayed	1.12	2.00	1.60	1.52	0.10	0.12

Values are expressed in g $100 g^{-1}$ of dry wt.

combined with atrazine. The decreased nitrogen content in our study indicates a reduced uptake of nitrate by the plants due to glyphosate treatment. Nitrogen metabolism is discussed further with respect to the enzymes nitrate and nitrite reductases afterwards.

2. PHOSPHORUS

Phosphorus content in *Cyperus* and *Celosia* (Table 13) though was reduced by the glyphosate treatment the amount did not differ much in control and the treated plants on both the days of analysis. In wheat and soybean the decrease in the phosphorus content was about 50% on both the days of analysis. In *Cyperus* the decline was less than 50% whereas in *Celosia* the content was not much affected.

Phosphorus is absorbed as dihydrogen phosphate ion (H_2PO_4) and is one of the three prominent elements which are absorbed as complex anions. Phosphorus is not reduced in cell to a lower oxidation state as in case of nitrogen and sulphur. It plays a key role in energy metabolism in organic form such as ADP, ATP and sugar phosphates. ATP is considered as the universal energy currency of living cells. Phosphorus is also a component of nucleic acids and phospolipids. It regulates many enzymatic and energy requiring processes.

Niklicek *et al*. (1983) have studied the effect of glyphosate on uptake and translocation of labelled phosphorus in quack grass (Elytrigia repens). They oserved a marked decrease in the quantity of phosphorus taken up by the test plant. In the present investigation also glyphosate has affected the phosphorus content is both the crops and weeds.

3. POTASSIUM

Potassium content also decreased in all plants treated with glyphosate and the effect was more evident on the X day after treatment in most of the plants. In both the weeds and soybean the content of potassium was reduced to about one half to one third as compared to the untreated plants. In wheat, only a marginal decline was noticed on the II and X day after treatment.

Potassium is the only monovalent cation essential for all higher plants. The principle role of the potassium is an activator of numerous enzymes. It is also involved in ion transport through its role in ATPase activity. Translocation of sugar is closely linked to potassium. It has an osmoregulatory function which helps to prevent loss of water from the cell under stressed conditions. Potassium along with ATP and ATPase interacts in the opening of stomata. Higher concentration of potassium is observed in the guard cells of opened stomata than in the closed ones.

In a few studies K-uptake has been shown to be affected by herbicidal treatments. A strong inhibition of potassium uptake was observed in maize root segments after 12 h pretreatment with chlorsulfuron (Marina and Giardina, 1984). Atrazine also inhibited potassium uptake efficiency in excised roots of maize (Giardina et al. 1983). According to Duke (1986), Demos et al. (1975) and Glass (1974), phenolic compounds can inhibit potassium uptake and many other physiological processes. The inhibition of potassium uptake observed in the present study may be due to increased levels of phenolic compounds by glyphosate treatment.

4. CALCIUM

Effect of glyphosate on calcium content has been depicted in Table 14. Glyphosate treatment increased calcium content in *Cyperus*, *Celosia* and soybean on both the days after treatment by only about 10% or less. (in *Celosia* 20%). In wheat accumulation of calcium was higher as compared to the other plants. Maximum concentration of Ca was observed on X day after treatment in wheat which exhibited a 54% increase.

Calcium acts as a structural component in different forms in different types of plants. In the plant cell wall it is present as a calcium pectate. It provides mechanical strength to the tissue. Calcium also plays an important role in membrane integrity and ion regulation, influencing the selectivity of the ion uptake. Calcium ions are required for the activity of enzyme α-amylase from

Table 14 Effect of glyphosate on calcium, magnesium and iron content in the leaves of *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species		Calcium		Magnesium		Iron	
		II day	X day	II day	X day	II day	X day
Cyperus rotundus	Control	0.620	0.572	1.000	1.080	0.148	0.200
	Sprayed	0.704	0.632	0.460	0.500	0.204	0.354
Triticum aestivum	Control	0.576	0.486	0.360	0.340	0.070	0.139
	Sprayed	0.752	0.740	0.120	0.080	0.085	0.228
Celosia argentea	Control	1.112	1.100	2.360	1.900	0.124	0.170
	Sprayed	1.144	1.320	1.040	0.900	0.166	0.250
Glycine max	Control	1.180	0.952	5.380	4.220	0.087	0.065
	Sprayed	1.284	1.024	3.400	1.040	0.123	0.108

Values are expressed in g 100^{-1} g dry wt.

higher plants. In leguminous plants more calcium is required for nodulation.

Glyphosate has been reported to reduce the uptake and translocation of calcium in a few studies (Duke et al. 1983 and Niklicek et al. 1983). The increase in calcium content observed in our study may be due to its accumulation in mature parts. In case of wheat Ca-content, increased on the II day and remained more or less constant thereafter. This suggests that in wheat, calcium translocation is greately affected as compared to other plants.

5. MAGNESIUM

An overall decrease in magnesium content was observed in all the plants sprayed with glyphosate. In *Cyperus* and *Celosia*, magnesium content was reduced to less than half when compared with the control values on the II as well as X day after treatment. The effect of glyphosate was evident from the II day with a marginal change in the content thereafter. The crops wheat and soybean were found more sensitive to the treatment of glyphosate as compared to the weeds. In wheat magnesium content was reduced to one third on the II day and then to one fourth on the X day after treatment. In soybean the decline was prominent on the X day after treatment showing about 25% reduction in the magnesium content.

Magnesium is important constituent of chlorophylls which contains an atom of megnesium in their structure. Magnesium is associated with many plant proteins and also required for the ribosomal integrity. As a cofactor, it is involved in all phosphorylation reactions. Deficiency of magnesium affects severely the synthesis of chlorophylls followed by a decrease in photosynthesis. Biosynthetic pathway get disturbed as a result of inibition of transphosphorylation reactions during magnesium deficiency.

Reports regarding the influence of herbicide on the uptake and concentration of magnesium were not available. Our results indicated that glyphosate severely affects the magnesium content in both the dicotyledonous and monocotyledonous plants. These results are suported by the reduced content of total chlorophylls in the treated plants.

6. IRON

Iron content in the plants treated with glyphosate increased on both the days of analysis as presented in Table 14. In the weeds Cyperus and Celosia the increase was noticeable on the X day as compared to the II day after treatment. In soybean also the amount of iron enhanced on both the days after treatment as compared to the control plants. In wheat the effect of glyphosate appeared later,

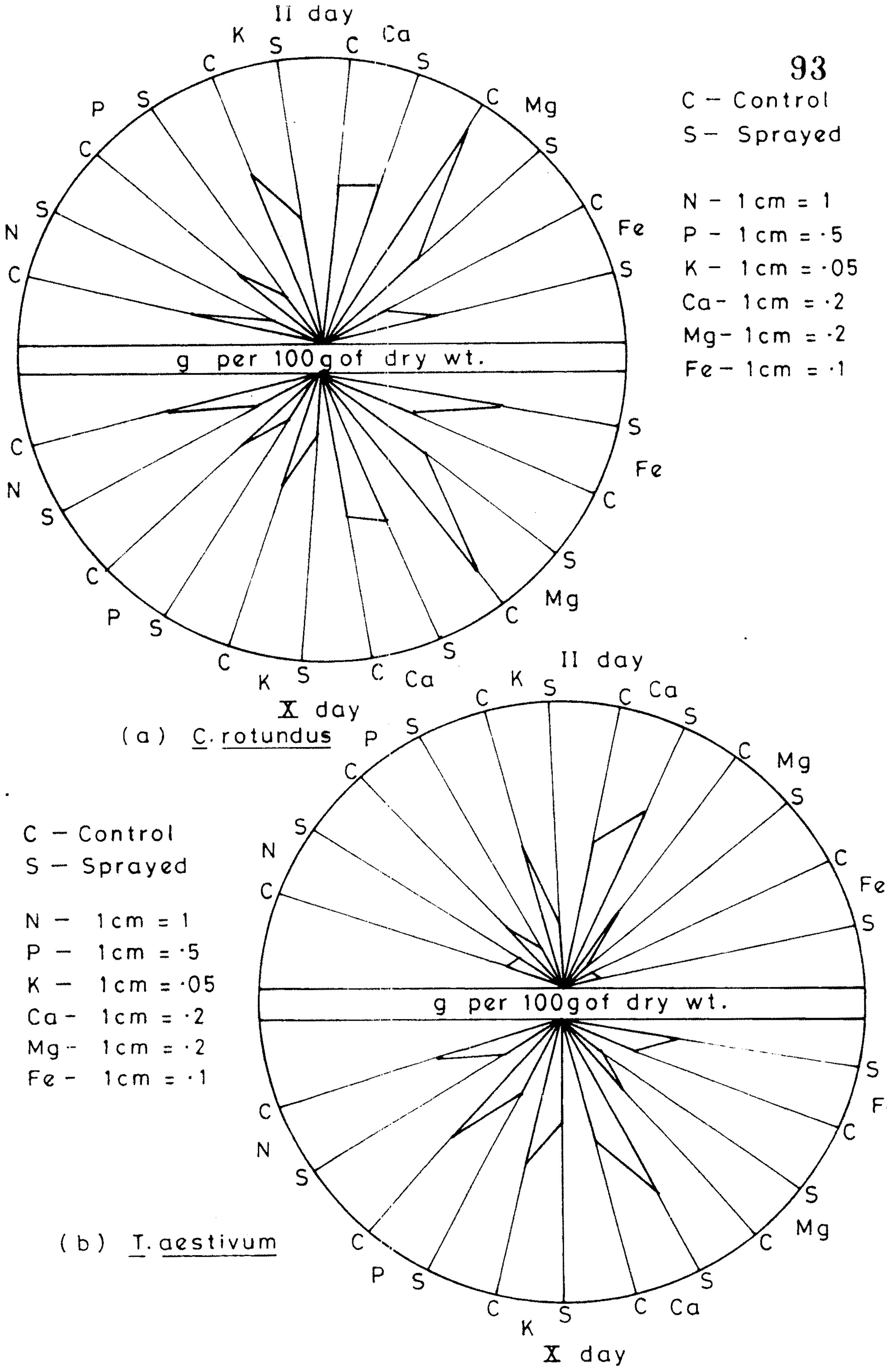


FIG. 7 (a & b) – EFFECT OF GLYPHOSATE ON MINERAL CONTENT IN *C. rotundus* AND *T. aestivum*.

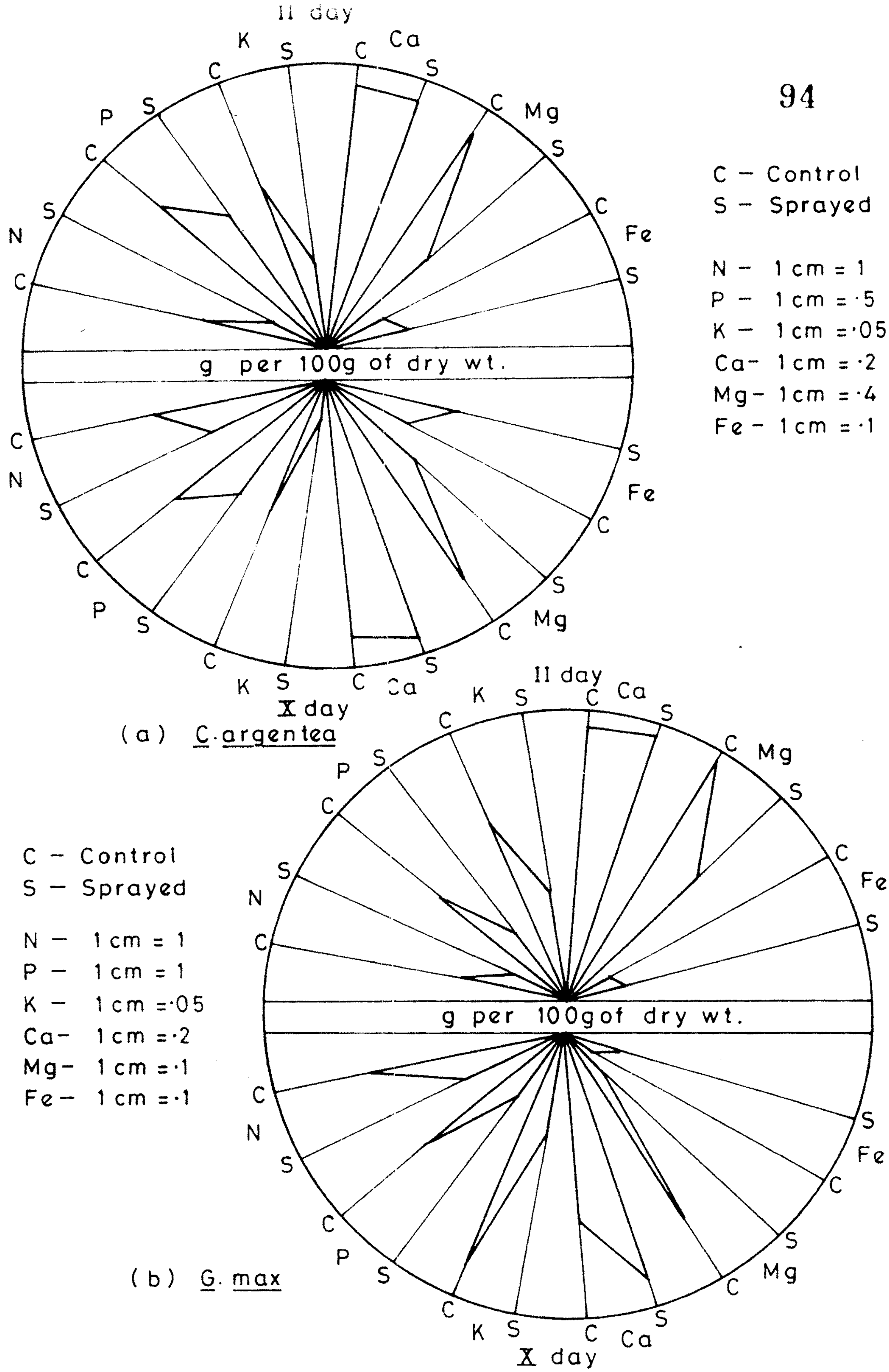


FIG.8 (a& b) – EFFECT OF GLYPHOSATE ON MINERAL CONTENT IN *C. argentea* AND *G. max* .

on the X day showing an increase in the content.

Iron functions as a structural component and also cofactor for many enzymatic reactions. Oxidation reduction reactions are commonly associated with iron containing system, as it can accept or donate electrons according to oxidation potential of the reactant. Iron has a specific role in chlorophyll synthesis also. In onion seedlings treated with propachlor an increase in iron content has been observed (Pospisilova and Janyska 1980). However they have not commented on the chlorophyll content. Increased iron content observed in the present study is somewhat confusing as a decline in chlorophyll content has been observed in all the plants treated with glyphosate. A further probe regarding the iron accumulation is necessary.

Overall mineral budget in various plants under study has been well demonstrated in figs.7 and 8. In Cyperus rotundus (fig.7 'a') nitrogen, phosphorus, potassium and magnesium decreased while iron content increased due to glyphosate treatment. A marginal increase in calcium content on both the days after treatment was also observed. In Triticum aestivum (fig.7'b') decrease in nitrogen content and increase in calcium content was prominent on the X day after glyphosate treatment. One interesting feature was that, in this plant iron content was very low initially in both the control as well as sprayed plants but it increased notabally in the gyphasate treated plants.

The total content of nitrogen, magnesium, potassium was less in the crop as compared to the weed in control as well as treated plants, particularly during the initial phase of treatment.

In both the dicotyledonous plants studied calcium content was higher and increased slightly in Celosia and markedly is soybean due to herbicidal treatment (fig.8 a and b). A sharp decrease in magnesium on X day was also noteworthy in case of soybean. Nitrogen, phosphorus, and potassium also exhibited a prominent decline in soybean as compared to Celosia.

Thus glyphosate eaused a change in mineral content in all the plants. Scybean appeared to be very sensitive to the glyphosate treatment. Wheat showed a moderate sensitivity to the herbicide. Celosia was also moderately sensitive to the treatment and Cyperus exhibited a degree of resistance to the herbicidal treatment.

D. **Enzymes**

Metabolic prccesses are closely associated with various enzymes involved therein. Effect of glyphosate on some hydrolytic and other enzymes was analysed in the weeds and crops selected for the present study. Results are discussed in the following pages.

1. HYDROLYTIC ENZYMES

Activities of some hydrolytic enzymes in the leaves of glyphosate treated and control plants have been demonstrated in Table 15. In *Cyperus*, *Celosia* and soybean -amylase activity enhanced due to glyphosate on both the days of analysis. The increase observed was about twice as compared to the activity represented by the untreated plants. In wheat amylase activity was more influenced showing aout a 4-5 fold increase in the activity in the treated plants on both the days after treatment.

Glyphosate also influenced greately the enzyme protease in different plants. Mostly a two fold rise in protease activity was observed in *Cyperus*, *Celosia*, wheat and soybean on both the days after glyphosate treatment. In control plants the enzyme level exhibited a slight increase from the II to the X day whereas in plants treated with glyphosate the rise in the enzyme activity was more prominent.

Table 15 also depicts the effect of glyphosate on acid phosphatase activity in crops and weeds taken for study. An overall increase in the enzyme activity was observed in all the treated plants, due to glyphosate treatment, the response being different with respect to the plant. Maximum level of enzyme activity was detected in wheat followed by *Celosia* for the X day after treatment. In both these plants about 4-6 fold increase in the enzyme

Table 15 Effect of glyphosate on α-amylase, protease acid phosphatase activities in the leaves of Cyperus rotundus, Triticum aestivum, Celosia argentea and Glycine max

Species		α-amylase (DB units)		Protease (ug of tyr. $h^{-1}g^{-1}$ fr.wt.)		Acid Phosphatase (ΔOD $h^{-1}g^{-1}$ fr.wt.)	
		II day	X day	II day	X day	II day	X day
Cyperus rotundus	Control	5.21	6.20	13.586	16.630	1.40	1.80
	Sprayed	9.00	10.06	18.158	34.920	4.40	5.00
Triticum aestivum	Control	1.33	1.90	4.442	5.960	1.00	1.00
	Sprayed	6.66	7.50	7.530	12.060	4.00	6.60
Celosia argentea	Control	3.63	4.34	4.442	5.900	1.00	1.20
	Sprayed	7.77	8.57	10.530	13.580	4.60	6.40
Glycine max	Control	5.88	7.36	9.010	12.060	1.80	3.40
	Sprayed	10.00	14.540	18.160	33.390	3.80	5.60

Values are mean of three determinations

activity was recorded. In Cyperus, acid phosphatase activity was enhanced three times as compared to the control on both the days of analysis. Enzyme activity in soybean increased twice as compared to the control and then reduced on X day of analysis.

Hydrolytic enzymes are present mostly in the lysosomes of plant cell. Which are broken down during stress condition releasing these enzymes. (Silva 1976). These enzymes catalyse, hydrolysis of cell metabolites like, starch, proteins and phosphate esters. Available literature regarding the herbicidal influence on the behaviour of these enzymes is scanty. Saeed and Duke (1990) have reported large increase in certain amylases in pea seedlings due to norflurozon-induced bleaching. As -amylase catalises random hydrolysis of 1-4 glucosidic linkages in both the starch components, a decrease in starch level accompanied with an increase in the reducing sugars has been recorded in glyphosate treated plants (Table 8 and 9). Thus the degradation of starch can be correlated with the increased α-amylase activities in the glyphosate treated plants.

Protease activity reflects the breakdown of proteins by the enzyme. A remarkable decrease in protein content has been recorded in our study (Table 10) which may be associated with the increased activity of protease due to glyphosate treatment. Similarly activity of acid phosphatase was also stimulated by the glyphosate treatment in the present study. The treatment of herbicide glyphosate might have imposed a general stress causing an increase in the levels of hydrolytic enzymes.

2. NITRATE REDUCTASE AND NITRITE REDUCTASE

Influence of glyphosate on the activity of nitrate reductase and nitrite reductase in various plants has been presented in Table 16. The levels of both these enzymes of nitrogen metabolism were found affected due to the herbicidal treatment. In the untreated plants the activity of NR was maintained more or less to the same level on the II as well as X day in *Cyperus*, *Celosia* and soybean and in wheat it was slightly reduced. Whereas a marked decrease in the enzyme activity was observed in all the plants treated with glyphosate. The reduction was more than half for the II day after treatment in case of wheat, soybean and *Celosia*. The enzyme activity declined further to one third in these plants on the X day after treatment. In *Cyperus* a one third and one fourth reduction was detected in NR activity respectively on II and X day of analysis.

Glyphosate also inhibited NIR activity in all the treated plants, the effect being more visible on the X day after treatment. In wheat, soybean and *Celosia*, the NIR activity was less affected on the II day as compared to the *Cyperus* where the activity was reduced on X day. In

Table 16 Effect of glyphosate on nitrate and nitrite reductase activities in the leaves of *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species		Nitrate reductase (μg of NO_2 formed $hr^{-1}g^{-1}$fr.wt.)		Nitrite reductase (μg of NO_2 reduced $hr^{-1}g^{-1}$fr.wt.)	
		II day	X day	II day	X day
Cyperus rotundus	Control	25.075	26.550	545.75	472.00
	Sprayed	7.375	5.900	265.50	177.00
Triticum aestivum	Control	23.600	18.437	501.50	324.50
	Sprayed	13.275	5.162	354.00	147.50
Celosia argentea	Control	25.812	23.600	560.50	531.00
	Sprayed	11.800	7.375	324.50	206.50
Glycine max	Control	28.025	26.550	767.00	472.00
	Sprayed	11.062	7.375	442.50	118.00

Values are mean of three determinations

soybean a maximum decrease in the enzyme activity was observed on the X day when the enzyme activity was found almost one fourth as compared to the control. In Cyperus about one third reduction whereas in wheat and Celosia more than one half decrease in the activity was recorded on the X day of analysis.

Nitrate reductase is the important enzyme for incorporation of Nitrate into amino acids. The enzyme is a molybdoflavoprotein and requires energy in the form of NADH or NADPH for its activity. Many external and internal factors such as the content of nitrate, availability of reducing power etc. affect the NR activity. During water stress NR activity gets reduced with an increase in the nitrate levels in leaf tissue (Ray and Sisson 1986). Irena and Duczek (1984) have studied the influence of simazine on nitrate uptake and NR activity in wheat seedlings. They found that simazine reduces nitrate uptake and the activity of NR. Munoz-Rueda et al. (1986) have also reported a reduction in NR and NIR activity in Medicago sativa and Trifolium pratense subjected to various glyphosate concentrations.

Our results also indicate the same influence of glyphosate on various weeds and crops studied. A decline in the nitrogen content has already reported in these plants (Table 13) indicating a reduced uptake of nitrate due to the herbicidal treatment. A decline in the substrate level can affect the NR activity in the glyphosate

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treated plants as compared to the untreated ones. As NR activity is inhibited, the amount of NO_2 produced is also decreased thereby causing a reduction in NIR activity.

Nitrate reductase is chloroplastic enzyme and is dependent on photosynthetic electron transfer for ferredoxin which is used as a reductant. Inhibitors of photosynthetic electron transport therefore can stop or reduce the reduction of nitrite to ammonium ion causing toxic accumulation of their levels. Herbicides which inhibit photosynthetic process cause a rapid accumulation of nitrate via this mechanism (Klepper 1988, 1974, 1975, 1976; Fedtke 1977). None of the herbicides has been reported to have significant direct effect on nitrate reductase. However they indirectly affect and inhibit photosynthesis through secondary effect and cause nitrate accumulation as tertiary effect. Thiobencarb has been known to cause nitrite accumulation in Barnyard grass (Echinochloa sp.) leaves which is associated with inhibition of photosynthesis followed by a decline in nitrite reductase activity. (Prakash et al. 1989).

Inhibition of carotenoid synthesis generally causes a complete absence or loss of extractable nitrate reductases. (Deane-Drummond and Johnson 1980; Schuster et al. 1988). Gluphosinate has also been reported to inhibit nitrate reductase by inhibiting uptake of nitrate (Trogisch et al. 1989). Uptake of nitrate as well as other anions is inhibited due to direct depolarisation of the plasmalemma and to the accumulation of ammonium ion which results in inhibition of NIR activity. This was observed by Lacuesta *et al.* (1990) in gluphosinate treated alfalfa. According to Munoz-Rueda *et al.* (1986) decreased transpiration caused by herbicide can limit nitrate uptake and ultimately can reduce nitrate induced nitrate reductase.

In the present study reduced transpiration rate, decreased carotenoid synthesis caused by glyphosate treatment might have reduced the nitrate uptake and also NR and NIR enzyme levels. Thus the herbicide seems to affect the overall nitrogen matabolism, in the crops as well as weeds selected for the study. The results also indicate susceptible nature of soybean to the herbicidal treatment.

3. POLYPHENOL OXIDASE AND IAA OXIDASE

Effect of glyphosate on the oxidising enzymes has been presented in Table 17. Glyphosate stimulated the activity of polyphenol oxidase as observed on both the days of analysis. A maximum increase in the activity was exhibited by soybean two and half times when compared with control plants. In *Cyperus* a two fold rise on the II day after treatment was observed which was slightly decreased on the X day. In wheat and *Celosia* the rate of enhancement of enzyme activity was more or less equal and it was lower as compared to soybean and *Cyperus*.

Polyphenol oxidase is localised on thylakoid of chloroplast and in vesicles of other bodies in non green

Table 17 Effect of glyphosate on polyphenol oxidase and IAA oxidase activities in the leaves of *Cyperus rotundus*, *Triticum aestivum*, *Celosia argentea* and *Glycine max*

Species		Polyphenol oxidase (ΔOD hr^{-1} g^{-1} fr.wt.)		IAA oxidase (mg of IAA broken down hr^{-1}g^{-1} fr.wt.)	
		II day	X day	II day	X day
Cyperus rotundus	Control	0.100	0.166	25.00	30.00
	Sprayed	0.200	0.266	45.00	82.50
Triticum aestivum	Control	0.133	0.133	17.50	20.00
	Sprayed	0.166	0.200	45.00	62.50
Celosia argentea	Control	0.133	0.166	15.00	15.00
	Sprayed	0.200	0.233	77.50	82.50
Glycine max	Control	0.100	0.133	22.50	22.50
	Sprayed	0.266	0.333	57.50	75.00

Values are mean of three determinations

plastids (Vaughn and Duke 1988). According to Butt (1985) oxygenases are involved in the hydroxylation of many phenolic compounds in higher plants. As PPO enzymatically carries out many of these reactions *in vitro* it has been assumed that it was involved in synthesis of these compounds *in vitro* A correlation between the presence of high levels of phenolic compounds and PPO or low levels of phenolic compounds and no PPO may be observed. (Kogima and conn 1982, Vaughn and Duke 1981, Bajaj *et al*. 1985). These correlations however do not always exhist as reported by Müller and Beckman (1978). In our results a high total polyphenol content has been recorded during the glyphosate treatment in various plants (Table 7) which can be positively correlated with a high PPO in these plants. In the untreated plants both the polyphenol content and the enzyme are lower than in the glyphosate treated plants. This suggests a stimulation in the PPO activity in the plants sprayed by glyphosate.

The effect of glyphosate on the synthesis of secondary compounds such as phenols, are well studied. However reports regarding PPO activity and its correlation with phenolics has not reported. Secondary phenolic compounds in developing tissues increase usually in the light (Creasy and Zucker 1974) and this increase is always accompanied by an increased PAL activity (Duke and Naylor 1974, 1976). Further glyphosate has been shown to stimulate PAL activity in light and dark grown soybean seelings

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(Duke *et al*. 1979 and Hoagland *et al*. 1979). Thus glyphosate affects significantly the secondary metabolism of the plants, Our results support these findings.

Enzyme activities of IAA oxidase in the various glyphosate treated and untreated plants have been depicted in Table 17. Glyphosate induced an increase in this enzyme in both the weeds and the crop plants analysed on the II and X day after treatment. In *Celosia* IAA metabolism was maximum as indicated by about five-fold increase in IAA exidase activity over the control. In soybean and wheat metabolic degradation of IAA as represented by the enzyme was at a medium level as compared to other plants. *Cyperus* exhibited the least stimulation in the oxidase activity by glyphosate treatment.

Glyphosate though acts by disrrupting shikimate pathway it is known to cause some influence on harmonal level in the plants also. In rapidly translocated herbicides disrruption of apical dominance occures at sublethel doses of herbicides (Parkar 1975, Lee 1984). Glyphosate has been reported to increase the IAA content in *Cyperus esculentus* (Canal *et al*. 1987). In soybean and pea it reduced free levels of indogenous IAA (Lee 1984). Increased metabolism of IAA in glyphosate treated tissues has been attributed to changes in IAA oxidase due to altered phenolic content (Lee 1984, 1982)). Further IAA oxidase activity can be inhibited or stimulated by different phenolic compounds and *in vitro* activity will be influenced by many phenolic compounds. (Lee *et al*. 1982, Grambow and Langenbeck-Schwich 1983). A positive correlation among the plants has been reported by Lee and Dumus (1985) between rates of IAA metabolism in untreated tissue and tolerance to glyphosate. According to the authors the plants having high rates of IAA metabolism might be less dependent on IAA and thus less affected by reduction in IAA caused by glyphosate. Glyphosate has also been reported to influence conjugation of IAA in the treated tissues (Lee 1982b, Lee *et al*. 1983). Thus absolute and relative rates of IAA oxidase, IAA conjugation activities and the content and type of phenolic compounds, all these may have an effect on IAA levels in the glyphosate treated plants which is complex and sometimes conflicting also.

Our results indicate an increased metabolism of IAA in glyphosate treated plants than in untreated plants. Further increased levels of both oxidases in soybean are suggestive of sensitive nature to the herbicidal treatment.

E. Residual analysis of glyphosate

The residual content of glyphosate in various crop and weed plants was analysed after II and X day after the treatment. The leaf material was harvested on the respective days and extracted in acetonitrile for TLC analysis. The results are demonstrated in plates VII and VIII.

Photoplate VII showing

Residual analysis of glyphosate in the leaves of

1. *Cyperus rotundus*

2. *Triticum aestivum*

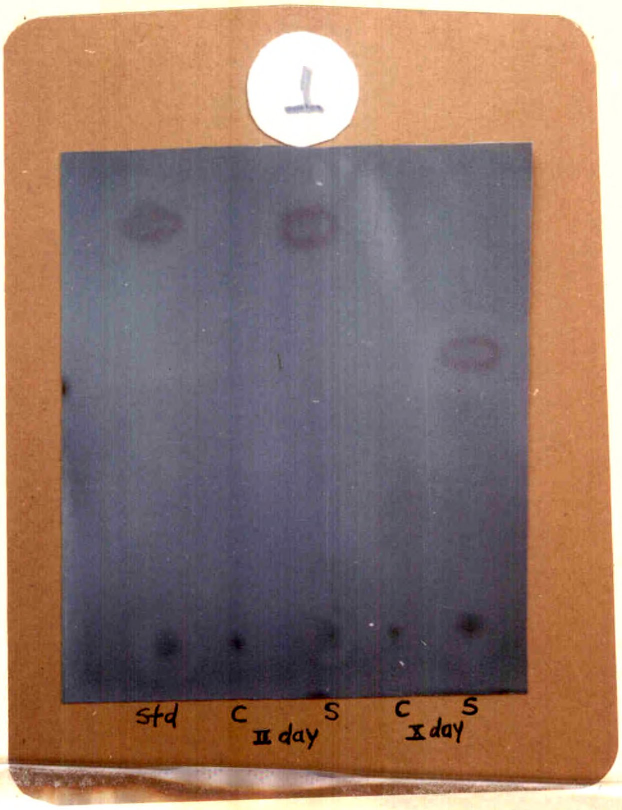
Photoplate VIII showing

Residual analysis of glyphosate in the leaves of

3. Celosia argentea

4. Glycine max

PLATE - VII



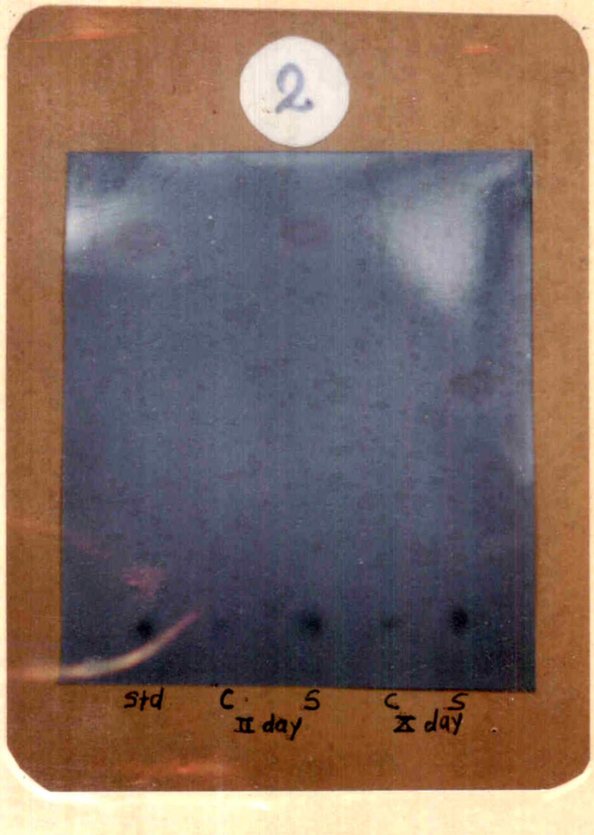
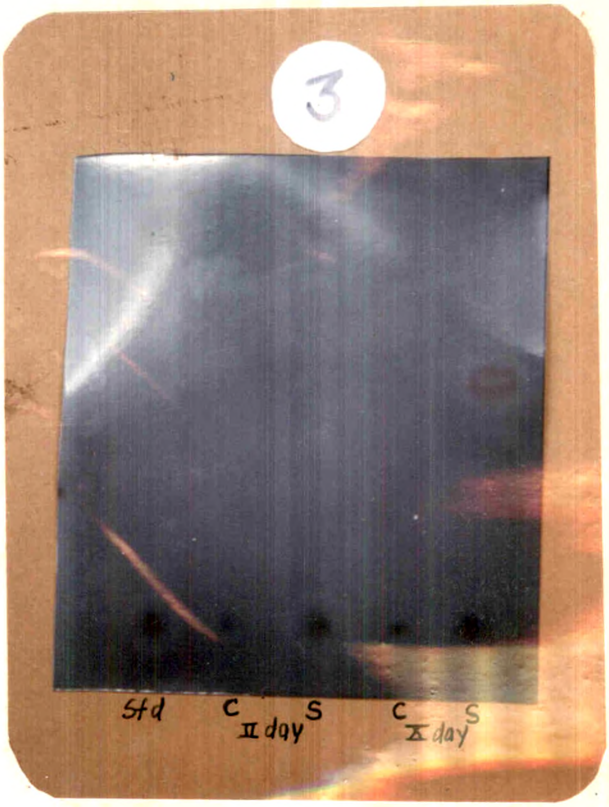
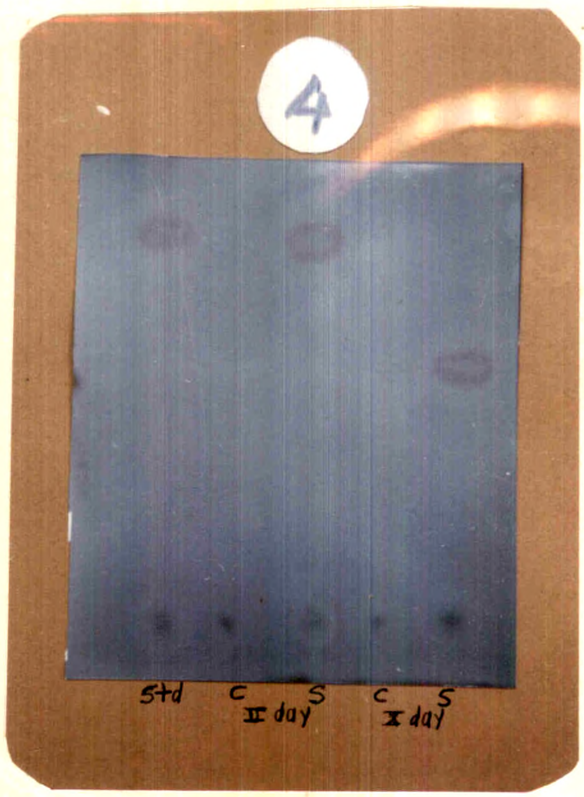


PLATE - VIII





In all the treated plants the residue of glyphosate was detected on TLC plates for both the II as well as X day after treatment. The Rf values recorded for the residual bands observed on the II day of analysis were found very close to the standard Rf values of glyphosate. This was noticed in all the glyphosate treated plants. The residual analysis of X day also revealed the presence of the herbicide, however the Rf values were much less as compared to those of standard glyphosate. The lower Rf values suggest a decressed rate of migration of glyphosate residue over the TLC plates. This may be due to modification or change in the herbicidal structure caused by the plant itself. The persistance of residue was clearly visible even ten days after the treatment of glyphosate.

The phosphate bond present in the glyphosate is of the phosphonate type and can not be easily split up by hydrolysing enzymatic reactions. Therefore metabolic degradation of glyphosate by most of the higher plants does not take place or is very slow (Duke 1988). Several soil micro-organisms have been reported to degrade and use glyphosate as a sole phosphorus source (Moore et al. 1983, Kishore and Jacob 1987, Jacob et al. 1988). In Equisetum sp. glyphosate is metabolised to AMP (Marshall et al. 1987). However in higher plants metabolic degradation of glyphosate is not possible.

The metabolism of herbicides in plants determines the herbicidal selectivity among weed and crop plants. Generally a crop plant or a tolerant weed can detoxify the herbicide at a faster rate to avoid its accumulation to phytotoxic levels in the tissue. This process involves removal of the herbicide by conjugation, detoxification, deposition etc.

Our results of residual analysis clearly indicate an accumulation of herbicide even on the X day after treatment in both the crops and weeds studied. The amount of herbicide left over may be different in the crops and weeds and requires a quantitative analysis of the residue. But it has negatively influenced the growth and metabolism in all the plants irrespective of the crop or the weed.

Thus glyphosate was found to persist in crops and weeds, affecting contents of various cell constituents and metabolic processes as discussed earlier. The degree of tolerance varied in the crops and weeds. Soybean being more susceptible as compared to wheat. Cyperus exhibited some degree of tolerance and Celosia was less sensitive than soybean to the herbicidal treatment.