

## Research Progress Report

**TITLE:** Control of *Xanthomonas* in Cabbage Seed

**Request change of title: Characterization of compost tea microbial communities and their effects on the spermosphere and phyllosphere of cabbage.**

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**DATE** (period which report covers): Nov. 2005 – October 2006

**KEYWORDS:** compost tea, bacterial disease, organic seed

### ABSTRACT:

Organic vegetable seed production continues to grow along with the demand for product, adding millions of dollars to Washington's agricultural economy. Production of organic vegetable seeds is hindered by numerous pathogens. Because there is a lack of reliable organic control methods for bacterial black rot (caused by *Xanthomonas campestris* pv. *campestris*) on crucifers, a biologically based solution would be readily adopted. Compost teas produce a large population of microorganisms in a short time with relatively accessible equipment -- microbial communities that are potentially suited to competitively and/or directly inhibit pathogens and stimulate plant growth and defense responses. This work will establish methods of application and describe the microbial profile of compost tea that provides control of *Xanthomonas* bacterial black rot on cabbage seeds at time of planting and in production. The first step in this work has been to establish materials, conditions, and protocols that reliably produce similar microbial profiles, since compost teas are notoriously variable in make-up. Four specialized compost teas with very different microbial communities are being tested.

### OBJECTIVES:

- 1) To assess compost teas of substantially different microbial makeup as a seed soak or soil drench for the control of *Xanthomonas* bacterial black rot of cabbage on seeds under organically managed field and greenhouse conditions.
- 2) To investigate the efficacy of compost tea for the control of *X.* bacterial black rot of cabbage on foliage and developing seeds under organically managed field conditions.
- 3) To determine microbiological characteristics of teas for use on organic seeds and/or plants.

### PROCEDURES:

This research depends on the production of compost teas with consistent chemical and microbiological properties. A primary hypothesis of this research is that the efficacy of disease suppression by compost tea is related to the microbial community cultured and active in each

brew. Four scenarios of pathogen suppression via competition, direct antagonism, stimulation of plant defense systems, and predation are dependent on activities of very different microbial communities. Thus our first priority was to develop and confirm methods for consistent production of compost teas with a predictable microbial profile.

Microbial populations have been assessed by plate counting total bacteria and fungi on selective plates, total activity level with a modified dehydrogenase test, and proportion of living bacterial cells using a fluorescent live/dead cell probe. Teas are made using traditional methods of microbial culture, still utilizing accepted organic materials as microbial starters and substrates, and limiting “over-engineering” that could prevent realistic scale-up to field volumes. We have worked to develop dependable brewing ingredients and schedules that meet the above descriptions in single lab batches. All teas a-d can now be made quite predictably following the recipes below.

#### **PROGRESS TOWARDS OBJECTIVES:**

Objective 1: Teas of substantially different microbial make-up have been developed for testing on infected seed lots.

#### Compost Tea Recipes

**a.** bacterial-dominated compost tea produced from vermicompost. Per 1L total solution:

Subler vermicompost: 50 ml volume

Whey powder: 5 g

Glucose: 2 g

Incubate 72 F with aeration 24 hr.

**b.** fungal-dominated tea. Per 1L total solution:

WSU bedding compost: 50 ml volume

Whey powder: 5 g

Incubate at 72 F for 4 days with aeration only 5 minutes every 24 hr.

**c.** protozoan-dominated tea. Per 1L total solution:

10 grams of chopped straw in 1 liter of deionized water is autoclaved, cooled and strained. Then the following are added:

¼ tsp Subler Vermicompost

10g single piece of organic red potato

Incubate at 72 F. Aerate for one hour. Then aerate 5 mins 2 times per day for 3-8 days.

Protozoans begin to appear in 3 days and reach a peak in population at 8 days. They are pH sensitive. Adding a sugar source will turn the protozoan tea into an acidic fungal tea.

**d.** tea with high bacterial diversity. Per 1L total solution :

Subler vermicompost: 50 ml

1 ml seaweed powder

2 ml liquid humic acids

3 g Azomite rock dust

Incubate at 72 F with aeration 24 hr.

Although the 2005 report stated the development of both teas a and c, subsequent incubations of tea c past day 4, in order to further increase the protozoan population, actually led to population

crashes. Redesign of tea c removed simple sugars and substituted raw potato as a more complex nutrient source for extended incubations and greater protozoan populations.

Fungal tea production has been enhanced by addition of whey powder. This tea is still highly populated with bacteria but also has much greater populations of fungi than any other teas evaluated. The continued prevalence of bacteria even in “fungal teas” has been observed by other researchers (S. Scheurell).

The researchers have obtained 2 lots of highly infected savoy cabbage seeds from our cooperator Bejo Seeds. The above teas are being tested in growth chamber trials along with sterilizing bleach treatment as seed soak and a soil drench to study the effects of tea microbial communities on the spermosphere and endosphere- dwelling bacterial pathogen, *X. campestris*. Reference strains and initial disease confirmation were obtained from Dr. Al Poplowski at University of Idaho, Moscow. Seed soaks are applied overnight (any effective teas also will be assessed for shorter soak times) before planting in potting mix (¼ Ritzville silt loam, ¼ sand, ½ Sunshine #2) in 10cm diameter pots and 24/20 C growth chamber.

Table 1. Preliminary results of compost tea seed soak effect on *Xanthomonas* population on highly infected seed.

<b>Treatment</b>	<b>log <i>Xanthomonas</i> cfu/g (20 seeds)</b>	<b>Seed germination</b>
Overnight water	7.2	100%
10 min 0.5% sodium hypochlorite (bleach)	1.1	95%
Overnight tea A	7.4	95%
Overnight tea B	6.9	100%
Overnight tea C	6.2	100%
Overnight tea D	6.9	100%

A duplicate set of tea-soaked seeds were planted in growth chamber pots and harvested after 21d. Plant material was macerated and diluted for population estimates. Data is not available at this time, but all treatments including bleach treatment have high populations of infection within the cabbage plant. This presents the probability that these very highly infected seeds contain viable propagules both ON and IN the seed, i.e. that even the surficial bleach treatment that dramatically reduces *X* populations from a seed wash, does not necessarily effectively reduce the *X* population IN the seed that can infect the growing seedling. We will continue investigating seed soak treatments, but these preliminary data may mean that any seed soak that doesn't also reduce seed viability may not effectively control *X*. blights on plants, but rather treatments on the infected plants themselves may be necessary. However the seed lot tested was also a very infected lot. Seeds with lower internal pathogen load may be treatable with a seed soak.

Objective 2: Field tests have not started, though they were planned for 2006. State approval of compost teas as an exploratory pesticide is needed, along with more laboratory and greenhouse tests, prior to field trials. The classification of compost tea as a pesticide has changed the way we frame and commonly discuss this work, to a more ecological study of microbial broth and changes to the existing microbial population of seed (spermosphere) and leaf (phyllosphere).

However now that the teas are well defined, simplified, and they can be produced by cooperators as well as undergraduate helpers, applications will be multi-site and multi-species. Existing research plots of snow mold (*Microdochium* and *Typhula*) -infected turf will be treated with teas B and D in summer 2007 in addition to *X.*-infected seed cabbage.

Objective 3: Significant progress has been made toward creating and characterizing defined teas of known microbial make-up. Our lab is running successful pcr with specificity for bacterial or fungal DNA. Several protocols using physical and/or chemical treatments were assessed this year for reliable high-yield DNA extraction from these very different teas. Working with a mixed community it is necessary to use some physical disruption (silica beads primarily for fungal cells) but remain gentle enough to prevent excessive shearing of DNA from more delicate bacterial and lower-fungal cells. The protocol now uses a lysozyme and brief silica bead beating before DNA precipitation. When extractions and pcr were shown to work well, a final run of pcr is made with fluorescent-labelled primer and the pcr products are cut with restriction enzymes. The first set of samples finished with fluorescent pcr and restriction enzyme cutting are currently ready for final cleaning and delivery to the WSU Bioinformatics Core Facility for TRFLP analysis.

**OUTPUTS:**

- Carpenter-Boggs, L. 2005. Diving into Compost Tea. Biocycle 46 (7): 61-62.
- Master Gardeners presentation Sept. 9, 2005, Pullman, WA.
- Washington Grape Society presentation and discussion, November 16, 2006, Grandview, WA
- This work will result in 1-2 peer reviewed articles, 1-2 articles in industry journals, and proceedings from the Soil Ecology Society.

**IMPACT:** Producer impact at this time remains low, although a great number of both growers and researchers are interested not only in the use of compost tea for disease control but the ability to gain better control of compost tea microbial composition, and methods to do this. High potential impact in improving the scientific and practical understanding of compost teas and their use.

**INSTITUTION:** Washington State University, Pullman

**STATE:** Washington

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