Use of Bioassays for Herbicide Persistence Studies in the Humid Tropics[1]

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ABSTRACT

The importance of bioassay techniques in monitoring herbicide persistence and soil residue levels is briefly reviewed. Factors that make for reliable bioassay procedures such as the choice of test organisms, appropriate monitoring of environmental variables, and relevant statistical procedures are highlighted. Examples are given of selected indices used in expressing bioassay results. Need for the development of simple, easily adaptable bioassay procedures for recommended herbicides in the humid tropics, especially where facilities for chemical analysis are inadequate, is emphasized.

COMPENDIO

Este artículo examina brevemente la importancia de técnicas de bio-ensayos en el Manejo de la Resistencia de los Herbicidas y del nivel de residuos del suelo. Factores que forman la selección de organismos-pruebas, el control adecuado de las variables del medio, y los procedimientos estadísticos apropiados se ponen de relieve. Asimismo, se dan ejemplos de índices seleccionados utilizados para expresar resultados de bio-ensayos y se pone de relieve la necesidad de desarrollar procesos de bio-ensayos sencillos, fácilmente adaptables a los herbicidas recomendados en la zonas tropicales húmedas, especialmente en las zonas donde las facilidades para análisis químicos son inadecuadas.

INTRODUCTION

The use of herbicides for weed control is a necessary technology in large-scale farming. An appropriate herbicide for a cropping situation must persist long enough to control the weeds and then dissipate to avoid being an environmental hazard. Extended persistence could endanger the environment by injuring the following crops, having deleterious effects on beneficial soil organisms, incorporation into the food chain, and water contamination. It is therefore necessary to monitor the persistence of any herbicide applied within a specific environment and quantify its residue after application.

Persistence has been defined as the length of time a herbicide remains active or remains in the soil (34). The two major methods of monitoring herbicide persistence are: chemical analysis and the use of living organisms, or bioassays. The former measures the chemical residue in treated soil, and can also differentiate between the parent compound and its metabolites. Chemical analysis has been used extensively to monitor or determine residue levels of herbicides in soils and plant materials.

Table 1 shows the list of some herbicides the residues of which were determined by various chemical methods and the limits of detection observed. However, there are constraints to using the chemical analysis method in monitoring herbicide persistence, especially in the humid tropics These include grossly inadequate technical expertise in the use of necessary equipment and chemicals, local unavailability of the equipment and materials, together with the problem of after sales service

Bioassay, the second method of determining herbicide residue in both plants and soil, has been defined by Santelmann (46) as the measurement of a biological response by a living organism to determine the presence and or concentration of a chemical in a substrate. This method has been used extensively either as a complement to chemical analysis or by itself (1, 6, 7, 12, 20, 21, 22, 27, 40, 48, 49, 52, 53). Bioassays are limited in scope and applicability as they only measure those herbicide residues which are biologically active, they do not differentiate between parent compound and bioactive metabolites, and are influenced by environmental variables. In spite of these limitations, they are still widely used because of their relative simplicity and versatility (31).

Most of the reported work in herbicide bioassays is carried out in temperate locations, and their ready applicability in the tropics is hindered by non-avail-

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ability and adaptability of test species, different climatic conditions (temperature, rainfall, relative humidity), and edaphic factors of soil organic matter, clay fractions, and microbial populations, among others. Under tropical conditions there is therefore a need to use locally-available plant species in the development of bioassays to monitor the persistence and residue levels of herbicides.

The purpose of this paper is to discuss the subject matter of bioassay in the tropical environment as it applies to herbicide research, emphasizing the choice of test organisms, choice of correct indices to express results, and the need to develop appropriate bioassays for herbicides commonly used in the tropics.

MATERIALS AND METHODS

Role of bioassays in measuring herbicide persistence

The major advantages of bioassays in persistence studies include their relative simplicity and versatility (31), their high sensitivity (24, 27), the detection of toxic metabolities which may be undetected by chemical assays (12), the low cost of execution as no sophisticated equipment and materials are required (31, 46), and the ability to quantify only the biologically active portion of a chemical which has direct application to field conditions (5). The basic assumptions when using bioassay are that the bioassay species will show an injury response in proportion to herbicide concentrations; the response obtained is reproducible (46); and the suitable indicator organisms should be sensitive to minute amounts of the chemical and respond by clear, easily-observable and measurable symptoms (31). In spite of the numerous advantages of bioassay, factors such as variations in environmental conditions, species heterogeneity and adaptability, soil properties and length of time required to complete the assay, could limit their usefulness and reproducibility.

Reproducibility of responses in bioassays

One common criticism of bioassay techniques is that responses obtained from specific techniques are often not easily reproducible. In a collaborative study by workers in 12 different laboratories in Europe, Myfeller *et al* (42) studied the reproducibility of bioassay techniques by comparison and statistical treatment of the ED50 values (g herbicide/g soil). The techniques used include two direct seeding methods (DSM), a transplanting method (TPM), and a shoot extension method (SEM). The herbicides used include atrazine, metribuzin, tri-allate and trifluralin while *Lepidium sativum* and *Brassica rapa* were test species. They reported that reproducibility was best in the SEM. They found good reproducibility between participating laboratories and concluded that their study demonstrated that bioassay techniques may be useful tools in herbicide residue studies. Such positive results should assure users of bioassay techniques that reproducibility could be appropriate if environmental conditions are carefully monitored.

Table 1. Chemical analysis of some herbicides in different substrates.

Herbicide	Method[1]	Substrate	Limit of detection (ppm)	References
Atrazine	GC	Soil	0 03	40
Atrazine	SPEC	Soil	1 17	3
Diuron	GC	Soil	0 06	40
Hexazinone	GC	Soil, plant material & animal tissue	0 04	30
Hexazinone	GC	Soil	0 004	33
Prometryn	SPEC, GC	Soil	0.3	47
Simazine	GLC	Plant	0.05	2
Simazine	GC	Soil	0 05	40

1 GC = Gas chromatography, GLC = Gas liquid chromatography, SPEC = spectrophotometry.

Choice of test organisms

Several test organisms could be used as bioassays for a specific herbicide and also many herbicides, even those bolonging to different chemical groups, could be tested by the same organism (31). The necessary criteria for the choice of test organisms include: sensitivity of the organism to minute quantities of the herbicide; species must exhibit a gradual increase in susceptibility with increasing herbicidal concentrations (46); and species must respond by clear, easily-observable and measurable symptoms (31)

It is proposed that the investigator take into conderation the following non-exhaustive list of factors before making a choice regarding the test organisms(s):

- Exclude crop plants known to be tolerant to the herbicide in question; for example, one should not use corn as a bioassay material for atrazine since the latter is readily degraded into non-herbicidal hydroxy-atrazine within the plant
- Carry on an extensive literature review to guide in the choice of plants, although considerable variations in susceptibility to chemicals within families and even within species, do occur.
- Note that certain species are known to be highly sensitive to a large number of herbicides, for example, tomato (*Lycopersicum esculentum*); *Cucumber* (*Cucumis sativus* L.) and oats (*Avena sativa* L.)
- Note that succulent or soft-tissued species are usually more susceptible than woody ones.
- Concerning the mode of action of herbicides, species with non-permeable or tough seed coats should not be used as test organisms for germination inhibitors.
- An initial application of the recommended dosage to a fairly large number of potential test plants could be helpful in eliminating tolerant species before real experiments are set up.
- Investigators should endeavour to use adapted or indigenous species as opposed to newly-introduced species to eliminate the effect of environmental non-adaptation. This was also emphasized by Kohn (35).

In most of the reported works, the investigators used temperate species and there is a need to use species that are indigenous to specific environments to permit easy adoption by farmers or extension workers who may wish to monitor the persistence of such herbicides in their immediate environments. A list of bioassay species used in selected herbicides biossays is presented in Table 2.

Table 2. Species used in selected herbicide bioassays.

Herbicide	Bioassay species and references[1]
Alachlor	Japanese millet (4), Chlorella (54).
Amiben	Cucumber (6), Soybean (14).
Atrazine	Oat (31, 40, 45), Cucumber, Foxtail millet and Sunflower (32), Rice (53), Tomato (53, 1).
Bromacil	Pumpkin (51), Watermelon (20), Mustard (31).
Dalapon	Millet (29), Oat (28), Cucumber (19).
Diuron	Barley (18), Cucumber (21, 31), Pumpkin (51).
Paraquat	Wheat (50), Bean (9), Watermelon (20), Duckweed (24)
Simazine	Oat (31, 48), Duckweed (43), Strawberry (16).
Trifluralin	Barley, Cucumber (8), Morning glory, Alfalfa, Velvetleaf, Foxtail (44), Oat (15).
2, 4-D	Duckweed (11), Chlorella (29), Tomato (37, 41), Cotton (38), Cucumber (22), Sorghum (31).

1 List is by no means exhaustive

DISCUSSION

Measurements of response in bioassay

Measurements of response in bioassays are of two major types, plant part response and whole plant response. Plant part response could be in the form of root or shoot-growth inhibition, chlorosis or necrosis, or various morphological changes. Total plant response could be in the form of inhibition of germination or emergence of seeds, chlorosis, suppression or inhibition of plant growth. Visual rating of injury symptoms, which involves the visual scoring of plant conditions, is also useful as a measurement of plant response to herbicide effect in bioassays. Water consumption pattern has also been used as a measurement of plant response in bioassays, which may in fact show greater sensitivity than plant weight and height measurement (46).

Factors in bioassay sensitivity

Several reviews in herbicide bioassay have highlighted the main factors in bioassay sensitivity (5, 10, 31, 46). These include test organism species, chemical and physical properties of soil, type of herbicides used, plant growth, environmental conditions, herbicide application methods, and the age or stage of development of test plants. All these factors must be

precisely specified in any bioassay procedure as any variation could lead to difficulties in reproducibility and reliability.

Indices for expressing bioassay response

Visual injury response: For visual estimation of injury due to herbicide application, it is most important that the two extremes of the rating scale be precisely defined. Usually, scales range from 0 or 1 to 5 or 10 upwards or downwards. In such a case each plant constituting a replication should be rated individually and in the control allowing calculations of an average rate.

Below is an example of a rating scale ranging from 0 to 7 to estimate the injury of strawberry (*Fragaria* x *Ananassa* Duchesne), blackcurrant (*Ribes nigrum* L.) and apple stocks (*Malus pumila* Mill) to some soil-applied herbicides (17).

0 = dead plant
1 = moribund
2 = alive with some green tissue, no growth
3 = very stunted but still making growth
4 = considerable (50%) growth inhibition
5 = readily-distinguishable growth inhibition
6 = some detectable adverse effects
7 = undistinguishable from control

It is important to define each injury level precisely and assign its numerical value appropriately. It is desirable that scores be transformed to probits before response curves are drawn, especially in dosage response assays (16, 17). Workers reported that visual scoring of plant conditions was the most informative method of assessing response of certain fruit crops to soil applied herbicides.

In visual injury response, symptoms which are typical of a certain group of herbicides or of a given compound could be used for qualitative assay and if the intensity of symptoms is dose-related, it may be used for quantitative determinations (31). Such symptoms include epinasty and stem malformations, chlorosis, necrosis and various degrees of wilting of plants. Even where the symptoms are dose-related, it is better to compare the response to other quantitative measurements, such as weight or plant height, before conclusions are drawn.

Objective measurements: Objective measurements include weight, length of plant or plant parts, oxygen evolution, photosynthesis and respiration.

These measurements are expressed as percentages of corresponding control. Also, transformation of data to logarithms is often necessary before drawing dose-response curves. Several workers have expressed plant sensitivity to a herbicide in terms corresponding to the LD50 values of animal toxicity studies (46).

Today, there are various simple ways of expressing plant sensitivity to a herbicide in bioassay studies:

- GR50 and ED50: These values represent the herbicide concentration required to inhibit plant growth by 50%. Upchurch (52) was the first to define GR50 as the amount of herbicide required to cause a 50% growth reduction in plants. He used dry shoot weight data expressed as a percentage of untreated control plants. Sheets (48) defined ED50 as the amount of herbicide in the soil which reduced the yield of a specific species by 50%. He obtained this by plotting the fresh weight expressed as a percentage of the untreated control against the logarithm of herbicide concentration.

- I50: Kraty and Warren (36) expressed the concentration of herbicides that caused 50% inhibition either of photosynthesis or respiration as I50.

- Selectivity Index (SI): Fryer and Makepeace (13) used selectivity index to express the difference in response of two plant species to one herbicide, and defined it as:

$$SI = \frac{\text{ED50 Species 2}}{\text{ED50 Species 1}}$$

- Tolerance Index (TI): Clay (16) used TI to assess the tolerance of strawberries to herbicides in different experiments, and defined it as:

$$TI = \frac{\text{ED value of test herbicide}}{\text{ED value of a standard herbicide}}$$

- Dose-Response Index (RI): Similarly used by Clay (16) to express the degree of response to increases in dose of a particular herbicide, and defined as:

$$RI = \frac{\text{ED50 value}}{\text{ED20 value}} \quad \text{for each herbicide}$$

- Speed of Action Index (SAI) used to express the length of time required for the herbicide injury to show with respect to a particular organism when compared with that of a standard herbicide, and defined as:

$$SAI = \frac{\text{Time to minimum ED value test herbicide}}{\text{Time to minimum ED value standard herbicide}}$$

- Disappearance Time (DT): expressed by Brown (13) as the time required for a percentage of the

herbicide to disappear from the soil. He designated them as DT_{10}, DT_{20} ... DT_{50} ... DT_{100} corresponding to time required for 10, 20, ... 50 or 100% of the chemical to disappear.

Finally, the choice of which index or indices to use will depend largely on the aims and objectives of the bioassay.

Need for data transformation in bioassay

Data transformation becomes necessary when data violate any of the four basic assumptions of the analysis of variance. These assumptions, according to Little and Hills (39) include:

- error terms are randomly, independently and normally distributed; sample variances are homogenous; non-correlation of variances and means of different samples; and additivity of main effects.

Most of the time, data emanating from bioassay tests do violate one or more of these assumptions, and transformation therefore becomes imperative. Examples of such data are:

- Data based on counts expressed as percentages or proportions of the total sample which normally have a binomial distribution. Variances in such data are related to the means in a way. The arc sine or angular transformation should be used if the range of percentages is greater than 40, if less, there is no need for transformation (39).

- Assumption of additivity is usually violated by data emanating from insect or disease studies and in dose response experiments. Logarithmic transformation is necessary to revert it to the additive model (26).

- Square root transformation is appropriate for data made up of small whole numbers, most especially data obtained in counting rare events like number of infested plants in a plot, insects caught in a trap or number of a particular weed species on a plot. In these sets of data, the variance tends to be proportional to the mean. The square root should first be taken prior to commencing the analysis of variance. If data contain small values in percentage from (0-30) then $\sqrt{x + \frac{1}{2}}$ should be used instead of $\sqrt{x}$, where x = the observed value to be transformed

CONCLUSION

Limitations in the use of bioassays have been extensively discussed elsewhere (5, 10, 31). However, in spite of their limitations, bioassays remain a quick means of monitoring herbicide persistence and quantifying herbicide residue in the soil which can complement or confirm chemical analysis. It may even be the only means of monitoring persistence of herbicides, especially where infrastructure for chemical analyses is inadequate. Considerable improvement in sensitivity of bioassays is possible if experimental conditions are standardised (42). Bioassay investigators should endeavour to use locally-available species as test organisms to enable easy adoption of such procedures by educated farmers and extension workers who may wish to use the method to monitor the persistence of a particular herbicide. Appropriate indices must be employed to express the results of bioassay tests to elucidate the inherent advantages in the test carried out.

Because of the potential environmental hazards posed by persistent herbicides, there is a need to develop bioassays for commonly used herbicides so as to provide a ready means of monitoring their persistence. Bioassay procedures should be simple enough to be followed by the average educated farmer or extension worker whose responsibility it is to encourage farmers to adopt herbicide technology in their farm operations.

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