AGRI-FACTS
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Plant Bioassay Techniques for Detecting and Identifying Herbicide Residues in Soil

A plant bioassay is 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

In all cases of herbicide use, refer to the manufacturer's guidelines to determine if herbicide residue is a concern.

Figure 1 shows a typical bioassay being performed on several species to detect bromacil residues in soil.

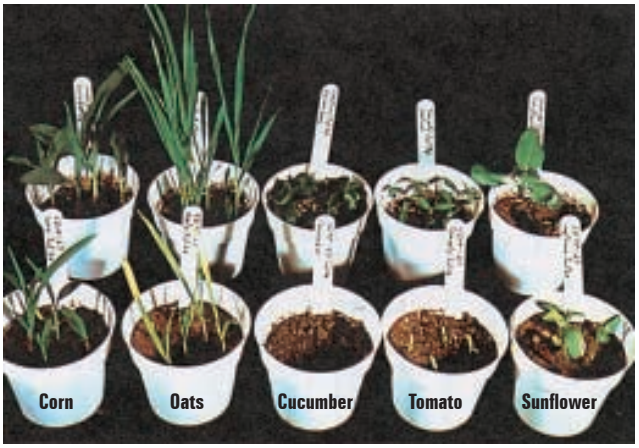

Figure 1. Front row: Bromacil *contaminated soil; Back row: Check soil. The photograph was taken 20 days after seedling emergence*

Collecting and submitting soil samples

The accuracy of a bioassay depends on two factors:

- sampling technique
- depth and area of soil sampled

For best results, soil samples should be collected and the test conducted about one month before seeding time.

To collect soil samples:

- sample soil from areas suspected of having herbicide residues as well as those areas believed to be free of herbicide. Keep samples separate.

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- take separate samples from high spots and low spots, including areas where sprayer overlap could have resulted in an overdose – particularly if the overlap is still visible in standing stubble.
- collect soil samples from the upper surface (0 to 8 cm/ 0 to 3 inches). Most residual herbicides are bound in the upper top of soil (5 cm/2 inches), unless the land has been cultivated to a specific depth, in which case, samples should be taken at the depth of cultivation, usually 10 to 15 cm/4 to 6 inches.
- using a spade, trowel or soil sampler, take several samples from the suspect area and combine them. Collect enough soil, about 2 kg or 4 lbs, to fill several 10 cm (4 inch) pots.
- along with the soil sample from the herbicide-free area, you may now take one to three additional soil samples, depending on land topography.
- store the samples in cool conditions. If the soil is wet, spread it out to dry, keeping different samples separate to avoid cross contamination. When dry, crush clods to pea-sized particles.

To submit soil samples

Clearly label the separately bagged samples with detailed information and send to:

Alberta Research Council
Crop & Plant Management
Hwy 16A & 75 Street,
Vegreville, AB Canada
T9C 1T4

Attn: Harold Feddema
or Sandi Checkel

If you require more information, contact Harold Feddema at (780) 632-8238 or Sandi Checkel at (780) 632-8217.

Cost – There is a charge for each sample assayed. For current rates, contact the Alberta Research Council.

Plant bioassay procedure

Selecting bioassay species

Bioassays are conducted by growing species of plants known to be sensitive to a specific herbicide or class of herbicides (see Table 1) in the sample soil, along with the crop to be planted. If the suspected herbicide contaminant is unknown, a broad range of crop species are grown to help identify the culprit.

If injury occurs to test bioassay plants, then the potential exists for significant field crop injury, particularly if the crops grown are sensitive to that particular herbicide.

Table 1. Selecting plant species for herbicide bioassay

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

Who can test for herbicide residues

Producers, crop specialists, industry representatives and others can all conduct bioassays for herbicide residues. Here are the steps.

Seeding and growing bioassay species

To minimize variables in plant growth, make three to four 10 or 15 cm (4 or 6 inch) pots for each soil sample. Assay pots should contain no drainage holes at the bottom to prevent the leaching of chemicals.

Seed 10 to 15 seeds of specifically sensitive bioassay species into the submitted "*clean*" and "*contaminated*" soil samples. A laboratory control, made up of sterilized herbicide-free potting soil, is usually added as a further check.

Make the soil to be bioassayed wet by adding water close to field capacity. Only one plant species is seeded per pot. Label the pot. Place the pots in a greenhouse with day/ night temperatures of 22/18°C and a photoperiod of 16hr day/8hr night.

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.

Evaluating plant growth and injury

To accurately diagnose herbicide injury, you need to be able to recognize herbicide-related symptoms (refer to Table 1), and have an understanding of herbicide modes of action, fate and behavior in the soil.

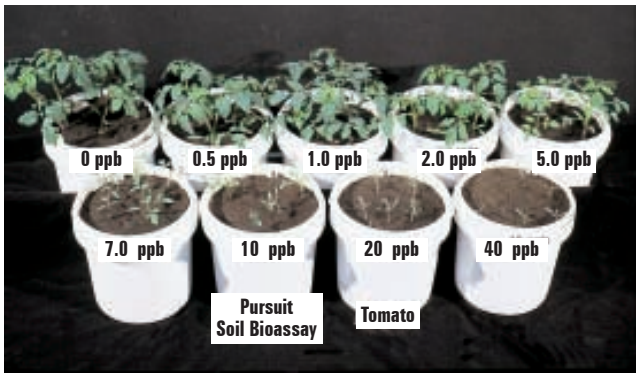

Figure 2. A soil bioassay conducted with tomato showing imazethapyr *dose-response. 1 ppb (parts per billion) = 1µg/litre (1X rate of* imazethapyr *=50 g a.i/ha = 42 ppb). Plants photographed 20 days after emergence*

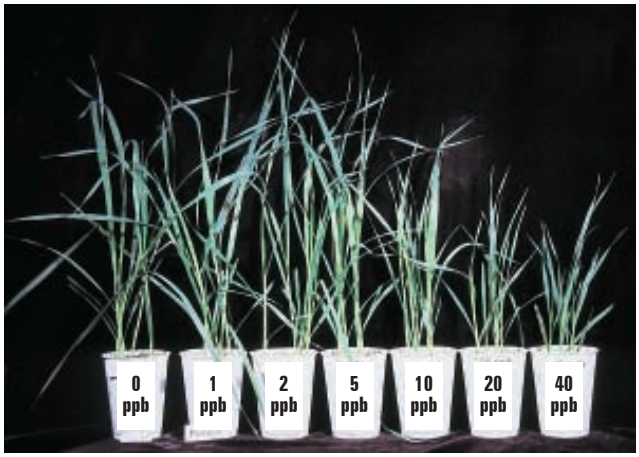

Figure 3. A soil bioassay conducted with tame oat showing imazethapyr *dose-response. Plants photographed 20 days after emergence*

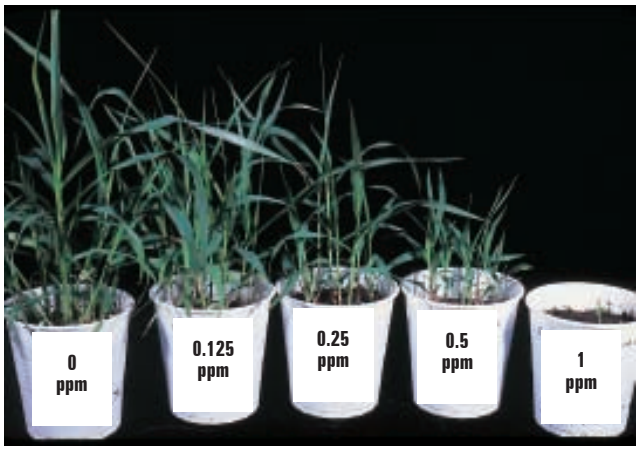

Figure 4. A soil bioassay conducted with green foxtail showing trifluralin *dose-response. 1 ppm (parts per million) = 1mg/litre*

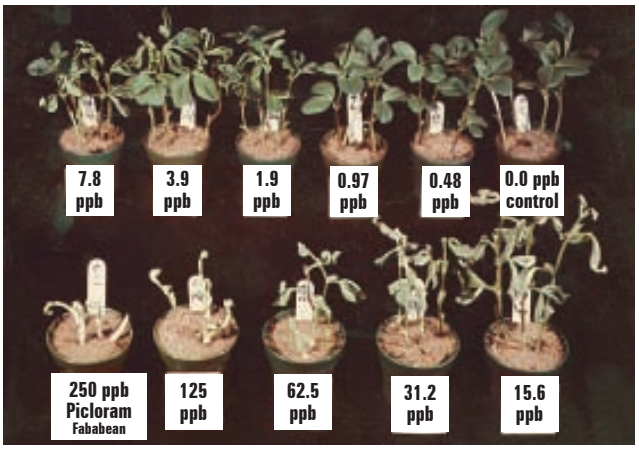

Figure 5. A soil bioassay conducted with faba bean showing picloram *dose-response in parts per billion (ppb)*

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. Four examples are shown in Figures 2 to 5.

Reporting bioassay results

After completing a bioassay for herbicide residue diagnosis, a written report should be prepared. If the bioassay is being done for someone else, provide the client with the written report.

If it is necessary to validate results, send comparable samples to a laboratory for chemical analysis. Chemical analysis confirms the presence and concentration of a herbicide in the soil. A partial list of laboratories that offer these services is available upon request.

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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