development, however, was considerable with potassium periodate.

Total and exchangeable Mn,[12,13] in a large number of samples of *Lathyrus* field soils were satisfactorily estimated by this wet-ashing procedure followed by oxidation with ammonium persulphate. In the case of soils, however, filtration during sample preparation was necessary.

This procedure was usefully employed for Mn estimations in various crop plants lathyrism endemic areas in Central In tissues of experimental animals fed La diet, and in sera, CSF fluids and fecal samples of lathyrism patients.

[Current Science

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INFLUENCE OF SIMAZINE ON CHLOROPLAST METABOLISM

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A CHLOROPLAST, the site of photosynthesis, acts as energy capturing, storing and transferring device in carbon assimilation. The photosynthetic pigment, the chlorophyll and carotenoids, are concentrated within the dense lamellæ of the grana containing chloroplasts and in the lamellar chloroplasts. In addition to these pigments it consists of protein and lipid layers. It has been reported that simazine [2-chloro-4, 6-*bis* (ethylamino)-*s*-triazine] a selective herbicide inhibits the photochemical activity of isolated chloroplasts.[4-6] Ashton *et al.*[2] reported the destruction of chloroplast structure as the result of atrazine treatment. As no result is available to indicate the changes in the concentration of chlorophyll and protein of the chloroplast the present investigation was undertaken to study the influence of simazine on the chlorophyll and protein metabolism in this very important organelle of the plant.

Methods and Materials

In this investigation, seminole variety of oats (*Avena sativa* L.) which is susceptible to simazine was grown in the acid washed sand treated with 2 ppm of simazine with the use of Hoagland-Arnon complete nutrient solution. The plants were grown under controlled temperature, photoperiod and light intensity for 12 days before their desiccation.

Determination of Chlorophyll.—Total chlorophyll, chlorophyll *a* and chlorophyll *b* of simazine treated and control plants were determined by the method of Association of Official Agricultural Chemists.[3] In all four samples in three replications were collected at two-day intervals starting six days after the treatment. The following equations were used to determine these chlorophylls:

Total chlorophyll (mgm per litre) $= 7 \cdot 12\ A\ 652 + 16 \cdot 8\ A\ 634 \cdot 5$

Chlorophyll *a* (mgm per litre) $= 9 \cdot 93\ A\ 652 - 0 \cdot 777\ A\ 634 \cdot 5$

Chlorophyll *b* (mgm per litre) $= 17 \cdot 6\ A\ 634 \cdot 5 - 2 \cdot 81\ A\ 652$.

where A = Absorbance.

Estimation of Chlorplast Protein.—Triplicate samples of plants from simazine and control treatments were collected at two-day intervals starting six days after treatment. The tissue was homogenized in $0 \cdot 5\ M$ glucose, $0 \cdot 02\ M$

magnesium chloride and 0·02 *M* phosphate buffer at pH 7·0. The homogenate was filtered through cheese cloth. The filtrate was centrifuged at 2,000 × g. for ten minutes to sediment chloroplasts. The protein in this fraction was precipitated by 5% cold TCA. The chlorophyll and lipids were removed by washing the protein pellet several times with undiluted acetone. The protein was dissolved in 0·01 *M* NaOH and determined by the method of Lowry *et al.*[5]

TABLE I

Changes in chlorophyll and chloroplast protein content of oat shoots caused by simazine treatments

Days after treatment	Simazine concentration (ppm)	Chlorophyll (mgm./gm. fr. wt.)		Chloroplast protein (mgm./gm. fr. wt.)
		Chlorophyll *a*	Chlorophyll *b*	
6	0	12·67	3·80	3·96
	2	7·81	2·33	2·85
3	0	1[illegible]·18	5·13	4·69
	2	7·01	2·52	2·47
10	0	1[illegible]·60	5·24	5·47
	2	6·32	2·55	2·19
12	0	13·43	6·62	4·88
	2	4·42	1·67	1·90

EXPERIMENTAL RESULTS

The concentration of chlorophyll *a* and *b* in the control plants increased upto eight days of the growth period, and decreased in later samples. Chlorophyll *b* increased in all the samples studied. On the other hand, the chlorophyll determinations in the simazine treated plants showed that chlorophyll *a* and chlorophyll *b* were drastically reduced as the age of plant advanced. In the last sample, the degree of reduction in chlorophyll *b* was more intense than in the chlorophyll *a* content.

The concentration of chloroplast protein increased in control plant samples collected upto 10 days after the treatment. The chloroplast protein content decreased as the period from simazine treatment progressed. The maximum reduction in protein content due to simazine treatment was observed in the later two samples.

DISCUSSION

A marked influence of simazine was observed on the chlorophyll contents of oat plants. These findings have revealed that in the earlier period of simazine toxicity chlorophyll *a* was reduced more than chlorophyll *b* but the results were reversed in the later samples. The observations of Schneider[7] demonstrate qualitative changes in the chlorophyll contents in simazine-treated plants. Ashton and Bisalputra[1] have also reported the reduction in the amount of *Chlorella* chlorophyll. The findings of this investigation clearly demonstrate the inhibition in chlorophyll *a* content in the earlier period of phytotoxicity.

Inhibition in the synthesis of chloroplast protein is obvious from the findings of this experiment. Therefore, on the basis of these results it may be postulated that simazine inhibits protein synthesis in the seat of synthesis —the chloroplast organelle. This may be the reason for the findings of Ashton *et al.*[2] that atrazine alters the chloroplast structure. The disintegration of chloroplast structure as the result of inhibition in protein synthesis from simazine toxicity may be the probable reason for reduction in the chlorophyll content.

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