Biocontrol Nematode Activity

Activity Overview

Students will collect soil from two areas of their school's athletic field (one that received an application of entomopathogenic nematodes (EPN), and one that did not). Working in groups, they will carry out a simple laboratory procedure to measure the "pest control potential" of EPN in the soil samples. The activity can be adapted in many ways to meet the interests of the teachers and students. For instance, soils can be collected from multiple locations on the school property (e.g. landscaping beds, playgrounds, lawns) to evaluate differences in pest control potential among different environments. The data generated from the activity will be gathered by our lab and used to assess the effectiveness of our nematode applications, and can also be used by the class to meet a variety of science learning standards.

Activity Goal - Evaluate differences in pest control potential on school property between areas where EPN were applied and where they were not.

Materials Checklist

[ ] Dissecting microscope

[ ] Bench/cabinet/closet space for storing Ziploc bags, deli cups and nematode traps (either out of direct sunlight, or in the dark, depending on what needs to be stored)

[ ] Squirt bottles filled with spring water (or other non-chlorinated water) (1 per group)

[ ] Trowels or other digging tools (1 per group, Figure 2A)

[ ]Tray or other work surface (1 per group, Figures 2C-E)

- School lunch tray would be ideal, but can use anything that will contain the soil and allow handling/mixing

[ ] 16 oz plastic deli containers with lids (Figure 3A; 1 per group for every area to be sampled)

[ ] Waxworm larvae (ordered online from Grubco, Figure 3C; 10 per group for every habitat to be sampled)

- avoid unhealthy-looking larvae (inactive when at room temp, major discoloration)
- http://www.grubco.com/store/store_product_detail.cfm?Product_ID=5

[ ] 5 oz Dixie cups or similar (2 per group)

[ ] Plastic spoons for mixing (2 per group if implements are rinsed and re-used, or more if they are not)

[ ] Sharpies for labeling (1 per group)

[ ] 1 gallon Ziploc bags (1 per group for every area to be sampled)

[ ] Nematode traps (1 per sampling area per group) - ***provided by Wickings lab; instructions for making your own also available***

Activity Instructions

In this activity, students will be collecting soil from different areas of the athletic field that have or have not received an application of EPN. Your students will collect soil samples and carry out a laboratory assay to quantify pest control potential and determine if our nematodes have established on your field.

Soil Collection – Day 1 (1-2 hours)

Materials needed:

- Sharpies for labeling (1 per group)

- 1 gallon Ziploc bags (1 per group for every area to sample)

- Trowels or other digging tools (1 per group)

- 5 oz Dixie cups or similar (1 per group)

- Bench/cabinet/closet space for storing Ziploc bags away from the light

1. Before going out to the field, use sharpies to label Ziploc bags with group name, date, and area to be sampled (e.g., EPNs applied or No EPNs applied, **Fig.**

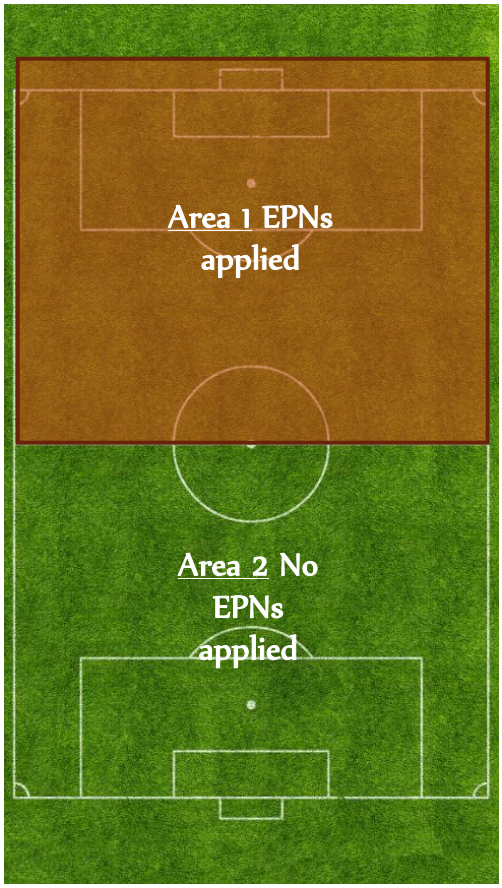

Figure 1. Soccer field sampling areas.

2. Once you are out in the field, use a hand trowel to dig up a trowel-sized amount of soil **(Fig. 2A, B)**.
3. Remove a small handful of soil from the bottom (enough to fill the Dixie cup, **Fig. 2C-E**). NOTE - avoid collecting debris such as rocks, sticks, and dead leaves.
4. Return all remaining soil to the hole, and please replace the live grass at the top of the hole **(Fig. 2F)**. This will help your school grounds manager, because the grass will "repair" naturally and not leave holes in the field.
5. Repeat this process so that each group collects 5 soil samples from within the same sample area (i.e., "Area 1" in **Fig. 1** is one sample area). These samples should be spread out across the entire area being sampled. At a minimum, the groups should each sample the two different sides of the athletic field (the area receiving EPN application and the EPN-free area, **Fig. 1**), but other areas can be added as desired and based on the class's interest!
6. Each group should combine all 5 of its soil samples from one sampling area into a single (pre-labeled) Ziploc bag. Mix the soil by gently massaging the bag and breaking up clumps.
7. Each group should repeat this process for each area sampled (at a minimum, each group will return to the classroom with two bags of soil – one from the EPN application area and one from the EPN-free area of the athletic field).
8. When you return to the classroom, check that all bags are sealed and labeled. Store them away from direct sunlight at room temperature for up to 48 hours before proceeding to the next step of the activity.

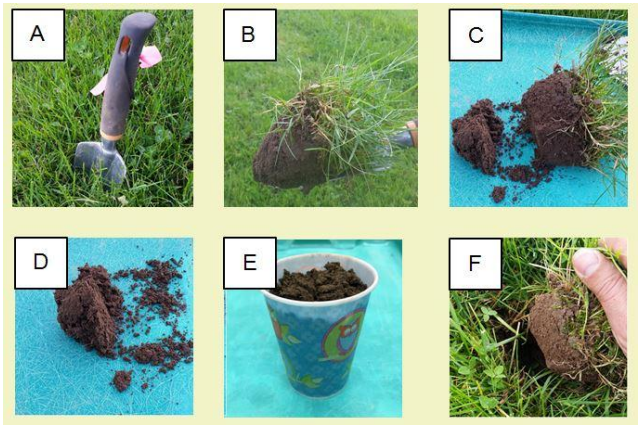

Figure 2. Soil sampling procedure.

<u>Nematode Infection Bioassay: Set-up</u> – Day 2 or 3 (~30 min, depending on how many soil samples each group collected)

Materials needed

- Sharpies for labeling (1 per group)

- 16 oz plastic deli containers with lids (Figure 3A; 1 per group for every habitat to be sampled)

- Plastic spoons for mixing (1 per group)

- Wax moth larvae (10 per group)

- Spring water for moistening soil

- Cabinet/closet space for storing deli cups in the dark

1. Each group should label one plastic deli cup (group name, assay start date, area name) per area they sampled (i.e., one cup for every Ziploc bag of soil they collected).
2. Fill the plastic deli cup half full with soil from the appropriate Ziploc bag. Make sure that the soil is well crumbled and

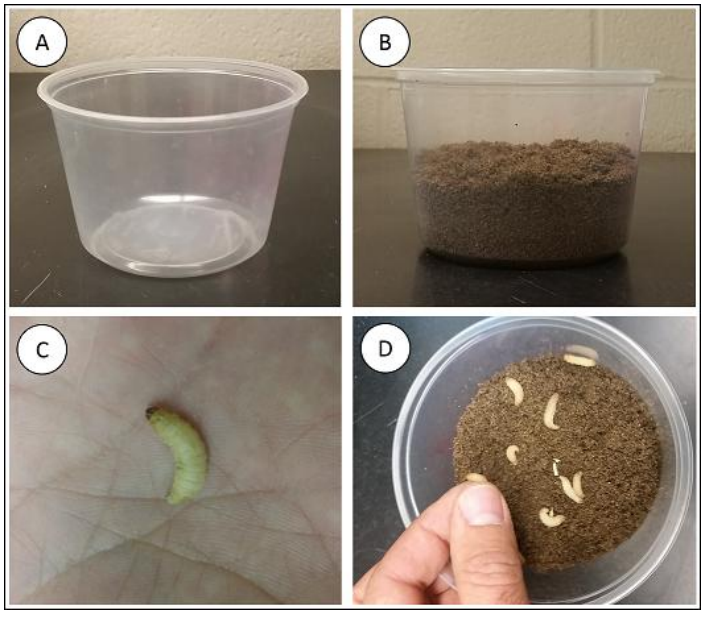

Figure 3. Nematode infection bioassay steps.

not clumped. If the soil is dry add a small amount of spring water (or other non-chlorinated water) to moisten it (but it should not be muddy!!) **(Fig. 3 A-B).**

3. Place 10 live wax worms into each cup. Students may use their hands or (if they prefer) plastic spoons to gently transfer the wax worms to their cups. Close the cup and slowly turn it end over end to bury the wax worms in the soil. Then, crack the lid open just a little to allow air circulation **(Fig. 3 C-D**).
4. Incubate all cups in a dark place at room temperature for 7 days. Turn end over end daily as in step 3.

Nematode Infection Bioassay: Removing dead waxworms – Day 9 or 10 (~30 min, depending on how many soil samples each group collected)

Materials needed

- Squirt bottles filled with spring water (1 per group)

- 5 oz Dixie cups or similar (1 per group)

- Plastic spoons for handling wax worms (2 per group)

- School lunch trays or other work surface (1 per group, Figures 2C-E)

- Sharpies for labeling (1 per group)

- Nematode traps (1 per sampling area per group)

- Datasheet for students to collect data

- Cabinet/closet space for storing nematode traps in the dark

1. Seven days after setting up the bioassay, each group should open their deli cup, find and retrieve all the wax worms, recording the number of dead and live wax worm larvae. Repeat for all deli cups and record data separately for each sample area (see example datasheet).

    Tip: Sometimes, wax worms can die and start to rot quickly, making them hard to find as they blend in with the soil. To make it easier to locate all the wax worms in a cup, try pouring the contents onto a school lunch tray and spreading the soil out.

Example datasheet

Group _____	**Area** _____		**Date** _____
Habitat (sample)	**# alive**	**# dead & plated**	**% EPN infected**
Soccer field inoculated (1)			
Flowerbed (1)			

2. Assemble white traps – each group should place a plaster disk into the center of a petri dish (indentation side facing up (**Fig. 4**). Add just enough spring water to completely saturate the disk and create a shallow layer of water in the dish.
3. Fill a Dixie cup about half full with spring water. Using a plastic spoon, dip the dead wax worms that you suspect of being infected with nematodes in the water individually to remove soil and debris. Note that highly decayed cadavers may be more difficult to clean, so be gentle.
4. Place clean dead wax worms on the prepared nematode trap (**Fig. 4**). All larvae suspected to be EPN-infected should be grouped onto a single trap. Place the trap lid on and label the lid with a sharpie; include group name, date larvae were added to the trap, and sample area. Label around the edges of the petri dish lid (to make it easier to observe the wax worms in the next part of the activity).
5. Place all traps in a dark place (closet, shelf or cabinet) at room temperature.

Figure 4. Nematode trap with infected wax worm.

Nematode Infection Bioassay: Observing traps – every 2-3 days starting on Day 12 or 13, and continuing through the next 2 weeks (~30 min each day, depending on how many soil samples each group collected and how many dissecting microscopes are available)

Materials needed

- Dissecting microscope

- Datasheet for students to collect data (or students can create their own)

1. Each group should observe their traps carefully every 2-3 days using the dissecting microscope to check for the presence of nematodes emerging from their dead wax worms.
2. Once a wax worm larva has confirmed nematode infection and has been recorded, remove the larvae and discard it.
3. Record "Nematode-Induced Mortality". First, tally the total number of wax worms with confirmed nematode infection (i.e., nematodes emerging from them). Then, determine the percentage of all 10 original wax worms placed in the deli cup that were infected by nematodes (i.e., divide by 10). Repeat for all soil samples. Students can then be asked to calculate an average percent of wax worms infected for each area sampled, and create a bar graph by hand or in Excel (See **Fig. 5** for an example).

Example datasheet

Group _____	Area _____		
	Date ___	Date ___	Date ___
Habitat (sample)	**# with EPN**	**# with EPN**	**# with EPN**
Soccer field inoculated (1)	1	5	1
Flowerbed (1)			

Date ____	
total # infected	**% of total infected**
7	70

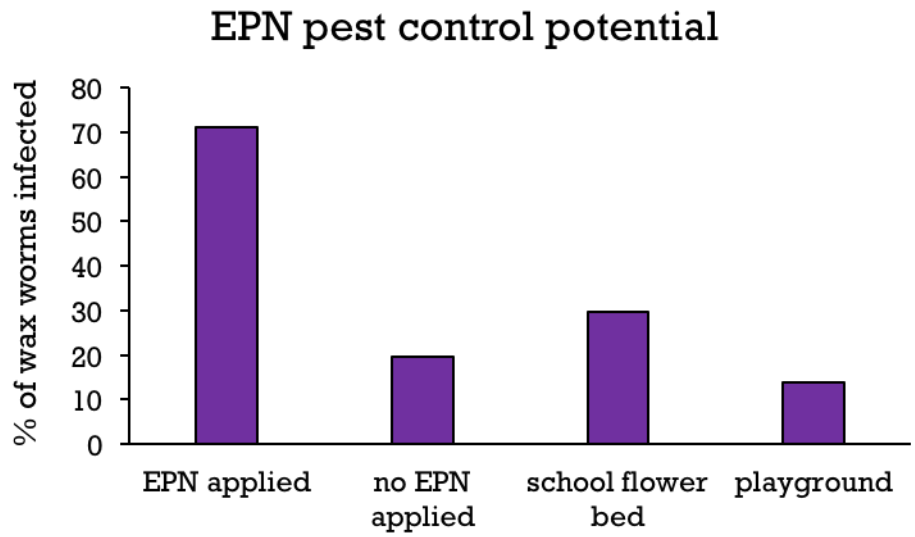

Figure 5. Example bar graph showing the percentage of infected wax worms (EPN pest control potential) from different sampling areas.