Bioassay Technique for Detecting Herbicide Carryover in the Soil


Bioassay

The bioassay can help predict potential crop injury. The test is inexpensive and can be done with a few simple supplies. A bioassay does not measure the amount of herbicide residue present in the soil, but it may indicate whether or not enough residue is present to injure a sensitive crop.

Soil Collection and Preparation

With the lab analysis or indoor bioassay, proper sampling of soil is the first step. The procedures for submitting a soil for laboratory analysis and for conducting an indoor bioassay are similar. These guidelines should be followed:

1. Before planting time, collect representative soil samples from the suspect field. Take samples from several locations in the field. For the bioassay, take 15 to 20 soil cores and combine them to make a composite sample. This sample should represent no more than 15 to 20 acres. Enough areas must be sampled to avoid missing locations with high herbicide-residue content.

Take separate samples from areas where excessive residues are suspected, such as sprayer turnaround points and end rows. Do not mix these samples with the others. Sample the soil to a 6-inch depth, and divide the samples into two sections for greater accuracy — those from 0 to 3 inches and those from 3 to 6 inches. Be sure to mark on the bags the depths from which the samples came. About 8 pounds of soil are needed for each bioassay.

2. Sample an area that is not suspected of having residues for use as a “check” sample. This soil may be taken from a nearby fencerow or another untreated area. Keep this sample separate from the others.

3. Bioassays should be run on the soil samples as soon as possible after they have been obtained from the field. If samples cannot be assayed immediately, store the soil in a refrigerator or freezer that is not used for food. If samples are stored in a warm environment, herbicide residue may break down over time.

Field Bioassay

A field bioassay is conducted by planting one or more strips of a species sensitive to the suspect herbicide in the field. This procedure can be done in the fall or spring, but it is more accurate when performed closer to the planting of the intended crop. Before planting the desired crop, allow the test plants to grow and develop symptoms of injury from any herbicide residues. Plant the strips in several locations, if possible, and include an area that is most suspect and an area that can serve as a check. Choose an appropriate species for the bioassay, such as one of the
more sensitive ones listed below. Include several species of differing sensitivity for greater accuracy.

**Indoor Bioassay**

The procedures for conducting an indoor bioassay vary, depending on what herbicide residue is of concern. However, for the indoor bioassay, the procedures for soil collection and preparation are the same.

1. For an indoor bioassay, collect the samples and allow them to air dry if needed until they can be worked readily. Do not overdry. If the soil is cloddy, crush the clods into pieces (the size of a pea or smaller). If the soil contains a high amount of clay, the addition of coarse sand (50 percent by volume) improves its physical condition. If sand is added, mix it thoroughly with the soil.

2. Clean tin cans, milk cartons, and cottage cheese containers are appropriate containers in which a bioassay can be conducted. Punch holes in the bottoms of the containers to allow water drainage. Fill two or more containers (a set) with soil from each sample. Additional containers increase the accuracy of the test. Place the soil samples obtained from depths of 0 to 3 inches in one set of containers; in another set, place the soil obtained from depths of 3 to 6 inches. Follow this procedure for the composite sample and the sample taken from areas where excessive residues are expected. In addition, fill a final set of containers with the check soil.

Suggested plant species for specific herbicide groups.

<table>
<thead>
<tr>
<th>Herbicide group</th>
<th>Plant</th>
<th>Brief instructions (see links below for more detailed instructions and injury symptoms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eg. atrazine, simazine</td>
<td>small grains, radish, cabbage</td>
<td>Place plants in sunny area. Sunlight is essential for inducing symptoms of triazine injury. Injury symptoms should be noticeable within 10-14 days of emergence.</td>
</tr>
<tr>
<td>eg. linuron, terbacil</td>
<td>small grains, radish, cabbage</td>
<td></td>
</tr>
<tr>
<td>Seedling growth inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eg. trifluralin, pendimethalin</td>
<td>sorghum, corn</td>
<td>Place seeds in moist paper towel at room temperature for 2-3 days. Plant 3-5 seeds per container and leave for 10-14 days. Remove the plants and inspect the roots for injury symptoms. Plant 10-15 seeds in each container.</td>
</tr>
<tr>
<td>eg. dimethamid, s-metolachlor, napropamide</td>
<td>small grains</td>
<td></td>
</tr>
<tr>
<td>Amino acid synthesis inhibitors</td>
<td>sugarbeet, radish, cabbage</td>
<td>Grow plants for 14-21 days after emergence. Examine shoots and roots for damage.</td>
</tr>
<tr>
<td>Growth regulators</td>
<td>beans, corn</td>
<td>Plant 3-5 seeds per container.</td>
</tr>
<tr>
<td>Pigment inhibitors</td>
<td>wheat, oats</td>
<td>Plant 10-15 seeds in each container.</td>
</tr>
</tbody>
</table>

A Quick Test for Herbicide Carry-over in the Soil ([http://www.ianrpubs.unl.edu/epublic/live/g1891/build/g1891.pdf](http://www.ianrpubs.unl.edu/epublic/live/g1891/build/g1891.pdf))

**TIP**

When herbicides are suspected to be present at low concentrations, it may be beneficial to keep the number of plants per container to about 3 for large-seeded species, more for small grains. With more plants per container, each plant will extract less of the herbicide, making the bioassay less sensitive.

From: A Quick Test for Herbicide Carry-over in the Soil ([http://www.ianrpubs.unl.edu/epublic/live/g1891/build/g1891.pdf](http://www.ianrpubs.unl.edu/epublic/live/g1891/build/g1891.pdf))
Testing EC of Transplant Plugs.

Source: Dr. Douglas Cox, Plant and Soil Sciences, University of Massachusetts—Amherst
How to Use pH and EC “Pens” to Monitor Greenhouse Nutrition
(http://www.umass.edu/umext/floriculture/fact_sheets/greenhouse_management/phecpens.html)

A number of inexpensive pen-like instruments are available which can be used for assessing pH and soluble salts (EC).

Supplies
In addition to the pens the following items are needed for pen maintenance and to do EC tests. Many of the items can be obtained for free from home or inexpensively from the grocery store. Of course more expensive and professional looking supplies can be purchased from a science lab supply company, but the tests won't turn out any better than using the cheaper alternatives. Here is the basic list:

1. EC standard solution to calibrate EC pen
2. Plastic funnel (5-6" diameter)
3. Distilled water
4. Basket-style coffee filters
5. Clean 1 qt. wide mouth jars
6. 3 oz. waxed paper cups
7. Plastic colander (min. 6" diam.)
8. Shallow baking pan or 8-10" diameter plant saucers

Calibration of Pens
All of the commonly available pens and more expensive types of pH and EC meters work on the same basic principles and if they are calibrated properly should give the same readings. All pens and meters must be calibrated or standardized to give consistent and dependable readings each time they are used. If a pen or meter has no means of being calibrated it is probably not going to give good results.

Calibration procedures differ somewhat depending on the brand of pen. On some you push a button, on others you turn a small screw to set display at the proper value. Calibration involves placing the EC pen in a 3 oz. cup of buffer solution, allowing a stable reading to develop, and then, if necessary, adjusting the displayed value to the EC of the buffer. After calibration the used solutions should be discarded.

Calibration is a must to get any useful information from pens and meters! How often it needs to be done depends on the type of meter and frequency of use. The readings of even the most expensive laboratory meters tend to "drift" over time and must be brought back to the proper reading fairly often. When you first start using the pens, plan on calibrating at the beginning of every testing session until you find out how much the readings drift between sessions.

Direct Measurements Using the Pens
On certain types of materials pens can be used directly without any special sample preparations.

The EC pens can be used to measure the EC of fertilizer solutions. Calibrate the pen first according to the instructions, rinse off the buffer, and then place the pen directly into a sample large enough to completely immerse the sensor. Agitate the pen slightly in the sample to dislodge any air bubbles and then allow a stable reading to develop. To check the operation of an injector, determine the EC of water without fertilizer and then check the fertilizer solution after the injector has run for several minutes. Subtract the EC of the water from the fertilizer solution EC. Compare the results to the table on the fertilizer bag or product literature. This will tell you whether or not you are getting the ppm you think you are.
Testing Growth Media

Greenhouse growth media are tested by extracting the sample with distilled water and measuring the EC of the filtered extract. Some low-priced testing meters claim that you can stick the sensor probes directly into the growth medium and take readings. This approach has no basis in scientific soil testing and it is not recommended for professional growers.

Growth medium samples. Take samples from the root zone or use all of the material in a plug. Never sample from the surface because nutrients and soluble salts are highest here and do not represent the fertility status of the root zone. Sampling is a good time to inspect the roots - a small or diseased root system can often be the best explanation of apparent fertility problems.

It is best to air-dry the sample at room temperature or below 80°F (27°C) on a greenhouse bench. Spread the sample out in the baking pan or plastic saucer and remove any large pieces of root and other debris. Unless the sample is very wet it should be dry enough in 24 hours to test. Screen the sample using the colander or similar sieve. The screened sample is ready to test.

Extraction. The extraction procedure described here is known as the "1:2 dilution method."

1. Combine one volume of air-dried growth medium with two volumes of distilled water. Using the items listed earlier, this means fill one 3 oz. cup with growth medium and two 3 oz. cups with distilled water and mix them together in the 15 oz. jar.
2. Mix the sample and distilled water thoroughly by swirling the jar and then allow it to stand for 30 minutes.
3. After 30 minutes pour the mixture into the funnel supporting the coffee filter (I find two filters put together best). Catch the filtered extract in another clean 15 oz. jar. The objective is to separate the liquid extract from the solids which are discarded. The extract is now ready to test for EC.
4. Properly calibrate pens.
5. Pour enough extract into a clean 3 oz. cup so that the sensor of the pen will be completely immersed in the extract. The pen should be swirled in the extract to dislodge any air bubbles and then leave the pen still until a stable (unchanging) reading appears. The stable EC reading is your result.
6. Compare your EC reading to the table, below. Use the 1:2 column for the extraction method described here.

Note: pH can be measured using this procedure as well.

Interpreting Test Data

Soluble salts. Measuring soluble salts provides a general indication of nutrient deficiency or excess. Excess soluble salts is very common and generally results from too much fertilizer in relation to the plant's needs, but inadequate watering and leaching, or poor drainage, are other causes. Sometimes high soluble salts levels occur when root function is impaired by disease or physical damage. Again, always check the condition of the root system when sampling soil for testing.

Seedlings and young transplants are less tolerant of excess soluble salts. Soluble salts above the normal range for prolonged periods may cause root injury, leaf chlorosis, marginal burn, and sometimes, wilting. Soluble salts below the normal range may indicate the need for increased fertilization.

Soluble salts levels by various methods and associated interpretation. Readings shown in mS or mmhos.¹


<table>
<thead>
<tr>
<th>Source</th>
<th>1:5</th>
<th>1:2</th>
<th>SME</th>
<th>Pour-through²</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 0.12</td>
<td>0 to 0.25</td>
<td>0 to 0.75</td>
<td>0 to 0.9</td>
<td>Very low. Nutrient levels may not be sufficient to sustain rapid growth.</td>
<td></td>
</tr>
<tr>
<td>0.12 to 0.35</td>
<td>0.26 to 0.75</td>
<td>0.76 to 2.0</td>
<td>1.0 to 2.6</td>
<td>Low. Suitable for seedlings, bedding plants and salt sensitive plants.</td>
<td></td>
</tr>
<tr>
<td>0.36 to 0.65</td>
<td>0.76 to 1.25</td>
<td>2.0 to 3.5</td>
<td>2.7 to 4.6</td>
<td>Normal. Standard root zone range for most established plants. Upper range for salt sensitive plants.</td>
<td></td>
</tr>
<tr>
<td>0.66 to 0.89</td>
<td>1.26 to 1.75</td>
<td>3.5 to 5.0</td>
<td>4.7 to 6.5</td>
<td>High. Reduced vigor and growth may result, particularly during hot weather.</td>
<td></td>
</tr>
<tr>
<td>0.90 to 1.10</td>
<td>1.76 to 2.25</td>
<td>5.0 to 6.0</td>
<td>6.6 to 7.8</td>
<td>Very high. May result in salt injury due to reduced water update. Reduced growth rates likely. Symptoms include marginal leaf burn and wilting.</td>
<td></td>
</tr>
<tr>
<td>&gt; 1.1</td>
<td>&gt; 2.25</td>
<td>&gt; 6.0</td>
<td>&gt; 7.8</td>
<td>Extreme. Most crops will suffer salt injury at these levels. Immediate leaching required.</td>
<td></td>
</tr>
</tbody>
</table>

1. 1.0 mmhos/cm = 1.0 dS/m = 1.0 mS/cm. Note: The decimal place for micro-siemens is 3 places to right when comparing to milli-mhos. Therefore 0.5 dS/m (milli-mhos) = 500 micro-siemens.
2. This method has not been described here. Due to the variability of pour-through results depending upon your methods and media, you should always compare your initial results to other methods before using this technique.

For more information:


Soil pH Quick Test

Soil pH is the measurement of the hydrogen ion activity or concentration in the soil solution. This has an impact on the availability of most nutrients. It can cause the concentration of some elements to rise to toxic levels i.e. aluminum. It also affects the activity of soil organisms that build soil structure, cycle organic matter or fix nitrogen in legumes nodules. For example, many bacteria such nitrogen fixing bacteria prefer a neutral to alkaline pH while fungi generally prefer a neutral to acidic pH. Soil pH also has a dramatic effect on the performance and breakdown of some pesticides i.e. Pursuit.

pH can be measured with a standard lab test using a pH electrode. Basically the electrode is a glass bulb that is porous to hydrogen ions. As the positive ions move into the electrode, a current is set up that is measured and given as pH values. In Ontario, the accredited method for pH uses a saturated paste. The paste is prepared by adding just enough de-ionized water to the soil sample to completely saturate the soil without leaving any free water. The measured pH tends to increase as the amount of water added to the soil sample increases. This is of particular concern in soils with the lowest buffering capacity i.e. coarse sands where low pH can be a crop management problem.

Soil pH can also be measured with hand held meters, particularly for diagnostic purposes. There is a large number of hand held meters available in a variety of price ranges. When selecting one, consider ease of use, accuracy, and cost. Ease of use includes storage and calibration. Often pH meters are used intermittently. Some meters require the electrode to be stored wet, often in a buffer solution. Re-wetting or soaking the electrode can take time and cause frustration when wanting to “just take some quick readings”. There are a number of meters that do not require a wet storage. Calibration is also an important component. Avoid meters that do not have some form of calibration. At least one calibration reading should be performed. Some meters require a high and low pH calibration. Accuracy with portable pH meters has improved with most meters being quite comparable to lab electrodes. However, any meter that is directly inserted into the soil in field is not going to give reliable readings that are comparable to our standard pH values (think of the variation that we get with soil moisture over the season – soil moisture carries the hydrogen ions, so pH is very difficult to measure in a dry soil).

Soil samples for pH analysis should be collected similar to soil fertility samples. Use a soil sampler core to collect soil to a consistent depth of 15 cm. Take a number of soil cores to well represent the area. Mix the sample well. Often it helps in diagnosing a problem if separate samples are collected from the affected area and an unaffected area. Ensure that the soil has been ground or broken up finely so that it will mix readily with water and form a smooth paste.

You will need a supply of small cups, stir sticks, de-ionized or distilled water and the pH meter (and any calibration supplies). Usually it is easiest to collect the samples and measure the pH at a counter or table. Follow the calibration directions for the particular meter and re-calibrate often.
Ontario Crop IPM
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Ontario CropIPM is an interactive educational tool to improve your knowledge of Integrated Pest Management in Ontario crops. Growers, scouts and consultants will be able to learn about insects, diseases, disorders, and weeds by:

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- comparing photos of often-confused pests,
- learning about soil diagnostics and herbicide injury,
- referring to glossaries,
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- Tomatoes

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Watch for more crops to be added soon!

These pages outline some quick tests that can help you narrow down a diagnosis, however be aware that in many cases, they may not be as accurate as laboratory analyses. Depending on the situation, you may choose to confirm your quick test results with laboratory analysis.

For more information:
Telephone: 1-877-424-1300
Email: ag.info.omafra@ontario.ca

www.ontario.ca/crops