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Bio-assay of soil samples for content of photosynthesis inhibiting herbicides

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Principle:

Test plants are grown in standard amounts of soil or on soil suspensions in nutrient solution of which the herbicide concentration has to be determined. Through visual observation and by determining the yield in organic matter the growth of these plants is compared to that of plants of the same species growing on solid soil samples or on soil suspensions containing increasing amounts of the herbicide (standard concentration series). In this manner the unknown concentrations of the herbicide in the test soil samples can be assessed.

1. Taking soil samples for residue analysis of herbicides

Generally only the soil layers of 0-5, 5-10 and 10-15 cm are sampled, deeper layers only if desired. The first two layers are sampled with a so called "potato planting auger" with a diameter of approx. 5 cm; the layer of 10-15 cm is sampled with a smaller auger of approx. 3.5 cm diameter. On each plot of the experimental field 10 samples are taken. The soil is collected in a bucket or on a plastic sheet and the whole amount is taken to the laboratory in a paper or plastic bag.

Each plot is sampled individually. Soil materials coming from replicated plots are not mixed.

For each soil layer approx. 1 kg of soil is required for the bio-assay. Approximately 4 kg of dry soil or 5 kg of wet soil for each soil layer of the untreated, herbicide-free sample, is needed for processing.

2. Preparation of the samples in the laboratory

2.1. Immediately after receipt of the samples in the laboratory (especially in the case of wet soil samples) they are placed in open containers in order to dry to "air-dry" conditions. In clay soils one usually cannot wait until considerable drying out has taken place, since crushing and sieving the soil will require too much work. In this case further drying out can take place later if so desired.

2.2. The dried-out soil is crushed and sieved through a sieve of 5 mm. Of the material remaining on the sieve the gravel and the coarse organic material are separated, since the latter may

contain residual herbicide. Separation can be done by putting the sieve rest in a cylinder with water; the organic material will float and can be taken off easily and dried. The dried organic material (crushed as finely as possible) is uniformly mixed through the sieved soil.
The soil material thus obtained can be kept in a cold room, although it is recommended to carry out the bio-assay as soon as possible after processing the soil, because of possible continuing breakdown of the herbicide.

3. The bio-assay procedure

(Growing of test plants in soil samples)

3.1. The soil samples

3.1.1. Soil moisture percentage

The moisture equivalent of the soil is determined and expressed as % moisture on dry soil basis.

Example: wet soil = 100 g = 125 %
dry soil = 80 g = 100 %

3.1.2. Experimental samples

Each bio-assay requires standardization of the treatments to be studied. Depending on soil type, the kind of container used and the objective of the bio-assay, the amount of dry soil to be used will vary. In most cases 200 g of dry soil seems to be an acceptable quantity.

The number of treatments per experiment is determined by the size, the number of soil samples (for each plot in the field 1 soil sample per soil layer) and the number of replicates.

Example: Experimental plot with 2 rates (0.1 and 2 kg/ha) and 3 replicates. Bio-assay carried out with 3 replicates.

Plots to be assayed (soil samples) : 6 =	18 pots
Standard concentration series 5 concentrations (see 3.1.3.)	15 pots
Total per soil layer to be studied	33 pots
3 layers	99 pots

3.1.3. The standard concentration series (= comparison series)

Herbicide-free soil of control plots is mixed with increasing amounts of the herbicide present in the soil samples to be assayed. The concentrations in these samples are therefore known.

Example: Each container: 200 g of soil

Concentration	Amount of active mat. in mg	ml of solution	ml of extra water
control	-	-	5
0.1 ppm	0.02	0.5	4.5
0.2 ppm	0.04	1	4
0.5 ppm	0.1	2.5	2.5
1 ppm	0.2	5	-

(Concentrated Stock solution of a 50 % commercial preparation: per ml 0.04 mg a.i. = 80 mg product per liter.)

The series of standard concentrations depends on the phytotoxicity of the herbicide studied. For simazine and lenacil it is recommended to use 0 - 0.1 - 0.2 - 0.4 - 0.8 - (1.2) ppm, for pyrazon
0 - 0.5 - 1 - 1.5 - 2 - 3 - 5 ppm

In order to avoid structural differences in the various soil samples, all samples, i.e. the control samples and the samples to be bio-assayed, are mixed with the same amount of water.
In most cases it is necessary to prepare a separate concentration series for each soil layer sampled (see 3.3.3.).

3.1.4. Calculation of herbicide concentration to be expected in the soil samples

Generally the following equation can be used in calculating the concentration present in the top 10 cm, if no breakdown or leaching has occurred:

$$\frac{\text{Dosage in kg a.m. per ha}}{\text{Apparent specific density}} = x \text{ ppm a.m.}$$

(1 kg a.m./ha = 10^6 mg a.m./10^6 dm^2 = 10^6 mg/10^6 dm^3)

At average soil density of 1.3 kg/dm^3, average concentrations at 1 kg a.m./ha are:

approx. 0.8 ppm in the 0-10 cm layer
1.6 ppm in the 0- 5 cm layer
8.0 ppm in the 0- 1 cm layer
} assuming no deeper penetration

3.2. The test species

Various species can be selected for this purpose. They should meet the following requirements:

1. rather uniform growth
2. relatively tolerant to differences in soil structure
3. synthesis rate of organic matter in the test plant should be reasonably high within a relatively short period of time
4. susceptible to the herbicide to be tested
5. easily available

In general, cereals (oats, barley) fulfill these requirements. They are susceptible to a rather wide range of concentrations. Moreover, they can be selected on uniformity by removing seeds that are abnormally large or small. However, they are less suitable for the concentration range < 0.1 ppm. For this range Brassica-species are more interesting (as was demonstrated by e.g. the Weed Research Organisation at Yarnton). In this respect Raphanus sativus var. oleïferus (Siletta) is also useful.

3.3. Growing methods

The test plants can be grown in the soil samples in various ways.

1. in small containers - cans or plastic cups - with closed bottom. The loss of water is regularly compensated for by adding water to the surface.
2. in small containers in which the soil is continuously remoistened (e.g. through a hole in the bottom, containing a cotton wool plug).
3. in a mixture of soil and nutrient solution, in which the suspended soil fraction settles on the bottom of the growth vessels.

3.3.1. Growing test plants in containers with closed bottoms

If no uniformly germinating seed material is available it is recommended to pregerminate the test plants for 24 hrs (at approx. 23°C, on wet filter paper).

The pregerminated kernels are planted in small holes at uniform depth, prepared with a small glass pencil. Only germinated kernels (small white radicle just visible) of a uniform size are used. After planting the small holes are filled with soil and slightly pressed.
After the herbicide has been mixed with the soil in the container it will not have its required moisture percentage as yet. The preferable moisture equivalent in small containers with 200 g is:

for a sandy soil	approx.	25 - 30 %
for clay soil	approx.	30 - 40 %
for peat soil	approx.	70 - 80 %

This moisture equivalent is obtained by adding to the soil surface in the containers the remaining quantity of water. With this technique the plant material can be selected three times for uniformity: a) by selecting uniform kernels; b) by selecting for uniform germination and c) by thinning out the number of plants (usually 8) to the final number of 5 per container.
After thinning to 5 per container, the test plants are fertilized with a complete nutrient solution. 10 mls of this solution (for composition/liter see below - v/d Weij) is added to each small container.

Nutrient solution acc. to van der Weij

20 cc	1	M NaCl	Mill.	eq.		
40 cc	1	M KNO_3	Na	20	NO_3	120
20 cc	0.5	M K_2SO_4	K	120	Cl	20
120 cc	0.5	M KH_2PO_4	Ca	80	SO_4	60
40 cc	1	M $Ca(NO_3)_2$	Mg	20	H_2PO_4	60
20 cc	0.5	M $MgSO_4$	NH_4	20		
20 cc	0.5	M $(NH_4)_2SO_4$		260		260

At least every 2 days the containers must be brought back to their original weight, at the end of the experiment this must be done every day. The evaporation loss can be recorded. Notes are made on visual differences between the plants growing in the test soils and those in the standard concentration series.
The containers are too small to carry out these experiments in the glasshouse. Under these conditions evaporation losses are too great.

It is therefore necessary to grow the plants under conditions of artificial light.

3.3.2. Growing the plants in soil moistened by continuous capillary action

This method is very similar to the previous one. The only difference is that the soil in the cups is connected through a hole in the bottom closed by a cotton wool plug with a bottom layer that is kept uniformly and continuously wet. In this way the experiment can be carried out in the glasshouse, although it is recommended to prevent high temperatures and direct bright sunlight.
Small plastic ice cups of approx. 130 ml are very convenient. A 7 mm hole in the bottom is closed with a cotton wool plug, with relatively long ends at each side of the hole. The cups with soil are placed on a water-saturated sheet of foam-plastic, covered with strong filter paper and spred in a shallow metal box.
At the beginning of the experiment the soil should be sufficiently moist to guarantee movement of capillary water from the sheet of foam plastic into the soil.
In peat and sandy soil, however, it is possible that under conditions of high evaporation, especially occurring in the glasshouse, the supply of moisture through the cotton wool plugs is not sufficient. Under these conditions corrections are made by light sprinkling on the containers with a hand-sprinkler.
Clay soils on the other hand can take up an excess of water. This, however, will have no serious practical consequences, because the small cups need not be removed for weighing etc. It is recommended to include in this system one extra replicate, because under these conditions a container may frequently differ too much from the other test cups.
Also other containers can be used, for instance porous clay cups, placed into moist peat, or small pots with filter paper between soil and the hole in the bottom, individually supplied with a shallow saucer holding water.

3.3.3. Bio-assay on a nutrient solution - soil suspension

200 g of soil of each sample to be tested or of the samples of the standard concentration series are stirred thoroughly several times with 850 ml of a nutrient solution (for instance $\frac{1}{2}$ - concentrated Hoagland I) in a 1 liter jar.

On this soil-settling suspension, pregerminated test plants are grown according to the procedure commonly used in nutrient-culture-techniques. At the moment of transplanting cereal plants should have a well-developed root system. Preferably the 1st leaf should have a reasonable size, but should not be unrolled. Young Raphanus seedlings must be in the cotyledon-stage. Several plants may be placed in each jar. After a few days more uniform plant material can be obtained through selection.
Pregermination and seedling development of young cereal plants can be done best in coarse riversand; for other crops a very porous, kernel shaped brick-product is used.
In the soil-nutrient solution system used in this technique, the herbicide attains equilibrium concentration in the nutrient solution. In order to make this equilibrium concentration in the jars containing the samples to be tested comparable to those of the standard concentration series, the following procedure is recommended:

a) the herbicide is added to the series of untreated soil samples (= standard concentration series). In order to facilitate this mixing of the herbicide is done by adding 100 ml of nutrient solution per 100 g of soil.
b) in the same way the same amount of nutrient solution is added to the soil samples to be tested.
c) after 24 hours the remainder of the required amount of nutrient solution (final volume approx. 1 liter) is added: stir thoroughly several times.
d) after at least another 24 hours the test plants are placed in the jars.

Adsorption and desorption of the herbicide in tested soil samples and in the standard concentration series lead to comparable equilibria due to the sufficiently long exposure time.
The system guarantees more uniformity. Irregularities due to structural and fertility differences between the soil samples are overcome. In many cases one can restrict the standard concentration series to one soil layer only.
After placing the test plants in the jars the experiment requires very little attention. It can be carried out in the glasshouse and under artificial light conditions. Another advantage is that also the reaction of the root systems can be easily studied.
Standard concentration series may not be restricted to one soil

layer when considerable differences exist between the soil layers in fertility and in adsorptive capacity to the herbicide (for instance differences in organic matter content).

3.4. Harvest of the test plants

After about 2 weeks the plants are harvested. Generally, in the bio-assay technique of photosynthesis inhibitors, only fresh weight data have to be collected.
Dry weight data of shoot or root production are of interest, however, since irregularities in the dry matter percentages may point to the presence of, for example, growth inhibition.
Yield data of the tested samples can be compared to those obtained on the concentration series. Thus an indirect estimation can be made of the herbicide concentrations, using graphical presentations.
From these concentrations the amount of herbicide present in the soil at the moment of sampling can be calculated in kg/ha (ppm per 10 cm x apparent soil density) c.f. calculation of the maximum concentration to be expected in the soil samples.

4. Some general remarks

An attempt should always be made to obtain a steep concentration curve, starting at the lowest possible concentration to allow the best interpolation of the yield data and the determination of very low concentrations in the soil samples.

The characteristics of the concentration series are dependent on:

1) the phytotoxicity of the product
2) the amount of product per jar (see below)
3) growth conditions
4) (susceptibility of) the test plant
5) duration of the experiment.

Depending on the importance of these individual factors the amount of soil per jar may be changed.

When working with a not too strong herbicide the amount of soil can be increased from 200 to 300 g or more. Thus more active material can be desorbed and enter the soil-nutrient solution system, giving steeper concentration curves.

Higher light intensities always result in steeper production curves. In many cases the differences between the control samples

(no herbicide present) and herbicide containing samples will be greater with time.

Frequently it is recommended to perform a preliminary experiment in order to establish the best concentrations for the standard concentration series.

Sometimes very high concentrations may be expected in the samples to be tested and consequently a good comparison of synthesis rates in organic matter will not be possible. In this case the samples can be diluted 3 or 10 x with untreated soil. Also a less susceptible test plant can be used (and a longer standard concentration series).

References

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