Implementing A Plant Growth Testing Program

US Composting Council®

www.compostingcouncil.org

A step-by-step guide for designing a simple, inexpensive, repeatable, and defensible plant growth test program to determine if **Persistent Herbicides** occur in your compost products. Please note that references to specific products are intended as visual examples and does not constitute endorsement by the USCC.

Background

Persistent Herbicides are invisible, and therefore, a compost producer's only assessment option is to conduct plant growth testing and/or chemical analysis to determine if your finished compost affects sensitive plants. Several of the Compost Analysis Proficiency (CAP) certified laboratories provide plant growth testing (i.e., Bioassay Testing) and other labs provide chemical testing for Persistent Herbicide active ingredients aminocyclopyrachlor, aminopyralid, clopyralid, and picloram. Certainly such testing is important but it is also important that a compost producer have direct knowledge of how your facility's compost products perform. A plant growth testing program is the best way to achieve that knowledge.

Although one growth test by itself provides limited data, multiple plant growth tests over years will provide important data on suitability of your compost to grow Persistent Herbicide-sensitive plant species. Plant growth testing along with other best management practices (*see Fact Sheet #2, Strategies to Mitigate Persistent Herbicide Contamination at Your Compost Facility*) also provides proof that your facility has made reasonable efforts to assess and manage Persistent Herbicide contamination. If you do find evidence of Persistent Herbicide contamination, it will become important to eliminate or segregate contaminated feed stocks. Consider implementing a plant growth testing program as a supplement to your regular testing. Finally, also consider that plant growth testing is scalable and can be as simple as a single grow bench in your office, or as elaborate as a greenhouse such as the program at Green Mountain Compost operated by the Chittenden Solid Waste District in Williston, Vermont.

Materials List

- ❏ **Plastic 5-gallon buckets,** HDPE (2)
- ❏ **Disposable gloves**
- ❏ **Distilled water**
- ❏ **Electrical Outlet.** Must have at least 2 outlets for this grow test, one for seedling heating mat and one for the timer and light. Use a multi-outlet electrical cord with its own circuit breaker if a wall outlet is not available.
- ❏ **Enclosure.** Your plant growth tests will be more successful if you provide an enclosure to keep the whole trial contained and to maintain temperatures of 65° – 85° F and humidity above 50%. An enclosure for your indoor growth bench combined with lights and seedling heat mat will be enough to maintain those conditions inside the enclosure. There are many types of manufactured enclosures on the market but one can easily build one. The enclosure can be as simple as some heavy mill clear plastic sheeting. Another option is to repurpose a plastic covering from a cheap wardrobe rack that fits over a wire rack (Figure1).

  If you opt to build an enclosure, the plant growth test detailed in these methods can be contained within a wooden framed box of dimensions ~24"(L) x ~12"(W) x ~24"(H) with clear plastic fastened to the sides.
- ❏ **Fava bean seeds** (sometimes referred to as "broad beans"). Many plants are sensitive to Persistent Herbicides but fava bean is a relatively large seed that is easy to handle, and that germinates and grows quickly.
- ❏ **Lighting.** A 24 inch two fluorescent lamp light will be sufficient. Example: Sun Blaze HO 22 http://growershouse.com/sun-blaze-t5-ho-fluorescent-light-fixture-2-ft-2-lamp. You will need a way

Figure 1. Use of a repurposed plastic wardrobe cover as a plant growth test enclosure (photo credit: Whitt Environmental Services)

to easily adjust the lights up and down and light duty chain and S hooks work well for that purpose (Figure 2).

- ❏ **Marking Labels.** These will be used to write the contents of a pot. Push the stake into the soil of the pot. Here is an example of a marking stake from Gempler's horticultural supply company: http://www.gemplers.com/product/G40580/Case-of-1000-4x5-8-Plastic-Plant-Labels.
- ❏ **Monitor for Temperature/Humidity.** It is important to maintain temperatures of 65° – 85° and 50% or greater relative humidity. A temperature and relative humidity monitor is useful to measure conditions inside the enclosure: http://www.amazon.com/AcuRite-00613A1-Indoor-Humidity-Monitor/dp/B0013BKD08.
- ❏ **Potting Containers.** These should have a volume of approximately one quart (~one liter). A round pot, 5" diameter (at top) by 3-3/4" tall holds 27 fl. oz. and such pots are called Azalea Pots by one manufacturer (http://www.beldenplastics.com/images/Belden_Catalog_2013.pdf). Whatever pot you choose, ensure that it will be readily available and that it has drainage holes to limit overwatering. Reused pots should be thoroughly washed between plant growth tests.
- ❏ **Potting Media.** The horticultural industry sometimes refers to this as "soil-less potting mix" that will mostly consist of sphagnum peat moss and no compost. The potting mix should provide synthetic fertilizer to ensure better plant growth. The most important considerations are that the mix should be readily available for future trials and that all pots use the same mix.
- ❏ **Saucers.** Choose a saucer that contains the pot with plenty of extra room on all sides to catch water that passes through the pot. Nearly any garden supply store or big box retailer with a garden supply aisle will have many clear plastic sizes.
- ❏ **Seedling Heat Mat.** All pots should be kept on heat mats until the seeds have germinated. In warmer parts of the U.S. where one can easily maintain temperatures near the upper end of the temperature range, a heating mat may not be necessary. In the 6 container trial detailed in this fact sheet, you will need one 10" × 20" mat (see Figure 3). Hydrofarm manufactures one such mat: https://www.hydrofarm.com/p/MT10006.
- ❏ **Timer.** The lights will be connected to the timer to provide 14 hours of daylight per day. Light is necessary once the first seedling germinates.
- ❏ **Trowels (2).** One for the compost media and one for the soil-less potting media. Stainless steel trowels are ideal.
- ❏ **Watering Can.** Pick a can with a small long spout for accurate watering of the pots.

Figure 2. Use of light-duty chain and S-hooks to adjust the height of the fluorescent lamp (photo credit: Whitt Environmental Services)

A Note On The Science

This experiment will help a compost producer answer the question, "Do Persistent Herbicides contaminate my compost?" The methods require carefully controlled conditions (e.g., lighting, temperature, humidity, nutrients, etc.) to answer the question. The "experimental unit" is the growing media and these methods compare two mixtures. One mixture consists of 50% compost and 50% soil-less (compost free) planting media. The second growing media is 100% soil-less potting media. These methods recommend fava bean as a test subject. Fava bean is in the Leguminaceae (i.e., nitrogen-fixing legume) family of plants and they are susceptible to Persistent Herbicides down to a few parts per billion. There are many other sensitive plants that one could use such as clover, tomato, peas, and other beans but they may not be ideal due to small seed size, longer germination times, and slower growth rates. It is a good idea to utilize other plants in a long term plant growth experiment but these should be additions and not substitutions to your program and every additional plant species requires at least 6 more pots, 3 containing soil-less media and 3 containing the amended compost and soil-less media.

Symptoms of Persistent Herbicide effects include leaf curling, malformed leaves, and lack of a central stem. Subtle symptoms usually result from low concentrations of herbicides or environmental factors but seeing these same traits over and over will build confidence over time. You may observe plant symptoms that indicate a deficiency in the growing media or growing conditions that are not related to Persistent Herbicide contamination. Such symptoms include yellowing leaves, poor germination, and differences in plant height.

Plant growth (i.e., bioassay) test results provide qualitative data but if you have ever seen a report from some CAP labs you will note that they often provide quantitative data or a "scale of phytotoxicity." They develop these data by evaluating plants during many bioassay experiments with known concentrations of Persistent Herbicides. Woods End® Laboratories provides a good explanation on their website (https://woodsend.org/compost/herbicide-bioassay). This plant growth test should supplement bioassay, chemical, and your regular nutrient testing from a laboratory.

Step-By-Step Plant Growth Test Instructions

1. You will need at least one gallon of your cured compost for this plant growth test. Obtain compost using an appropriate sub-sampling procedure in a high-density polyethylene (HDPE) five gallon bucket. First, fill a one-gallon Zip-lock bag, two-thirds full, label the bag with the date and any other information to identify this sample and plant growth test trial and store the sample in a freezer. Plan to keep the sample for 6 – 12 months. You can use this sample to retest or to send it to a lab for bioassay or chemical testing if you observe a problem. It would be a great idea to collect an additional sample at this time and send it to your testing lab as part of your regular nutrient testing

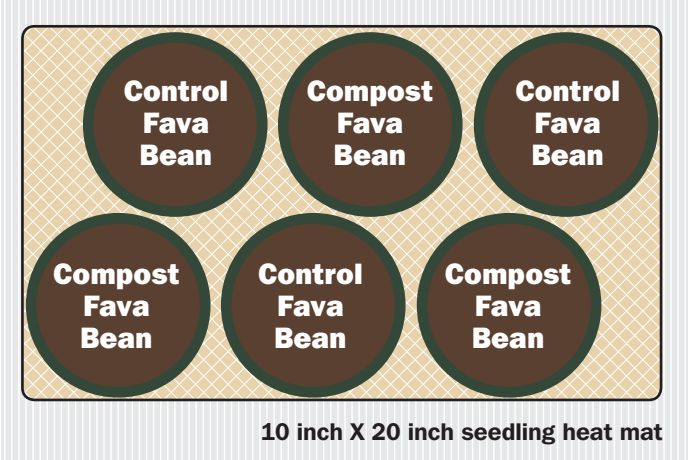


Figure 3. Sample layout for a six pot plant growth test experiment.

2. In a small clean dish, soak at least 24 fava bean seeds in distilled water
3. Wear disposable gloves and mix your compost with the same volume of potting media to obtain a 50% compost: 50% potting soil mixture. Thoroughly blend the mixture using a trowel. The proper moisture will be when all water is absorbed in the media and firmly squeezing a handful produces some drops of water. Do not overwater. It may take some time for the potting mix to absorb water. Add small amounts of water until you achieve the proper moisture. Persistent herbicides are water soluble, and therefore, it is best to maintain the soil on the slightly dry side, fill individual pots (Step 6), and then water the pots rather than allow water to leach through the media and collect in the bottom of the bucket. Leave the second trowel in the bucket. Discard the gloves.
4. Wear a new set of disposable gloves. Fill another five gallon bucket approximately one third full with the soil-less potting media and thoroughly wet the media using distilled water and mix using a trowel. The proper moisture will be when all water is absorbed in the media and firmly squeezing a handful produces some drops of water. Do not overwater. It may take some time for the potting mix to absorb water. Add small amounts of water until you achieve the proper moisture. Although there should not be any persistent herbicides in the soil-less potting mix, it is important that both treatments are treated the same in all respects. Again, it is best to maintain the soil on the slightly dry side, fill individual pots (Step 7), and then water the pots rather than allow water to leach through the media and collect in the bottom of the bucket. Leave the second trowel in the bucket. Discard the gloves.
5. Prepare 6 tags as follows:
   - Compost fava bean 1, 2, and 3 (3 pots)
   - Control fava bean 1, 2, and 3 (3 pots)
6. Wear a new set of disposable gloves. Fill 3 pots with the compost soil mixture. Tap the bottom of each pot on a table to compress the material and place one of the 3 "Compost" tags into each pot. Plant four (4) fava bean seeds in four quadrants in each of the three "Compost Fava Bean" pots slightly deeper than one inch. Cover the seeds and lightly pack the soil with your fingers. Discard the gloves
7. Repeat Step 6 with the soil-less potting media
8. Place a clean saucer under all six pots and arrange the pots with saucers in an order similar to the diagram (Figure 3). It is important to position the pots in a way that spreads them throughout the array similar to the diagram. This helps ensure that other variables such as humidity, temperature, and light are relatively uniform for all pots.
9. Plug in the heating mat. Place the temperature and humidity monitor in the middle front of the experiment on a scrap piece of wood so that it is not sitting on the heating mat
10. Take daily records and water the pots every day such that they are thoroughly moist but do not drain water to the saucers. Be careful not to over-water by dispensing small amounts of water until a minute amount of water exits the bottom of the pot into the saucer. Take care to avoid splashing water from one pot into another pot. **IMPORTANT: Any excess water that drains through the pots and collects in the saucer should be used to water that same pot. Record seedlings as they germinate in daily notes (see sample data sheet).**
11. After the first seed germinates, plug the lights into the timer set for 14 hours of light per day. Unplug the heating mats when no additional seeds germinate in 3 days. Keep in mind that failure to germinate is one possible effect of high concentrations of Persistent Herbicides and that you should keep track of seeds that fail to germinate. Adjust the height of the lights to approximately 12 inches above the height of the plants as they grow until the end of the experiment
12. Allow another 2 – 3 days for the seedlings to show their relative vigor and then remove the two smallest seedlings such that only two seedlings remain in each pot. Note on the data sheet when you culled the seedlings
13. Continue to water pots every day and take daily notes for approximately 30 days. Take pictures throughout the experiment because they will be useful to compare in future trails and because you can send them to experts that have seen thousands of plant experiments. Use a naming convention for

Symptoms of Persistent Herbicide effects include leaf curling, malformed leaves, and lack of a central stem. Subtle symptoms usually result from low concentrations of herbicides or environmental factors but seeing these same traits over and over will build confidence over time.

the picture files that you can easily decipher that includes the treatment (Compost or Control), the pot number (1, 2, or 3), and the date such as: Compost Bean1 = CstBean1_31Jan15.

14. At the end of the experiment, visually compare the Compost Fava Bean pots to the Control Fava Bean pots. Leaf curling, malformed leaves, and lack of a central stem are indicators of herbicide contamination. Take pictures of all pots including close-up pictures of suspected symptoms. Pictures with compost fava bean pots next to control fava bean pots will be especially useful for later comparison. Assess observed damage on some sort of relative scale such as severe damage (see Photos 4 and 5), moderate damage (see Photo 6), slight damage (see Photo 7), and no damage (see Control pot in Photos 4, 5, 6, or 7).
15. It is possible that you may observe nutrient effects that have nothing to do with Persistent Herbicide contamination. Your regular nutrient testing and your testing laboratory can assist you to assess this possibility. Symptoms such as leaf yellowing, marked differences in plant height, and deficient numbers of mature leaves are usually due to other factors such as nutrient deficiency or perhaps high electrical conductivity in the potting media If you sent a sample of your compost to your soil lab at the beginning of the experiment, you will have results and this will allow an assessment of symptoms that may not be related to Persistent Herbicides.

For more information, go to
http://compostingcouncil.org/persistent-herbicides

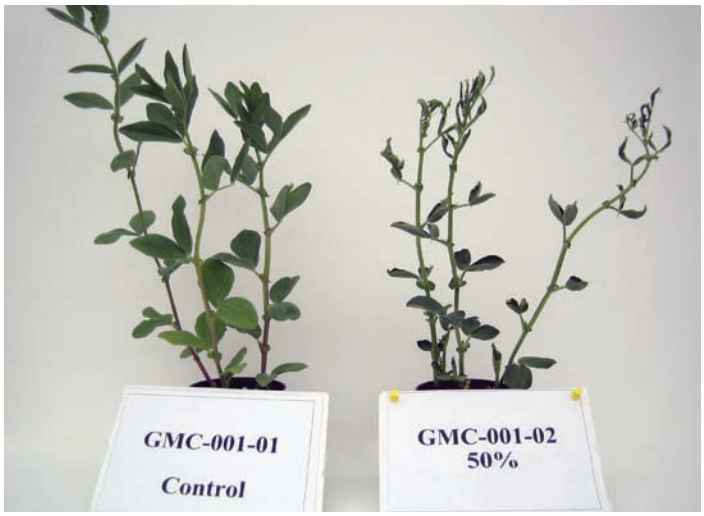


Figure 4. Severe leaf curling and malformed leaves in fava bean plants as a result of Persistent Herbicide contamination. (photo credit: Green Mountain Compost)

Figure 5. Severe leaf curling and malformed leaves in fava bean plants as a result of Persistent Herbicide contamination (photo credit: Green Mountain Compost)

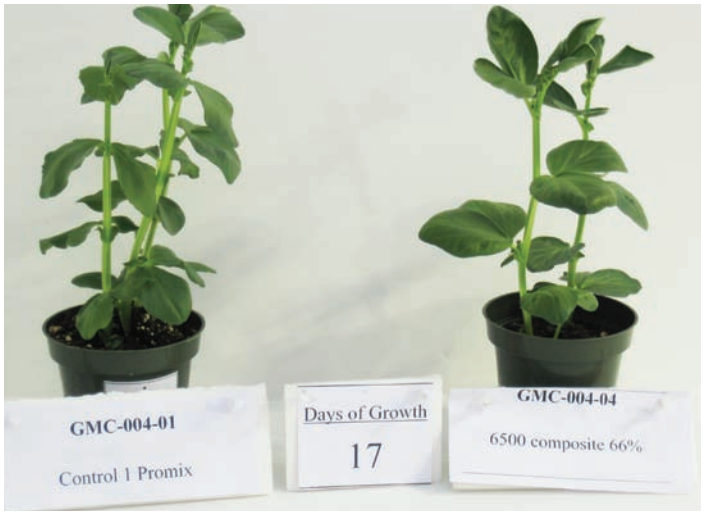


Figure 6. Moderate damage indicated as leaf curling and malformed leaves in fava bean plants as a result of Persistent Herbicide contamination (photo credit: Green Mountain Compost)

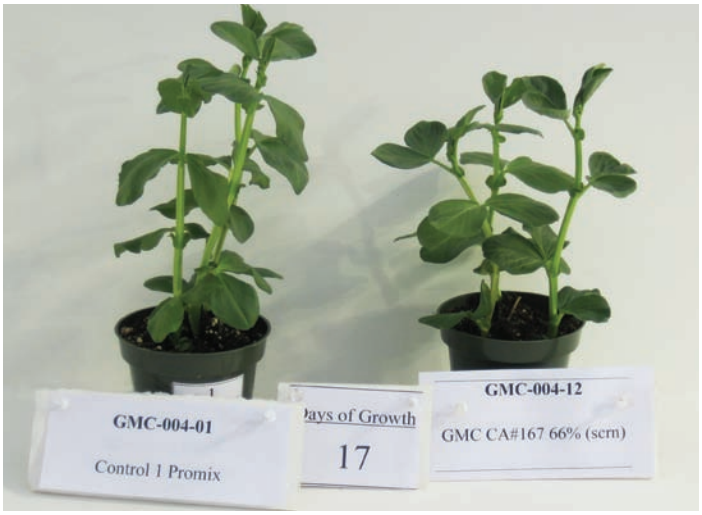
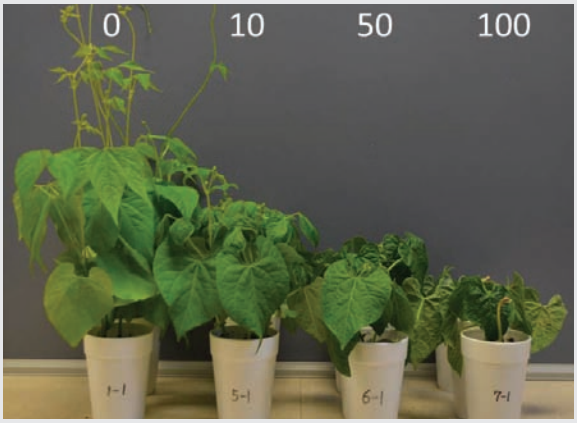
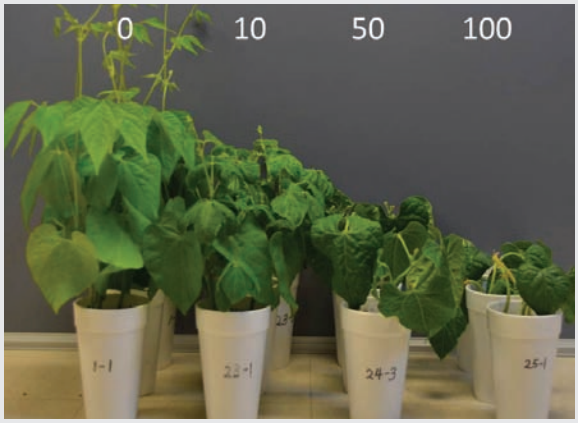


Figure 7. Slight leaf curling and malformed leaves in fava bean plants as a result of Persistent Herbicide contamination (photo credit: Green Mountain Compost)

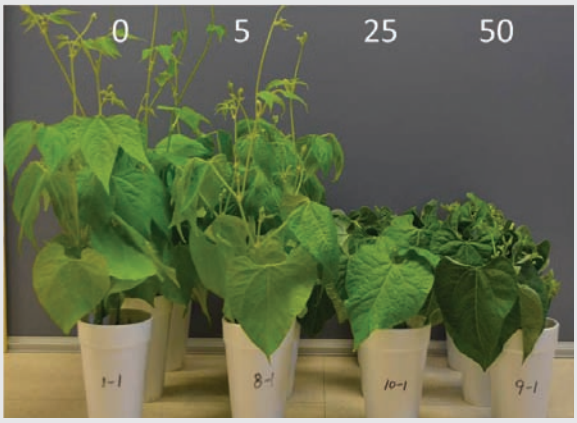
Concentration values at the top of the photos are the overall parts per billion concentration in the media.



Aminopyralid in Yard Trimming Compost

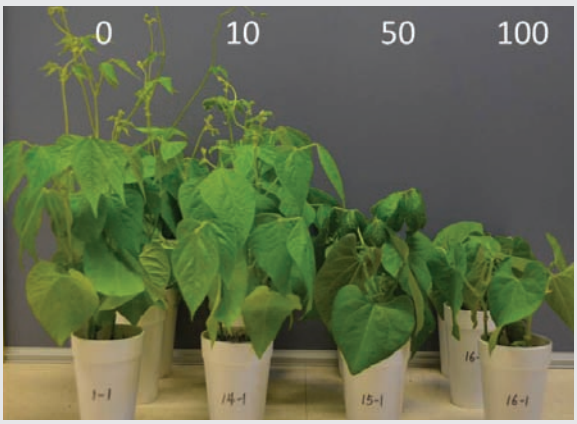


Aminopyralid in Dairy Manure Compost

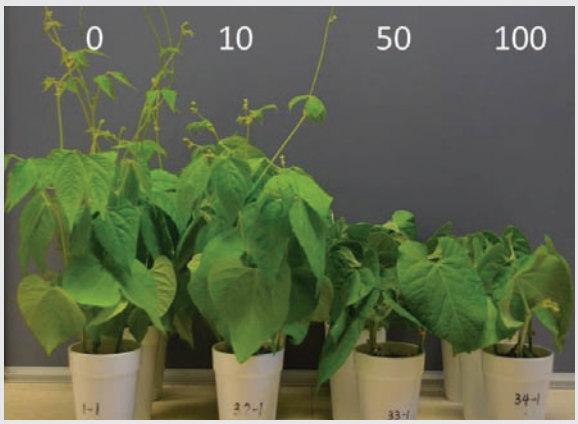


Aminocyclopyrachlor in Yard Trimming Compost

Aminocyclopyrachlor in Dairy Manure Compost



Clopyralid in Yard Trimming Compost



Clopyralid in Dairy Manure Compost

PLANTGROWTH TEST DATA SHEET

Date	Initials	Humidity	Temp	Compost Bean 1	Compost Bean 2	Compost Bean 3	Control Bean 1	Control Bean 2	Control Bean 3

Acknowledgments

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About the USCC: The United States Composting Council (USCC) is a national not-for-profit organization dedicated to the development, expansion and promotion of the composting industry. For more information visit www.compostingcouncil.org

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