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COMPARISON OF OATS AND LEMNA OLIGORRHIZA
FOR DIURON BIOASSAY

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SUMMARY

A rapid bioassay using thin duckweed (*Lemna oligorrhiza*) was found to be more sensitive than oats for detecting diuron in soil.

I. INTRODUCTION

Studies in north Queensland have confirmed the effectiveness of diuron (N¹—(3,4-dichlorophenyl)NN-dimethylurea) for weed control in tea (*Camellia sinensis*), reported by Rochecouste and Pilot (1966). A recent experiment studied the effect of repeated applications of diuron to the soil around mature tea trees (Bailey, unpublished), and the purpose of this paper is to compare the indicator plants used in bioassays of this investigation. Preliminary results have already been briefly reported elsewhere (Bailey 1968).

Because oats are widely accepted as an indicator plant for diuron detection (Hill *et al.* 1955; Harris and Sheets 1965; Schweizer and Holstun 1966; Dalton, Evans and Rhodes 1966; Bayer 1967; Dawson, Bruns and Clore 1968) they were used initially, but were later discarded in favour of a rapid bioassay technique described by Parker (1965). Italian ryegrass (*Lolium multiflorum*) was also used in a few instances.

II. MATERIALS AND METHODS

Soil.—An alluvial silty clay-loam showing little change in clay, silt or sand content down to 2 ft was used (Table 1). Samples were taken from several sites in 6 in. intervals to 2 ft, composited, dried to *c.* 5% moisture, passed through a 2 mm sieve, and then thoroughly mixed.

Preparation of standards.—Weighed amounts of soil (oven-dry basis) were placed in polythene bags and known amounts of diuron added in 2–10 ml of water. Du Pont "Karmex" was the diuron source. The herbicide was incorporated by inflating the bags and shaking them vigorously by hand.

TABLE 1
PROPERTIES OF THE SOIL USED IN THE BIOASSAYS

Depth (in.)	Organic Carbon (%) [*]	Clay (%)	Silt (%)	Sand (%)
0-6	1.80 ± 0.19 [†]	42	24	34
6-12	1.44	41	26	33
12-18	0.82	43	25	32
18-24	0.51	42	25	33

^{*} Walkley and Black.

[†] Mean of 6 samples ± S.D.

Oat bioassay.—In the first test one plant of the variety (cv.) Saia and one plant of Bentland were grown in free-draining waxed-paper cups containing 220 g of soil. Cups were surface-watered with a nutrient solution (A.C.F. and Shirleys "Liquifert"), care being taken to avoid leaching. Each treatment was replicated five times and green weights of oat shoots were recorded 38 days after planting ungerminated seed.

In an endeavour to find a variety more sensitive to diuron, Saia and Bentland were compared with seven other varieties in a factorial experiment of four replicates (Table 2). Soil was from the 0-6 in. layer, and experimental procedures were similar to the first test, with two seedlings being grown per cup. Green weights of shoots were taken 25 days after planting germinated seed.

Italian ryegrass bioassay.—Ungerminated seed from commercial seed lots was used in both tests. Soil was from the 0-6 in. layer in the first test and the 12-18 in. and 18-24 in. layers in the second. Treatments were replicated five times and experimental methods were the same as for the oat bioassay. Six seedlings were grown per cup in the first test and five in the second. In the first, green and oven-dried (95°C) weights of shoots, together with total seedling height per cup, were recorded 31 days after planting. Oven-dried shoot weights were recorded in the second test 28 days after planting.

Rapid bioassay.—Parker (1965) has described the method in detail and has given the background theory. In this particular case, it relied upon the bleaching action of paraquat (1,1'-dimethyl-4,4'-bipyridylium-2A) being inhibited when diuron was present in the indicator plant.

Procedures were similar to those of Parker (1965), but thin duckweed (*Lemna oligorrhiza*) was substituted for *L. minor*. Thin duckweed was usually taken direct from the field but in some instances it was maintained in water culture for 1 or 2 months before use. Slurries were prepared by mixing 100 g of soil with tap-water for 1 min with a mechanical stirrer. To ensure sufficient free water at the top of the slurry, it was found necessary to use the following amounts of water for each sampling depth: 0-6 in., 100 ml; 6-12 in., 100 ml; 12-18 in., 110 ml; and 18-24 in., 120 ml. Initially waxed-paper cups were used to contain the slurries, but because these leaked slightly, plastic cups were used to contain the slurries in later experiments. The number of thin duckweed fronds placed in each cup varied from experiment to experiment, but generally there were about 30 in each. Pre-paraquat and post-paraquat light exposures consisted of 8 hr in a shaded glasshouse, which transmitted about 40% of full sunlight, and 16 hr (5.00 p.m. to 9.00 a.m.) 18 in. below six 40W fluorescent

tubes. Paraquat was applied at 1 lb ion/ac with an Oxford Precision Sprayer, using Teejet 730116 nozzles giving an output of 14 gal/ac. "Gramoxone" was the paraquat source and no wetting agent was used with the spray.

As a rule, the fronds were bleached sufficiently to permit scoring 24 hr after spraying. They were rated on a 0-5 scale similar to that outlined by Parker (1965), but because of their small size, a pair of watch-maker's magnifying glasses (x5) were used for assessment. Each frond was rated separately and the average score per cup calculated.

There were four replicates in the first test and five in all others.

Statistical analyses.—All data were subjected to analysis of variance without transformation.

III. RESULTS

Table 2 shows the response of nine oat varieties to six rates of diuron.

TABLE 2
RESPONSE OF NINE OAT VARIETIES TO DIURON IN SOIL FROM 0-6 IN. DEPTH
Green shoot weight (g)

Variety	Diuron (p.p.m.)						Mean
	0	$\frac{1}{2}$	1	2	4	8	
Saia	1.95	1.94	1.75	1.55	1.18	0	1.40
Bentland	2.65	3.22	2.71	2.90	2.26	0.56	2.38
Klein	2.27	1.95	2.32	1.63	2.00	0.57	1.79
Santa Fe	2.19	2.40	2.49	2.26	1.63	0.18	1.86
Landhafer	2.73	3.16	2.65	2.68	2.10	1.23	2.42
Algerian (Q2831)	1.96	1.88	2.06	2.14	1.39	0.54	1.66
Minhafer	3.27	3.54	2.76	3.74	3.14	1.24	2.95
Ballidu	2.20	1.83	2.05	1.62	1.93	0.33	1.66
Fulghum	2.60	2.05	2.19	2.81	1.25	0.30	1.87
Mean	2.42	2.44	2.33	2.37	1.88	0.55	

	Within Table	Rate Means	Variety Means
Necessary differences for { 5% significance	0.94 1.24	0.31 0.41	0.38 0.50
Variety x Rate interaction	n.s.		

Shoot weights of all varieties were significantly ($P < 0.01$) reduced by diuron at 8 p.p.m., and Fulghum was the only variety to show significant reduction ($P < 0.01$) at 4 p.p.m. The variety x diuron rate interaction was, however, not significant, indicating an overall similar varietal response. Averaged over all varieties, shoot weights were only significantly ($P < 0.01$) reduced by 4 and 8 p.p.m. of diuron. In terms of overall shoot yield, Minhafer was the most productive variety and Saia the least.

The effects of diuron on oats (first test), Italian ryegrass (first test) and thin duckweed in soil from the 0-6 in. layer are shown in Table 3. Oats were insensitive to diuron at rates up to 4 p.p.m. in the 0-6 in. layer, whereas Italian ryegrass was more sensitive. In seven of the eight tests thin duckweed detected ($P < 0.01$ or $P < 0.05$) diuron at 4 p.p.m. and at 2 p.p.m. in five tests.

TABLE 3

RESPONSE OF OATS, ITALIAN RYEGRASS AND THIN DUCKWEED TO DIURON IN SOIL FROM 0-6 IN. DEPTH

Indicator Plant	Diuron (p.p.m.)						
	0	$\frac{1}{4}$	$\frac{1}{2}$	1	2	4	8
Oats (Saia and Bentland) (a)	2.01	2.57	1.69	2.01	1.58	2.32	†
Italian ryegrass (a)	0.51	†	0.38*	0.39*	0.42	0.40	0.13**
(b)	66	†	54	53	56	53	16**
(c)	37.6	†	34.7	33.5	35.0	32.5*	19.5**
Thin duckweed (d)†	3.90	†	3.80	2.95**	1.58**	0.22**	0.06**
(d)	4.07	4.05	4.15	4.06	3.88	3.50	1.70**
(d)	2.79	3.18	3.00	2.58	2.58	1.42**	†
(d)	3.84	3.23	2.78**	2.94**	1.14**	0.73**	†
(d)	4.20	3.92	3.75	4.27	3.56	2.76**	†
(d)	2.02	2.29	1.97	1.50	0.78**	0.59**	0.69**
(d)	3.71	3.77	3.51	3.24	2.95*	1.76**	0.80**
(d)	4.49	4.62	4.59	4.55	3.84*	1.38**	0.52**

† Not included in test. ‡ First test (see text). * Less than 0 p.p.m. at $P < 0.05$.
 ** Less than 0 p.p.m. at $P < 0.01$. (a) Green shoot weight in g. (b) Dry shoot weight in mg. (c) Total height of shoots per cup in in. (d) Paraquat damage score.

TABLE 4

RESPONSE OF OATS AND THIN DUCKWEED TO DIURON IN SOIL FROM 6-12 IN. DEPTH

Indicator Plant	Diuron (p.p.m.)						
	0	$\frac{1}{4}$	$\frac{1}{2}$	1	2	4	8
Oats (Saia and Bentland) (a)	1.89	1.84	1.64	1.61	0.89**	†	†
Thin duckweed (b)	3.06	3.01	1.96**	1.87**	1.50**	1.55**	1.17**
(b)	3.37	3.01	2.50**	2.19**	1.32**	1.20**	†
(b)	2.84	2.84	2.64	1.87	1.03**	0.95**	†
(b)	3.51	2.33**	1.19**	1.18**	0.84**	0.98**	1.01**
(b)	4.11	4.50	4.31	3.37	1.83**	0.89**	1.10**

† Not included in test. * Less than 0 p.p.m. at $P < 0.05$. ** Less than 0 p.p.m. at $P < 0.01$. (a) Green shoot weight in g. (b) Paraquat damage score.

The response of oats (first test) and thin duckweed in soil from the 6-12 in. layer is shown in Table 4. Both plants responded ($P < 0.01$) to diuron at 2 p.p.m. in the 6-12 in. layer, and in three of the five tests thin duckweed detected ($P < 0.01$) diuron at $\frac{1}{2}$ p.p.m.

The effects of diuron on oats (first test), Italian ryegrass (second test) and thin duckweed in the 12-18 in. and 18-24 in. layers are presented in Tables 5 and 6. Table 5 shows that Italian ryegrass was more responsive than oats in the 12-18 in. layer and that thin duckweed was equivalent to Italian ryegrass in the first test, superior in the second and inferior in the third. In the 18-24 in. layer all three indicator plants detected ($P < 0.01$) diuron at $\frac{1}{4}$ p.p.m., and Italian ryegrass and thin duckweed responded ($P < 0.01$) at $\frac{1}{2}$ p.p.m. Oats were not tested at rates below $\frac{1}{4}$ p.p.m.

TABLE 5

RESPONSE OF OATS, ITALIAN RYEGRASS AND THIN DUCKWEED TO DIURON IN SOIL FROM 12-18 IN. DEPTH

Indicator Plant	Diuron (p.p.m.)						
	0	1/16	1/8	1/4	1/2	1	2
Oats (Saia and Bentland) .. (a)	1.52	†	†	1.82	0.67*	†	†
Italian ryegrass (b)	0.30	†	0.21	0.13*	0.05**	0.003**	†
Thin duckweed (c)	3.37	†	2.67	2.32*	1.81**	†	†
	3.15	1.69**	1.17**	0.66**	0.57**	0.74**	†
	4.43	4.67	4.40	3.81	2.67**	1.03**	0.78**

† Not included in test. * Less than 0 p.p.m. at $P < 0.05$. ** Less than 0 p.p.m. at $P < 0.01$. (a) Green shoot weight in g. (b) Dry shoot weight in g. (c) Paraquat damage score.

TABLE 6

RESPONSE OF OATS, ITALIAN RYEGRASS AND THIN DUCKWEED TO DIURON IN SOIL FROM 18-24 IN. DEPTH

Indicator Plant	Diuron (p.p.m.)					
	0	1/16	1/8	1/4	1/2	1
Oats (Saia and Bentland) (a)	1.88	†	†	0.61*	†	†
Italian ryegrass (b)	0.25	†	0.07**	0.04**	†	†
Thin duckweed (c)	3.05	2.00**	1.93**	1.46**	1.33**	†
	2.26	1.56*	1.08**	0.75**	0.50**	0.77**
	4.12	3.38	1.68**	1.53**	1.37**	1.15**

† Not included in test. * Less than 0 p.p.m. at $P < 0.05$. ** Less than 0 p.p.m. at $P < 0.01$. (a) Green shoot weight in g. (b) Dry shoot weight in g. (c) Paraquat damage score.

IV. DISCUSSION

Thin duckweed was generally more sensitive than oats for diuron detection and had the advantage that a bioassay could be completed in 4 days as opposed to 4 or 5 weeks for oats. A bioassay involving up to 70 cups could be handled satisfactorily by two persons working with sieved soil to the following schedule: first day (9.00 a.m.-4.00 p.m.), preparation of standards and slurries; second day (10.00 a.m.-12.00 noon), placement of fronds on the free water of the slurries using a camel-hair brush; third day (11.30 a.m.-12 noon), application of paraquat; fourth day (1.00 p.m.-3.00 p.m. approx.), assessment of fronds for paraquat bleaching.

Italian ryegrass was more sensitive than oats to diuron but was found difficult to grow satisfactorily in this environment and consequently was not persevered with.

A feature of this work was the poor sensitivity of all test plants to diuron in the 0-6 in. layer, the ED₅₀ value for oats (average of varietal experiment) being 6.3 p.p.m. Harris and Sheets (1965) studied the response of oats (var. Markton) to diuron in 32 soils and found ED₅₀ values ranging from 0.14 p.p.m. to 3.06 p.p.m., the higher value being for a soil containing 6.90% organic matter and the lower for a soil containing 0.14%. It appears therefore that the organic matter in the soil used in these present studies may have been unusually effective in rendering diuron unavailable to the indicator plants.

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