

# Persistent Herbicides in Compost - Bioassay Method

## Goal

- Implement a site-specific compost bioassay testing program to monitor persistent herbicides in compost.
- Control the narrative surrounding your compost product

## Bioassay Objective

- Execute a quick and easy (17 day) bioassay to test for persistent herbicides in finished compost

## Materials

### Herbicide Control

1. Lontrel – Clopyralid herbicide
2. mL dropper
3. 0.5 L container
4. Spray bottle
5. 5 gallon bucket
6. Latex Gloves
7. Safety Glasses
8. Long Sleeves

### Bioassay

1. Mixing Bin
2. Small (4 inch) pots
3. Potting Soil
4. Clover Seeds
5. Tweezers
6. Markers or Labels
7. Growing Space (Greenhouse/grow light)

## Methods

### Treatments

1. Positive Control
2. Negative Control
3. Test Composts (as many as desired)

### Replicates

5 replicates (pots) per treatment

### Duration

17 Days

### Damage Rating / Analysis

Rate damage within each pot on a scale of 0-4.

## Procedure

**Herbicide Control** – Compost that is artificially contaminated with Clopyralid herbicide to assist in correctly identifying and quantifying herbicide damage.

**Clopyralid Stock Solution** – Make and store for trials. Will contaminate 294 CF of compost and be enough for 5500 rounds of testing!

**Background Math:** Lontrel is 31.1% Clopyralid. The stock solution will be 499.69 mL H<sub>2</sub>O, and 0.31 mL Clopyralid, which is 620 ppm OR 620,000 ppb OR 0.0621%.

To make stock solution, mix 1 ml Lontrel in 499 mL H<sub>2</sub>O (1/2 liter).

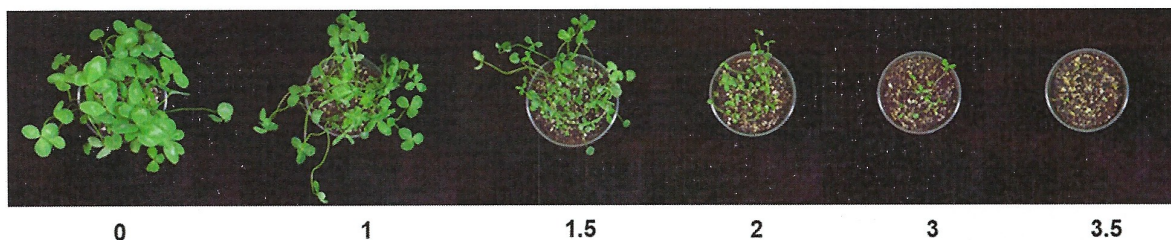
**Clopyralid Spray Bottle** – Make spray bottle to contaminate one 5 gallon bucket (0.67 CF) of compost, which will be enough for 12 rounds of testing.

**Background Math:** To make 5 gallons (0.67 CF) of compost with 80 ppb clopyralid:  
(0.000008 g clop/100g compost) \* (453.59 g compost/ 1 lb soil) \* (30 lb/1 CF) \* (1 ml stock / 0.0621 g clop) = 1.752 ml stock / 1 CF soil or 1.17 mL / 0.67 CF soil

To make 5 gallons of compost contaminated with 80 ppb clopyralid, mix 1.17 mL of stock solution with 100-500 mL H<sub>2</sub>O. Spray entire volume onto 5 gallon bucket of compost, mix well.

*Bioassay Method – To monitor for the presence of persistent herbicides in finished compost*

1. Blend the appropriate volume of each test compost as well as the negative control at a 1:1 ratio with potting mix
  - Some composts have high EC, which can inhibit germination
  - Better approximates real world usage rates
  - Easier to control soil moisture to ensure trial success
  - Mix thoroughly and consistently! We are dealing with ppb, so thorough mixing is important
3. Fill 5 labeled pots per treatment
4. Fill positive control pots with 100% potting mix
5. Seed with 10 clover seeds per pot
  - Use tweezers
  - Place seed just below surface (1-2mm). Don't stress about covering
6. Water pots and put in growing space
7. Water as necessary for 17 days
8. Rate damage per pot, take photos if desired



Injury Rank	Observed Symptoms of Red Clover Seedlings at 17 DAP
None ( 0 )	Cotyledons fully emerged and either flat or very slightly curved downward. First true leaf (single) fully emerged, 8-12 mm diameter, round, and very flat, with 2-4 cm erect petiole. First compound leaf open and flat, or beginning to open.
Slight ( 1 )	Cotyledons and leaves normal size but slightly down-curved. Continued growth normal in size.
Moderate ( 2 )	Cotyledons normal size but curled downward into half circle. Most first leaves strongly curled. Petioles normal size but curved and leaning. Little compound leaf development. Poor continued growth.
Severe ( 3 )	Cotyledons stunted, curled into half or full circle. Leaves few, stunted, tightly curled, not erect. No further growth.
Extreme ( 4 )	Germination not reduced, but very stunted curled cotyledons. Most prostrate. No leaves. No further growth.

Brinton et al., (2006) Compost Science and Utilization, Vol. 14:4, 244-251

**How much damage is too much?**

The answer to this is a judgement call. You need to consider the severity of damage you observe, and whether it is in every pot or just a few. You also need to consider the intended use of the product. Finally, depending on pile size and feedstock variability, you may consider retesting from a different part of the pile to gain more clarity on the scale of the problem.