Bioassay Test for Herbicide Residues in Compost: Protocol for Gardeners

Purpose

This protocol is intended to answer the following question using simple, relatively inexpensive procedures:

"Will this test material (compost, manure, or topsoil blend) cause plant growth symptoms consistent with auxinic herbicide damage in susceptible plants?"

Using the Bioassay – General Procedures

This bioassay is intended for use by homeowners and gardeners. It has been modeled after the bioassay developed by Washington State University and Washington State Department of Ecology.

In this protocol, peas are planted and allowed to grow for two to three weeks until three sets of leaves have appeared. The plants are compared to control plants grown at the same time and evaluated for herbicide damage.

Photographs of plants grown in media contaminated by aminopyralid are available in Figures 1 and 2 as an aid to identifying plant damage consistent with auxinic herbicide damage.

Materials

- Test material (compost, manure, or soil mixture)
- Potting mix (or some other growing media known to be herbicide free)
- 4" plastic pots with saucers (preferably new or well cleaned)
- Garden pea seeds (variety not specified)

Procedure

1. Evaluate test material (compost, manure, topsoil mix)
   - Record observations of odor and general condition of compost to be tested. For example, if the material still smells like manure or you get hints of ammonium, then it may not be finished composting.

2. Set up a control pot
   - Fill **3 pots** with just potting mix or some other material that is known to be pesticide free. Mix well and tap several times on a countertop to settle.
   - Label pots accordingly.

3. Prepare test pots and label pots. The mix will vary depending on garden scenario:
   - If testing soil or topsoil mix where plants grown exhibited suspicious symptoms in the previous growing season: fill **3 pots** with straight soil/topsoil mix.
   - If testing compost received for the current growing season you can use it straight, or mix it to the same ratio you plan on incorporating into your garden beds. For example, you could mix 1-part compost to 1-part garden soil (**The ratio is 1:1 *by volume*, not by weight**). Fill **3** pots with the compost or compost blend and label accordingly.

4. Plant at least **3 seeds** in each pot, pushing them into mixture so they are just under the surface.

5. Grow plants
   - Position pots in random order, and place saucers underneath.
   - Space pots far enough apart to avoid splashing media from one pot to the next during watering.
   - **Water each pot carefully**. Keep the media uniformly moist; minimize water leaching into saucer. If excess water drains into saucer, allow it to be re-absorbed back into the pot.
   - Maintain consistent growing conditions with 12 hours light, supplemented with grow lights as necessary. Temperature should not drop below 50 F at night.

6. Evaluate plant growth
   - Record germination from each pot. In order to consider the results valid, at least two seeds in each of two pots from each replicate must germinate. (That's a total of 4 out of the nine seeds planted.)
   - Grow plants **until three sets of leaves appea**r, from **14 to 21 days**, depending on growing conditions.
   - Compare plants from compost-blend pots to control.
   - Determine level of auxinic herbicide damage. Use Figures 1 and 2 below as a guide.

A. Three-week-old Pea plants with severe damage. Note: leaf curling and stunted plants

B. Note leaf curling on plant grown in 5ppb (parts per billion) aminopyralid and death of plants at higher levels.

Table 1: A list of auxinic herbicides and their brand names.

Brand Names	Active Ingredient(s)
2,4-D	2,4-D
2,4-DB	2,4-DB
Banvel	dicamba
Banvel-K + Atrazine	dicamba + atrazine
Butoxone	2,4-DB
Butyrac	2,4-DB
Celebrity, Celebrity Plus	nicosulfuron + dicamba
Celsius	Iodosulfuron + thiencarbzone + dicamba
Cimarron Max	metsulfuron + 2,4-D + dicamba
Clarity	dicamba
Clopyr AG	clopyralid
Confront	Clopyralid + triclopyr
Crossbow	2,4-D + triclopyr
Diablo	dicamba
Dicamba	dicamba
Distinct	dicamba + diflufenzopyr
Drive, Drive XLR8	quinclorac
Escalade	2,4-D + dicamba + fluroxypyr
ForeFront	aminopyralid + 2,4-D
Garlon	triclopyr
Grazon P+D	2,4-D + picloram
Gunslinger	2,4-D + picloram

Brand Names	Active Ingredient(s)
Lontrel	Clopyralid
Marksman	dicamba + atrazine
Milestone	aminopyralid
One-Time	dicamba + MCPP + quinclorac
Outlaw	2,4-D + dicamba
PastureGard	Triclopyr + fluroxypyr
Q4	2,4-D + dicamba + quinclorac + sulfentrazone
Quincept	2,4-D + dicamba + quinclorac
Remedy	triclopyr
Spotlight	fluroxypyr
Status	dicamba + diflufenzopyr
Sterling	dicamba
Stinger	clopyralid
Surge	2,4-D + dicamba + MCPP + sulfentrazone
Surmount	picloram + fluroxypyr
Transline	clopyralid
Turflon Ester	triclopyr
Vanquish	dicamba
Vision	dicamba
Weedmaster	2,4-D + dicamba
Yukon	halosulfuron + dicamba

Table 2. Test species that work best for specific classes of herbicides.

Herbicide Families or Mode of Action	Recommended Test Species	Expected Symptoms
Acetanalides and similar (s-metolachlor, dimethenamid-p)	Oat, ryegrass	Stunting, malformed leaves
Dinitroanilines and similar (trifluralin, prodiamine, pendimethalin, oryzalin, dithiopyr,)	Oat, ryegrass	Stunting, swollen and shortened or "clubbed" roots
Isoxaben	Mustard, Chinese cabbage (not grasses)	Reduced emergence. If plants emerge, roots are swollen and stunted
Indaziflam	Ryegrass, mustard	Reduced emergence, stunted root system, chlorotic foliage and growing points
PPO inhibitors (oxadiazon, oxyfluorfen, flumioxazin, sulfentrazone, others)	ryegrass, tomato, mustard	Stunted shoot growth, roots less affected. Foliage necrotic where contacted by herbicide treated soil
Sulfonylureas and imidazolinones (metsulfuron, sulfosulfuron, imazapyr, imazapic, others)	Tomato, cucumber, spinach	Stunting and general yellowing of the new growth
Triazines (atrazine, simazine, others)	Oats	Stunting, yellow leaves
	Cucumber, tomato	Stunting, interveinal yellowing of new leaves (starting with about the third true leaf)
Synthetic auxins (dicamba, 2,4-D, aminopyralid, clopyralid, picloram, others)	Cucumber, tomato, beans	Malformed, twisted shoot growth (epinasty)