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Herbicide Bioassays

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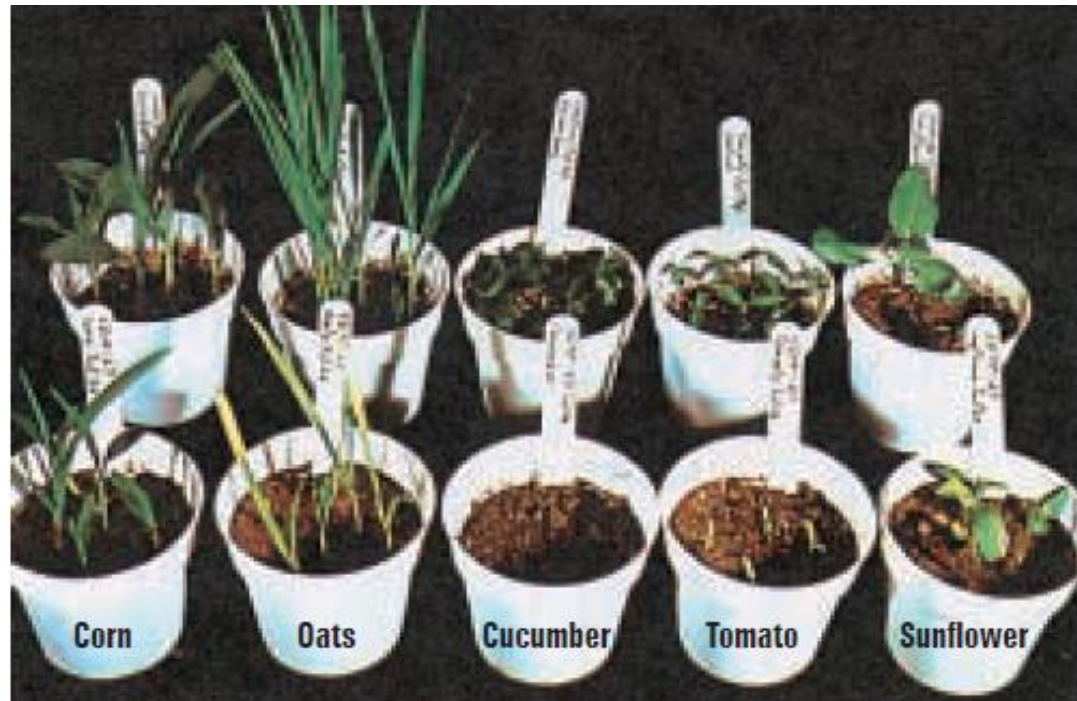
2018

Plant bioassay:

- “ Bioassays are experiments that use living things to test the toxicity of chemicals.
- “ A simple, inexpensive, accurate and direct method of determining if it is safe to grow crops on farmland previously treated with known herbicides or on cropland with an unknown history of herbicide use.
- “ A bioassay can detect if herbicide or chemical residues are present in the soil at concentrations high enough to adversely affect crop growth, yield and quality.

Bioassays should be conducted:

- when newly seeded or established plants exhibit abnormal growth / injury
- when seeding or planting on areas previously treated with herbicides known to be residual, such as *imazethapyr*
- when using abandoned farmland, which may contain herbicide residues such as *picloram* or *triazines*
- when using non-cropland, such as railways, roadsides, storage areas, and industrial sites that may contain residues of *chlorsulfuron* and *picloram*
- when purchasing livestock manure or topsoil of unknown origin



Front row: Bromacil contaminated soil

Back row: Check soil

The photograph was taken 20 days after seeding

Advantages

- “ major tool for qualitative and quantitative determination of herbicides
- “ phototoxic activity of the herbicide molecule is being measured
- “ not generally necessary to extract the herbicides from the substrate
- “ more economical, less difficult to perform and do not require as much expensive equipments as in chemical analytical methods

Plant bioassay procedure

Selecting bioassay species

The accuracy of a bioassay depends on two factors:

1. Sampling technique
2. Depth and area of soil sampled

For best results, the test must be conducted about one month before seeding time.

Table 1. Selecting plant species for herbicide bioassay		
Herbicide Group	Test Species	Common Injury Symptoms
Group 2: <i>imazethapyr,</i> <i>chlorsulfuron</i>	Sugar Beet, Canola	Stunting, purpling, general yellowing of new growth, terminal bud death
Group 3: <i>trifluralin,</i> <i>ethalfluralin</i>	Green Foxtail, Oat	Stunting, swollen and shortened roots
Group 4: <i>picloram,</i> <i>clopyralid</i>	Faba Bean, Flax	Malformation; bending and twisting of stem, leaf cupping and curling
Group 5: <i>triazines, bromacil</i>	Oat, Cucumber	Stunting, interveinal yellowing of new leaf
Group 8: <i>triallate</i>	Tame Oat, Wild Oat	Lack of emergence, brittle and dark green coleoptiles, shoots may form loops

Test species for various groups of herbicides

Herbicide group	Bioassay species
Aliphatic halogenated acids (Dalapon)	Oats, millets, cucumber, barley, wheat, rice
Acetamides (diphenamide)	Crabgrass, oats, barley, ryegrass, pigweed
Acetanilide (alachlor, metolachlor)	Cucumber, ryegrass, crabgrass
Benzoic acid	Cucumber, oats, foxtail, pigweed
Dicamba	Beans, sorghum, cucumber
Chlorpropham	Cucumber, oats, ryegrass
Phenoxy derivatives (2,4-D)	Cotton, pigweed, tomatoes, mustard
Substituted ureas (diuron, isoproturon)	Cucumber, ryegrass, oats, barley, millet, sorghum
Thiocarbamates (EPTC)	Ryegrass, oats
Dinitroanilines (trifluralin, fluchloralin)	Oats, sorghum, rice, cucumber
Triazines (atrazine, simazine)	Oats, cucumber, sugarbeets

Seeding and growing bioassay species

- i. To minimize variables in plant growth, make three to four 10 or 15 cm pots for each soil sample.
- ii. Assay pots should contain no drainage holes at the bottom to prevent the leaching of chemicals.
- iii. Seed 10 to 15 seeds of specifically sensitive bioassay species in the pot (*clean & contaminated* Soil samples).
- iv. A laboratory control, made up of sterilized herbicide-free potting soil, is usually added as a further check.
- v. Make the soil to be bio assayed wet by adding water close to field capacity. Only one plant species is seeded per pot. Label the pot.
- vi. Place the pots in a greenhouse with day/night temperatures of 22/18°C and a photoperiod of 16hr day/8hr night.
- vii. Let the plants grow for at least three weeks and continue to observe them regularly. Water as required, taking extreme caution to avoid over-watering.

Assessment Parameter

- “ Germination tests
- “ Assessment of plants
- “ **Physiological and Morphological Effects**
- “ **Symptoms**

A) Germination Tests:

- ❖ Many herbicides strongly inhibit the germination of sensitive species but few tests have been based simply on germination numbers.
- ❖ Sublethal concentrations frequently produce an inhibition of radical or shoot elongation which is dose related in a range sufficiently large to allow valid measurements.
- ❖ Typically, the root or shoot elongation is observed after a period of 24 ó 96 hours.
- ❖ Cucumber, sorghum and oat are the main species used in germination tests but they are not sensitive to photosynthetic inhibitors (Kratky and Warren 1971).0

B) Assessment of Plants:

Determination of dry weight is most common assessment used in bioassay.

- I. Generally, the tops only are weighed since the separation of roots from soil is laborious.
- II. Observation based on fresh weight and dry weights generally give good estimations.
- III. For many photosynthesis inhibitors shoot growth is reduced before the appearance of the injury symptoms.
- IV. Measurement of plant height or leaf length provides an assessment of herbicidal activity which may replace weighing.
- V. While leaf length is easily measured on monocots, other measurement may be considered on the other species.

C) Physiological and Morphological Effects:

Reduction of photosynthetic activity by photosynthesis inhibitors has been measured on leaf discs (De Silva et al. 1976).

Chlorosis may be evaluated by determining chlorophyll content through spectrophotometer (Horowitz 1970). Duffy (1972).

Estimation of the decrease in viability of root tissue with a modified tetrazolium test.

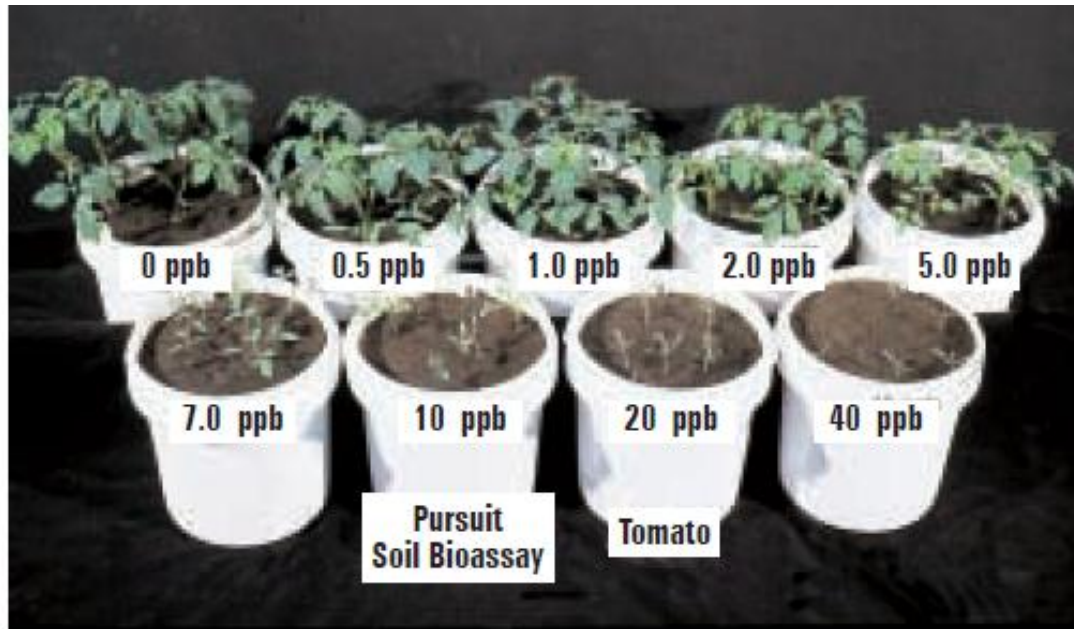
D) Symptoms:

Symptoms which are typical of a certain group of herbicides or of a given compound can be used for qualitative assay and if the intensity of symptoms are dose related, it can also be used for quantitative determinations.

Ex. Epinasty of cotton have been used to measure the effect of 2, 4-D.

Evaluating plant growth and injury

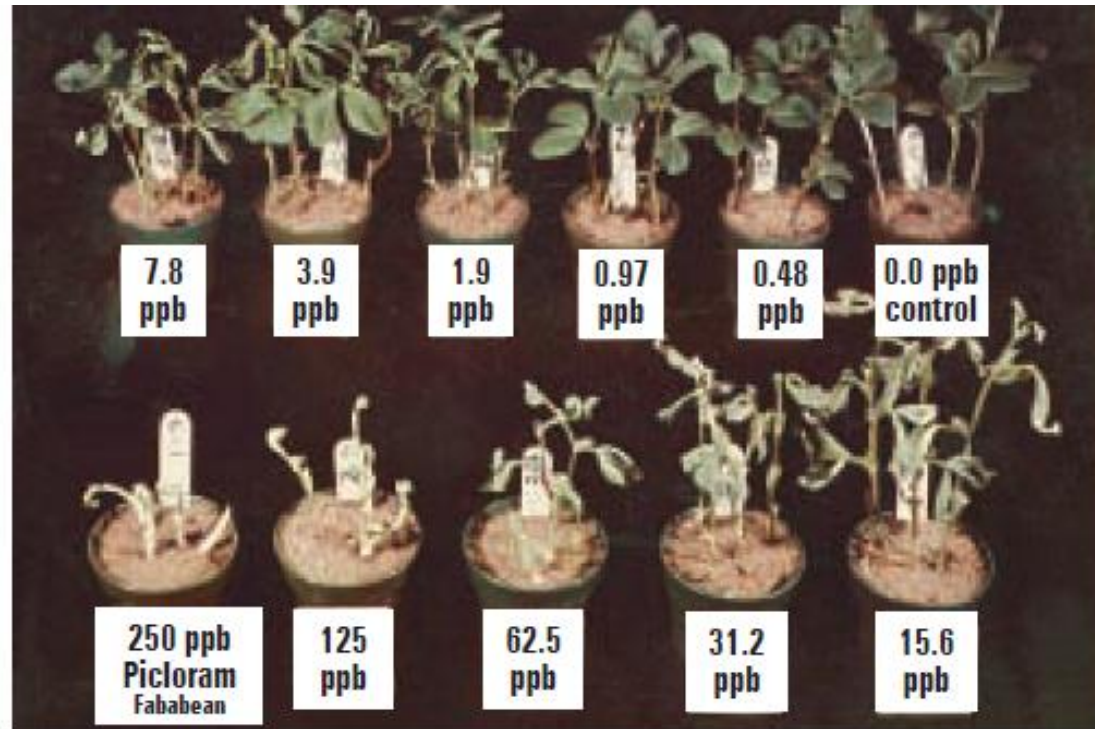
- “ **To accurately diagnose herbicide injury, you need to be able to recognize herbicide-related symptoms .**
- “ **Depending on the type and/or concentration of residues, injury symptoms usually appear within 10 to 20 days after plant emergence.**
- “ **Examine the plants of each species for stunting of growth, yellowing or discoloration of leaves or stems, abnormal leaf and stem growth, and root swelling or stunting.**
- “ **The plants may be photographed two to three weeks after emergence.**
- “ **The approximate concentration of herbicide residues in a soil is estimated by comparing the test plants with existing standard dose-response bioassays.**



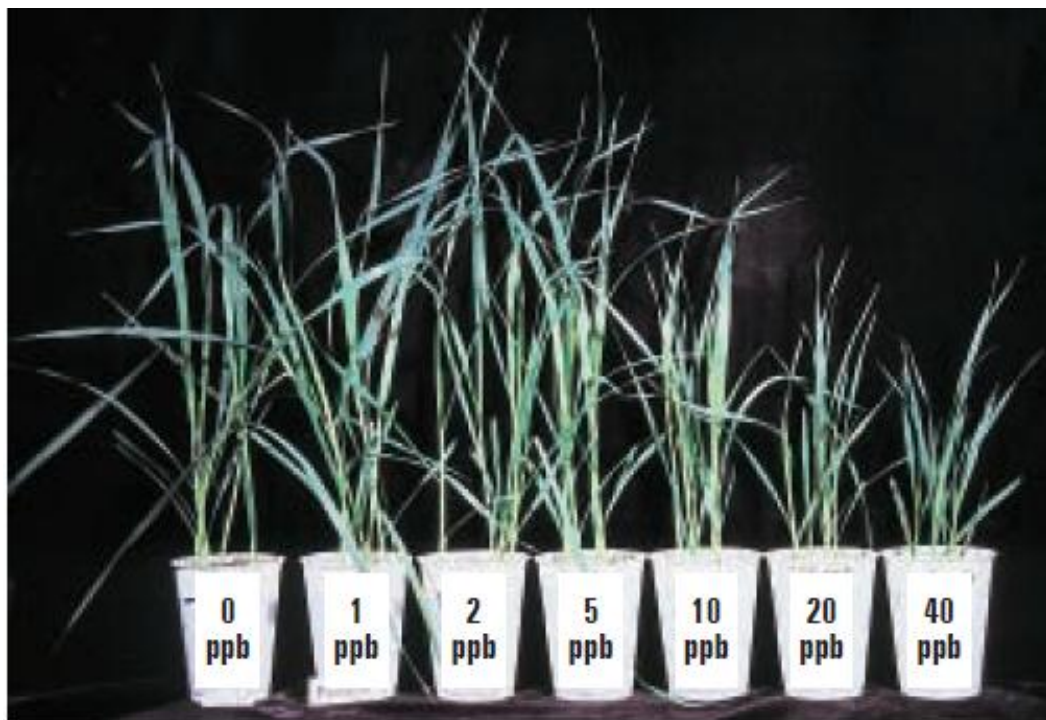
A soil bioassay conducted with tomato showing imazethapyr dose-response

1 ppb (parts per billion) = $1\mu\text{g}/\text{litre}$ (IX rate of imazethapyr = 50 g /ha = 42 ppb).

Plants photographed 20 days after emergence

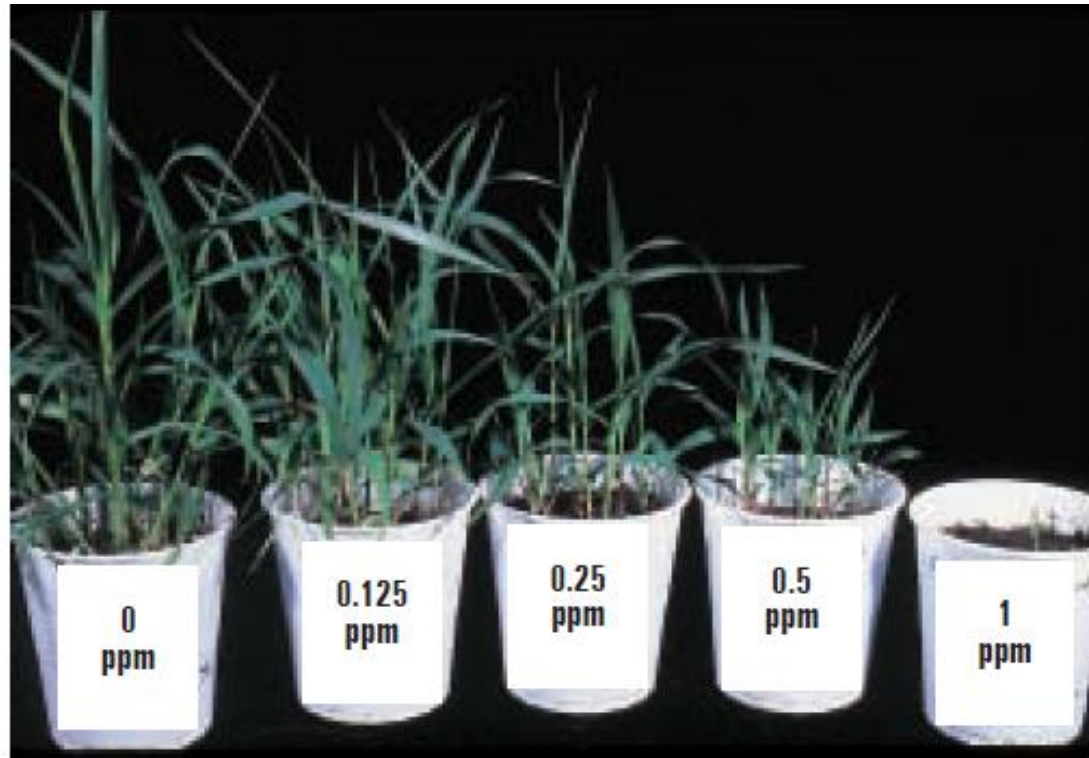


A soil bioassay conducted with faba bean showing picloram dose-response in ppb



A soil bioassay conducted with tame oat showing imazethapyr dose response

Plants photographed 20 days after emergence



A soil bioassay conducted with green foxtail showing trifluralin dose response

1 ppm (parts per million) = 1 mg/litre

Table. Common injury symptoms on sensitive species

<i>Herbicide mode of action</i>	<i>Representative products</i>	<i>Typical injury symptoms on sensitive crop species</i>
Growth regulators	2,4-D, dicamba (Banvel®), Clarity®, Status), clopyralid (Stinger™), fluroxypyr (Starane™)	<p>Broadleaf species: leaf cupping or strapping, stem twisting or epinasty, callus tissue on stems, brittle stems, necrosis of meristematic tissue</p> <p>Grass species: onion leafing, leaf rolling, brace-root fusion, brittle stems, stalk bending</p>
ALS inhibitors	imazaquin (Scepter®), imazethapyr (Pursuit®), chlorimuron (Classic®), thifensulfuron (Harmony®), nicosulfuron (Accent®), flumetsulam (Python®)	<p>Broadleaf species: chlorosis, stunted plants or shortened internodes, red or black veins on underside of leaf</p> <p>Grass species: stunted plants, interveinal chlorosis, purpling of</p>

<p>Seedling growth</p>	<p>trifluralin (Treflan), pendimethalin (Prowl®), metolachlor (Dual®), acetochlor (Harness®), dimethenamid (Outlook®)</p>	<p>Broadleaf species: swollen hypocotyls, callus on stem tissue at base of stems, root growth inhibited, inhibitors heart-shaped leaves, rough, crinkled leaves</p> <p>Grass species: stunted, thickened, red or purple in color, may appear drought stressed, “buggy whipping.” onion leafing or improper leaf unfurling</p>
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Photosynthesis inhibitors	Atrazine (AAtrex®), metribuzin (Sencor®), linuron (Lorox)	Broadleaf species: chlorosis and necrosis at leaf tips and margins; interveinal chlorosis; brown speckling near leaf edges Grass species: chlorosis and necrosis at leaf tips and margin
Cell membrane disruptors	fomesafen (Reflex®/Flexstar®), flumioxazin (Valor®), sulfentrazone (Spartan®)	Broadleaf species: stunting; chlorosis and necrosis; leaf crinkling Grass species: stunting; veinal chlorosis; necrosis
Pigment inhibitors	clomazone (Command®), isoxaflutole (Balance®), mesotrione(Callisto™), topramezone (Impact), tembotrione (Laudis®)	Broadleaf species: white, chlorotic, then necrotic tissue Grass species: translucent, white, chlorotic, then necrotic tissue

Application of bioassays

- A) Soil Effects
- B) Dissipation from Soil Surface
- C) Movement in Soil
- D) Degradation and Persistence
- E) Residual Effect

Recommendations

When residues of any herbicide or herbicide group are detected (confirmed) in a submitted soil sample, growers can follow these guidelines:

- **Leave the soil fallow for one or more growing seasons, depending on the level of residues, before planting sensitive crops.**
- **Plant a crop species that is tolerant of the detected herbicide residue. incorporate activated carbon into the soil to a depth of 15 cm (6 inches) if for a small area or on a horticultural crop.**
- **Conduct a bioassay before planting high-priced, sensitive crops such as potatoes or genetically modified (GM) canola, particularly if you suspect herbicide residue.**
- **Consult with an agricultural crop specialist if herbicide residue is a concern.**
- **Remember, if you incorporate a soil-applied herbicide and end up with a crop failure in a given year, you can only fallow the land, or time permitting, replant with the same or similar crop.**

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