and Therapeutical Activity, Springer-Verlag, Berlin. 1977, p. 209.
10. Ramwell, P. W., Sherratt, H. S. A. and Leonard, B. E., In: *Biochemistry of Phenolic Compounds*, (ed.) J. B. Harborne, Academic Press, London, 1964, p. 493.
11. Suter, C. M. (ed.), *Medicinal Chemistry*, John Wiley & Sons, London, 1951, vol. 1, pp. 232.

INHIBITION OF PHOTOSYNTHESIS BY OXYFLUORFEN

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OXYFLUORFEN, a diphenyl ether (DPE) group herbicide, has been found to be very effective against broad- and micro-leaved weeds[1,2]. The mechanism of herbicidal injury induced by oxyfluorfen is still unclear. Most of the DPE herbicides have a light-dependent mode of action but some do not[3,4]. Oxyfluorfen requires light for herbicidal action[4,5] and the evidence suggests that the photo-oxidative damage caused by oxyfluorfen involves photosynthetic electron transport[6–8]. Data in support of oxyfluorfen action independent of photosynthetic electron transport are also available[9–11]. Recently, Haworth and Hess[11] have shown that oxyfluorfen-induced herbicidal injury by generation of singlet oxygen is independent of photosystem I (PS I) electron transport, in agreement with the previous findings on the requirement of oxygen for DPE herbicidal activity[12,13].

In the present communication, we report that photo-oxidative damage to chloroplast membranes is due to the inhibition of photosystem II (PS II) electron transport by oxyfluorfen, which may involve binding of oxyfluorfen to pigment complex(es) or any other component of the membrane.

Seeds of *Oryza sativa* (var. CSR-4) obtained from the College of Agriculture, Indore, were soaked in water for 24 h and grown under laboratory conditions in plastic containers. Oxyfluorfen (1, 3, 5 and 7 ppm) was sprayed on 15-day-old rice plants, and studies were carried out every alternate day.

Chloroplasts were isolated from freshly cut leaves of rice plants according to Karabourniotis *et al.*[14] in ice-cold isolation medium containing 400 mM sucrose, 20 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$, 10 mM KCl, 150 mM NH_4Cl, 2 mg/ml BSA (fraction V) and 4 g/l polyvinylpyrrolidone (PVP-10). The homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged for one min at 3000 *g*. The supernatant was discarded and the pellet was suspended in minimal volume of isolation medium, but without PVP.

Chlorophylls were extracted in cold 80% acetone under dim green light and the amount of chlorophylls in the extract was estimated according to Mackenny[16].

Heat treatment of spinach chloroplasts (to destroy water splitting complex) was carried out by incubating chloroplasts at 48°C for 4 min and thereby stopping electron flow to PS II. Diphenylcarbazide (DPC) was used (final concentration 0.5 mM) as donor of electrons on oxidizing side of PS II.

Type C spinach chloroplasts were used in experiments to study the mode of action of oxyfluorfen and to calculate I_{50} values at different chlorophyll concentrations. For other experiments type C rice chloroplasts were used.

The photoreduction of 2,6-dichlorophenol indophenol (DCIP) (PS II activity, $H_2O \rightarrow DCIP$) was measured spectrophotometrically (Shimadzu model UV–VIS 160) according to Mohanty *et al.*[17] The chloroplasts were illuminated for 30 sec with saturating white light (50 W/m^2). The incident beam was passed through a water filter to cut off the infrared radiation. The reaction mixture in a final volume of 3 ml contained chloroplasts equivalent to 10 μg chlorophyll/ml, 20 μM DCIP, 20 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$ and 5 mM NH_4Cl. The photoreduction of DCIP was measured spectrophotometrically at 605 nm and the rate of DCIP reduction was calculated using an extinction coefficient of 21 mM^{-1}.

Spraying of oxyfluorfen resulted in visible chlorosis, curling of leaf and appearance of irregular burnt patches. The visible injury was apparently more at higher concentrations (5 to 7 ppm) of oxyfluorfen. The effect of different concentrations of oxyfluorfen on total chlorophyll content of rice leaves is shown in figure 1. At low concentration of oxyfluorfen (1 ppm), there was a slight decrease in chlorophyll content up to 24 h after spray, followed by recovery. However, at higher concentrations of oxyfluorfen, there was no recovery but a further decrease. There was about 80% decrease in total chlorophyll content of leaves of rice plants sprayed

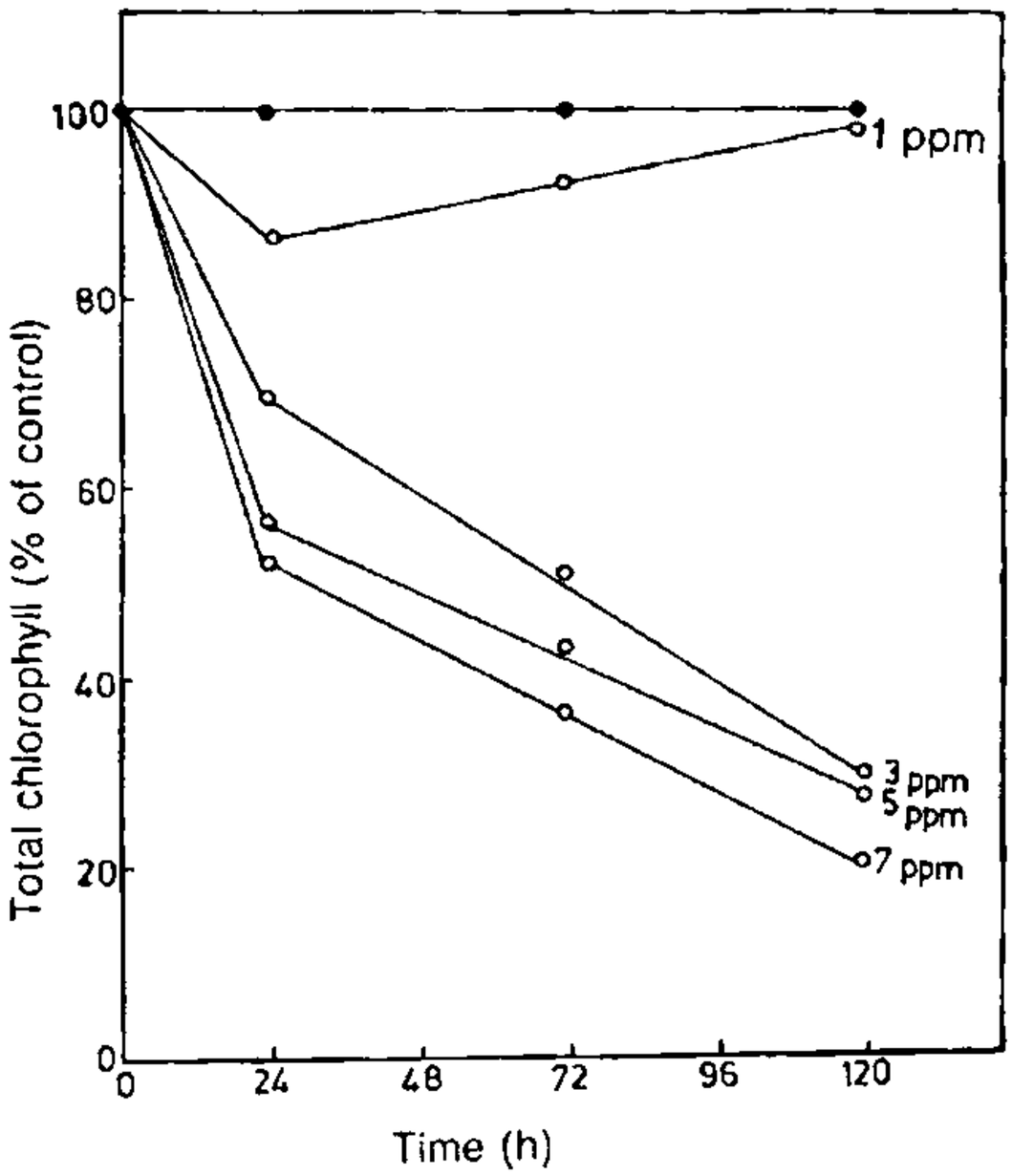

Figure 1. Effect of oxyfluorfen on total chlorophyll content (○) of rice plants (100% (control, ●) is equal to 1.734 mg total chlorophyll per/g fresh wt).

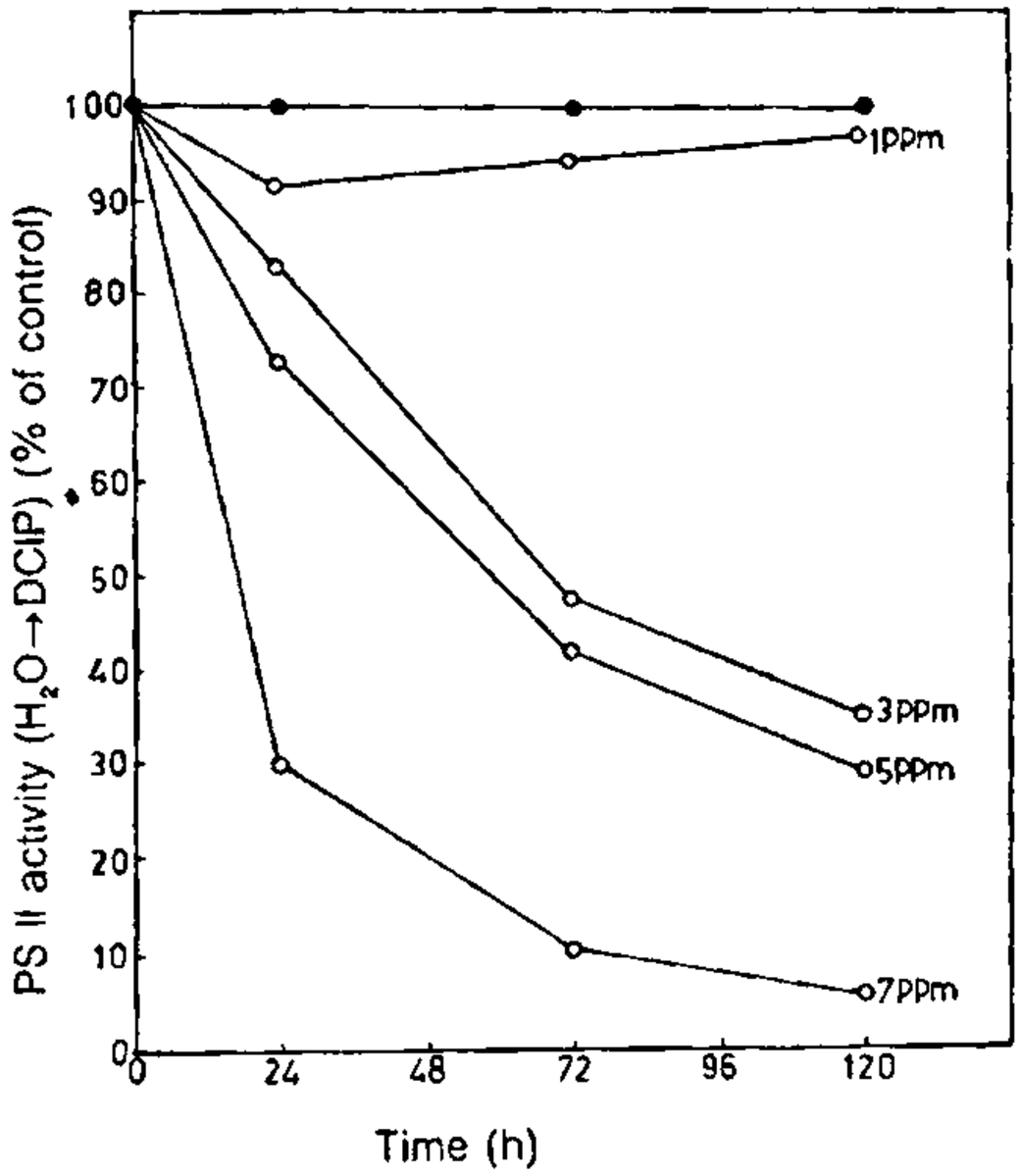

Figure 2. Effect of oxyfluorfen on PS II activity (○) of rice plants (100% (control, ●) is equal to 94.8 μmol DCIP reduced/mg chl/h).

with 7 ppm of oxyfluorfen 5 days after spray.

Oxyfluorfen also inhibited photosynthesis. Chloroplasts isolated from sprayed leaves showed inhibition of DCIP Hill reaction (figure 2). There was continuous decline in the rate of DCIP Hill activity of chloroplasts isolated from plants sprayed with 3, 5 and 7 ppm of oxyfluorfen but little and temporary inhibition by oxyfluorfen at low concentration (1 ppm). DCIP reduction 5 days after spray was inhibited by about 90% in chloroplasts isolated from rice leaves treated with 7 ppm oxyfluorfen.

In order to understand the mode of action of oxyfluorfen, isolated spinach chloroplasts were incubated with different concentrations (0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 ppm) of oxyfluorfen for one min and the rate of DCIP reduction was measured (figure 3). The I_{50} values for inhibition of DCIP reduction increased with increase in chloroplast concentration. The I_{50} value for 10 μg chl/ml was 0.44 ppm while for 30 μg chl/ml I_{50} was 0.72 ppm (inset of figure 3). The inhibition of PS II activity by oxyfluorfen was not reversed by the addition of DPC (table 1).

These results suggest that oxyfluorfen causes photo-oxidative damage to chloroplast membrane by inhibiting PS II activity and that oxyfluorfen inhibits electron transport chain beyond the DPC

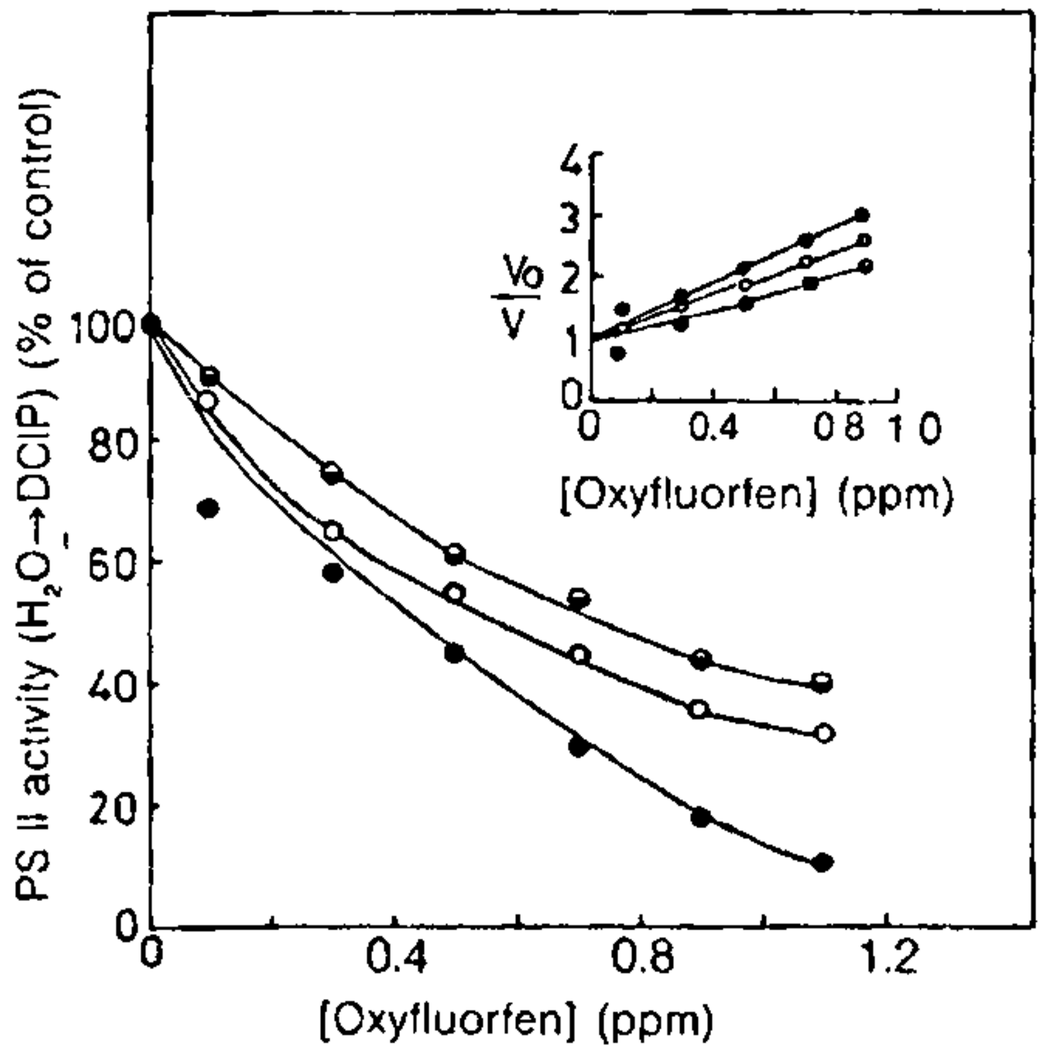

Figure 3. Effect of oxyfluorfen on PS II activity of isolated spinach chloroplasts (100% is equal to 390, 372 and 309 μmol DCIP reduced/mg chl/h by chloroplasts equivalent to 10 (○), 20 (●) and 30 (◒) μg chl/ml respectively). Inset shows a plot of V'_0/V' as a function of concentration of inhibitor. V'_0 is rate of DCIP reduction in absence of inhibitor, V' is rate in presence of inhibitor.

Table 1 *Effect of oxyfluorfen on PS II activity of normal and heat-inactivated spinach chloroplasts*

Treatment	PS II activity* (μmol DCIP reduced/mg chl/h) Control	Heat-treated
—	380 ± 67	48 ± 2
Oxyfluorfen	8 ± 1.2	0
Oxyfluorfen + DPC	4 ± 0.6	0
DPC	—	172 ± 3

*Each value is mean ± SD of triplicate samples. The data are representative as rate of DCIP reduction in control chloroplasts varied from 350 to 400 μmol DCIP reduced/mg chl/h in different preparations. Other conditions are as in text.

electron-accepting site on the oxidizing side of PS II. Increase in I_{50} with increase in chloroplast concentration suggests that oxyfluorfen may bind to pigment complex(es) or some other membrane component. The binding of oxyfluorfen affects PS II photochemistry[18].

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1. Yin, R. Y., *J. Agric. Food Chem.*, 1975, **23**, 592.
2. Gorske, S. F. and Hopen, H. J., *Weed Sci.*, 1978, **26**, 585.
3. Matsunaka, *J. Agric. Food Chem.*, 1969, **17**, 171.
4. Fadayomi, O. and Warren, G. F., *Weed Sci.*, 1976, **24**, 598.
5. Vanstone, D. E. and Stobbe, E. H., *Weed Sci.*, 1977, **25**, 352.
6. Boger, P., *Z. Naturforsch.*, 1984, **39C**, 468.
7. Alscher, A. and Strick, C., *Pest. Biochem. Physiol.*, 1984, **21**, 248.
8. Gillman, D. J., Evans, M. W. and Dodge, A. D., *Proc. Br. Crop. Protect. Conf. Weeds*, 1985, **7**, 1195.
9. Duke, S. D. and Kenyon, W. H., *Z. Naturforsch.*, 1987, **42C**, 813.
10. Ensminger, M. P. and Hess, F. D., *Plant Physiol.*, 1985, **78**, 46.
11. Haworth, P. and Hess, F. D., *Plant Physiol.*, 1988, **86**, 72.
12. Hess, F. D. and Orr, G. L., *Abstr. Weed Soc. Am. Meet., nos.*, 1981, p. 246.
13. Kunert, K. J. and Boger, P., *Weed Sci.*, 1981, **29**, 169.
14. Karabourniotis, G., Menetas, Y. and Gavrees, N. A., *Plant Physiol.*, 1985, **77**, 300.
15. Hall, D. O., *Nature (London)*, 1972, **235**, 125.
16. Mackenny, G., *J. Biol. Chem.*, 1941, **140**, 315.
17. Mohanty, P., Papageorgiou, G. and Govindjee, *Photochem. Photobiol.*, 1971, **14**, 667.
18. Sharma, D., Bhardwaj, R., Maheshwari, V. and Nagar, S., *Plant Sci.*, 1989, **63**, 1.

ELECTRICAL CONDUCTIVITY AS AN INDICATOR OF ORTHODOX AND RECALCITRANT SEED PHYSIOLOGY IN TROPICAL FOREST TREES

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THE majority of seeds used in agriculture show orthodox behaviour, that is to say, over a wide range of environmental conditions, their longevity may be increased in specific and predictable ways by decreasing temperature and moisture content. Recalcitrant seeds are those that do not obey these rules since they are killed when their moisture content is reduced below some relatively low value[1]. Such seeds cannot be preserved for more than a few months and some can only survive for a few weeks even when desiccation is prevented[2,3]. Diagnosis of the recalcitrant condition is not always straightforward, and there are many cases of seeds initially classified as recalcitrant and now known to be typically orthodox[4]. In the case of tropical forests where a number of forest tree species are recalcitrant, a quick and reliable method for identification of such species is absent.

Incorrect diagnosis of orthodox and recalcitrant seeds can affect decisions on methods of genetic conservation. The present study is the first attempt to find a measure for the identification of these two seed categories, taking seeds from truly recalcitrant and orthodox species. Experiments were conducted on freshly collected mature seeds of three truly recalcitrant species, viz. *Shorea robusta* Gaertn f., *Eugenia jambolana* Lam. and *Saraca indica* Linn., and four orthodox species, viz. *Bauhinia variegata* Linn., *Bombax malabarcium* D. C., *Dalbergia sissoo* Roxb. and *Melia azadirachta* Linn. Germination of seeds in the seven species was found to be 80 to 100%. Electrical conductivity was determined[5] hourly up to 24 h after immersing seeds in water at 20°C.

Figure 1 shows that electrical conductivity in recalcitrant seeds ranged from 1.36 to 14.72 $\mu s\,cm^{-1}\,g^{-1}$.