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Development of bioassay techniques/ field persistence of trifluralin, simazine and diuron. Calotropis procers and Parkinsonia aculeata.

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WESTERN AUSTRALIA

EXPERIMENTAL SUMMARY 1981

1. Development of bioassay techniques for trifluralin, simazine and diuron.

2. Field persistence of trifluralin, simazine and diuron in Wongan Hills soils.

3. Taxonomic studies of *Calotropis procera*, a declared weed of the North-West of Western Australia.

4. Germination studies of *Calotropis procera* and *Parkinsonia aculeata*.

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1. DEVELOPMENT OF BIOASSAY TECHNIQUES FOR TRIFLURALIN, SIMAZINE AND DIURON

OBJECTIVE

To develop a sensitive method to be used in subsequent studies for the detection and measurement of trifluralin, simazine and diuron persistence and movement in soils.

INTRODUCTION

The use of plant material as a means of detecting herbicide concentration in solution and in soils has become a standard technique in weed control investigations. Measurement of plant responses under the influence of various known concentrations of herbicide provides a standard for determination of the amount of herbicide in unknown samples. Various growth responses may be used; fresh weight, dry weight, length of primary root, coleoptile or hypocotyl, and height of plant above the soil, to name a few. In this investigation, a few plant species known to be sensitive to trifluralin, simazine and diuron were screened for their utility as bioassay material.

EXPERIMENTAL

A modification of the petri dish technique first developed by C. Parker of the W.R.O. was used for the trifluralin bioassay. Oat, Japanese millet and sorghum were tested for their sensitivity to trifluralin. All plant materials used were carefully selected; soil moisture content and weight of air-dry soil per petri dish were standardised, i.e., 12 ml herbicide solution per 100 gm air-dry soil; and the environmental factors carefully controlled to ensure reproducibility of results. Four replications were used for each treatment rate per species and five seeds per replicate were used.

Both roots and shoots were evaluated for sensitivity to trifluralin by estimating graphically from plots of the dosage-response curves for each species, using the GR_{50} technique. The GR_{50} value is the concentration of trifluralin required to inhibit the growth response by 50% and was used as the susceptibility index for each species. Plotting the relative growth response (as a percentage of untreated control) on a probit scale against log dose, proved to be a suitable transformation converting the sigmoid curves into straight regression lines. The best fit for the resultant probit line was calculated by regression.

For simazine and diuron, oats and turnip were tested out for their use as bioassay materials. The testing was done in plastic pots containing 125 gm air-dry soil, treated with 15 ml of either simazine or diuron of increasing concentrations. The various treatments were run in four replicates, with 5 seeds per treatment sown at a uniform depth of 13 mm. The pots were arranged in a randomised complete block design in a glasshouse under natural light and a controlled temperature regime of 20°C 12 hr, 15°C 12 hr.

Assessment of the relative phytotoxicity to simazine and diuron was made in terms of the oven-dry weight per plant, 12 (turnip) and 15 (oat) days after sowing. Data were analysed using the GR_{50} technique.

RESULTS

Table 1 The root growth of oat and Japanese millet, expressed as per cent of the control, in various concentrations of trifluralin.

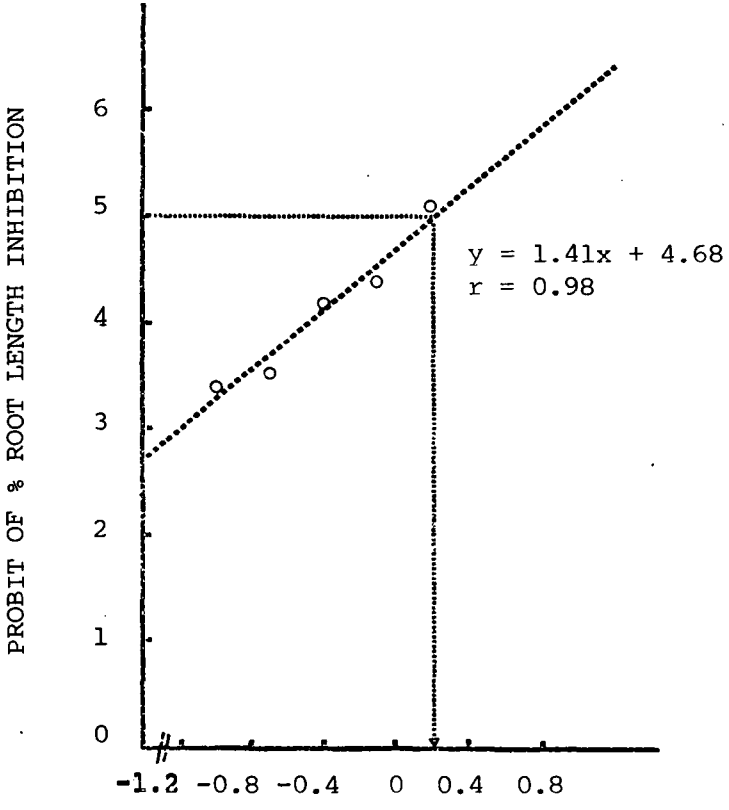
Concentration (ppmw)	Root elongation growth as per cent of control	
	Oat[a]	Japanese millet[b]
0.0125	102.47	101.32
0.025	102.22	104.66
0.05	100.94	98.98
0.1	94.57	92.71
0.2	92.91	38.50
0.4	79.61	40.03
0.8	73.62	17.50
1.6	46.65	13.76

a = 30 hours in incubator in the dark at 20°C
b = 4 days in incubator in the dark at 20°C

FIGURE 1

Probit of response against dose on logarithmic scale to determine 50% growth reduction (GR_{50}) values.

(a) Trifluralin bioassay with oat



(b) Trifluralin bioassay with Japanese millet.

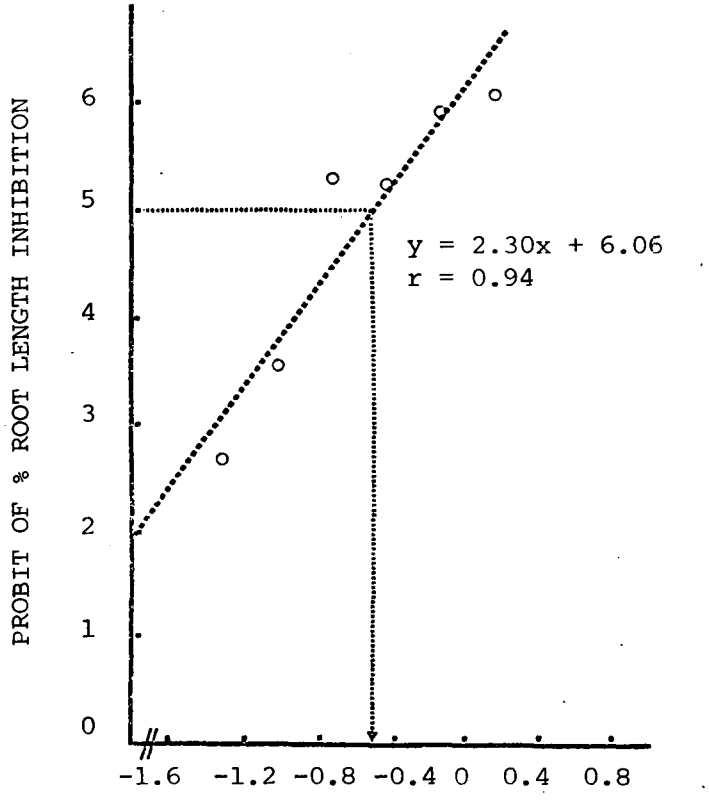


Table 2 GR_{50} concentration (ppmw) for trifluralin on oat and Japanese millet (Petri dish assay).

Assay plant	GR_{50} root length (ppmw)
Oat	1.66
Japanese millet	0.35

Table 3 The dry weight of oat and turnip, expressed as per cent of control, in various concentrations of simazine and diuron.

Con. (ppmw)	Dry weight per plant as per cent of control: Simazine Oat	Simazine Turnip	Diuron Oat	Diuron Turnip
0.0125	*Nd	109.26	Nd	97.69
0.025	Nd	119.84	Nd	113.39
0.05	92.79	88.05	101.58	105.17
0.1	66.49	45.43	101.73	101.95
0.2	39.21	23.47	97.51	72.37
0.4	34.79	13.64	87.85	27.75
0.8	27.83	12.20	56.13	9.25
1.6	29.29	8.89	35.57	10.04
3.2	21.47	8.26	28.25	8.22

*Nd = not determined

100

FIGURE 2

Probit of response against dose on logarithmic scale to determine 50% growth reduction (GR_{50}) values.

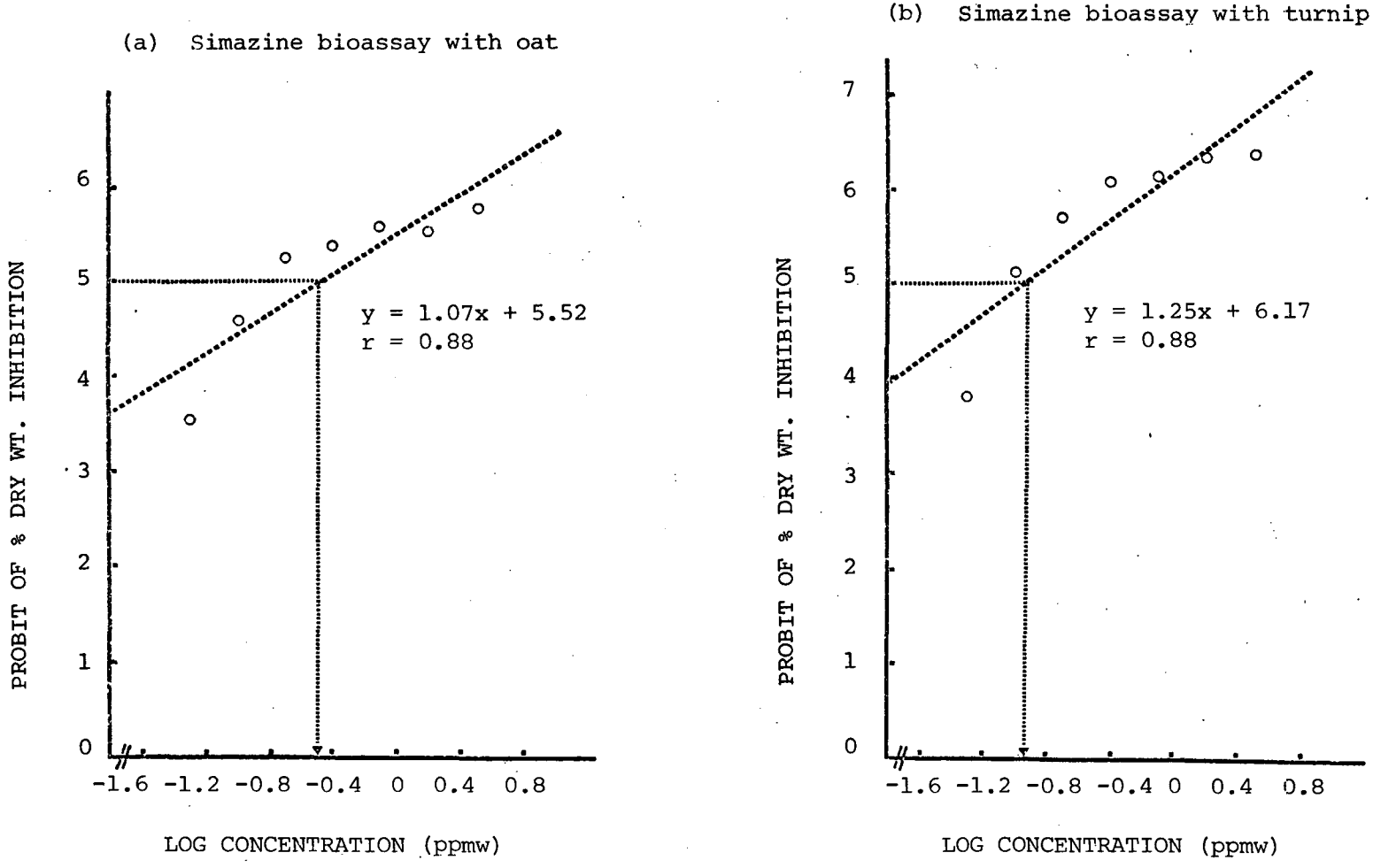
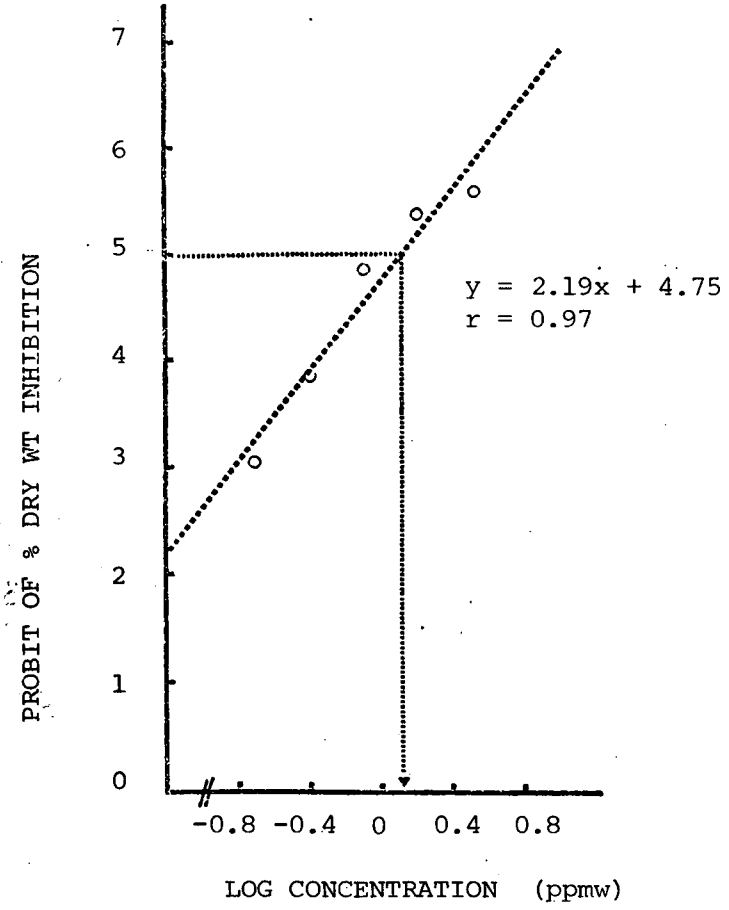


FIGURE 3

Probit of response against dose on logarithmic scale to determine 50% growth reduction (GR_{50}) values,

(a) Diuron bioassay with oat



(b) Diuron bioassay with turnip

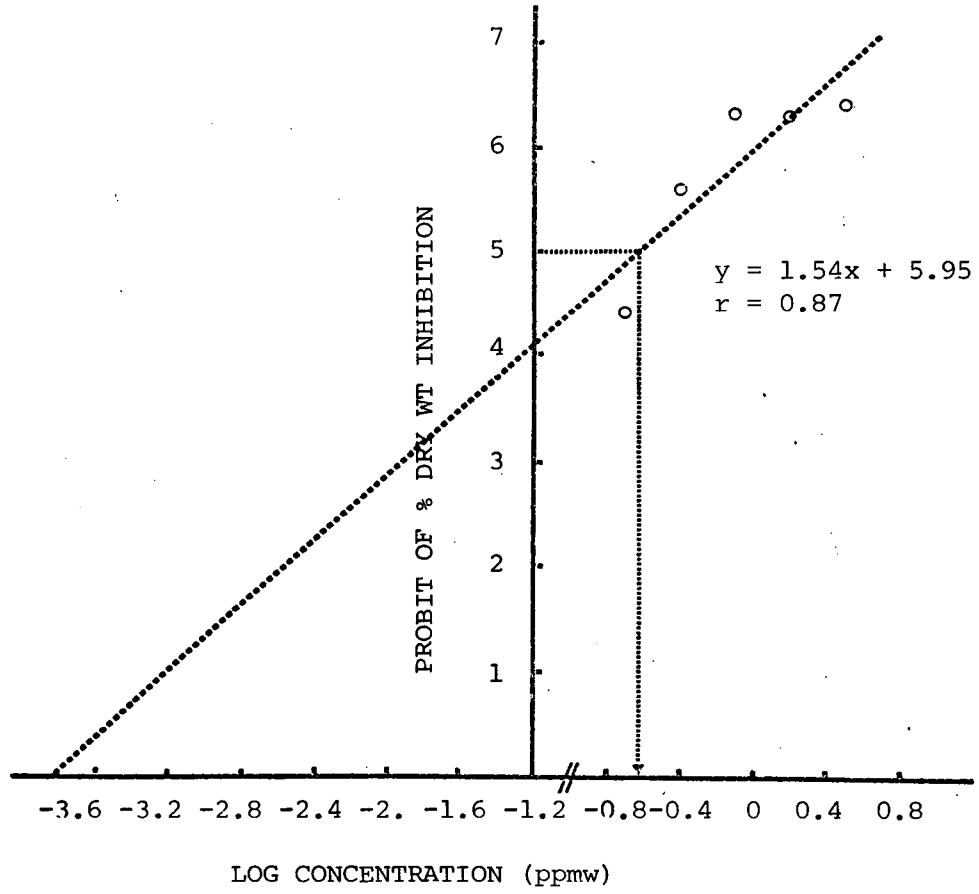


Table 4 GR_{50} concentration (ppmw) for simazine and diuron on oat and turnip (pot assay).

Assay plant	GR_{50} dry weight per plant (ppmw)	
	Simazine	Diuron
Oat	0.32	1.29
Turnip	0.11	0.25

COMMENTS

Trifluralin assay

The inhibition of root elongation growth is the most reliable criterion for rapid detection of trifluralin with oat, Japanese millet and sorghum. This is in agreement with the well known fact that the inhibition of roots is the primary action of trifluralin in weed control.

Growth in root length is more susceptible than growth in weight. This is probably due to the fact that there is a larger increase in weight of affected tissues than in their length because of the hypertrophy of cells. Therefore, in all subsequent studies and interpretations, growth in root length was used as the criterion for comparing sensitivity to trifluralin.

Japanese millet is a more sensitive species than oat. The limit of detection was 0.1 ppm for oat and 0.05 ppm for Japanese millet (Table 1).

Using the GR_{50} technique, the root length GR_{50} value for oat was about five times the concentration required for the same growth response in Japanese millet (Table 2).

Simazine and diuron assays

With simazine and diuron, the best criterion to use for assessing sensitivity to the chemicals is the dry weight of the treated plant. This criterion was used in all subsequent studies and interpretations.

Turnip was more sensitive than oat irrespective of the herbicide or concentration used (Table 3). With both turnip and oat, the sensitivity range for the detection of simazine was 0.05 to 3.2 ppm, and for diuron was 0.2 to 3.2 ppm.

With simazine, the GR_{50} value for oat was three times the concentration required for the same growth response in turnip, whereas with diuron, oat was five times less susceptible than turnip.

RECOMMENDATIONS

The recommended bioassay species for trifluralin are oat and Japanese millet, and for simazine and diuron, oat and turnip.

2. FIELD PERSISTENCE OF TRIFLURALIN, SIMAZINE AND DIURON IN WONGAN HILLS SOILS

OBJECTIVE

To follow the persistence of trifluralin, simazine and diuron under field conditions using bioassays.

INTRODUCTION

When used for residues in soil, a bioassay provides a direct measurement of the available herbicide in the substrate, information which is frequently more useful than knowledge of the total amount that can be extracted and estimated by instrumental methods. Using the bioassay techniques developed for the estimation of trifluralin, simazine and diuron as reported earlier, two experiments were conducted to follow the persistence of trifluralin and diuron in a wheat crop and simazine in a lupin crop under two different techniques of planting, viz., conventional district practice and direct drilling.

EXPERIMENTAL

Site: Paddock 2 HC, Wongan Hills Research Station.

Experimental design: Each experiment was laid out in a split-plot design with planting techniques as main plots and herbicide treatments as subplots. Each treatment was replicated three times.

Plot size: 3 m x 30 m for Expt. 1 and 3 m x 10 m for Expt. 2.

Herbicide treatment: Experiment 1 at recommended rates - trifluralin 0.4 kg/ha, simazine 0.75 kg/ha, and diuron 0.25 kg/ha. Experiment 2 at five times recommended rates - trifluralin 2 kg/ha, simazine 3.75 kg/ha and diuron 1.0 kg/ha. Experiment 2 was included mainly to ensure the availability of enough residues for detection when using bioassays.

Herbicide application: With a boom sprayer at 75 l/ha. Trifluralin as a pre-plant incorporated treatment; simazine a pre-plant pre-emergent treatment; and diuron a post-emergent treatment, 41 days after sowing.

Soil sampling: Nine cores were taken at random from each plot per layer of soil, i.e., 0 to 7.6 cm layer and 7.6 cm to 15.2 cm layer. Each set of cores was bulked, air-dried, sieved through 2 mm sieve and thoroughly mixed.

Setting-up of assay: Two subsamples from each bulked sample were used for bioassay. In most instances, 2 subsamples were sufficient but in some cases 4 subsamples were used. The bioassay species used were oat and Japanese millet for trifluralin and oat and turnip for simazine and diuron. The choice of species depended upon the quantities of the herbicide remaining in the soil. The quantity of herbicide present in each subsample was estimated from the standard curve prepared for each soil at each of the soil depths as described in an earlier report (1).

RESULTS

See Tables 1, 2 and 3.

COMMENTS

At the time of writing, only results for soil samples collected up to either three or four months after spraying were available. All results refer to the 0 to 7.6 cm soil layer. Residues from the 7.6 cm to 15.2 cm soil depth were rarely detected.

Results in gm ai/ha/7.6 cm soil were calculated on the basis that one hectare of soil to a 7.6 cm depth weighs 1,066,650 kg (Expt. 1) and 1,160,896 kg (Expt. 2).

Comparing conventional and direct drilling, at the recommended rate of applicaton, about twice as much trifluralin was retained in conventional plots than in direct-drilled plots, irrespective of whether the soil samples were obtained 1 or 4 months after spraying. Under the conventional system, a greater incorporation of trifluralin into the soil is expected, thus minimising the rapid dissipation from the soil surface due to volatilisation and photodecomposition.

In direct drilling, the higher dose of trifluralin took longer than the recommended dose to achieve 50% disappearance, contrary to the usual similarity between doses. No explanation could be provided.

The greater retention of simazine in conventionally treated plots than in direct drilled plots could probably be attributed to the relatively less disturbed top soil with its layer of organic matter acting as an efficient trap to the applied simazine molecules in the direct drilled plots. It is common knowledge that adsorption of simazine onto organic matter accounts for a significant loss of toxicity. In the present situation, it was unlikely that microbial breakdown was an important avenue of loss. If microbes had played a major role, one would have expected a greater breakdown in the conventionally treated plots, because cultivation generally enhances microbiological activity.

At the recommended rate, the time taken for 50% disappearance of simazine was about 3 months in the conventional plots and between 1 and 2 months in the direct drilled plots.

In the simazine high-rate experiment, the herbicidal activity was too high for accurate measurement with the oat bioassay and after 3 months, the concentrations were still near the upper limits of the oat bioassay, therefore, the values obtained are possibly low.

More diuron residues also remained in the conventional plots compared with the direct drilled. The same explanation as in the case for simazine could possibly be used to explain this differential persistence.

At the recommended rate, no diuron could be detected in samples obtained 3 months after spraying. Therefore, at recommended rate, annual applications of diuron would decompose rapidly enough that quantities toxic to most crops are unlikely to accumulate in the soil. However, in the case of trifluralin and simazine, no similar conclusion could be drawn from the limited data available. It would be useful to note the latest time at which a detectable residue remained in the trifluralin and simazine plots. However, according to the literature, repeated annual applications of trifluralin and simazine at recommended rate do not result in increasing accumulations of residues.

TABLE 1

Estimated concentrations of applied trifluralin remaining in soil following application to wheat at (a) 0.4 kg ai/ha and (b) 2.0 kg ai/ha. Determinations in both (a) and (b) were for the 0 to 7.6 cm soil depth, using Japanese millet bioassays for (a) and oat bioassays for (b).

Date of spraying	Months after spraying	Residue (gm ai/ha/7.6 cm soil)		Remaining (%)	
		Conventional	Direct drilling	Conventional	Direct drilling
(a) 3 June 1981	1 4	384 352	192 160	96 88	48 40
(b) 3 June 1981	1 2 4	1 980 1 880 1 780	1 640 1 500 837	99 94 89	82 75 42

TABLE 2

Estimated concentrations of applied simazine remaining in soil following application to lupin at (a) 0.75 kg ai/ha and (b) 3.75 kg ai/ha. Data for (a) are the mean over the concentrations obtained using both oat and turnip bioassays, data for (b) were determined by oat bioassays. Determinations in both (a) and (b) were for the 0 to 7.6 cm soil depth.

Date of spraying	Months after spraying	Residue (gm ai/ha/7.6 cm soil)		Remaining (%)	
		Conventional	Direct drilling	Conventional	Direct drilling
(a) 3 June 1981	1	735	675	98	90
	2	608	277	81	37
	3	384	235	51	31
(b) 3 June 1981	1	964	859	26	23
	2	917	697	24	19
	3	766	499	20	13

TABLE 3

Estimated concentrations of applied diuron remaining in soil following application to wheat at (a) 0.25 kg ai/ha and (b) 1.25 kg ai/ha. Determinations in both (a) and (b) were for the 0 to 7.6 cm soil depth, using turnip bioassays for (a) and oat bioassays for (b).

Date of spraying	Months after spraying	Residue (gm ai/ha/7.6 cm soil)		Remaining (%)	
		Conventional	Direct drilling	Conventional	Direct drilling
(a) 27 July 1981	1	243	96	97	38
	2	43	9	17	4
	3	+RND	RND	-	-
(b) 27 July 1981	1	1 225	1 030	98	82
	3	498	370	40	30

+ RND = Residues not detected.

3. TAXONOMIC STUDIES OF CALOTROPIS PROCERA, A DECLARED WEED OF THE NORTH-WEST OF WESTERN AUSTRALIA

OBJECTIVE

To study the fruit characters of Calotropis procera in the East Kimberley and the Northern Territory to establish whether the taxon found in Australia is distinctly different from the subspecies, Calotropis procera (Ait.) Ait. f. subsp. procera and Calotropis procera subsp. hamiltonii (Wight) S. I. Ali.

INTRODUCTION

The genus Calotropis R. Br. (Asclepiadaceae) consists of four species, distributed from the Indo-Pakistan subcontinent to Africa, the West Indies and tropical South America, South East Asia and Australia. One of the species, C. procera (Ait.) Ait. f. has been recently subdivided further into two subspecies, viz., C. procera (Ait.) Ait. f. subsp. procera and C. procera subsp. hamiltonii (Wight) S. I. Ali. In the present studies, a large number of fruit specimens from the East Kimberley and the Northern Territory has been examined in order to determine whether the Australian taxon is distinctly different from the other two known subspecies. The identification of a weed is of paramount importance in order to establish a firm basis for studies on distribution, physiological-ecological requirements and susceptibility to herbicides.

EXPERIMENTAL

Living fruit materials of Calotropis were studied during my 1980 December visit to the Northern Territory and the East Kimberley. The lengths and breadths of the fruits were measured, the fruit shapes noted, and the widths of the air space of the loculus and the central seed-bearing area and the placenta of dissected fruits were recorded.

RESULTS

No significant difference was noted in fruit characters of those from the East Kimberley and the Northern Territory. The taxon found in Australia is probably a distinct new subspecies. The fruit characters are so distinct that it seems best to regard the Australian form as a different taxon. The differences are best illustrated with the help of diagrams (Fig. 1).

COMMENTS

C. procera (Ait.) Ait. f. subsp. procera:

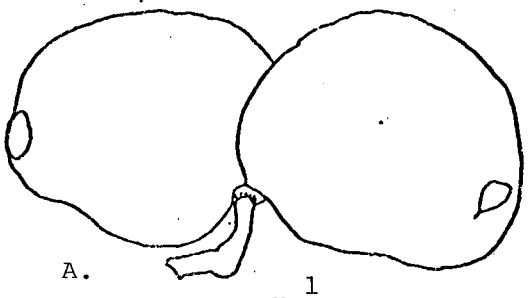
The length of the fruit varies from 10.8 cm to 14.5 cm with a mean of 12.2 cm and the breadth from 9.7 cm to 11.7 cm with a mean of 10.7 cm. The fruit is almost globose with the tip invaginated at maturity (Fig. 1A). Width of air space is greater than the central seed-bearing area and the placenta (Fig. 1B).

C. procera subsp. hamiltonii (Wight) S. I. Ali:

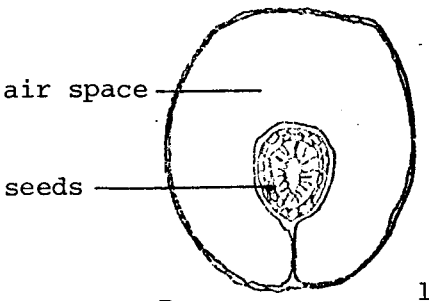
The length of the fruit varies from 6.5 cm to 9.5 cm with a mean of 7.1 cm, and the breadth from 3 cm to 5.1 cm with a mean of 3.8 cm. The fruit has the shape of a neckless inverted body of a duck; the tip is not invaginated at maturity (Fig. 1C). Width of air space is less than the width of the central seed-bearing region and the placenta.

C. procera of Australia:

Fruit length varies from 10.6 cm to 13.5 cm with a mean of 12. 0 cm; breadth from 6.7 cm to 8.7 cm with a mean of 7.9 cm. The fruit shape is very similar to C. procera subsp. hamiltonii, but the width of air space is greater than the width of the central seed-bearing region and the placenta. Therefore, it appears that the Australian form is an intermediate form. Further contacts with international experts on Calotropis will be made.

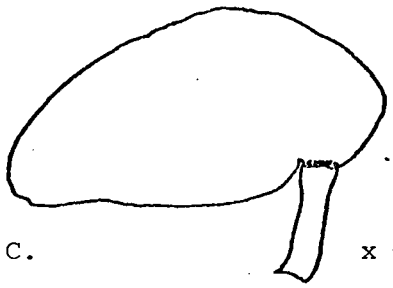


A. x $\frac{1}{3}$

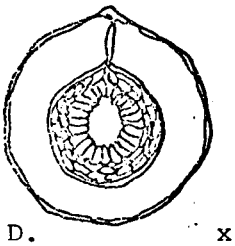


B. x $\frac{1}{3}$

Distribution: Africa, Mediterranean region, Jordan, Arabia, Palestine, Abu Dhabi, W. Indies, tropical S. and C. America.

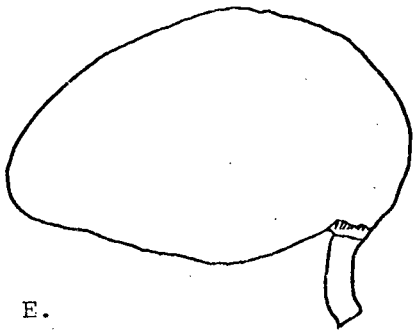


C. x $\frac{5}{7}$

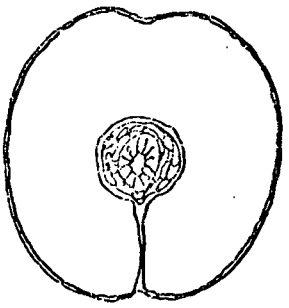


D. x $\frac{5}{7}$

Distribution: Pakistan, India, Afghanistan, Iran (?) Iraq(?), Oman.



E. x $\frac{5}{12}$



F. x $\frac{5}{12}$

Distribution: Northern Australia, ie, East Kimberley, Northern Territory and between Cairns and Normanton, Queensland.

Figure 1. Calotropis procera (Ait). Ait.f. subsp. procera: A, fruits; B,T.S. fruit. C. procera subsp. hamiltonii (Wight) S.I. Ali: C, fruit, D,T.S.fruit. C. procera (Australian form): E, fruit; F,T.S. fruit.

4. GERMINATION STUDIES OF CALOTROPIS PROCERA AND PARKINSONIA ACULEATA

OBJECTIVE

To determine the germination capacity of Calotropis and Parkinsonia seeds collected from different localities.

INTRODUCTION

Very little is known about the pattern of germination of Calotropis and Parkinsonia seeds. Such knowledge is essential to ensure the highest chances of success when one is planning any control strategy. From field observations, I have noted that seeds of Calotropis germinate readily and the number of seeds per pod varies from 350 to 500. However, Parkinsonia seeds do not germinate readily, the majority of pods contain 1 to 2 seeds but the number can vary from 1 to 8 seeds per pod. In this report some preliminary data for Calotropis are presented and work on Parkinsonia is in progress.

EXPERIMENTAL

Calotropis seeds, ripe, half ripe and green seeds collected from various parts of Australia were germinated in the dark in a germination chamber with alternating temperatures, 12 hr at 30°C and 12 hr at 20°C. This temperature regime was in close agreement with field conditions.

The number of seeds that germinated were counted each day and to obtain a clear picture of the germination rate, the germination rate index (GRI) was calculated using the formula:

$$GRI = (G_1/T_1 + G_2/T_2 + \quad + G_n/T_n) - (\% G)$$

where, G_1 = number germinated at T_1;

G_2 = number germinated between T_1 and T_2;

G_n = number germinated at T_n minus number germinated at $T_n - 1$;

T_1 = days to first count;

T_2 = days to second count;

T_n = days to final count;

$T_n - 1$ = days to the count immediately preceding the last count;

and % G = germination percentage obtained. The germination percentage obtained was used as the denominator of the equation in order to base the germination rate index on 100% germination.

RESULTS

Table 1 Per cent germination of Calotropis seeds# at various time intervals after sowing in complete darkness at 20°C 12 hr, 30°C 12 hr.

Seed samples (collection date)	Days from sowing					
	1	2	3	4	5	6
1. Kununurra ripe seeds (30/11/81)	0	15.5	84.0	92.5	96.0	96.0
2. Kununurra half-ripe seeds (6/12/81)	0	23.5	58.5	63.0	66.0	67.0
3. Kununurra green seeds (6/12/81)	0	3.5	33.5	43.5	46.5	49.5
4. Katherine ripe seeds (26/11/81)	0	57.5	96.0	97.0	97.0	97.0
5. Katherine half-ripe seeds (26/11/81)	0	34.5	93.5	98.0	98.0	99.5
6. Katherine green seeds (26/11/81)	0	55.5	96.5	99.5	99.5	100.0

Data expressed as cumulative percent germination out of a total of 200 seeds.

COMMENTS

Calotropis procera showed very rapid and almost synchronous germination. Germination commenced 2 days after sowing and within a week, almost all the seeds had germinated. Such germination trend was observed in both the Kununurra and Katherine collections. In the Katherine collections, even the half-ripe and green seeds germinated readily, reaching 99.5 and 100% germination respectively, 6 days after sowing. There was no significant difference in the germination percentage maxima for the Kununurra ripe seed and Katherine ripe seed collections.

The order of the germination rate for the different seed samples as indicated by the GRI values (shown within brackets) is as follows: Katherine ripe seeds (0.43)> Katherine green seeds (0.42)> Katherine half-ripe seeds (0.38) = Kununurra half-ripe seeds (0.38)> Kununurra ripe seeds (0.35)> Kununurra green seeds (0.31).

The laboratory results support my observations that Calotropis seeds germinate readily in the field. This rapid, almost synchronous germination can be a disadvantage in certain species. For example, it is common knowledge that staggered germination has ecological advantages in that even if transitory adverse environmental conditions are lethal to early germinating seeds, more seedlings may appear after conditions have again become favourable. However, Calotropis produces large quantities of seeds throughout the year and this serves as an important compensatory factor. This is one of the reasons why the infestation by Calotropis is fairly widespread in the East Kimberley and the Katherine region.