Interaction of Herbicides with Pea Chloroplasts

K. N. Singh, J. Prakash, A. K. Agarwal, and G. S. Singhal
School of Life Sciences, Jawaharlal Nehru University, New Mehrauli Road, New Dehli-110067, India

Z. Naturforsch. **39 c**, 464–467 (1984); received January 20, 1984

Pea, Chloroplast, Herbicides, Metribuzin, Pyrazon, Triazole

We have studied the effect of three herbicides viz., triazole, pyrazon and metribuzin on total chlorophyll, carotenoids and cell protein in leaves, and PS II and PS I activity in the chloroplasts isolated from control and herbicide treated pea plants. Pyrazon and metribuzin at 10 μM concentration affect these parameters resulting in the chlorosis of the leaves.

Introduction

Herbicides not only kill the weeds but also affect cell metabolism of economic crops, specifically their photosynthetic machinery [1]. They interfere with the development and structure of chloroplasts, thereby influencing light reactions of photosynthesis [2]. We report in this communication on the interaction of pyrazon, triazole and metribuzin with pea chloroplasts during greening in terms of morphological changes and photochemical activities.

Materials and Methods

Pea (*Pisum sativum* var. Bonneville) seeds were soaked in water for 24 h and germinated on water soaked filter sheets. The seedlings with 1 cm long roots were transferred to petri plates containing half strength Hoagland solution, and kept in dark for 5 days before they were transferred to continuous light (2200 lux) for further growth. For treatment with herbicides, seedlings were transferred to various herbicide solutions of desired concentration in half-strength Hoagland solution one day prior to their transfer to continuous light (4 days old seedlings in dark). The herbicide concentration was maintained by keeping a constant level of the solution in the petri plates.

Chloroplasts from the leaves were isolated essentially according to [3]. These chloroplast preparations were mostly of Type C according to the classification of Hall [4]. Hill activity was determined by measuring the photoreduction of ferricyanide as described [5]. Oxygen evolution by isolated chloroplasts was assayed using a Clark type oxygen electrode according to [6]. Measurement of electron transport from ascorbate DCPIP to MV was assayed polarographically using O_2 electrode as described in [7]. Protein was determined after [10].

Results

Figure 1 shows the effect of the herbicides on the morphology of pea leaves. Leaves showed chlorosis within 5 to 7 days of herbicide treatment, however, chlorosis appeared different with different herbicide. Chlorosis started from the margin of lamina in triazole-treated seedlings, while it appeared from the midribs in the case of pyrazon. Although the youngest leaf became fully white with pyrazon and triazole treatment, the first four leaves never showed any sign of chlorosis. The entire seedling became pale when treated with 10 μM metribuzin.

Pigment content

Chlorophyll *a*, chlorophyll *b* and carotenoids of control and herbicide-treated plants were measured after 6 and 9 days of growth in continuous light (the period when the leaves showed sign of chlorosis). There is virtually no difference in the pigment concentration of the leaves grown in presence of triazole. However, pyrazon significantly reduced Chl *a*, Chl *b* and carotenoid content of the leaf. Although, leaves growing in 10 μM metribuzin showed severe sign of chlorosis, it affected Chl *a* and carotenoids only and not Chl *b* (Table I).

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; metribuzin, 4-amino-6-isopropyl-3-methyl-thio-1,2,4-triazin-5-one; MV, methyl viologen; PS, photosystem; pyrazon (chloridazon), 5-amino-4-chloro-2-phenylpyridazine-3-one; triazole, aminotriazole.

0341-0382/84/0500-0464 $ 01.30/0

Table I. Effect of herbicides on pigment content of pea leaves[a].

Treatment	Light periods (days)					
	6			9		
	Chlorophyll[b]		Carotenoids[b]	Chlorophyll[b]		Carotenoids[b]
	Chl *a*	Chl *b*		Chl *a*	Chl *b*	
Control	2.60	0.85	6.1	2.18	0.74	5.7
Triazole (10 μM)	2.40	0.83	6.1	2.04	0.70	6.3
Metribuzin (10 μM)	2.10	0.81	5.8	1.56	0.88	4.4
Pyrazon (10 μM)	1.72 (34)	0.55 (35)	4.1 (33)	1.17	0.39	2.9

[a] The plants were grown and treated with herbicides as described in Methods. Leaves were harvested after 6 or 9 days in light. Chlorophyll and carotenoids in 80% acetone were measured according to [8, 9]. Figures in brackets indicate percent reduction vs. control.
[b] mg/g fresh wt. of the leaf.

Fig. 1. Effect of different herbicides (10 μM) on the development of pea leaves. The plants were grown for 5 days in dark as described in the Methods and transferred to continuous light for 8 days. (a) control, (b) pyrazon, (c) triazole, (d) metribuzin.

Total leaf protein

Total cell protein is considered as the indication of the growth conditions of the cells. Thus, we measured total leaf protein in control and herbicide-treated 14 days (5 days dark + 9 days light) old pea leaves. All the three herbicides inhibited total leaf protein (Table II).

Table II. Effect of herbicides on total leaf protein.

Treatment	Leaf protein (mg/g fresh wt)
Control	16.1
Triazole (10 μM)	10.5
Metribuzin (10 μM)	10.4
Pyrazon (10 μM)	10.3

Leaves were harvested from the seedlings grown for 9 days in light as described in Methods.

Photochemical activities

Effect on photosystem II (ferricyanide reduction and oxygen evolution) and photosystem I in the chloroplasts isolated from control and triazole and metribuzin treated seedlings was measured. The data for ferricyanide reduction and oxygen evolution are shown in Fig. 2a and 2b. Metribuzin at a concentration higher than 2.5 μM significantly affected both ferricyanide reduction and oxygen evolution, compared to the control. Lower concentrations of metribuzin (2.5 μM and lower) stimulate ferricyanide reduction by 20–35 per cent and oxygen evolution by 18–32 per cent. Triazole at all the concentrations tested did not inhibit either ferricyanide reduction or oxygen evolution.

Discussion

Several papers have appeared in the literature describing the effects of herbicides on the plants.

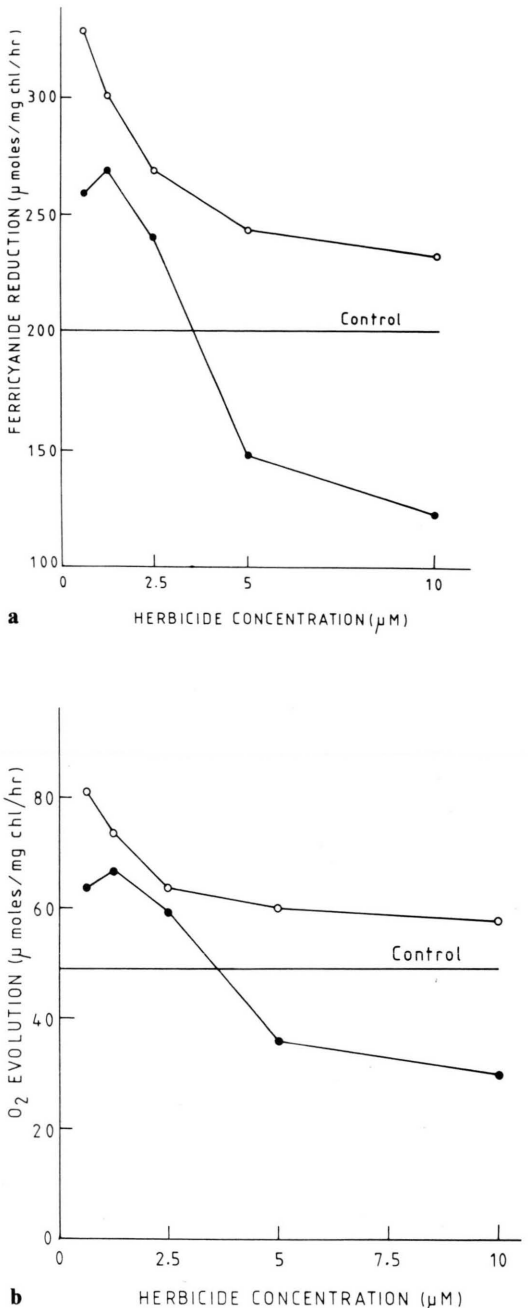

Fig. 2. a) Ferricyanide reduction, b) oxygen evolution in chloroplasts isolated from control and treated plants. The plants were grown and treated with herbicides as described in Methods. Chloroplasts were isolated after 9 days in light. (○), triazole; (●), metribuzin.

Most of these reports describe the effects with isolated chloroplasts. It is possible that their effects on the crop plant *in vivo* are different than observed with isolated chloroplasts. Thus, we have studied the effect of three different herbicides (metribuzin, pyrazon and triazole) on morphology, pigments and protein content of the leaf and photochemical activities of the chloroplasts isolated from the leaves grown in presence of herbicides.

There is a significant decrease in Chl *a*, Chl *b* and carotenoid content of the leaves growing in presence of 10 μM herbicide specially with metribuzin and pyrazon (Table I). This is reflected as a sign of chlorosis in treated seedlings (Fig. 1). Effect of these herbicides on chlorophyll content of the leaf were noticed within 12 h of transferring the plants to light (data not shown). This suggests that these herbicides also affect chlorophyll, carotenoids and leaf protein accumulation besides acting as electron transport inhibitor [11]. Effect on the accumulation of chloroplast pigments by triazole and pyrazon [12] and by DCMU and many other herbicides have been shown earlier [13]. Spectral characteristics of chloroplasts isolated from 14 days old control and herbicide-treated leaves were very similar indicating that overall integrity of the chloroplasts has not been changed. We do not know uptake and accumulation rate of these herbicides in our system. Difference in transport rate to the leaves would perhaps account for the difference of chlorotic symptoms [14].

It was interesting to observe that while PS II activity was significantly affected (Figs. 2a, 2b), there was no change in PS I activity of the chloroplasts isolated from the herbicides treated leaves (data not shown). This observation is similar to earlier observation made by many others who demonstrated that most of the herbicides are inhibitors of PS II activity and this is because there are reception sites present in PS II [15]. We do not understand why triazole-treated seedlings show chlorosis, while it does not inhibit either accumulation of pigments or PS II activity. Grumbach and Bach [16] also found that though San 6706 or amitrole induced chlorosis, chlorophyll and carotenoid biosynthesis was not inhibited.

[1] D. E. Moreland, Annu. Rev. Plant Physiol. **31,** 597 to 638 (1980).
[2] D. E. Moreland and J. L. Hilton, Herbicides: Physiology, Biochemistry, Ecology (L. I. Audus, ed.), **Vol. 1,** pp. 493–523. Academic Press, New York and London 1976.
[3] U. Prasad, Ph.D. thesis, 1977, Jawaharlal Nehru University, New Dehli, India.
[4] D. O. Hall, Nature **235,** 125–126 (1972).
[5] M. Avron, Biochim. Biophys. Acta **40,** 257–272 (1960).
[6] J. S. Boyer and B. L. Bowen, Plant Physiol. **45,** 612 to 615 (1970).
[7] G. Kulandaivelu and H. Senger, Physiol. Plant. **36,** 157–164 (1976).
[8] D. I. Arnon, Plant Physiol. **24,** 1–15 (1949).
[9] S. Liaaen-Jensen and A. Jensen, Methods in Enzymology (A. San Pietro, ed.), **Vol. XXIII,** 586–602 (1971).
[10] A. N. Misra and U. C. Biswal, Photosynthetica **16**(1), 22–26 (1982).
[11] A. Trebst, Proc. Fifth Int. Cong. Photosynthesis (G. Akoyunoglou, ed.), BISS, Philadelphia, **Vol. VI,** 507–520 (1981).
[12] G. Sandmann, K. J. Kunert, and P. Böger, Z. Naturforsch. **34c,** 1044–1049 (1979).
[13] K. H. Grumbach, Proc. Fifth Int. Cong. Photosynthesis (G. Akoyunoglou, ed.), BISS, Philadelphia, **Vol. VI,** 625–636 (1981).
[14] C. Fedtke, Pest. Sci. **4,** 653 (1972).
[15] J. J. S. Van Rensen, Physiol. Plant. **54,** 515–521 (1982).
[16] K. H. Grumbach and T. J. Bach, Z. Naturforsch. **34c,** 941–943 (1979).