BIOASSAY TECHNIQUES FOR THE DETERMINATION OF HERBICIDE RESIDUES

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Summary

Some background is given to the extensive use of bioassays in herbicide investigations. Two techniques in current use by the Research Division of the Ministry of Agriculture and Fisheries, one for trifluralin and the other for metribuzin, are described in detail.

INTRODUCTION

The use of bioassays to determine the concentration of a herbicide in soil or other growing medium has deservedly received much attention and has been of immense importance to research in weed science. Although chemical methods of detecting herbicides can often determine smaller amounts with greater precision, bioassays offer several advantages:

(a) The presence of a toxic metabolite is likely to be detected by a bioassay but bypassed by a chemical method.

(b) Bioassays distinguish between the herbicide and possible biologically inactive products of degradation while chemical methods may not.

(c) A chemical method may give a misleading result if its extraction technique removes residues which are normally too firmly absorbed on the soil to affect plants.

(d) Expensive analytical equipment or elaborate extraction procedures are normally not required for bioassays.

Early bioassay studies, using dry weights of plant foliage and roots as an indication of arsenic pesticides in soils were described by Stewart and Smith (1922). Crafts (1935) suggested an assay for sodium arsenite and sodium chlorate in soils, making use of oat plants to estimate chemical concentration. During the subsequent 40 years many workers have modified this basic procedure to their particular needs.

Bioassays of herbicides which involve seedling growth (e.g. Holly and Roberts, 1963) need 3 to 6 weeks to obtain sufficient shoot growth for assessment purposes. Detailed directions for the conduct of this type of assay have been described by Reisler (1972). There is the possibility of herbicide loss by microbiological activity during the test if very low concentrations are being assayed but the technique, as described below, to estimate trifluralin with a very sensitive plant is unlikely to suffer from this disadvantage. For herbicides which inhibit photosynthesis, the rapid duckweed (*Lemna* spp.) assay, as described for metribuzin estimation, may be preferable to slower methods. Parker (1965) and Damanakis (1970) have described similar Lemnid techniques which also give results in only a few days.

Other examples of rapid tests are the buckwheat *(Fagopyrum esculentum)* assay for chlorpropham (Roberts and Wilson, 1962) and the Stanford-De Ment determination of atrazine residues (Tompkins *et al,* 1968). A quick method suitable for carbamate, dinitroaniline and possibly other herbicides which inhibit germination has been described by Parker (1966).

METHOD AND RESULTS

Trifluralin bioassay

Initial tests were carried out with five plant species, viz. German millet *(Setaria italica),* Russian wild ryegrass *(Elymus junceus),* sorghum *(Sorghum vulgare),* oats *(Avena sativa)* and sugar beet *(Beta vulgaris).* A Horotiu sandy loam soil was used consisting of 15% organic matter, 21% clay, 18% silt and 46% sand with a pH of 5.9 and field capacity of 44%. Trifluralin solutions were thoroughly mixed with the soil to give a series of concentrations ranging from 0.1 to 1.0 ppmw. Plastic pots were half filled with untreated air-dry soil and treated soil added to give a top layer 5 cm deep in which seeds were placed at 2.5 cm depth. The soil was watered to field capacity and maintained thereafter between 75 and 100% field capacity. Five plants were established in each pot and the pots arranged in a four replicate, randomised block layout on a glasshouse bench. After five weeks, the plants were cut off at soil level and shoot dry weights were determined.

The response of the five test species to the trifluralin treatments, as percentage reduction in shoot dry weight, is shown in Fig. 1. On this basis, and also on the observed morphological damage, German millet was chosen as the most suitable test species.

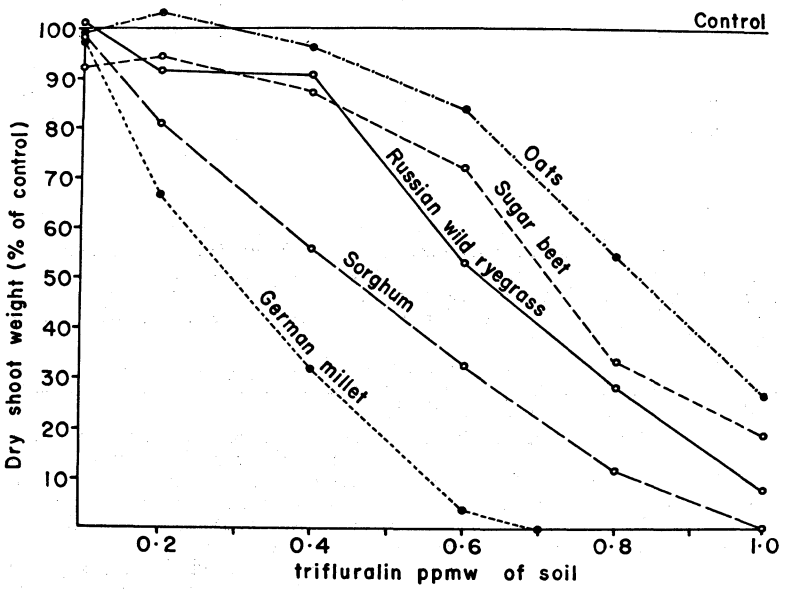

Fig. 1: Effects of different concentrations of trifluralin incorporated in top 5 cm of Horotiu sandy loam soil on the shoot growth of different species.

Field samples collected with a 7 cm diameter tube to a depth of 10 cm were taken from Horotiu sandy loam soil previously treated with trifluralin at rates of 0.5, 0.75, 1, 2 and 4 kg/ha. Plastic pots, 10 cm in diameter were filled with the thoroughly mixed soil and ten caryopses of German millet were planted 2.5 cm deep in each. At the same time, a standard series was prepared, as described previously. After emergence the seedlings were thinned to five plants per pot. Water was applied as necessary and shoot dry weights compared with the standards at the conclusion of the experiment.

Results of assays of the soil treated six months previously with trifluralin are given in Table 1. Data show that about 15% of an initial application of 2 kg/ha remained in the soil at the end of this period — enough to cause injury to susceptible crops. Residues from an initial 1 kg/ha were small (less than 0.15 kg/ha) and not likely to cause trouble. An amount equivalent to at least 0.5 kg/ha remained from an initial 4 kg/ha application. This amount, which killed the test plants, was too great to estimate precisely. This could probably be done, however, by diluting the field samples with untreated soil to bring the chemical concentrations into the range of sensitivity of the test species.

TABLE 1: GERMAN MILLET BIOASSAY OF SOIL RESIDUES OF TRIFLURALIN IN SAMPLES COLLECTED SIX MONTHS AFTER APPLICATION

trifluralin conc. kg/ha	*DM weight of German millet — % of control*	*Approximate amount of residue*	
		ppmw	*kg/ha*
0.50	119	<0.2	<0.15
0.75	104	<0.2	<0.15
1.0	91	<0.2	<0.15
2.0	37	0.4	0.30
4.0	0	>0.7	>0.50

Metribuzin bioassay

Initial comparisons were made with cultures of the duck weeds *Lemna minor, Spirodela polyrhiza* and *S. oligorrhiza.* The latter was adopted as the most suitable test plant as it was very sensitive to metribuzin, easily handled and readily obtained locally. It is recognised easily by the purple colour of the underside of the frond which also carries several root hairs.

A stock of plants can be maintained in nutrients provided by a one-tenth strength Hoagland's solution with added iron (see Damanakis, 1970 for details of growing method).

The test is carried out in duplicate in 200 ml capacity disposable polystyrene drinking cups, 6.5 m diameter at the rim. The duckweed is grown on the surface of solutions in the cups which are placed on a turntable 40 cm below a bank of four 80W fluorescent tubes for 16 hours per day. The temperature is maintained at about 19°C and under these conditions the fresh weight of the control plants increases 2½ times in 5 days. Fronds from the stock are patted dry on a paper tissue and accurately weighed in until about 75 mg of plant material has been added to each cup. This covers about one third of the surface area and at the conclusion of the test when the plants are reweighed, control treatments almost cover the surface.

The prepared standard series consists of a range of concentrations of the herbicide in the soil between 0.01 and 0.16 ppmw. To each cup is added 50 gm of air dry soil, herbicide and nutrients in a total of 150 ml water. The assay soil samples are prepared similarly and the contents of the cups allowed to equilibrate for 24 hours, stirring several times during this period. The duckweed is then weighed in and, after 5 days, weighed again and treated samples are compared against the standard curve. Fig. 2 shows that in Levin silt loam soil (5% organic matter, 20% clay, 23% silt and 52% sand) a 50% reduction in duckweed growth is caused by a metribuzin concentration of 0.09 ppmw approximately.

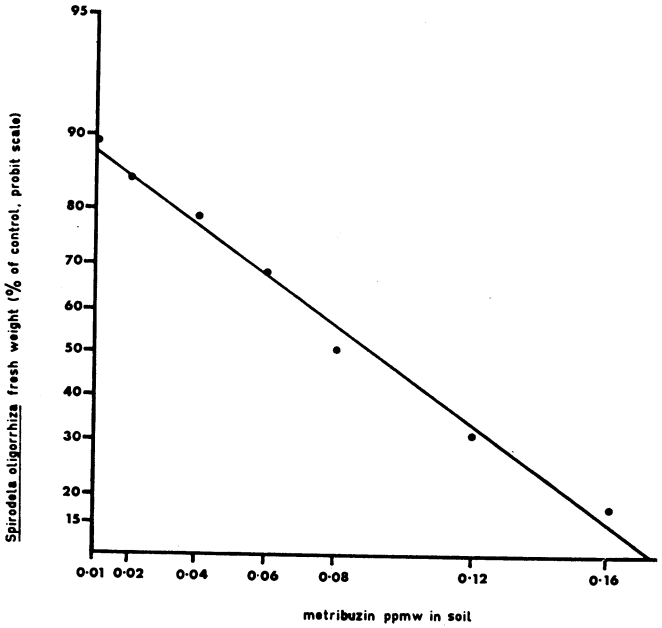

Fig. 2: Fitted curve showing sensitivity of *Spirodela oligorrhiza* to metribuzin in Levin sandy loam soil.

These two bioassays are examples of simple, repeatable techniques which have been used successfully by the authors for the estimation of trifluralin and metribuzin, respectively. The methods have wider application and can be adapted for use with a number of other herbicides.

REFERENCES

Crafts, A. S., 1935. The toxicity of sodium arsenite and sodium chlorate in four California soils. *Hilgardia,* 9: 461-468.

Damanakis, M., 1970. A bioassay for the determination of low concentrations of paraquat. *Weed Res.,* 10: 77-80.

Holly, K. and Roberts, H. A., 1963. Persistence of phytotoxic residues of triazine herbicides in soil. *Weed Res.,* 3: 1-10.

Parker, C., 1965. A rapid bioassay method for the determination of herbicides which inhibit photosynthesis. *Weed Res.*, 5: 181-184.

———, 1966. The importance of shoot entry in the action of herbicides applied to the soil. *Weeds*, 14: 117-121.

Reisler, A., 1972. Bioassay of soil samples for content of photosynthesis inhibiting herbicides. Verslagen nr. 61. IBS, Wageningen, The Netherlands.

Roberts, H. A. and Wilson, B. J., 1962. Note on the bioassay of chlorpropham in soil. *Weed Res.*, 2: 60-65.

Stewart, J. and Smith, E. S., 1922. Some relations of arsenic to plant growth: Part 2. *Soil Sci.*, 14: 119-126.

Tompkins, G. A., McIntosh, T. H. and Dunigan, E. P., 1968. Use of Stanford-De Ment bioassay to study atrazine — soil reactions. *Soil Sci. Soc. Amer. Proc.*, 32: 373-377.