INVESTIGATIONS ON CHLOROPHYLL FLUORESCENCE ASSAY TECHNIQUES USING PHOTOSYNTHETIC INHIBITOR-TYPE HERBICIDES

By

DAVID RAY SHAW

Bachelor of Science
Cameron University
Lawton, Oklahoma
1981

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1983

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
May, 1985

INVESTIGATIONS ON CHLOROPHYLL FLUORESCENCE ASSAY TECHNIQUES USING PHOTOSYNTHETIC INHIBITOR-TYPE HERBICIDES

Thesis Approved:

Thomas F. Peeper
Thesis Adviser

Howard A. L. Greer

Robert L. Westerman

Eddie Basler

Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to his major adviser, Dr. Thomas Peeper, for his advice, helpful criticism, time, and training during the course of this research. Appreciation is also extended to Dr. Eddie Basler, Dr. Robert Westerman, and Dr. David Nofziger for their suggestions and assistance as members of the author's graduate committee, and to Dr. Howard Greer for serving in Dr. Nofzigers absence.

I would like to offer special thanks to my wife, Sherri, for her patience, love, and support during the course of study, and for her assistance in the preparation of this thesis. Sincere thanks is also expressed to the author's family and friends for their interest and encouragement, making this accomplishment possible.

Appreciation is extended to the Department of Agronomy at Oklahoma State University for the research assistantship, and for the use of the facilities and equipment which made this research possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Each of the three parts of this thesis is a separate manuscript to be submitted for publication; Parts I and III in Weed Science, the journal of the Weed Science Society of America, and Part II in Crop Science, a Crop Science Society of America publication.

PART I

COMPARISON OF CHLOROPHYLL FLUORESCENCE AND FRESH WEIGHT AS HERBICIDE BIOASSAY TECHNIQUES

Comparison of Chlorophyll Fluorescence and Fresh Weight as Herbicide Bioassay Techniques

Abstract. The sensitivities of chlorophyll fluorescence and fresh weight as bioassay techniques for the determination of metribuzin {4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one}, diuron {3-(3,4-dichlorophenyl)-1,1-dimethylurea}, and atrazine {2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine} concentrations in soil were compared. The ratio of the initial inflection point (I) to the variable fluorescence maximum (P) of intact oat (Avena sativa L. 'Chilocco') leaves was determined for plants seeded directly into herbicide-treated soil and for those transplanted into treated soil after 14 days of growth in nutrient solution. Using the chlorophyll fluorescence of transplanted oats bioassay, 0, 0.13, 0.25, and 0.50 ppm concentrations could be distinguished from one another within 8 h for metribuzin, within 24 h for diuron, and within 48 h for atrazine. These distinctions between rates could not be made 17 days after seeding into treated soil when using fresh weight as the bioassay indicator. Chlorophyll fluorescence of oats seeded directly into treated soil was also a reliable technique, but required much more time to attain sufficient plant size for convenient chlorophyll fluorescence determinations.
Additional index words. Avena sativa, metribuzin, diuron, atrazine.

INTRODUCTION

A small portion of the light intercepted by a plant is absorbed by the photosynthetic pigments, producing electronic excitation energy. The majority of this energy is used in photosynthesis, whereas the

remainder is lost as heat or is emitted as fluorescence (12). Photosynthesis-inhibiting herbicides increase chlorophyll fluorescence because of dissipation of absorbed radiant energy in the absence of useful photochemistry. Chlorophyll fluorescence, measurable with commercially-available fluorometers, has been suggested as a tool for the investigation of photosynthetic mechanisms and herbicide resistance in plants. As early as 1973, Miles and Daniels (10) used a simple color filter system and color photography to detect changes in leaf fluorescence resulting from photosynthesis inhibition. Schreiber et al. (14), in 1975, described a portable, solid-state fluorometer for the measurement of chlorophyll fluorescence in intact plants. Smillie and Nott (17) used a data acquisition, storage, and retrieval system in conjuction with a portable fluorometer from which they could calculate mean values for any particular fluorescence curve parameter.

Prompt and delayed chlorophyll induction curves have characteristic parameters that can be used to make mathematical comparisons between the response curves of plants treated under various conditions. Papageorgiou (12) tentatively assigned the induction phases of chlorophyll fluorescence to the photochemical and physiological events that intervene between states of light and dark adaptation. These include a rapid rise to an inflection point (I) which represents the fluorescence level at the intermediary maximum, a slower increase to a maximum fluorescence level (P), and a subsequent gradual decay to a relatively stable yield (S). Papageorgiou indicated that the gradual rise from I to P accounts for the major portion of variable fluorescence, resulting from a buildup of reduced carriers on the acceptor side of photosystem II. When electron transport has been inhibited in some way, chlorophyll

fluorescence rises very quickly to a higher level. Schreiber et al. (15) found that the I-P-S transient was the portion of the induction curve most influenced by ozone injury, with 0.3 µl/l ozone almost totally abolishing this transient. Components of the initial peak fluorescence curves have been used by many investigators (3, 5, 7, 11, 18) as criteria for analyzing photosystem II electron transport. Ahrens et al. (1) and Bowes et al. (2) investigated the use of chlorophyll fluorescence as a potential screening system for triazine resistance in various crop and weed species. Fischer (6) reported a difference in fluorescence decay between tolerant and susceptible varieties of wheat treated with metribuzin.

A primary advantage of chlorophyll fluorescence assays over plant-growth bioassay or other in vitro techniques is the rapidity and simplicity with which fluorescence can be monitored (4). Chlorophyll fluorescence-induction assays accurately predicted ozone-induced injury to plants, and did so at least 20 h before any visible signs of leaf necrosis appeared (15). Richard et al. (13) reported that chlorophyll fluorescence measurements indicated significant electron transport inhibition of leaves treated with atrazine and diuron for 0.5 and 1 h, respectively. Fresh-weight bioassays, on the other hand, require a minimum of 14 to 30 days for the full effects of the herbicide to become evident (9, 16). Therefore, it should be possible to employ chlorophyll fluorescence assay techniques to estimate potential injury in plants long before visual injury symptoms appear. The objectives of this research were to compare chlorophyll fluorescence and fresh-weight change as bioassay techniques, and to determine the relative sensitivity range of chlorophyll fluorescence over time using the

herbicides metribuzin, diuron, and atrazine.

MATERIALS AND METHODS

Commercial formulations of metribuzin, diuron, or atrazine were added to 2 kg quantities of air-dried Kirkland loam soil (Abruptic Paleustoll, 1.1% organic matter, pH 7.0) to establish concentrations of 0, 0.13, 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 ppm ai (w/w) of the herbicides. The soil was mixed thoroughly and subdivided into 250-g portions. These portions were placed into a series of 237-ml perforated, polystyrene-foam pots. One-half of these were used in the transplanting experiments, and the remainder were used for fresh weight bioassay by planting eight oat seeds with the basal portion inserted into the soil, after which the pots were sub-irrigated daily. After germination, the oats were thinned to five plants per pot.

In the experiments using transplanted oats, the plants were germinated and grown in perlite watered with one-half strength Hoagland's (8) solution. All plants were grown under continuous illumination at a light intensity of 300±10 $\mu E \cdot m^{-2} \cdot sec^{-1}$. The temperature was maintained at 28±2°C. After 14 days the oat seedlings were transplanted into the treated soil. This was accomplished by subirrigating each cup to capacity, splitting the cup and soil vertically, and replacing the halves around the roots of the plant.

In the initial experiments using the transplanting technique, chlorophyll fluorescence curves of each plant were recorded 24 and 48 h after transplanting. In subsequent experiments, fluorescence 4 and 8 h after transplanting was also monitored. Chlorophyll fluorescence was also measured from one plant of those seeded directly into the treated

soil 10 days after seeding. For all fluorescence measurements, a portable plant productivity fluorometer[1] was used. The sensing probe of this instrument had been modified by partially covering the probe opening to leave a 1- by 3-mm slit which facilitated its use on the narrow oat leaves. The modified sensing probe was placed over the adaxial surface of the second leaf. The probe-covered area remained in this dark environment for 3 min. The light-emitting diode of the probe, producing light at wavelengths around 670 nm, was adjusted to provide an intensity of 7 $\mu E \cdot m^{-2} \cdot sec^{-1}$. The leaves were illuminated for 30 s, and chlorophyll fluorescence signals were collected every 10 msec, stored, and displayed graphically using a high-speed, computer-controlled data acquisition system. The ratio of the inflection point (I) to that of the variable fluorescence peak (P) was determined for each plant at all time intervals. Seventeen days after seeding, fresh weights of oat plants seeded directly into the herbicide-treated soil were determined, and expressed as a percent of the untreated check. A randomized complete block design with eight replications was used in each experiment, and all experiments were repeated.

RESULTS AND DISCUSSION

When dark-adapted oat leaves were exposed to light from the fluorometer probe, characteristic changes in the chlorophyll fluorescence yield were observed. Figure 1 compares typical fluorescence curves from leaves of control and herbicide-treated oat plants. Chlorophyll fluorescence of an untreated plant typically begins at a low initial level

---

[1]Model SF-10, Richard Brancker Research Ltd., Ottawa, Canada.

(O) and increases rapidly to an inflection point (I). Fluorescence emission continues to rise to a maximum peak (P), and then declines to a lower level (S). More slowly, fluorescence increases again to a second maximum (M) and gradually decreases to a terminal level (T). Treatment with a herbicide that inhibits photosynthesis significantly influences these parameters. At the onset of illumination, a very rapid rise to a maximal level is observed, with very little difference between the height of I and P, and little decay from the maximal (P) level. Therefore, a ratio of I:P can be a good indicator for photosynthetic inhibition.

Within 4 h, 0.50 ppm or higher rates of metribuzin in soil caused a significant increase in the I:P ratio of oat plants transplanted into treated soil (Table 1). Following 4 h of contact, significant differences could be detected between the 0, 0.50, 1.0, 2.0, and 8.0 ppm concentrations of herbicide. Within 8 h after transplanting, even the lowest rate of 0.13 ppm of metribuzin increased the I:P ratio, and each of the lower concentrations could be distinguished. After 24 and 48 h contact, plants exposed to any metribuzin rate of 0.50 ppm or higher had large increases in the level of I in relation to P, thus giving an I:P ratio at or near 1.0.

Chlorophyll fluorescence of direct-seeded oats was not monitored until 10 days after planting because, prior to that time, plant leaves were too short to fit conveniently under the fluorescence probe. After 10 days differences in the I:P ratio were observed between the 0.25 ppm or less, 0.50, 1.0, and 2.0 or greater concentration levels (Table 1). Therefore, chlorophyll fluorescence of direct-seeded oats could not distinguish as many metribuzin concentrations as chlorophyll fluores-

cence of transplanted oats after 4 h of contact.

Chlorophyll fluorescence of transplanted oats also exhibited greater sensitivity to metribuzin than did fresh-weight reduction of direct-seeded oats (Table 1). No differences in fresh weight were observed between metribuzin treatments of 0.25 ppm or less, or between the two highest rates of metribuzin. In comparison, chlorophyll fluorescence 8 h after transplanting distinguished between all except the four highest metribuzin rates. Variability in the chlorophyll fluorescence data was also much lower than that of the fresh-weight bioassay data, with coefficient of variation (CV) values of 7.8% or less, compared to a CV of 17.6% for the fresh-weight bioassay data.

In contrast to metribuzin, only the 4.0 and 8.0 ppm rates of diuron significantly increased the I:P ratio of oats 4 h after transplanting (Table 2). However, after 8 h marked differences at the higher diuron concentrations were recorded. After the 24 and 48 h time intervals, each increase in diuron concentration up to 0.5 ppm significantly increased the I:P ratio. Concentrations above 0.5 ppm could not be distinguished. Chlorophyll fluorescence of direct-seeded oats 10 days after seeding did not distinguish between diuron concentrations of 1.0 ppm and above, or detect the lowest concentrations. The fresh-weight bioassay for diuron failed to detect 0.13 or 0.25 ppm of diuron in the soil, and did not distinguish between the three highest concentrations. The fresh-weight data also had a CV that was more than twice that of any chlorophyll fluorescence assay (Table 2).

The I:P ratio of plants treated with atrazine at 2 ppm or higher was significantly increased within 4 h of transplanting (Table 3), and the three highest concentrations of atrazine could be distinguished.

After the 24 and 48 h time periods, rates of 1.0 ppm or higher caused I:P ratios approaching 1.0. However, after 48 h of contact with the soil, significant differences were recorded with increasing atrazine rates up to 1.0 ppm, indicating good sensitivity with the lower herbicide rates. Chlorophyll fluorescence of oats seeded directly into atrazine-treated soil was able to detect 0.25 ppm atrazine but not 0.13 ppm (Table 3). The fresh-weight bioassay of oat plants 17 days after seeding provided a high degree of rate sensitivity at higher atrazine concentrations, but did not distinguish between 0.13 and 0.50 ppm atrazine. Variability for the fresh-weight bioassay was greater than that for the chlorophyll fluorescence assays for atrazine at any time interval (Table 3).

The I:P ratio of oats transplanted into treated soil can be obtained quite rapidly, and provides an excellent in vivo system for the determination of inhibition of photosynthetic activity. Determining the value of I:P proved to be much more rapid and less variable than conventional fresh-weight bioassay. The fresh-weight bioassay for atrazine showed one more significant difference than did any single chlorophyll fluorescence assay for atrazine. However, using 2 or more time intervals after transplanting made possible precise determinations of both high and low herbicide levels in soil. For example, 4 h after transplanting into atrazine-treated soil all rates above 1.0 ppm could be differentiated. Eight hours after transplanting 2 ppm could be distinguished from 1 ppm and, after 48 h, each concentration below 1 ppm could be distinguished. Chlorophyll fluorescence of oats seeded directly into treated soil was also a reliable technique, but required maintaining pots for a much longer time before measurements could be

made, and was not as precise as chlorophyll fluorescence of transplanted oats. Research has shown that chlorophyll fluorescence can be effectively used to assess qualitative herbicide injury (13). The present research, however, demonstrates that the I:P parameter can be used to accurately distinguish specific quantities of photosynthetic-inhibitor herbicides. This could be especially useful as a rapid, nondestructive bioassay system capable of determining unknown quantities of a given herbicide when compared to a known standard.

LITERATURE CITED

1. Ahrens, W.H., C.J. Arntzen, and E.W. Stoller. 1981. Chlorophyll fluorescence assay for the determination of triazine resistance. Weed Sci. 29:316-322.

2. Bowes, J., A.R. Crofts, and C.J. Arntzen. 1980. Redox reactions on the reducing side of photosystem II in chloroplasts with altered herbicide binding properties. Arch. Biochem. Biophys. 200:303-308.

3. Brewer, P.E., C.J. Arntzen, and F.W. Slife. 1979. Effects of atrazine, cyanazine, and procyazine on the photochemical reactions of isolated chloroplasts. Weed Sci. 27:300-308.

4. Devlin, R.M., A.J. Murkowski, I.I. Zbiec, S.J. Karczmarczyk, and E.M. Skorska. 1983. Influence of buthidazole, diuron, and atrazine on some light reactions of photosynthesis. Weed Sci. 31: 879-883.

5. Etienne, A.L., C. Lemasson, and J. Lavorel. 1974. Quenching de la chlorophylle in vivo par le m-nitrobenzene. Biochim. Biophys. Acta 33:288-300.

6. Fischer, M.L. 1983. Investigations on the differential tolerance of wheat cultivars to metribuzin. Ph.D Diss., Okla. State Univ., Diss. Abstr. Int. 44:2033.

7. Forbush, B. and B. Kok. 1968. Reaction between primary and secondary electron acceptors of photosystem II of photosynthesis. Biochim. Biophys. Acta 162:243-253.

8. Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 547.

9. Marriage, P.B. 1975. Detection of triazine and urea herbicide residues by various characteristics of oat seedling in bioassays.

Weed Res. 15:291-298.

10. Miles, C.D. and D.J. Daniels. 1973. A rapid screening technique for photosynthetic mutants of higher plants. Plant Sci. Lett. 1:227-240.

11. Murata, N., M. Nishimura, and A. Takamiya. 1966. Fluorescence of chlorophyll in photosynthetic systems. Biochim. Biophys. Acta 120:23-33.

12. Papageorgiou, G. 1975. Chlorophyll fluorescence: an intrinsic probe of photosynthesis. Pages 319-371 in Govindjee, ed. Bioenergetics of Photosynthesis. Academic Press, New York.

13. Richard, E.P. Jr., J.R. Goss, C.J. Arntzen, and F.W. Slife. 1983. Determination of herbicide inhibition of photosynthetic electron transport by fluorescence. Weed Sci. 31:361-367.

14. Schreiber, U., L. Groberman, and W. Vidaver. 1975. Portable, solid-state fluorometer for the measurement of chlorophyll fluorescence induction in plants. Rev. Sci. Instrum. 46:538-542.

15. Schreiber, U., W. Vidaver, V.C. Runeckles, and P. Rosen. 1978. Chlorophyll fluorescence assay for ozone injury in intact plants. Plant Physiol. 61:80-84.

16. Sheets, T.J., A.S. Crafts, and H.R. Drever. 1962. Influence of soil properties on the phytotoxicity of the s-triazine herbicides. J. Agric. Food Chem. 10:458-462.

17. Smillie, R.M. and R. Nott. 1982. Salt tolerance in crop plants monitored by chlorophyll fluorescence in vivo. Plant Physiol. 70:1049-1054.

18. Van Assche, C.J. and P.M. Carles. 1982. Photosystem II inhibiting chemicals. Pages 1-21 in D.E. Moreland, J.B. St. John, and F.D.

Hess, eds. Biochemical Responses Induced by Herbicides. Am. Chem. Soc., Washington, DC.

Table 1. Relative fluorescence of oats transplanted into metribuzin-treated soil, and fluorescence and fresh weight of oats seeded directly into metribuzin-treated soil.[a]

Metribuzin concentration	Fluorescence of transplanted oats — Hours after transplanting				Direct-seeded oats	
	4	8	24	48	Fluorescence[b]	Fresh weight[c]
(ppm)	(I:P ratio)				(I:P ratio)	(% check)
0.00	0.56 e	0.63 e	0.55 c	0.54 c	0.61 d	100 a
0.13	0.59 e	0.71 d	0.96 b	0.98 b	0.61 d	101 a
0.25	0.60 de	0.79 c	0.96 b	0.99 ab	0.62 d	93 ab
0.50	0.65 d	0.89 b	0.97 ab	0.99 ab	0.72 c	89 b
1.00	0.74 c	0.97 a	0.98 a	1.00 a	0.92 b	66 c
2.00	0.88 b	0.98 a	0.98 a	1.00 a	1.00 a	33 d
4.00	0.91 ab	0.98 a	0.98 a	0.99 ab	1.00 a	21 e
8.00	0.95 a	0.98 a	0.98 a	1.00 a	1.00 a	15 e
CV (%)	7.8	7.8	3.8	2.9	8.9	17.6

[a]Within each column, values followed by the same letter are not significantly different at the 5% level according to LSD test.

[b]10 days after seeding.

[c]17 days after seeding.

Table 2. Relative fluorescence of oats transplanted into diuron-treated soil, and fluorescence and fresh weight of oats seeded directly into diuron treated soil.[a]

Diuron concentration	Fluorescence of transplanted oats — Hours after transplanting				Direct-seeded oats	
	4	8	24	48	Fluorescence[b]	Fresh weight[c]
(ppm)	(I:P ratio)				(I:P ratio)	(% check)
0.00	0.58 c	0.65 e	0.59 e	0.60 e	0.58 d	100 a
0.13	0.59 c	0.65 e	0.65 d	0.65 d	0.60 cd	101 a
0.25	0.59 c	0.68 de	0.71 c	0.79 c	0.74 c	99 a
0.50	0.61 c	0.72 cd	0.90 b	0.96 b	0.88 b	64 b
1.00	0.61 c	0.76 bc	0.94 ab	0.97 ab	0.98 a	22 c
2.00	0.63 c	0.83 b	0.96 a	0.98 ab	0.99 a	13 d
4.00	0.85 b	0.95 a	0.97 a	0.99 ab	0.99 a	9 d
8.00	0.93 a	0.96 a	0.97 a	1.00 a	1.00 a	10 d
CV (%)	8.6	8.6	7.3	6.7	8.7	20.7

[a]Within each column, values followed by the same letter are not significantly different at the 5% level according to LSD test.

[b]10 days after seeding.

[c]17 days after seeding.

Table 3. Relative fluorescence of oats transplanted into atrazine-treated soil, and fluorescence and fresh weight of oats seeded directly into atrazine-treated soil.[a]

Atrazine concentration	Fluorescence of transplanted oats, Hours after transplanting				Direct-seeded oats	
	4	8	24	48	Fluorescence[b]	Fresh weight[c]
(ppm)	(I:P ratio)				(I:P ratio)	(% check)
0.00	0.49 d	0.56 d	0.55 d	0.55 e	0.63 e	100 a
0.13	0.48 d	0.59 d	0.58 d	0.61 d	0.63 e	87 b
0.25	0.53 d	0.60 d	0.64 c	0.73 c	0.69 d	84 b
0.50	0.51 d	0.62 cd	0.80 b	0.90 b	0.71 d	83 b
1.00	0.57 cd	0.69 c	0.92 a	0.96 a	0.84 c	74 c
2.00	0.62 c	0.84 b	0.96 a	0.96 a	0.91 bc	51 d
4.00	0.76 b	0.94 a	0.96 a	0.96 a	0.98 ab	23 e
8.00	0.93 a	0.96 a	0.96 a	0.97 a	0.99 a	14 f
CV (%)	13.8	10.6	7.8	8.1	12.8	15.4

[a]Within each column, values followed by the same letter are not significantly different at the 5% level according to LSD test.

[b]10 days after seeding.

[c]17 days after seeding.

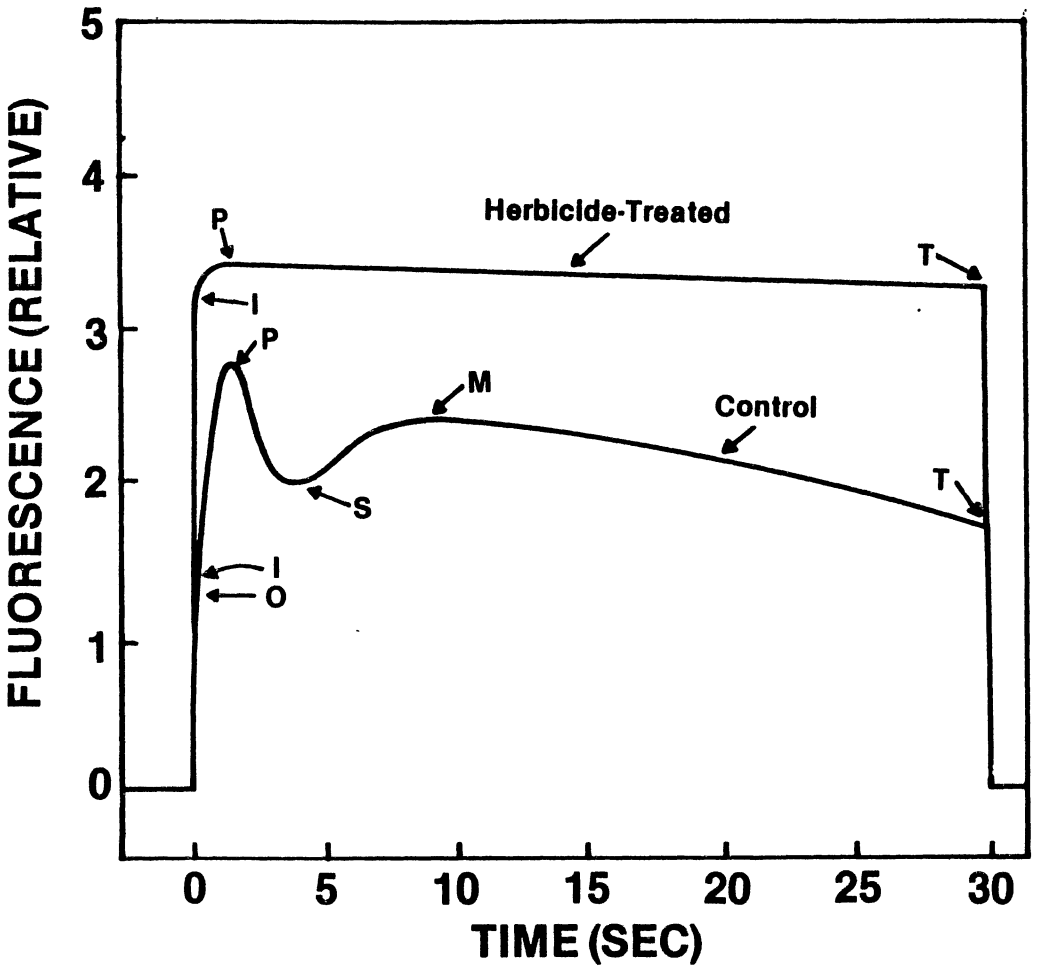

Figure 1. Relative chlorophyll fluorescence induction transients of oat leaves from photosynthesis-inhibiting-herbicide treated and control plants, illustrating the increase of I in relation to P, the lack of the secondary S-M-T transient, and the elevated level of T of herbicide-treated as compared to untreated (control) plants.

PART II

EVALUATION OF CHLOROPHYLL FLUORESCENCE PARAMETERS FOR AN INTACT-PLANT HERBICIDE BIOASSAY

Evaluation of Chlorophyll Fluorescence Parameters for an Intact-Plant Herbicide Bioassay

ABSTRACT

The correlations between various chlorophyll fluorescence induction curve parameters and metribuzin {4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one}, diuron {3-(3,4-dichlorophenyl)-1,1-dimethylurea}, and atrazine {2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine} concentrations were compared. The ratio of the initial inflection point (I) and the initial peak (P) was the most precise indicator of photosynthetic inhibition over time intervals of 4 to 48 h after an oat (*Avena sativa* L. 'Chilocco') plant was transplanted into herbicide-treated soil. The height of the initial inflection point (I), the peak level (P), and the fluorescence level 30 s after the onset of illumination (T) were also useful at 4 and 8 h after transplanting but correlated poorly with herbicide concentration at later time intervals. Other variables examined included the slopes from I to P and from P to S, the percent decay from P to T, and the complementary area. These parameters did not correlate to herbicide concentration as well, and had a much higher degree of variability associated with them.

Additional index words. Metribuzin, diuron, atrazine, photosynthetic inhibitor.

When a plant leaf intercepts light, its photosynthetic system is not activated in full force immediately. In movement toward a steady state, this photosynthetic apparatus must first pass through several transitory stages. These events, known as the induction phenomena or "Kautsky effect" after Kautsky (7), can easily be studied using chlorophyll fluorescence measurements (10). Chlorophyll fluorescence induction provides a simple, rapid, and nondestructive method to observe the influence of various agents on plant photosynthesis (2). Miles and Daniels (9) suggested a simple screening technique which used color filters and photography to detect changes in leaf fluorescence of photosynthetic mutants in higher plants. Their research provided only a qualitative method to visually estimate herbicide injury to the photosynthetic system in plants. However, both prompt and delayed chlorophyll fluorescence induction curves exhibit characteristic parameters which are useful in making mathematical comparisons between plants maintained under different treatment regimes. Papageorgiou (10), in 1975, reviewed the tentative assignments made to various fast and slow change components of the induction curve. At the onset of illumination, fluorescence begins at an initial low level (O), which is insensitive to inhibitors. From this point, there is an initial, rapid rise to an inflection point (I). Fluorescence continues to increase more slowly to a maximum peak (P) level, then declines to a lower level (S). From S, slow changes are observed in fluorescence yield with an increase to a secondary maximum (M) and a gradual decline to a steady-state, terminal level (T). Various investigators have examined induction parameters influenced by photosynthetic inhibitors in in vitro systems. Devlin et al. (4) use a ratio of relative fluorescence and relative

postluminescence from Chlorella to evaluate the phytotoxicity of three herbicides. Brewer et al. (3) suggested a parameter which compared the rapid fluorescence component to that of the variable fluorescence change in isolated chloroplasts. The I_{50} values generated by this parameter were quite similar to those determined by electron transport assays for three triazine herbicides. Etienne et al. (5), also using isolated chloroplasts, suggested the use of a complementary area designated "S", which is calculated as the area above the inflection point (I) and to the left of the peak value (P). Van Assche and Carles (15) also used this area, called A_{max}, and found that this varied with inhibitor concentration so that a half-effect can be calculated and termed pA_{max50}. These values correlated closely to pI_{50} values for various inhibitors in both Chlorella and isolated chloroplasts.

Recently, electronic detection equipment has been developed which allows a more accurate assessment of chlorophyll fluorescence parameters in intact-plant systems. Schreiber et al. (12) evaluated the influence of ozone injury on various parameters associated with the fast phase of chlorophyll fluorescence in intact bean (Phaseolus vulgaris L.) plants. Of the variables examined, the I to P amplitude was most sensitive to ozone injury, with injury prediction occurring at least 20 h prior to visual symptoms. Fischer[1] used the percent decay from P to T 30 s after the onset of illumination to evaluate hard red winter wheat (Triticum aestivum L.) cultivars for metribuzin tolerance. Smillie and Nott (14)

---

[1]Fischer, M.L. 1983. Investigations on the differential tolerance of wheat cultivars to metribuzin. Ph.D. Dissertation, Oklahoma State University, Stillwater, OK 74078.

also evaluated a number of induction parameters in intact plants, including the rise from I to P, the rise from O to I, the rate of increase from I to P, the rate of decrease after P, and the maximal rate of the O to I rise. Of these, the most useful indices of salinity were the rise from I to P and the rate of decrease after P. Other researchers (1, 8, 11) used the terminal, steady-state level of fluorescence to detect injury by chilling or herbicides. Shaw et al. (13) found that the ratio of the initial inflection point to the peak value (I:P ratio) of oats transplanted into herbicide-treated soil was a rapid and sensitive indicator of herbicide levels in soil.

The objective of this research was to compare the I:P ratio to other parameters of chlorophyll fluorescence induction curves for their usefulness as an indicator of photosynthetic-inhibitor herbicide concentrations in soil, using an intact-plant chlorophyll fluorescence bioassay.

MATERIALS AND METHODS

Fluorescence determinations were made in the manner previously described by Shaw et al. (13), in which chlorophyll fluorescence induction curves were obtained from oats transplanted into herbicide-treated soil. Commercial formulations of metribuzin, diuron, or atrazine were added to 2 kg quantities of air-dried Kirkland loam soil (Abruptic Paleustoll; 1.1% organic natter, pH 7.0) to establish concentrations of 0, 0.13, 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg kg^{-1} ai of the herbicide. After thorough mixing, 250-g portions were placed in a series of 237-ml polystyrene-foam pots which were perforated at the bottom. A randomized complete block design with eight replications was used in each experiment, and all experiments were repeated. Prior to transplanting, oats were grown in perlite saturated with Hoagland's nutrient solution (6). These plants were grown under constant illumination, with a light intensity of 300±10 μmol photon m^{-2} s^{-1} and a temperature of 28±2°C. Fourteen days after seeding, an individual oat plant with four leaves was transplanted into a pot containing treated soil. The transplanting procedure was completed by subirrigating the soil to capacity, splitting the pot and soil vertically, placing the roots of the plant into this split, and replacing the halves back around the roots. These pots and plants were maintained under the illuminated environment for 23.5 and 47.5 h in the initial experiments, and for 3.5, 7.5, 23.5, and 47.5 h in the subsequent experiments. Plants were then moved to a darker environment (10 μmol photon m^{-2} s^{-1}) for 0.5 h to allow the photosystem to move to a lower state of activity. A portable plant productivity fluorometer[2]

---

[2]Model SF-10, Richard Brancker Research Ltd., Ottawa, Canada.

linked to a high-speed, computer-controlled data acquisition system was used to obtain chlorophyll fluorescence induction curves from these plants. The sensing probe of this instrument had been modified by partially covering the probe opening to leave a 1- by 3-mm slit in order to facilitate its use on the narrow oat leaves. The sensing probe was placed on the adaxial surface of the second leaf, and this probe-covered area remained in this darkened environment 3 minutes. The light-emitting diode of the probe, producing light at wavelengths around 670 nm, was adjusted to provide a light intensity of 7 μmol photon m^{-2} s^{-1}. The leaves were illuminated for a 30 s interval, and relative chlorophyll fluorescence intensity signals were collected every 10 msec, stored, and displayed graphically from the microcomputer. Software developed for this system allowed the user to automatically begin data collection at the onset of illumination from the fluorometer. Data collected was then displayed immediately after the 30 s illumination interval, and the computer automatically selected and displayed parameters of interest from the curve, with a manual override capability. After data collection was completed, a supplemental program was then used to calculate specific parameters of interest from each data file and stored these values in a composite file for data analysis.

RESULTS AND DISCUSSION

Characteristic changes in chlorophyll fluorescence from dark-adapted oat leaves can be observed when exposed to light from the fluorometer probe. Specific, observable points from these fluorescence curves are illustrated in Figure 1. Chlorophyll fluorescence typically begins at an initial low level (O), and increases rapidly to the initial inflection point (I). Fluorescence continues to increase at a slower rate to an initial peak (P), then declines to a lower level (S). Fluorescence then increases more slowly to a second maximum value (M), and subsequently decreases gradually to a terminal, steady-state value (T). From these points, various parameters can be calculated for use in evaluation of photosynthetic activity and injury. These include the height of I, P, and T, the slope from I to P and from P to S, the percent decay from P to T, the complementary area as described by Etienne et al. (5), and the I:P ratio.

When chlorophyll fluorescence induction curves are obtained from oats transplanted into herbicide-treated soil, characteristic changes in these curves are evident. Figure 2 depicts fluorescence curves obtained from plants transplanted into 0, 2, and 8 mg kg^{-1} metribuzin-treated soil, 4 h after transplanting. In comparison to the untreated plant's chlorophyll fluorescence curve, the curve from a plant in 2 mg kg^{-1} metribuzin has a higher level of the inflection point (I) in relation to the height of the peak level (P). The secondary fall and rise is not as prominent, and the terminal level (T) is somewhat elevated. At 8 mg kg^{-1}, there is little difference between the height of I and P, with both elevated much higher than the untreated plant. None of the secondary transients are evident, and T is much higher. Therefore, various

chlorophyll fluorescence parameters outlined may be valuable in making mathematical comparisons between photosynthetic-inhibitor treatments.

Within 4 h after transplanting oats into metribuzin-treated soil, the height of I, P, and T, the I:P ratio, and the percent decay from P to T were all highly correlated with metribuzin concentration (Table 1). The I:P ratio gave the highest correlation coefficient (r=0.92), whereas there was little correlation between metribuzin concentration and the slope from I to P, the slope from P to S, and the complementary area. After 8 h of exposure to metribuzin, these same fluorescence parameters were still well correlated with herbicide concentration. However, 24 h after transplanting, only the I:P ratio, percent decay, and the complementary area demonstrated a good correlation to metribuzin level in soil. The heights of I, P, and T actually became negatively correlated. This was due primarily to the fact that, after extended exposure to a photosynthetic inhibitor, chloroplasts within the plant are destroyed and begin to lose their ability to fluoresce, driving the chlorophyll fluorescence curve downward. Fluorescence parameters which involve a relationship between two or more points are advantageous under these circumstances, since they are not dependent on one simple height measurement.

The I:P ratio of oats transplanted into diuron-treated soil was well correlated to diuron concentration at all time intervals (Table 1). The height of I, P, and T correlated well with diuron concentration at 4 and 8 h after transplanting, whereas the slope from I to P and P to S, the percent decay, and the complementary area were better correlated at the later time intervals. As was the case with metribuzin, the later time intervals with diuron gave either no significant positive correla-

tion with I, P, or T height and diuron concentration or actually developed a tendency to become negatively correlated.

As with diuron, the I:P fluorescence parameter correlated well with atrazine concentration in soil at 4, 8, 24, and 48 h after transplanting. The height of I at 4 h, and the height of I, P, and T at 8 h provided sensitivity comparable to that of the I:P ratio. However, response to atrazine was similar to that for metribuzin and diuron, with I, P, and T either having no correlation or a negative correlation to atrazine concentration at 24 and 48 h after transplanting. At 24 and 48 h after transplanting the percent decay and complementary area parameters were useful indicators of atrazine phytotoxicity. The I to P slope and P to S slope were inferior to other parameter at each time interval with atrazine.

For metribuzin, 4 h after transplanting fluorescence data collected provided the best correlation between herbicide concentration and the parameters of fluorescence induction (Table 2). The I:P ratio was capable of distinguishing slightly lower metribuzin concentrations than was any other fluorescence parameter. However, the height of I also provided excellent delineation between the higher metribuzin concentrations, with significant differences between 8, 4, 2, 1, and 0.5 mg kg^{-1} or less. The heights of P and T were not as sensitive, with only three concentration ranges distinguishable from one another. The percent decay and complementary area were also able to distinguish the highest concentrations from the lower. However, variability for these parameters was much higher than for the I:P ratio, I, P, or T. The I to P and P to S slopes provided little distinction between metribuzin concentrations, and also had coefficients of variation (CV) values which were much

higher than those for I, P, T, or the I:P ratio.

With diuron, the highest average correlation between chlorophyll fluorescence parameters and herbicide concentration occurred after 8 h of herbicide exposure to oat roots. Eight hours after transplanting, the I:P ratio, I, P, and T all provided excellent sensitivity between diuron concentrations in soil (Table 3). However, at this time interval the heights of I, P, and T were already significantly lower at 8 mg kg^{-1} compared to 4 mg kg^{-1}, indicating a reduction in the overall height of the chlorophyll fluorescence curve as mentioned previously. As was the case with metribuzin, the I to P and P to S slopes and the complementary area could not distinguish as many concentration ranges for diuron, and CV values were higher than those for the height or I:P ratio parameters. The percent decay gave significant differences between 4, 2, and 1 mg kg^{-1} diuron, but variability again was higher.

As with diuron, the chlorophyll fluorescence parameters of oats in atrazine-treated soil correlated best, on average, at 8 h after transplanting. With atrazine, the I:P ratio and the height of I 8 h after transplanting were the most sensitive parameters to atrazine concentration, and could distinguish exactly the same concentrations (Table 4). The height of P and T, the P to S slope, and percent decay were not as sensitive, but could distinguish 4, 2, and 1 mg kg^{-1} from one another. Variability for the I:P ratio, height of I, height of P, and height of T was again much less than that of the other fluorescence parameters.

Fluorescence measurements from indicator species transplanted into treated soil can be obtained quite rapidly, and are useful tools in measuring inhibition of photosynthesis in plants. The I:P ratio suggested by Shaw et al. (13) has demonstrated a greater overall

correlation to herbicide concentration at all time intervals than the other chlorophyll fluorescence parameters examined in this research. However, the height measurements for P and T - and particularly I - did provide a great deal of concentration sensitivity at the earlier time intervals, with low coefficients of variation. Also, recent advances in electronic fluorescence detection equipment has provided the user with the capability of digital display of the peak and terminal levels of chlorophyll fluorescence, thereby circumventing the need for the more expensive data acquisition system. The primary objection to the use of one simple point measurement from the fluorescence induction curve is the decrease in overall chlorophyll fluorescence at the longer exposure times. The other parameters examined in this research, slope of I to P, slope of P to S, percent decay, and complementary area, were not as sensitive to a wide range of herbicide concentrations, and variability associated with them was higher. Therefore, as a broad spectrum bioassay technique, the I:P chlorophyll fluorescence parameter is highly desirable in determining specific concentrations of photosynthesis-inhibiting herbicides in soil.

REFERENCES

1. Ahrens, W.H., C.J. Arntzen, and E.W. Stoller. 1981. Chlorophyll fluorescence assay for the determination of triazine resistance. Weed Sci. 29:316-322.

2. Bohme, H., K.J. Kunert, and P. Boger. 1981. Sites of herbicidal action on photosynthesis: A fluorescence assay study. Weed Sci. 29:371-375.

3. Brewer, P.E., C.J. Arntzen, and F.W. Slife. 1979. Effects of atrazine, cyanazine, and procyazine on the photochemical reactions of isolated chloroplasts. Weed Sci. 27:300-308.

4. Devlin, R.M., A.J. Murkowski, I.I. Zbiec, S.J. Karezmarczyk, and E.M. Skorska. 1983. Influence of buthidazole, diuron, and atrazine on some light reactions of photosynthesis. Weed Sci. 31:879-883.

5. Etienne, A.L., C. Lemasson, and J. Lavorel. 1974. Quenching de la chlorophylle in vivo par le m-nitrobenzene. Biochim. Biophys. Acta 33:288-300.

6. Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 547.

7. Kautsky, H. and A. Hirsch. 1931. Neue versuche zur Kohlensaure-assimilation. Naturwissenschaften 19:964.

8. Melcarek, P.K. and G.N. Brown. 1977. The effects of chilling stress on the chlorophyll fluorescence of leaves. Plant Cell Physiol. 18:1099-1107.

9. Miles, C.D. and D.J. Daniels. 1973. A rapid screening technique for photosynthetic mutants of higher plants. Plant Sci. Lett. 1:227-240.

10. Papageorgiou, G. 1975. Chlorophyll fluorescence: An intrinsic probe of photosynthesis. Pages 319-371 in Govindjee (ed.) Bioenergetics of Photosynthesis. Academic Press, New York.

11. Richard, E.P., Jr., J.R. Gross, C.J. Arntzen, and F.W. Slife. 1983. Determination of herbicide inhibition of photosynthetic electron transport by fluorescence. Weed Sci. 31:361-367.

12. Schreiber, V., W. Vidaver, V.C. Runeckles, and P. Rosen. 1978. Chlorophyll fluorescence assay for ozone injury in intact plants. Plant Physiol. 61:80-84.

13. Shaw, D.R., T.F. Peeper, and D.L. Nofziger. 1985. Comparison of chlorophyll fluorescence and fresh weight as herbicide bioassay techniques. Weed Sci. 33:29-33.

14. Smillie, R.M. and R. Nott. 1982. Salt tolerance in crop plants monitored by chlorophyll fluorescence in vivo. Plant Physiol. 70:1049-1054.

15. Van Assche, C.J. and P.M. Carles. 1982. Photosystem II inhibiting chemicals. Pages 1-21 in D.E. Moreland, J.B. St. John, and F.D. Hess (eds.) Biochemical Responses Induced by Herbicides. Am. Chem. Soc., Washington, D.C.

Table 1. Simple correlation coefficients (r) comparing log of metribuzin, diuron, and atrazine concentrations and various chlorophyll fluorescence induction curve parameters.

Herbicide	Time after transplanting (h)	I:P† ratio	I	P	T	I-P slope	P-S slope	Percent decay	Complementary area
Metribuzin	4	0.92	0.89	0.84	0.85	-0.22	0.50	-0.79	-0.46
Metribuzin	8	0.68	0.71	0.69	0.72	-0.51	0.54	-0.72	-0.62
Metribuzin	24	0.60	-0.01	-0.37	-0.08	-0.38	0.46	-0.55	-0.52
Metribuzin	48	0.60	-0.34	-0.61	-0.44	-0.59	0.42	-0.48	-0.52
Diuron	4	0.73	0.74	0.59	0.67	-0.49	0.30	-0.60	-0.29
Diuron	8	0.84	0.86	0.79	0.82	-0.45	0.51	-0.76	-0.34
Diuron	24	0.86	0.50	0.12	0.49	-0.42	0.61	-0.86	-0.66
Diuron	48	0.84	0.07	-0.34	0.05	-0.66	0.54	-0.82	-0.76
Atrazine	4	0.78	0.72	0.38	0.55	-0.59	0.54	-0.54	-0.68
Atrazine	8	0.85	0.87	0.73	0.82	-0.60	0.67	-0.78	-0.51
Atrazine	24	0.88	0.71	0.39	0.64	-0.45	0.49	-0.79	-0.83
Atrazine	48	0.72	0.31	-0.07	0.25	-0.37	0.52	-0.72	-0.57

†From Shaw et al. (13).

Table 2. Relative chlorophyll fluorescence curve parameters from oats transplanted into metribuzin-treated soil, 4 h after transplanting.

Metribuzin concentration (mg kg^{-1})	I:P† ratio	I	P	T	I-P slope	P-S slope	Decay (%)	Complementary area
0.00	0.56 e‡	106 e	190 c	128 c	1.17 a	-0.14 b	32 a	4074 ab
0.13	0.59 e	112 e	190 c	125 c	0.93 a	-0.13 b	34 ab	5440 ab
0.25	0.60 de	116 e	194 c	122 c	0.95 a	-0.13 b	37 a	5590 ab
0.50	0.65 d	124 e	191 c	132 c	0.93 a	-0.12 b	30 bc	3989 ab
1.00	0.74 c	158 d	213 c	153 c	0.48 b	-0.04 a	28 c	5769 a
2.00	0.88 b	259 c	292 b	251 b	0.52 b	-0.03 a	15 d	2654 bc
4.00	0.91 ab	289 b	317 a	292 a	1.13 a	-0.04 a	8 e	673 c
8.00	0.95 a	315 a	332 a	308 a	0.74 ab	-0.03 a	7 e	344 c
CV (%)	8	15	10	15	48	-89	26	84

†From Shaw et al. (13).

‡Column means followed by the same letter were not significantly different at the 5% level according to LSD test.

Table 3. Relative chlorophyll fluorescence curve parameters from oats transplanted into diuron-treated soil, 8 h after transplanting.

Diuron concentration (mg kg^{-1})	I:P† ratio	I	P	T	I-P slope	P-S slope	Decay (%)	Complementary area
0.00	0.65 e‡	116 ef	168 d	118 de	0.97 ab	-0.19 b	30 a	1665 abc
0.13	0.65 e	117 ef	180 cd	118 de	1.19 a	-0.24 b	35 a	2176 ab
0.25	0.68 de	106 f	159 d	108 e	0.71 b	-0.17 b	32 a	3280 a
0.50	0.72 cd	131 de	183 c	120 de	0.86 ab	-0.22 b	34 a	1848 abc
1.00	0.76 bc	148 d	194 c	134 d	0.83 ab	-0.20 b	31 a	1563 abc
2.00	0.83 b	195 c	234 b	184 c	0.70 b	-0.16 b	22 b	1312 bc
4.00	0.95 a	273 a	289 a	265 a	0.51 b	-0.04 a	8 c	318 bc
8.00	0.96 a	246 b	257 b	231 b	0.54 b	-0.07 a	10 c	198 c
CV (%)	9	12	11	13	47	-50	18	123

†From Shaw et al. (13).

‡Column means followed by the same letter were not significantly different at the 5% level according to LSD test.

Table 4. Relative chlorophyll fluorescence curve parameters from oats transplanted into atrazine-treated soil, 8 h after transplanting.

Atrazine concentration (mg kg^{-1})	I:P[†] ratio	I	P	T	I-P slope	P-S slope	Decay (%)	Complementary area
0.00	0.56 d[‡]	121 d	220 c	136 c	1.52 a	-0.29 c	38 a	3865 ab
0.13	0.59 d	124 d	214 c	138 c	1.46 ab	-0.26 c	34 a	3499 ab
0.25	0.60 d	130 d	219 c	142 c	1.36 ab	-0.25 c	35 a	4961 a
0.50	0.62 cd	133 cd	217 c	146 c	1.27 ab	-0.27 c	32 a	3401 ab
1.00	0.69 c	159 c	230 c	153 c	1.12 b	-0.25 c	24 b	2633 abc
2.00	0.84 b	230 b	274 b	210 b	0.68 c	-0.13 b	23 b	1809 bc
4.00	0.94 a	309 a	329 a	303 a	0.72 c	-0.04 a	8 c	439 c
8.00	0.96 a	326 a	338 a	315 a	0.72 c	-0.03 a	7 c	182 c
CV (%)	11	14	13	16	33	-43	26	94

[†]From Shaw et al. (13).

[‡]Column means followed by the same letter were not significantly different at the 5% level according to LSD test.

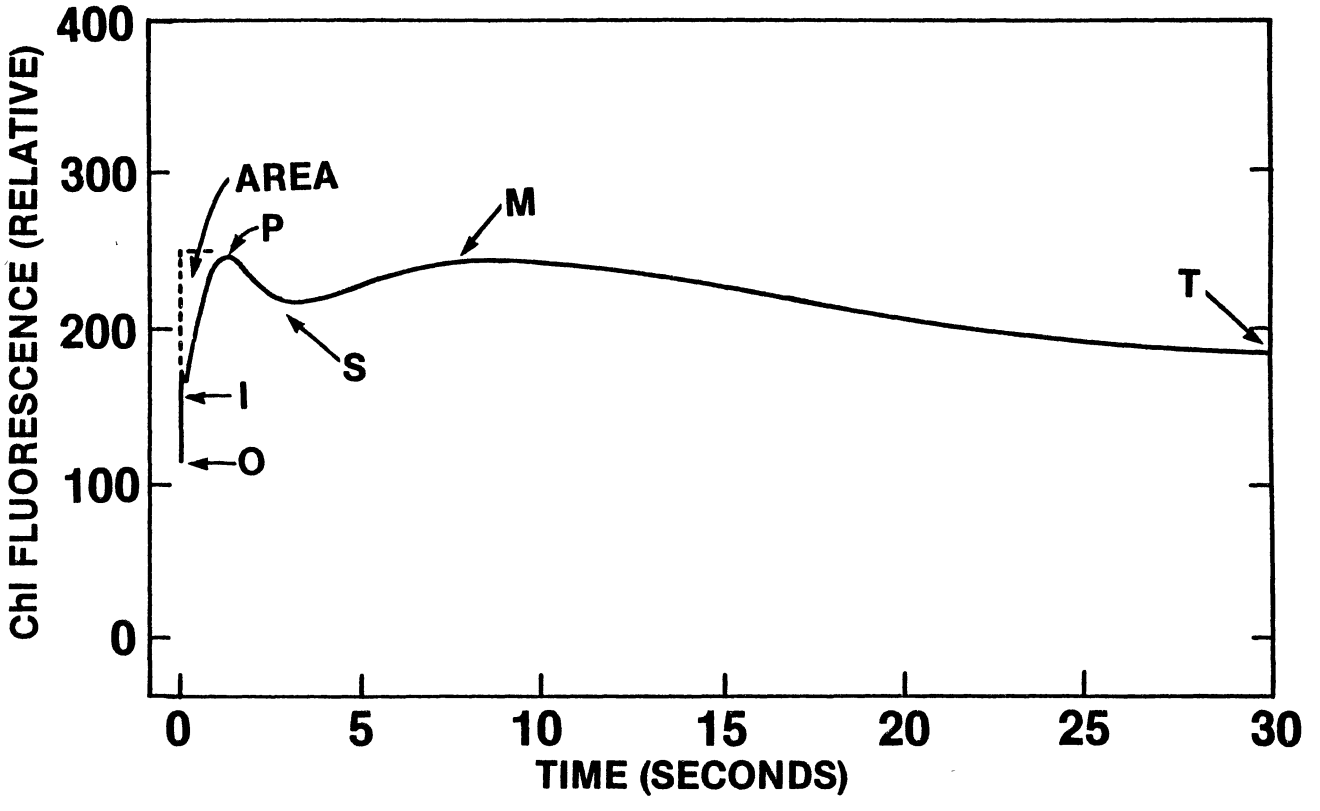

Figure 1. Relative chlorophyll fluorescence induction curve from oat, illustrating specific, observable parameters of induction transients.

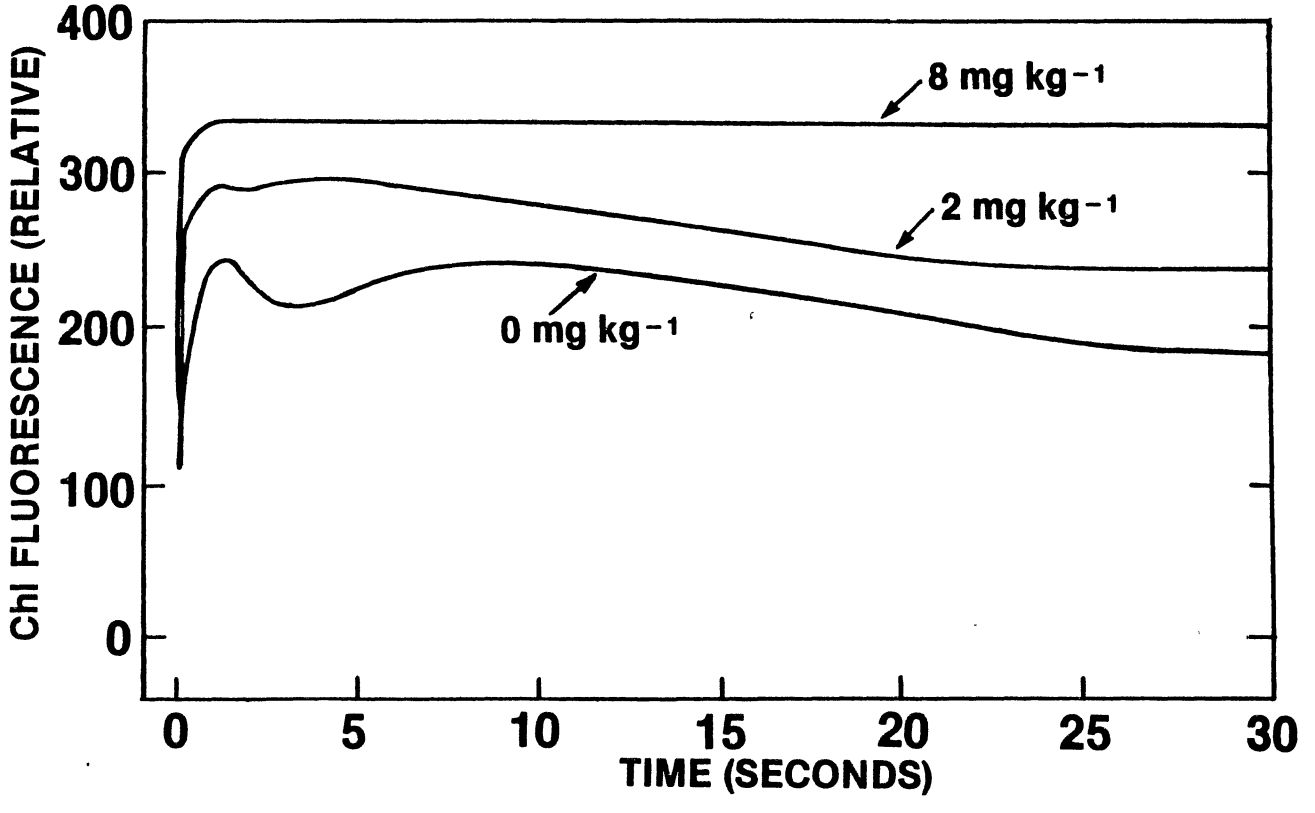

Figure 2. Relative chlorophyll fluorescence induction curves taken from oat leaves after 4 h exposure to 0, 2, and 8 mg kg^{-1} metribuzin.

PART III

PERSISTENCE OF PHYTOTOXICITY OF METRIBUZIN AND ITS ETHYLTHIO ANALOG

Persistence of Phytotoxicity of Metribuzin and Its Ethylthio Analog

Abstract. The persistence of biologically active metribuzin {4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one} and its ethylthio analog {4-amino-6-tert-butyl-3-(ethylthio)-as-triazin-5(4H)-one} were compared using an intact-plant chlorophyll fluorescence bioassay technique. Degradation of metribuzin phytotoxicity was linear over time, with a half-life of 8 days at 35°C. Initial degradation of the biologically active ethylthio analog was much more rapid than for metribuzin, with a decrease in rate at later time intervals. Therefore, quadratic regression analysis best described this degradation pattern. The initial degradation rate of phytotoxicity for the ethylthio analog indicated a half-life of 4 days at 35°C. Soil pH had no significant influence on the activity or persistence of either herbicide within the pH range used in this research.

Additional index words. Degradation, biological activity, chlorophyll fluorescence bioassay.

INTRODUCTION

Metribuzin is currently the only herbicide labeled for cheat (Bromus secalinus L. #[1] BROSE) control in hard red winter wheat (Triticum aestivum L.) in Oklahoma. However, due to differential variety tolerance (11), it can only be applied to four hard red winter wheat

---

[1]Letters following this symbol are a WSSA-approved computer code from Weed Sci. 32 (Supp. 2), 1984.

cultivars. There are also soil pH, textural, and organic matter restrictions associated with metribuzin application. Due to these varietal and soil limitations, other compounds are under investigation for cheat control. One such compound is the ethylthio analog of metribuzin, BAY SMY 1500 or DPX R 7910. Preliminary research indicates that this herbicide is effective as a preplant or early postemergence treatment for selective cheat and Italian ryegrass (Lolium multiflorum Lam. # LOLMU) control in winter wheat (9). Other field studies have indicated that metribuzin's ethylthio analog can be applied early postemergence to several winter wheat cultivars with little or no crop injury (10). The reduction in phytotoxicity observed with the ethylthio compound may be attributed to decreased root uptake because of the lower water solubility of this analog (350 ppm[2] versus 1220 ppm for metribuzin) (15). A similar methylthio-to-ethylthio substitution was made with prometryn {2,4-bis(isopropylamino)-6-(methylthio)-s-triazine} to obtain dipropetryn {2-(ethylthio)-4,6-bis(isopropylamino)-s-triazine}. The resulting decrease in water solubility, from 48 to 16 ppm (15), enabled the use of dipropetryn on much coarser textured soil. However, little is known about the effect of the methylthio-to-ethylthio substitution on persistence.

The persistence of metribuzin has been evaluated under various environmental conditions, with its half-life reportedly varying according to temperature, soil texture, soil pH, and detection technique (4, 6, 8, 12). Savage (12), using chemical extraction, found that the

---

[2]Technical Information Sheet - BAY SMY 1500. Mobay Chemical Corporation, Kansas City, MO, 6 pp.

half-life of metribuzin varied from 17 to 29 days in six soils. Hyzak and Zimdahl (6) also used chemical extraction, and reported that the half-lives of metribuzin, an isopropyl analog {4-amino-6-isopropyl-3-(methylthio)-as-triazin-5(4H)-one}, and a cyclohexyl analog {4-amino-6-cyclohexyl-3-(methylthio)-as-triazin-5(4H)-one} were similar, with values of 44 and 16 days at temperatures of 20 and 35°C, respectively, in a sandy loam soil. Bouchard et al. (3), using a dry-weight bioassay, reported that the half-life of metribuzin at 23 and 37°C in a Taloka silt loam soil, pH 5.2, was 18 and 17 days, respectively. Ladlie et al. (8), using a chemical extraction technique, reported that the half-life of metribuzin decreased as soil pH increased. However, this decrease was only 14% between soil pH 4.6 and 6.7. Ladlie et al. (7) also reported that the activity of metribuzin, as detected by a corn (Zea mays L.) and soybean (Glycine max L. Merr.) bioassay, increased as soil pH increased. However, later research by Ballerstedt and Banks, who used oats (Avena sativa L.) (1) and grain sorghum (Sorghum bicolor L. Moench) (2), indicated that the initial activity of metribuzin was not influenced by soil pH.

Although the persistence of molecular integrity is of regulatory interest, the persistence of the biologically active portion of soil-applied herbicides may be of more interest for practical weed control purposes. The objectives of this research were to compare the persistence of biological activity of metribuzin and its ethylthio analog using a chlorophyll fluorescence bioassay technique (13), and to evaluate the influence of soil pH on the decrease in biological activity over time.

MATERIALS AND METHODS

Standard curve development. Soil from a long-term liming study established in north-central Oklahoma in 1978 was collected in March 1984, for use in all experiments. The Pond Creek silt loam (Fine, silty, mixed, thermic Pachic Argiustoll, 0.8% organic matter, CEC=19.0 meq/100 g) was limed in 1978 with 0 to 21.5 metric tons$\cdot$ha^{-1}, which resulted in pH values of 4.9, 5.4, 5.7, 6.4, and 6.9 for the five lime treatments at the time of sampling. Nitrogen (as urea-N) at a rate of 224 kg$\cdot$ha^{-1} actual N was applied in 1978, 1979, and 1980 to all treatments. Individual soil cores from the upper 10 cm of each treatment were thoroughly blended for uniformity. Since a preliminary bioassay indicated that soil pH had no influence on the initial activity of metribuzin or the ethylthio analog, only the high pH soil (pH=6.9) was used in the standard curve development. The lack of pH effect on activity was anticipated, since Ballerstedt and Banks (1, 2) had reported that soil pH had no influence on the initial activity of metribuzin.

Appropriate quantities of metribuzin (75 DF) or BAY SMY 1500 (50 WP) were added to 4 kg quantities of air-dried, screened soil to establish concentrations of 0, 0.13, 0.25, 0.5, and 1.0 ppm (w/w) ai of the herbicides. After thorough mixing, 250-g portions were placed into a series of 237-ml polystyrene-foam pots which were perforated at the bottom.

The bioassay species oats ('Chilocco') and wheat ('TAM W 101') were grown for 14 days in perlite saturated with half-strength Hoagland's nutrient solution (5). All plants were grown under constant illumination with a light intensity of 300±50 $\mu E\cdot m^{-2}\cdot s^{-1}$ and a temperature of 35±3°C. Fourteen days after seeding, an individual wheat or oat plant

with four leaves was transplanted into each pot of treated soil. Transplanting was accomplished by subirrigating each pot to capacity, splitting the pot and soil vertically, and replacing the halves around the roots of the plant. These pots and plants were removed from their illuminated environment 23.5 h later. After one-half hour, during which the photosystem moved to a lower state of activity, chlorophyll fluorescence induction curves from each plant were recorded in a manner similar to that described by Shaw et al. (13). All fluorescence measurements were obtained with a portable plant productivity fluorometer[3]. The sensing probe of this fluorometer was placed on the adaxial surface of the second leaf. The probe-covered area remained in this darkened environment 3 min. The light-emitting diode of the sensing probe, centered around 670 nm, was adjusted to provide a light intensity of 24 $\mu E \cdot m^{-2} \cdot s^{-1}$. The leaves were illuminated for a 30 s interval, and relative chlorophyll fluorescence intensity signals were collected every 10 msec, stored, and displayed graphically using a high-speed, computer-controlled data acquisition system. The ratio of the initial inflection point (I) to that of the variable fluorescence peak (P) was determined from each chlorophyll fluorescence induction curve. A randomized complete block design with three replications was used for each herbicide-bioassay species combination, and each study was repeated.

Persistence. Formulations of metribuzin or the ethylthio analog were added to 9.5 kg quantities of the soil from each liming treatment in the pH study to establish an initial concentration of one ppm (w/w) ai for each herbicide. After thorough mixing, the soil was potted as

---

[3]Model SF-20, Richard Brancker Research Ltd., Ottawa, Canada.

previously mentioned. In this work, a randomized complete block design with a factorial arrangement of treatments was employed, with five soil pH levels, two herbicides, two bioassay species, five weekly time intervals, and four replications. All pots were watered twice weekly to maintain approximate field moisture conditions. At weekly intervals, one-fifth of these pots were removed from the previously described growth room. These pots then received transplanted wheat or oat plants, in a manner exactly as described in the standard curve development. Chlorophyll fluorescence measurements were obtained from those transplanted bioassay species as described previously. The I:P ratios calculated from fluorescence induction curves could then be entered into the appropriate standard curve equation (Table 1), and the actual herbicide concentration indicated from the curve was calculated.

RESULTS AND DISCUSSION

Standard curve development. The standard curve for each species was developed by plotting the I:P fluorescence parameter versus the log of herbicide concentration (Figures 1 and 2). With oats as the indicator species, each increase in the herbicide concentration increased the I:P ratio obtained from plants in pots with both metribuzin and the ethylthio analog. Linear and quadratic regression analysis was used, and quadratic regression provided the best fit for both herbicides. Correlation coefficients (r) of curves for metribuzin and the ethylthio analog using oats as an indicator species were 0.97 and 0.98, respectively (Table 1). Curves constructed for each herbicide were quite similar in shape. However, with oats the standard curve for the ethylthio analog was shifted to the right somewhat, indicating that a greater

concentration of the ethylthio analog was necessary to cause the same degree of inhibition of photosynthesis. This would agree with field research (10), which indicates that 2 to 3 times more of the ethylthio analog is necessary to provide activity comparable to metribuzin.

When a metribuzin-tolerant hard red winter wheat cultivar was used as the indicator species in the standard curve development, response to metribuzin was still similar to the response of oats (Figure 2). Thus with each concentration increase, metribuzin increased the I:P fluorescence parameter, which indicates a lack of high levels of tolerance. In contrast, however, only the higher concentrations of the ethylthio analog influenced the I:P ratio of this wheat cultivar. Quadratic regression analysis again provided the best-fitting curve for both herbicides, with correlation coefficients of 0.96 and 0.86 for metribuzin and its ethylthio analog, respectively (Table 1). As was the case with oats, wheat as the bioassay species indicated that much higher concentrations of the ethylthio analog than of metribuzin were necessary to give the same level of injury to the photosystem of the plant.

In comparing the standard curves developed with oats and wheat as the bioassay species, both species were comparable in their sensitivity to metribuzin. However, the oat bioassay was much more sensitive to a wider range of concentrations of the ethylthio analog than was wheat. For this reason, only the standard curves developed with the oat bioassay were used in the degradation calculations. In these calculations, the I:P ratio of oat leaves could be obtained from plants transplanted into soil which had been allowed to degrade the herbicide for a specified length of time. This I:P ratio could be used in the appropriate standard curve equation from Table 1 to determine the actual concentra-

tion of biologically active herbicide in the soil at that time interval.

Persistence. Soil pH had no significant influence on the persistence of phytotoxicity of either metribuzin or the ethylthio analog. Therefore, degradation curves shown for each herbicide are averaged over both replicate and soil pH level (Figure 3). The biological activity degradation pattern of metribuzin was essentially linear within the time frame examined, with a linear correlation coefficient for the equation of 0.89 (Table 2). The half-life of biologically active metribuzin derived from this linear equation was 8 days. This half-life value is somewhat less than that reported by Hyzak and Zimdahl (6). However, their research involved a chemical extraction and analysis technique. Our research, on the other hand, involved a plant bioassay system, and therefore measured herbicide actually available for plant uptake.

In comparison to the degradation of phytotoxicity of metribuzin, the ethylthio analog had a much more rapid initial dissipation rate (Figure 3), with a gradual decrease in the rate over time. Thus, a quadratic relationship best described its degradation pattern, with a correlation coefficient of 0.88 (Table 2). The initial half-life derived from this degradation curve for the ethylthio analog was 4 days. The decreasing rate of herbicide degradation with the ethylthio analog may be due in part to adsorption factors. Weber (14) has suggested that herbicides normally decomposed by soil microorganisms may be somewhat protected when adsorbed on the soil colloid. Hyzak and Zimdahl (6) theorized that this phenomenon also occurred with metribuzin under certain circumstances. Since the water solubility of the ethylthio analog is 3.5 times less than that of metribuzin, the soil activity of the ethylthio analog would tend to be influenced more by the colloid

adsorption, and therefore show more of a decreasing rate of degradation over time.

The persistence of herbicides is of great interest in the development of herbicide programs for rotational cropping systems, as well as double-crop situations. Chemical extraction and analysis is useful in reporting the total herbicide concentration in soil, but usually cannot be used to indicate the amount of herbicide actually available for plant uptake. However, a rapid, intact-plant chlorophyll fluorescence bioassay technique such as that used in our research provides a means to accurately monitor biologically active levels of photosynthetic-inhibitor herbicides in soil.

LITERATURE CITED

1. Ballerstedt, P.J. and P.A. Banks. 1981. Herbicidal activity and persistence in soils as affected by soil pH. Proc. So. Weed Sci. Soc. 34:246.

2. Ballerstedt, P.J. and P.A. Banks. 1983. Behavior of alachlor and metribuzin as affected by soil pH. Proc. Weed Sci. Soc. Am. 35:89.

3. Bouchard, D.C., T.L. Lavy, and D.B. Marx. 1982. Fate of metribuzin, metolachlor, and fluometuron in soil. Weed Sci. 30:629-632.

4. Hance, R.J. and R.A. Haynes. 1981. The kinetics of linuron and metribuzin decomposition in soil using different laboratory systems. Weed Res. 21:87-92.

5. Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 547.

6. Hyzak, D.L. and R.L. Zimdahl. 1974. Rate of degradation of metribuzin and two analogs in soil. Weed Sci. 22:75-79.

7. Ladlie, J.S., W.F. Meggitt, and D. Penner. 1976. Effect of pH on metribuzin activity in the soil. Weed Sci. 24:505-507.

8. Ladlie, J.S., W.F. Meggitt, and D. Penner. 1976. Role of pH on metribuzin dissipation in field soils. Weed Sci. 24:508-511.

9. O'Bryan, K.A. and T.F. Peeper. 1984. Control of annual grasses in winter wheat with DPX 5882 and SMY 1500. Proc. So. Weed Sci. Soc. 37:109.

10. Ratliff, R.L. and T.F. Peeper. 1985. Bromus control in winter wheat with BAY SMY 1500. Proc. So. Weed Sci. Soc. 38:in press.

11. Runyan, T.J., W.K. McNeil, and T.F. Peeper, 1982. Differential tolerance of wheat (Triticum aestivum) cultivars to metribuzin.

Weed Sci. 30:94-97.

12. Savage, K.E. 1977. Metribuzin persistence in soil. Weed Sci. 25:55-59.

13. Shaw, D.R., T.F. Peeper, and D.L. Nofziger. 1985. Comparison of chlorophyll fluorescence and fresh weight as herbicide bioassay techniques. Weed Sci. 33:29-33.

14. Weber, J.B. 1970. Mechanisms of adsorption of s-triazines by clay colloids and factors affecting plant availability. Pages 93-127 in F.A. Gunther and J.D. Gunther, eds. Residue Reviews, vol. 32. Springer-Verlag, New York.

15. Weed Science Society of America. 1983. Herbicide Handbook, 5th Edition, 515 pp.

Table 1. Standard curve equations and correlation coefficient (r) values for metribuzin and the ethylthio analog with two bioassay species.

Bioassay Species	Herbicide	Equation	Correlation Coefficient (r)
oat	metribuzin	$Y^a = 1.004 - 0.039 \times X^b - 0.223 \times X^2$	0.97
oat	ethylthio analog	$Y = 0.972 + 0.097 \times X - 0.139 \times X^2$	0.98
wheat	metribuzin	$Y = 0.980 + 0.022 \times X - 0.149 \times X^2$	0.96
wheat	ethylthio analog	$Y = 0.832 + 0.314 \times X + 0.113 \times X^2$	0.86

[a]Y = I:P fluorescence ratio.

[b]X = log of herbicide concentration (ppm).

Table 2. Degradation equations, correlation coefficient (r) values, and half-lives ($t_{1/2}$) of metribuzin and the ethylthio analog.

Herbicide	Degradation Equation	Correlation Coefficient (r)	$t_{1/2}$ (days)
metribuzin	$Y^a = 0.177 - 0.259 \times X^b$	0.89	8
ethylthio analog	$Y = 0.231 - 0.573 \times X + 0.071 \times X^2$	0.88	4

[a] Y = log of herbicide concentration (ppm).

[b] X = time in weeks.

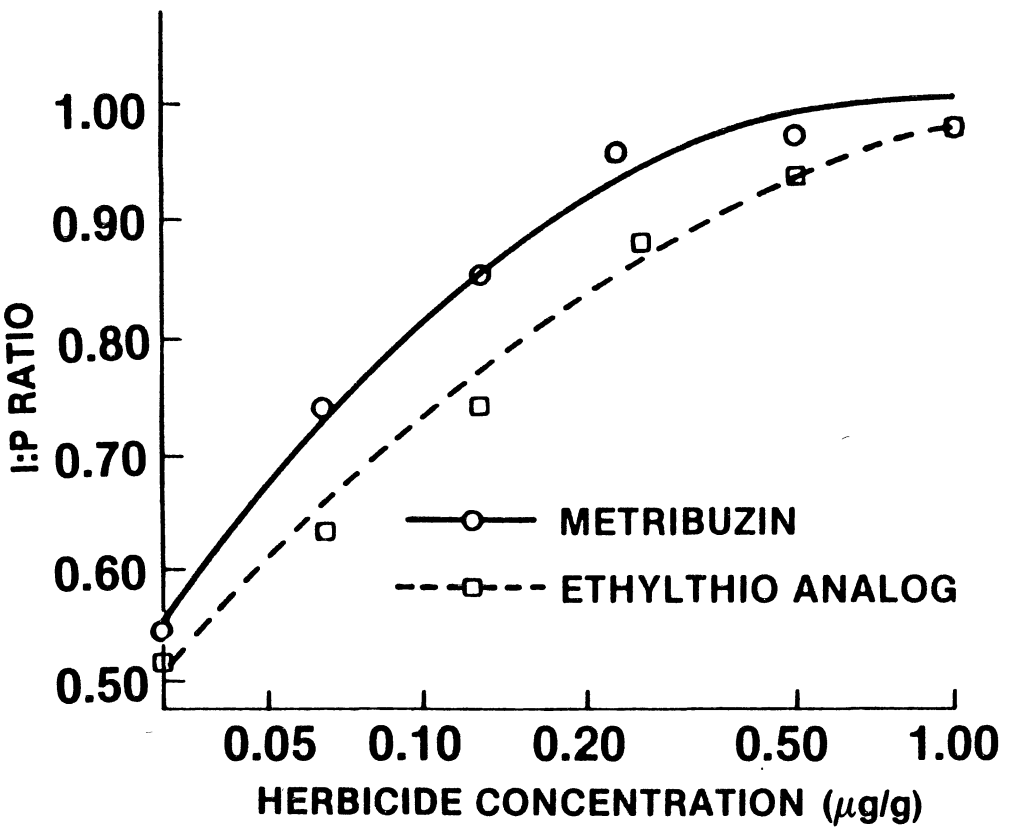

Figure 1. Standard curves for metribuzin and its ethylthio analog with oats as the indicator species in the chlorophyll fluorescence bioassay.

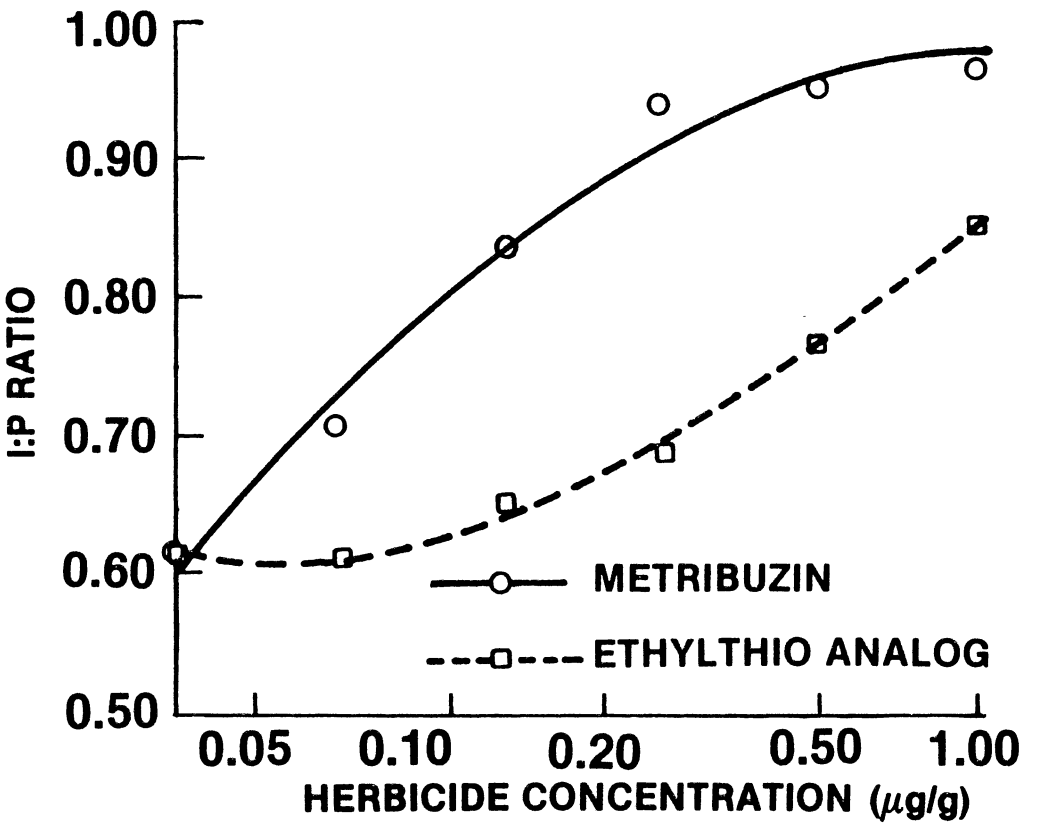

Figure 2. Standard curves for metribuzin and its ethylthio analog with wheat as the indicator species in the chlorophyll fluorescence bioassay.

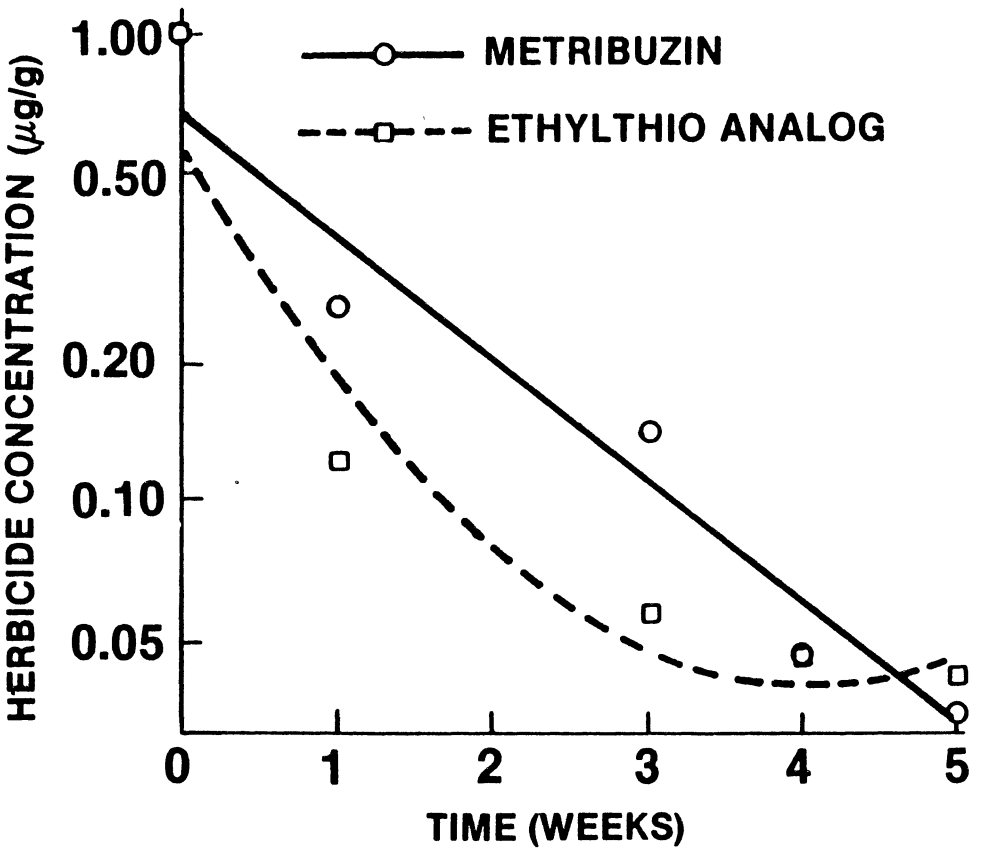

Figure 3. Rate of degradation of biologically-active metribuzin and its ethylthio analog derived from the chlorophyll fluorescence bioassay.

VITA 2

David Ray Shaw

Candidate for the Degree of

Doctor of Philosophy

Thesis: INVESTIGATIONS ON CHLOROPHYLL FLUORESCENCE ASSAY TECHNIQUES USING PHOTOSYNTHETIC INHIBITOR-TYPE HERBICIDES

Major Field: Crop Science

Biographical:

Personal Data: Born in Water Valley, Mississippi, October 18, 1959, the son of Bobby and Rose Ann Shaw.

Education: Graduated from Rush Springs High School, Rush Springs, Oklahoma, in May 1977; received Bachelor of Science degree from Cameron University, Lawton, Oklahoma, with a major in Agriculture in May 1981; received Master of Science degree from Oklahoma State University, with a major in Agronomy in May 1983; completed the requirements for Doctor of Philosophy degree with a major in Crop Science at Oklahoma State University in May 1985.

Experience: Raised and worked on family farm near Rush Springs, Oklahoma; farmed full-time March 1977 through May 1981; graduate research assistant, Oklahoma State University, June 1981 through May 1985.

Professional Memberships: Weed Science Society of America, Crop Science Society of America, Southern Weed Science Society, American Society of Agronomy, Council for Agricultural Science and Technology.