Physiological Changes in *Matricaria inodora* Following Ioxynil and Bromoxynil Treatment

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Z. Naturforsch. **39c**, 505–509 (1984); received December 8, 1983

Matricaria inodora, Photosynthesis, Ioxynil, Bromoxynil, Herbicide Symptoms

A range of biochemical and physiological changes were monitored in *Matricaria inodora* following field rate applications of ioxynil and bromoxynil. Bromoxynil showed greater phytotoxicity with decreased reducing sugars, amino acids and proteins occurring within 28 days of treatment, when plant death was apparent. After an initial decline these parameters increased as plants appear to recover from ioxynil treatment. CO_2 fixation was completely inhibited within 4 days in leaves treated with ioxynil and bromoxynil. Ultrastructural effects of both herbicides were similar with chloroplast swelling, decrease in starch grains and thylakoid disruption prior to cellular destruction. *In vitro* activity of the herbicides on isolated chloroplasts revealed ioxynil to be slightly more inhibitory than bromoxynil toward electron transport and approximately one hundred times more effective as an uncoupler of PMS cyclic photophosphorylation.

Introduction

Ioxynil and bromoxynil are post emergence contact herbicides which exhibit differential activity amongst certain weed species [1–3].

The hydroxybenzonitriles were classified by Moreland and Hilton [4] as inhibitory uncouplers because of their apparent ability to inhibit chloroplast electron transport and uncouple photophosphorylation, and oxidative phosphorylation in mitochondria. However, *in vitro* studies have indicated chloroplast electron transport to be 100–1000 × more sensitive than plant mitochondria or cyclic photophosphorylation suggesting photosynthetic inhibition to be the primary site of action [5, 6].

Following the application of photosynthetic inhibitor herbicides various other metabolic processes may be directly or indirectly effected. For example, nitrate reductase activity was limited in wheat following methabenzthiazuron treatment [7], and simazine increased protein synthesis in barley [8].

In this report, the physiological basis for the development of symptoms is investigated in *Matricaria inodora*, a weed species which is very susceptible to bromoxynil and exhibits some recovery after ioxynil treatment.

Abbreviations: DCPIP, dichlorophenol indophenol; PMS, phenazine methosulfate.

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0341-0382/84/0500-0505 $ 01.30/0

Materials and Methods

Treatment of plants

Plants were grown as previously reported [3] and used for experimentation when 3–4 leaves had developed. In all spray experiments, ioxynil-Na and bromoxynil-K salts were applied at a dose rate equivalent to the field rate (560 g a.i. ha^{-1}) using a hydraulic laboratory pot sprayer (Mardrive Marine Engineering Co. Ltd.) fitted with an 80° T-jet nozzle.

Assay of metabolic symptoms

7, 14, 21 and 28 days after treatment plants were harvested and fresh weight, total chlorophyll, reducing sugars, amino acids and proteins were extracted and determined by established methods [Sanders 1984, in preparation].

CO_2 fixation

Net photosynthesis was measured with an infrared CO_2 analyser (G.P. instruments Ltd.) in differential mode. Six replicate chambers were sampled automatically at 5 minute intervals by connection to an autoanalyzer (The Analytical Development Company Ltd.). For the duration of measurements the chambers were uniformly irradiated with saturating 750 μ $E.m^{-2} \cdot sec^{-1}$ light from a Camrex Solarcolour LGH, PS/U sodium fluorescent tube, and maintained at 25 °C by a constant temperature water jacket.

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Electron microscopy

Representative samples of leaves four days after herbicide treatment were processed for transmission electron microscopy as reported by Pallett and Dodge [9].

Chloroplast isolation

Chloroplast fragments [Type E, 10] were isolated from *M. inodora* by modifying established techniques [11, 12] until optimum photochemical activity was maintained for 45 minutes. Assay conditions for photochemical activity using dichlorophenolindophenol (DCPIP) as electron acceptor were those reported previously [3].

The uncoupling activity of ioxynil and bromoxynil was investigated by monitoring PMS mediated cyclic photophosphorylation in isolated chloroplast fragments [13]. Initial experiments reported in this paper, were carried out using chloroplast fragments isolated from 10–14 day pea seedlings.

Results

3–4 days after foliar application of the hydroxybenzonitriles the first visual symptoms appeared as wilting and necrosis of treated leaves. Within 14 days of bromoxynil treatment, *M. inodora* exhibited complete necrosis whereas only the treated leaves developed necrotic symptoms after ioxynil treatment. In the latter, the apex and lateral shoots continued to grow reaching approximately 60% of the control height after 28 days.

This apparent recovery is reflected in the fresh weight and chlorophyll content of treated foliage (Fig. 1a and b). Immediately following treatment there was a decline in the total protein content of the aerial shoots, with corresponding increases in soluble amino acids and proteins (ioxynil only, Fig. 1c, d and e). As the plant recovered from ioxynil application, the total protein content rapidly increased and amino acids and soluble proteins declined. 7 days after bromoxynil treatment all parameters investigated declined as plant death gradually occurred.

Net photosynthesis was rapidly inhibited reaching 80% inhibition within 48 hours (Fig. 2). No recovery of photosynthetic capacity was detected and by the end of the experimental period, leaves treated with both herbicides were totally necrotic. Complete inhibition of CO_2 uptake within 4 days

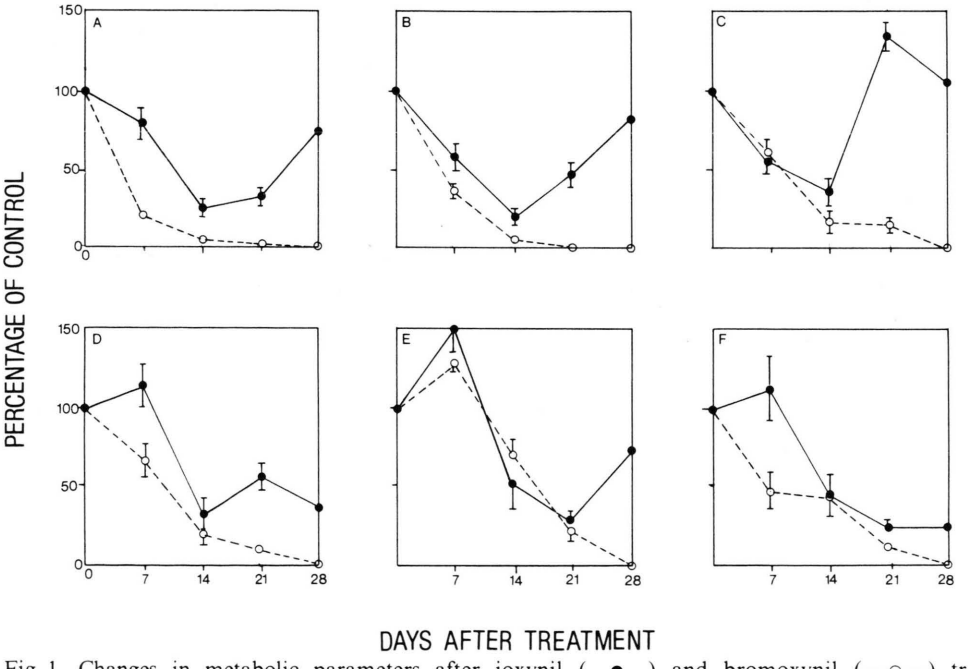


Fig. 1. Changes in metabolic parameters after ioxynil (—●—) and bromoxynil (--○--) treatment of *Matricaria inodora*. (A) fresh weight, (B) chlorophyll content, (C) total proteins, (D) soluble proteins, (E) soluble amino and (F) reducing sugars. Each point is the mean of 6 replicates and standard error bars are included where they exceed the symbol size.

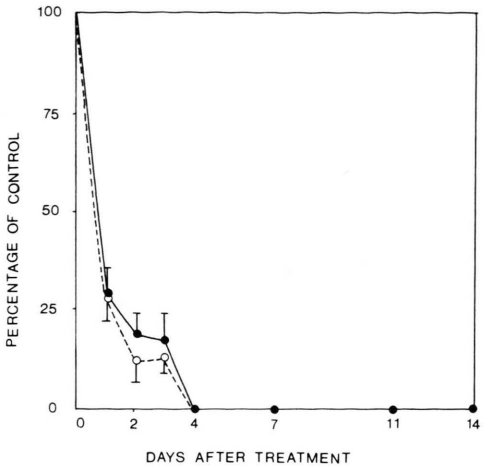


Fig. 2. Inhibition of CO_2 uptake by field rate ioxynil (—●—) and bromoxynil (--○--). Each point is the mean of 6 replicates with standard error bar.

was accompanied by ultrastructural changes in the chloroplast.

Plate A shows a typical disc shaped chloroplast from a control treatment. Both herbicides caused a progressive swelling of the chloroplast, decrease in starch and swelling of the thylakoid system. Plate B shows the early stages of chloroplast deterioration as a result of ioxynil treatment, and plate C reveals later deterioration with bromoxynil. The development of these ultrastructural symptoms appeared slightly more rapid with bromoxynil treatment, reflecting the development of visual symptoms.

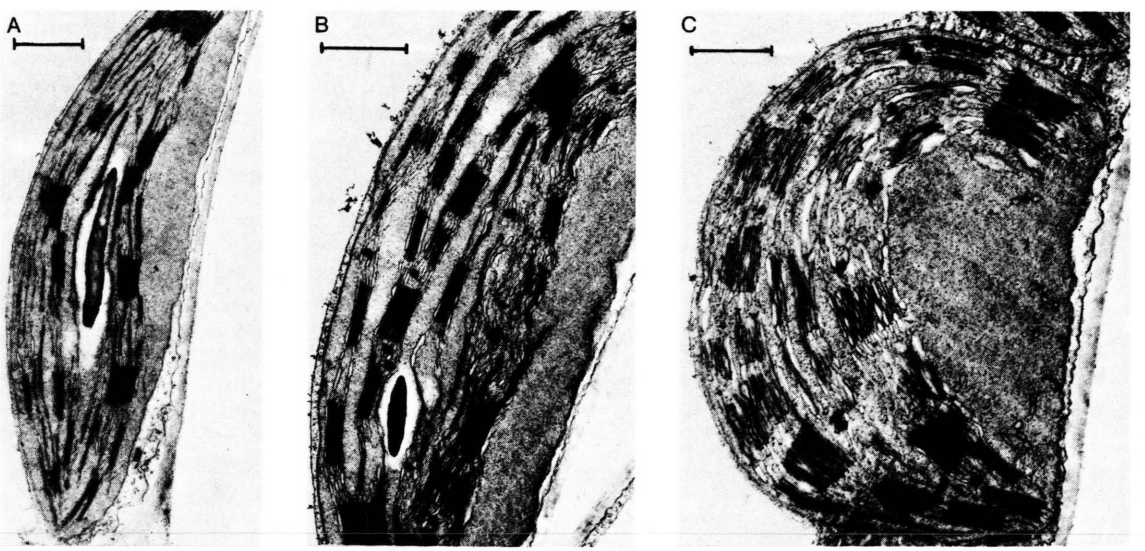
Table I shows ioxynil to be slightly more inhibitory than bromoxynil towards electron transport in chloroplasts isolated from *M. inodora*. Both herbicides uncouple PMS-mediated cyclic photophosphorylation in pea chloroplasts (Table II). Ioxynil

Table I. Inhibition of DCPIP reduction in chloroplasts isolated from *M. inodora* (each figure is the mean of 4 replicates).

	% of control	
Conc^n [µm]	Ioxynil	Bromoxynil
1.0	80.5	93.0
10.0	26.0	40.0
100.0	15.0	17.5

Table II. Inhibition of PMS-mediated photophosphorylation in isolated pea chloroplasts (each figure is the mean of 4 replicates).

	% of control	
Conc^n [µm]	Ioxynil	Bromoxynil
0.1	186.1	–
1.0	23.4	–
10.0	0.0	262.1
30.0	–	150.0
60.0	–	49.3
100.0	–	6.5



Plates A, B, C: Typical chloroplasts 4 days after treatment (A) control (× 12 200), (B) ioxynil (× 14 500) and (C) bromoxynil (× 13 100). Internal marker = 1 µm.

was approximately one hundred times more active than bromoxynil. At lower concentrations photophosphorylation was stimulated by both herbicides.

Discussion

The observed wilting and necrosis of treated foliage are indicative of a rapid herbicide action in this species. Within 4 days inhibition of CO_2 fixation was complete and chloroplasts had undergone marked ultrastructural changes. Chloroplast disruption has been proposed to result from an overloading of the protective carotenoid system inducing singlet oxygen and free radical formation as a result of electron transport inhibition [9, 14]. This, coupled with possible free radicals formed by degradation of ioxynil within the leaf [13, 15] may explain the rapid loss of turgidity in treated leaves.

Within 48 hours starch grains were less apparent in treated chloroplasts indicating rapid metabolic breakdown as a consequence of low CO_2 fixation rates. Inhibition of net photosynthesis was complete by 96 hours with both herbicides, at which time chloroplasts typically exhibited increased vacuolation of intergranal thylakoids and plastoglobuli content. There was also an increased tendancy for the thylakoids to be bowed against the chloroplast envelope. This phenomena is commonly reported [9, 16, 17] and was considered by Dodge and Lawes [18] to be the likely result of decreased osmotic potential of the cell vacuole.

As a consequence of photosynthetic inhibition minimal levels of reducing sugars were anticipated within seven days. Kinetic studies with potato and spring wheat suggest this parameter is the first to be effected following metribuzin and methabenzthiazuron treatment of roots [19, 20]. It is possible an earlier measurement would have detected such a decline. However, in bromoxynil treated plants, with the exception of soluble amino acids, all of the primary metabolites studied declined at a similar rate to fresh weight. During the recovery phase of ioxynil treatment, reducing sugar levels did not increase at the same rate as fresh weight implying a reduced photosynthetic rate newly developing foliage.

The interaction between total protein, soluble protein and soluble amino acids, particularly apparent after ioxynil treatment is commonly reported, especially for more resistant herbicide:plant interactions [7, 8, 19, 20]. Fedtke [21] reports enhanced N_2-metabolism at sublethal herbicide concentrations and suggests increased nitrite formation may contribute to the toxic action of ioxynil. This interaction may also be explained by autolysis of structural proteins resulting in increased free amino acids and proteins, and possibly by selective inhibition of protein synthesis [22].

Therefore *in vivo* studies have shown that ioxynil and bromoxynil rapidly inhibit CO_2 uptake. The consequences are physiological changes in chloroplast ultrastructure and the development of necrotic tissue from which there is limited recovery from ioxynil treatment. *In vitro* studies, however have indicated that ioxynil is slightly more inhibitory than bromoxynil at the thylakoid level (Table I). A similar *in vitro* response occurred in *Stellaria media* and *Viola arvensis* which exhibit some resistance to the two herbicides [3]. This supports previous reports that electron transport inhibition by the hydroxybenzonitriles depends on substitution and decreases in the order I > Br > Cl [23].

The uncoupling action of these two herbicides on photophosphorylation may also contribute to phytotoxicity (Table II), particularly ioxynil, which uncouples at concentrations lower than those which inhibit electron transport. Inhibition of electron transport by ioxynil and bromoxynil was similar for chloroplasts isolated from *M. inodora* (Table I) and peas (data not presented). The marked stimulatory effect at low concentrations (Table II) is a common feature of many herbicides interfering with biochemical processes [21].

The greater susceptibility of *M. inodora* to bromoxynil cannot be explained by this *in vitro* data for photosynthetic interference. Other factors are likely to be involved such as penetration, translocation and metabolism of the herbicides, which are currently under investigation.

Acknowledgements

The authors would like to acknowledge Mr. R. H. Hewett and May and Baker Ltd. for technical support, and to the organising committee for providing finance to attend the workshop in Wageningen.

[1] K. Carpenter, H. J. Cottrell, W. H. De Silva, B. J. Heywood, W. G. Leeds, K. F. Rivett, and M. L. Soundy, Weed Res. **4,** 175–195 (1964).
[2] L. Somerville, Ph.D. thesis, University of Strathclyde, Glasgow (1972).
[3] G. E. Sanders and K. E. Pallett, Proc. Brit. Crop Prot. Conf. – Weeds **1,** 325–332 (1982).
[4] D. E. Moreland, Ann. Rev. Plant Physiol. **31,** 597–638 (1980).
[5] Z. Gromet-Elhanan, Biochem. Biophys. Res. Comm. **30,** (1) 28–31 (1968).
[6] M. N. Kerr and R. L. Wain, Ann. Appl. Biol. **54,** 441–446 (1964).
[7] C. Fedtke, Pestic. Sci. **4,** 653–664 (1973).
[8] E. L. Pulver and S. K. Ries, Weed Sci. **21,**(3), 233–237 (1973).
[9] K. E. Pallett and A. D. Dodge, J. Exp. Bot. **31,** 1051 to 1066 (1980).
[10] D. O. Hall, Nature **235,** 125–126 (1972).
[11] K. E. Pallett and A. D. Dodge, Phytochemistry **16,** 427–429 (1977).
[12] S. G. Reeves and D. O. Hall, Methods in Enzymology **69,** 85–94 (1980).
[13] K. E. Pallett, Ph.D. thesis, University of Bath (1978).
[14] S. M. Ridley, Plant Physiol. **59,** 724–732 (1977).
[15] M. A. Zaki, H. F. Taylor, and R. L. Wain, Ann. Appl. Biol. **59,** 481–491 (1967).
[16] J. Geronimo and J. W. Herr, Weed Sci. **18**(1), 48–53 (1970).
[17] N. Harris and A. D. Dodge, Planta **104,** 201–209 (1972).
[18] A. D. Dodge and G. B. Lawes, Weed Res. **14,** 45–49 (1974).
[19] C. Fedtke, Pest. Biochem. Physiol. **2,** 312–323 (1972).
[20] C. Fedkte, Pest. Biochem. Physiol. **4,** 386–392 (1974).
[21] C. Fedtke, Biochemistry and Physiology of herbicide action, p. 202, Springer-Verlag 1982.
[22] J. D. Mann, L. S. Jordan, and B. E. Day, Plant Physiol. **40,** 840–843 (1965).
[23] A. Trebst, S. Reimer, W. Draper, and H. J. Knops, Z. Naturforsch. **34c,** 831–840 (1979).