WASHINGTON STATE UNIVERSITY

WASHINGTON STATE DEPT. OF ECOLOGY

Bioassay Test for Herbicide Residues in Compost:

Protocol for Gardeners and Researchers in Washington State

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Introduction

Herbicide residues in compost have raised concern in Washington State since plant damage occurred in two separate eastern Washington incidents beginning in 1999. One herbicide ingredient, clopyralid, has since been found in yard debris composts and manure-based products in Washington and other states at levels which could cause damage to susceptible plants.

In response, the Washington State Department of Agriculture (WSDA) adopted new rules prohibiting the use of clopyralid-containing herbicides on lawns and turf (except golf courses) in March 2002. Though the rule is expected to diminish clopyralid contamination in yard debris compost in the near future, tools for evaluating compost for herbicide residues are still needed. The bioassay test is one such tool, and has been used by university researchers, composters, and gardening groups since the clopyralid problem first became apparent. However, since the bioassay activity began, three or more different protocols have been used, making it difficult to compare results between sampling and analysis projects.

In a study released in July 2002[1], King County and Seattle Public Utility recommended that the state departments of Ecology and Agriculture convene a testing protocol forum to address variations in bioassay methodology. The initial forum meeting was held July 31, 2002. Participants agreed on the details in this bioassay protocol as standard operating procedures to evaluate compost for herbicide damage to plants.

Important note: Plant damage during any bioassay test can occur for several different reasons, including high salt concentrations in the growth medium, immature compost, insect pests, diseases and environmental conditions during the test. Plant damage from phenoxy herbicide has distinct characteristics and can be recognized by "a trained eye." The symptoms include leaf cupping and distortion or curling of stems. Poor germination is not a symptom of phenoxy herbicide damage.

Purpose

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

> *Will this test material (compost) cause plant growth symptoms consistent with phenoxy herbicide damage in susceptible plants?*

Using the Bioassay – General Procedures

This bioassay is intended for use by homeowners and gardeners, composters, research institutions, and commercial laboratories. General procedures for the bioassay are the same for all users. However, the protocol has been divided into two "tiers" in order to incorporate additional quality assurance/quality control procedures for situations requiring data comparability and increased scientific rigor.

The first tier, "Protocol for Gardeners," involves growing plants in compost-amended potting mix and comparing them to plants grown in plain potting mix (called a negative control).

The second tier, "Protocol for Researchers" involves the same basic test that compares plants grown in compost-amended potting mix with plants grown in plain potting mix. In addition, the "Protocol for Researchers" includes a "positive control" (plants grown in compost known-to-be contaminated with clopyralid at a specific concentration in the growth medium) It also includes tests for moisture content of the compost and potting mix.

In both protocols, 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. A scoring is assigned to indicate whether or not, or to what degree, plant damage has occurred.

Photographs of plants grown in compost known-to-be contaminated by clopyralid are available in Appendix 1 as an aid to identifying plant damage consistent with phenoxy herbicide damage.

Protocol for Gardeners - Materials

- Test material (compost)
- Potting mix (compost-free; peat-based commercial mix with fertilizer included; brand of mix not specified)
- 4" plastic pots (new, manufacturer not specified; volume = 0.75 liters)
- Plastic saucers
- Garden pea seeds (variety not specified)
- Plastic bags
- Disposable gloves
- Photographs of plants showing herbicide damage (See Appendix 1)

Protocol for Gardeners – Specific Procedures

(1) Evaluate compost (test material)
- Record observations of odor and general condition of compost to be tested.

(2) Set up control pots (negative control)
- Fill **3 pots** with potting mix, tapping several times on the counter top to settle mix uniformly.
- Label pots.
- Plant **3 seeds** in each pot, pushing seeds into potting mix so they are just under the surface.

(3) Prepare compost blend
- Mix 2 parts compost to 1 part plain potting mix in a clean plastic bag. (**The ratio is 2:1 *by volume*, compost to potting mix**.) Use separate, new 4" pots to measure compost and potting mix. Measure 2 pots of compost and 1 pot of potting mix for each compost to be tested.

(4) Set up compost-blend pots
- Fill **3 pot**s with the compost blend, tapping several times on the counter top to settle blend uniformly.
- Label pots.
- Plant **3 seeds** in each pot, using the same procedure as with the control.

Note: Additional composts may be tested simultaneously using one set of controls. Prepare 3 pots (replicates) for each compost tested. Use clean plastic bags to mix each compost tested to avoid cross-contamination.

(5) Grow plants
- Position pots in random order on bench **with plastic saucers underneath each one**.
- Space pots far enough apart to avoid splashing media from one pot to the next during watering.
- **Water each pot carefully**. Keep potting mix 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 fluorescent 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 negative control.
- Assign a scoring label to each material tested. (See "Scoring – Evaluating Bioassay Results.")

Protocol for Researchers - Materials

- Test material (compost)
- Potting mix (compost-free; peat-based commercial mix with fertilizer included; brand of mix not specified)
- 4" plastic pots (new, manufacturer not specified; volume = 0.75 liters)
- Plastic saucers
- Garden pea seeds (variety not specified)
- Plastic bags
- Disposable gloves
- Photographs of plants showing herbicide damage
- Positive control materials (See Appendix 3)

Protocol for Researchers - Specific Procedures

(1) Evaluate compost (test material)
- Record observations of odor and general condition of compost to be tested.
- Sample compost to determine moisture content. Dry samples at 70 $\pm$ 5 C for 48 hours. Record moisture percent. (TMECC 03.09 Total Solids and Moisture[2])

(2) Prepare potting mix for negative control
- Sample potting mix to determine moisture content. Dry samples at 70 $\pm$ 5 C for 48 hours. Record moisture percent. (TMECC 03.09 Total Solids and Moisture[3])

(3) Set up negative control pots
- Fill **3 pot**s with potting mix, tapping several times on the counter top to settle mix uniformly.
- Label pots.
- Plant **3 seeds** in each pot, pushing seeds into potting mix so they are just under the surface.

(4) Prepare compost blend
- Mix 2 parts compost to 1 part plain potting mix in a clean plastic bag. (**The ratio is 2:1 by volume, compost to potting mix.**) Use separate, new 4" pots to measure compost and potting mix. Measure 2 pots of compost and 1 pot of potting mix for each compost to be tested.
- Record total wet weight of compost and record total wet weight of plain potting mix.

(5) Set up compost-blend pots
- Fill **3 pots** with the compost blend, tapping several times on the counter top to settle blend uniformly.
- Label pots.
- Plant **3 seeds** in each pot, using the same procedure as with the control.

Note: Additional composts may be tested simultaneously using one set of controls. Prepare 3 pots (replicates) for each compost tested. Use clean plastic bags to mix each compost tested to avoid contamination.

(6) Prepare compost mix for **positive control** (compost known-to-be contaminated with clopyralid)
- Mix 2 parts contaminated compost with 1 part plain potting mix in a clean plastic bag. Take precautions to avoid cross-contamination.
- Record total wet weight of contaminated compost and record total wet weight of plain potting mix.

(7) Set up positive control pots
- Fill **3 pots** with the positive control compost mix, tapping several times on the counter top to settle material uniformly.
- Label pots.
- Plant **3 seeds** in each pot, using the same procedure as with the other pots.

(8) Grow plants
- Position pots in random order on bench **with plastic saucers underneath each one**.
- Space pots far enough apart to avoid splashing media from one pot to the next during watering.
- **Water each pot carefully**. Keep potting mix 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 fluorescent grow lights as necessary. Temperature should not drop below 50 F at night.

(9) 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 appear**, from **14 to 21 days**, depending on growing conditions.
- Compare plants from compost-blend pots to negative control and positive controls.
- Assign a scoring label to each material tested. (See "Scoring – Evaluating Bioassay Results.")

Protocol for Researchers – Notes on Measuring Moisture Content

Comparing data between composts and between research projects is an important aspect of using a uniform protocol. Knowing the moisture content of the compost and the total weight of compost used in the bioassay allows researchers to calculate the percentage of compost actually used in each replicate. This is especially important when evaluating plant damage from bioassay results together with chemical analysis of the compost.

Scoring – Evaluating Bioassay Results

The scoring scale for this protocol uses descriptive labels ranging from "no symptoms" to "severe damage." When evaluating each replicate, use best judgment to determine the extent of any damage to plants. Refer to photographs of known clopyralid damage in Appendix 1 as a guide.

"No symptoms" –Leaves lie flat before opening. Leaves do not cup or curl upward at all.

"Slight damage" - Leaves of new growth are somewhat cupped. Leaves do not lie flat before opening.

"Moderate damage" – Leaves are obviously cupped.

"Severe damage" – Most leaves are cupped. Stems are twisted.

Appendix 1 – Photographs of herbicide damage in peas grown in compost known-to-be contaminated with clopyralid.

(See Washington State University web site for color photos.)

Appendix 2 – Report Form – Protocol for Gardeners

Appendix 2 will provide forms that can be used to record important information about compost samples being evaluated in conjunction with local gardening programs.

Appendix 3 – Positive Control Materials

Appendix 3 will include an explanation of why the bioassay protocol for researchers includes a positive control. It will also include a description of two materials appropriate for use as a positive control:

1) Contaminated compost that has been verified by laboratory analysis to contain a concentration of clopyralid approximately 50 ppb; or

2) Plain potting media that has been applied with an appropriate amount of clopyralid-containing herbicide to bring the potting media to 25 - 50 ppb of clopyralid.

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[1] Clopyralid Sampling & Testing Report, July 2002. King County Dept Natural Resources and Seattle Public Utilities.

[2] Thompson, W., P. Millner, P. Leege, and M. Watson. 2002. TMECC on CD: Test Methods for the Examination of Composting and Compost (TMECC). 523pp. The US Composting Council. New York. http://tmecc.org/tmecc/index.html

[3] Ibid.