Name_____ Date_____ Hour_____

Experimental Design

1. What question will you investigate? Why is this of interest?

2. Which technique(s) will you use to collect data? How many replicates will you have?

3. Where will you do your sampling? (You may want to mark sites on a map.) Why are you choosing those locations?

4. When will you do your sampling? Over what period of time? Why?

5. Do we need to take any precautions when walking out to or working in the sampling area? If so, please describe them and explain their necessity.

6. What is your hypothesis? Explain what you predict will occur and why, using solid scientific reasoning.

7. Describe the types of evidence you will collect and how they will be recorded. Use another piece of paper if necessary.

Name_____ Date_____ Hour_____

Data Analysis and Discussion

1. After you analyze the results of your experiment as indicated by your teacher, summarize the evidence you collected in the space below and describe whether or not your hypothesis was supported.

2. Describe any unexpected events or problems that occurred that may have affected the results of your experiment. Are there any ways to avoid those issues and improve your methods?

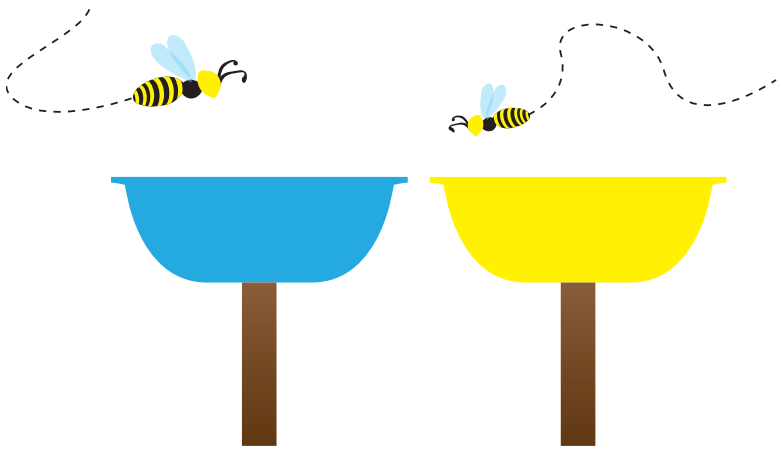
3. Sketch or describe the role invertebrates play in the communities you investigated. Use morphospecies names where possible.

4. Farmers are starting to consider using fields to grow crops for biofuels. How might we use the information from these field investigations to make recommendations about growing crops for biofuels?

Field Instructions: Bee Sampling using Bee Bowls

Objective

To investigate bee abundance and diversity for a given location as a measurement of pollinator activity.



Sample Questions

How does bee abundance and diversity vary across potential bioenergy crops (corn fields, soybean fields, canola fields, managed grassland, restored prairie)?

Do soybean fields located next to a prairie have more pollinator activity than those next to a corn field?

Safety warning: Bee sting hazard. Anyone who is allergic to stings should take appropriate precautions. Students will not handle live animals, but fieldwork exposes them to sting potential.

Materials

- 18 bowls (6 yellow, 6 blue, 6 white): roughly 1.5 inches deep, 4 inches diameter, to hold 5 oz of liquid. Ideally, find white bowls that can be spray painted fluorescent yellow and blue. Disposable or reusable.
- Gallon of water
- 1 tablespoon unscented dish soap
- Mesh strainers
- Collection jars with lids (equal numbers to mesh strainers)
- Rubbing alcohol (70% isopropyl or ethyl alcohol)
- Tape measure (50 m)
- Compass
- Optional: 18 (bamboo) poles cut to height of vegetation
- Optional: Tape or other method to secure bowls to poles
- Optional: Marker flags

General notes on procedure

Colored bowls containing soapy water are placed on the ground or fastened to posts in the field for 2 to 7 days. Passing bees see the bowls as giant flowers and try to visit them for pollen or nectar. When they land on the soapy water, they sink to the bottom. Then you collect, identify, and count them to assess bee abundance and diversity.

The response of bees to flower color varies by species. This procedure gives the bees three colors to choose from, which will increase the diversity of bees in your catch.

Bee bowls should be placed in the field so that passing bees can see them. In open habitats (sparse grassland, open forest), they are often placed on the ground. In closed habitats (dense grassland, cornfield) they can be raised to the top of the vegetation in any way that is practical (bee bowls can be taped to bamboo poles).

Steps

1. Spray paint bowls if necessary.
2. Choose your study location. One bee bowl will be placed every five meters along two, perpendicular 45 meter transects that cross at the center of the habitat patch (fig. 1).
3. Complete site description form for each location.
4. Use the compass and measuring tape to determine where the bowls will be placed in the field.
5. Randomly choose the order to place the three colors bowls and set them out every 5 meters in the transect (yellow-blue-white, or white-yellow-blue alternating, etc)
6. Carefully add 1 tbsp. soap to a gallon of water, gently mix the container, but avoid foam if possible.
7. Add soapy water to bowls until they are 3/4 full.
8. Place bowls (and poles if necessary) in the field at the proper locations.
9. Return at the end of the sampling period and dump the contents of the bee bowl through the strainer to separate bees from soapy water.
10. Place all bees from site in a collection jar in 70% alcohol.
11. Return to classroom to look at bees using a hand lens or microscope.
12. Separate bees to *morphospecies* using whatever physical characteristics you would like. Count the number of morphospecies and the number of individuals in each category. Compare your results to the other plots/areas you chose.

13. Bees can be stored in alcohol indefinitely or can be dried and pinned using standard insect pinning techniques.
14. Create a bar graph of your data.

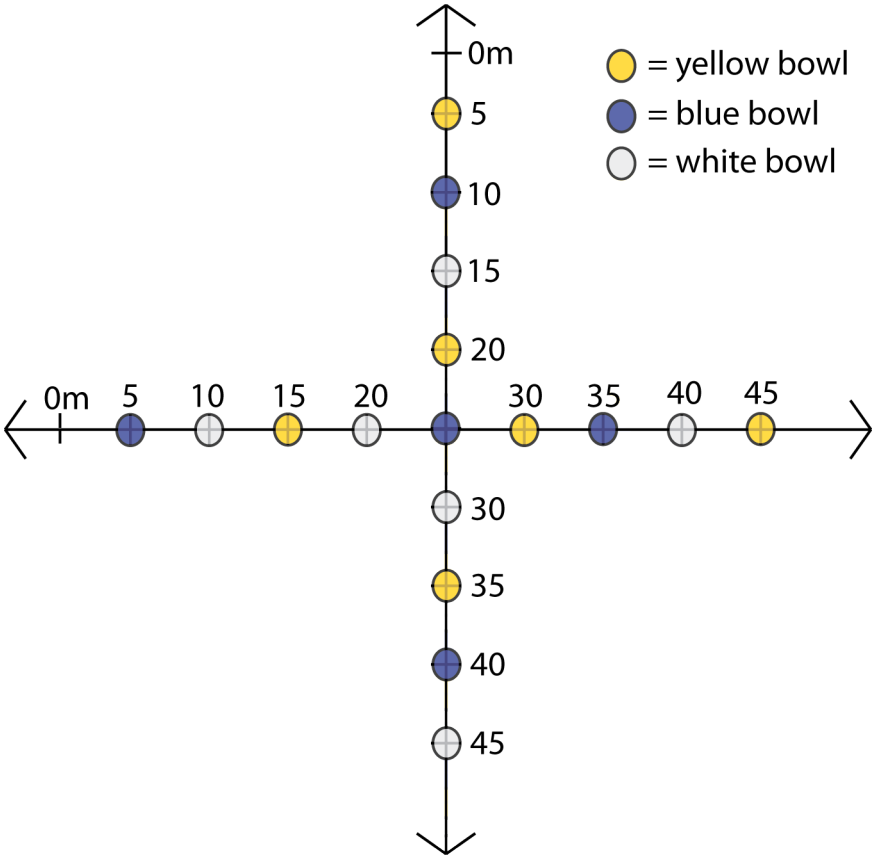
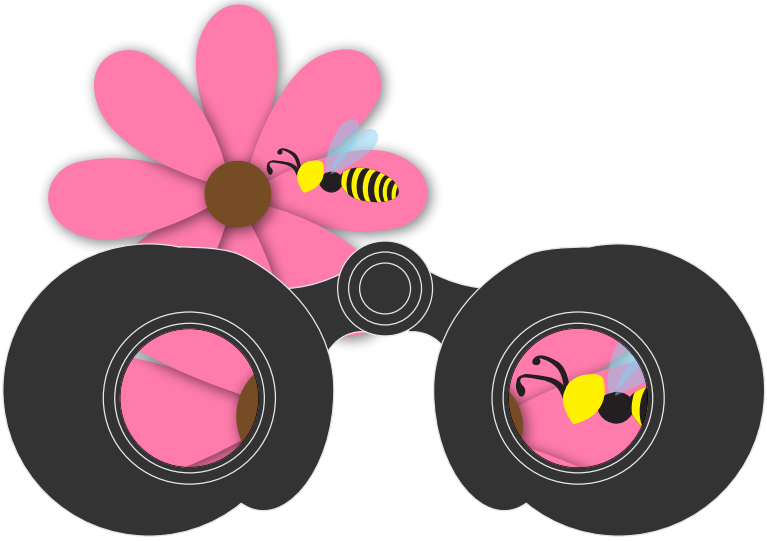


Figure 1. Suggested bee bowl configuration on transects.

Field Instructions: Estimating Pollinator Activity with Flower Observations



Purpose

To obtain an indicator of pollinator activity for a given location

Sample Questions

How does pollinator activity vary across potential bioenergy crops (corn fields, soybean fields, canola fields, managed grassland, restored prairie)? Do soybean fields located next to a prairie have more pollinator visits than those next to a corn field?

Safety warning: Bee sting hazard. Anyone who is allergic to stings should take appropriate precautions. Students will not handle live animals, but fieldwork exposes them to sting potential.

Materials

- Notebooks and/or data table
- Watch
- Optional: digital camera
- Optional: binoculars

General notes on procedure

Possible plants to observe include goldenrod, sunflowers, cone flowers, white and yellow sweet-clover. If you would like to measure flower visitation rates in potted plants, then see the protocol entitled *estimating pollinator activity with potted plants*.

It is best to make observations when it is sunny and warm, because pollinators are most active under these conditions.

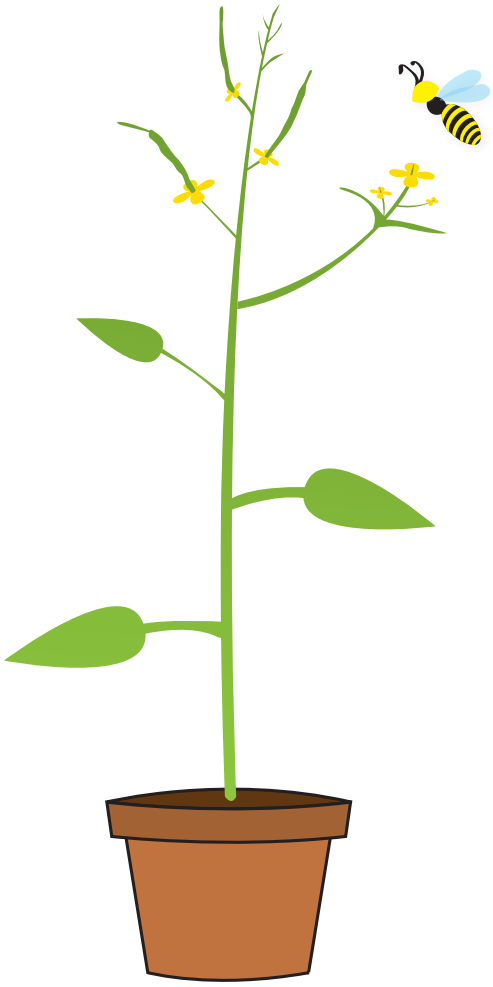
Sometimes, voucher specimens of pollinators are also collected for later identification. To do this, you will need a net, a killing jar, and supplies for preserving insects on mounting pins.

The distribution of flowers in a field has a strong effect on visitation rates. Thus, it is best to make observations in parts of focal habitats that have similar flower densities. Flower densities can be manipulated by clipping flowers in dense areas.

Steps

1. Complete site description form for each location.
2. Chose an individual plant common to all study sites to observe.
3. Sit quietly with a partner about 2 meters from a flower for 10 minutes. Count the number of unique visitors to the flower. If possible, tally the number of visitors by type (bee, fly, butterfly, etc). You should be at least 2 meters away from next nearest group.
4. If possible, have a partner take photos of all pollinators (do not approach the flower to do this!). If you do not have a camera, use the binoculars to get a good look at the pollinator and describe each one in writing or a drawing.
5. Calculate the average number of visitors for each field site and compare.
6. If you can identify morphospecies of pollinator, create a comparison by field as well.

Field Instructions: Estimating Pollinator Activity with Potted Plants



Purpose

To measure seed set as an indicator of pollination success in a given location.

Sample Questions

How does pollinator activity vary across potential bioenergy crops (corn fields, soybean fields, canola fields, managed grassland, restored prairie)?

Do soybean fields located next to a prairie have better seed set than those next to a corn field?

Safety warning: Bee sting hazard. Anyone who is allergic to stings should take appropriate precautions. Students will not handle live animals, but fieldwork exposes them to sting potential.

Materials

- 6 insect pollinated plants per field
- Thread
- Compass
- Measuring tape (30 m)
- Optional: (Bamboo) poles cut to height of vegetation
- Optional: Tape or other method to secure plants to poles
- Optional: Marker flags

General notes on procedure

Mustard, radish or Wisconsin Fast Plants grow fast and are pollinated by a variety of insect species. Sunflower seeds are used on occasion, but take longer to flower. Potting soil should be fertile and hold water so that plant reproduction is less likely to be limited by resource limitation.

Once seedlings have emerged, it is helpful to expose them to wind from a fan, as this causes them to toughen up their stems so that plants don't blow over in the field. Some plants may need to be staked.

Potted plants should be placed in the field so that passing bees can see them. In open habitats (sparse grassland, open forest), they are often placed on the ground. In closed habitats (dense grassland, cornfield) they can be raised to the top of the vegetation in any way that is practical (pots can be taped to poles driven into the ground).
Potted plants can be left in the field for any duration. However, it is common to leave them out for 3 to 10 days. Know your plant's flowering cycle and watch the weather.

After field work
Be careful to collect all materials out of the field when you are done. Collected pots should be carefully transported back to the indoor growing area and cared for at least until fruits ripen.

Steps

1. Grow plants in pots indoors until they flower.
2. Choose your study location. One plant will be placed every ten meters along two, perpendicular 30 meter transects that cross at the center of the habitat patch (fig 1).
3. Complete site description form for each location.
4. Use the compass and measuring tape to determine where the bowls will be placed. in the field.
5. Fill a gallon jug with water, collect your pots, and head for the field.
6. Locate two perpendicular transects.
7. Mark all open flowers by tying a thread at its base.
8. Set out plants (and poles).
9. Water the pots.
10. Return at the end of the sampling period, collect pots, and carefully transport them back to the indoor growing space.
11. Rear plants until fruits mature, you may air or oven dry them if desirable.
12. Plant fruit set can be calculated as the percent of marked open flowers that turned into fruit.
13. Seeds can be counted to calculate seed set, or the number of seeds per fruit. Be careful when opening seed pods – some species shoot their seeds long distances when dried fruits pop open. Calculate fruit set and seed set and compare habitats.

Extension
Plant mature seeds to calculate germination rates and pollinator success.

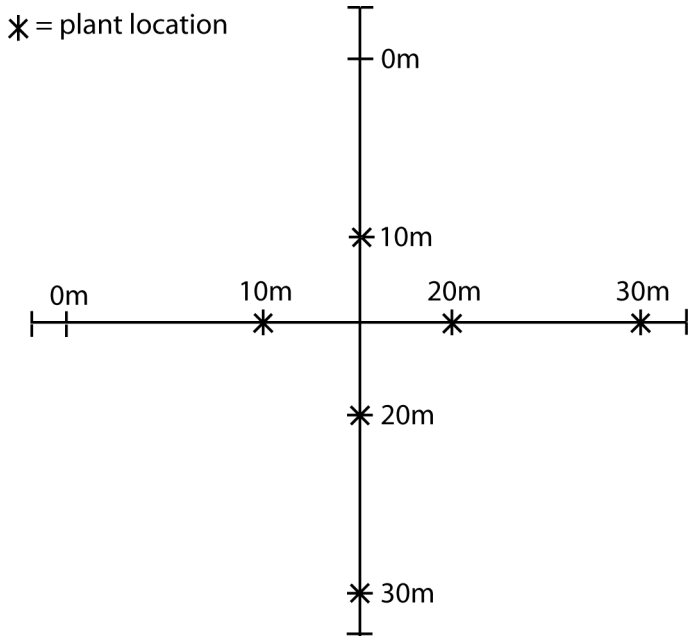
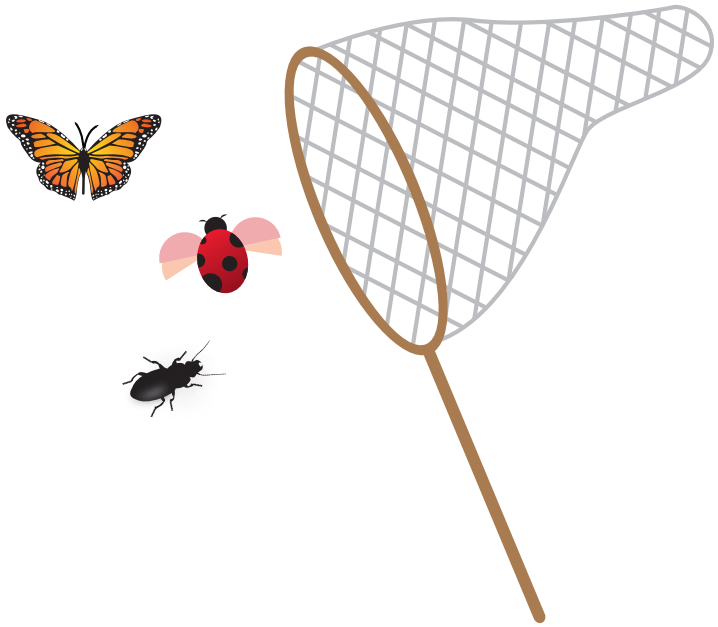


Figure 1. Suggested layout of potted plants on transects.

Field Instructions: Sampling Invertebrates with Sweep Nets

Purpose

To obtain an indicator of invertebrate abundance and diversity at a given site.



Sample Questions

How does invertebrate abundance and diversity vary across potential bioenergy crops (corn fields, soybean fields, canola fields, managed grassland, restored prairie)?
How does harvesting biomass affect invertebrate abundance and diversity?
Which field has a greater abundance and variety of spiders, a corn field or an old field?

Safety warning: Bee sting hazard. Anyone who is allergic to stings should take appropriate precautions.

Materials

- Sweep nets (one per group)
- Gallon size ziplock bag (one per group)
- Cooler of ice
- Optional: Collecting jars (one per group)
- Optional: Killing jars (one per group)
- Watch
- Hand lenses or dissecting scopes
- Freezer

General notes on procedure

Sweep netting should be conducted at a standard time of day and for a constant duration in all habitats of interest. This technique is not effective when vegetation is wet.

Samples should be on ice for at least 2 hours before formal counting if you want to temporarily immobilize your catch. They can be left on ice all day if necessary. One hour in the freezer should kill the invertebrates.

Samples can be stored in a freezer or in 70% alcohol. For long term preservation, samples can be dried and pinned using standard insect mounting practices.

Steps

1. Complete site description form for each location.
2. Begin sampling in the middle of the field (everyone should walk single file into the field along your starting line to avoid trampling vegetation and then space yourselves at 5 m intervals. When you sample, walk perpendicular to the line you walked to get to the middle of the field (fig 1).
3. Sample with a sweep net. Take one 180 degree sweep with each step, first on your right, then on your left. Tilt the net opening so the lower edge of the rim is slightly ahead of the upper rim. Sweep deeply enough to keep the upper edge of the sweep net opening even with the top of the plants.
4. Take 20 sweeps (with 20 steps) in one minute.
5. Shake your catch to the bottom of your net, and stick into a killing jar, or transfer into a Ziploc bag.
6. Place sample in jar or bag on ice.
7. Return to classroom and store sample in jar or in freezer.
8. Separate invertebrates into morphospecies using whatever physical characteristics you would like. Count the number of morphospecies and the number of individuals in each category. Compare your results to the other plots/areas you chose.
9. Graph your results.

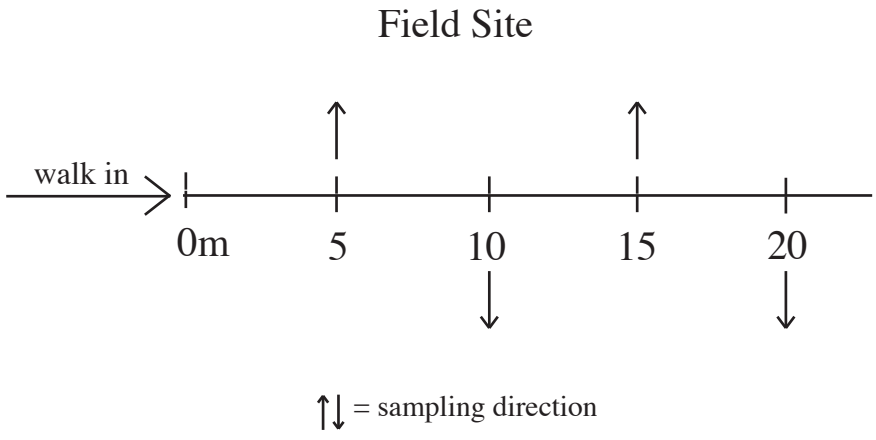
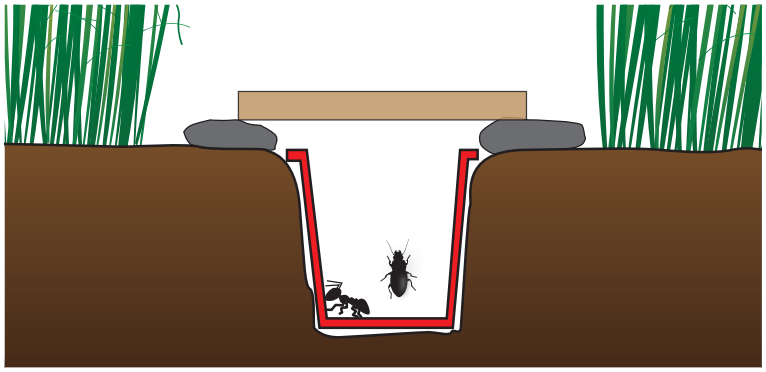


Figure 1. Suggested approach to conducting sweep net sampling with multiple groups. This method minimizes trampling of vegetation.

Field Instructions: Sampling Ground Invertebrates with Pitfall Traps



Purpose

To obtain an indicator of ground invertebrate abundance and diversity at a given site.

Sample Questions

How does ground invertebrate abundance and diversity vary across potential bioenergy crops (corn fields, soybean fields, canola fields, managed grassland, restored prairie)?

How does tilling the soil affect ground invertebrate abundance and diversity?

Safety warning: Bee sting hazard. Anyone who is allergic to stings should take appropriate precautions. Students will not handle live animals, but fieldwork exposes them to sting potential.

Materials

- Spade, post-hole digger, or bulb planter (one per group)
- Multiples of 9 cups for pitfall traps (16 oz disposable cups, yogurt container or steel soup cans with tops cut off)
- One gallon soapy water (1 tbsp unscented dishwashing soap/gallon)
- Board to cover each trap
- Strainer (one per group)
- Collecting jars (one per group)
- Rubbing alcohol 70% (ethanol or isopropyl)
- Tape measure (10 m)
- Hand lens or dissecting scopes
- Multiples of 9 marker flags.

General notes on procedure

Pitfall traps can be left in the field for two days to two weeks. For short sampling periods, soapy water is an adequate capture solution. For longer periods, a preservative, such as 70% alcohol with an evaporation retardant like glycol, should be used for a capture solution so that samples do not rot. Alternatively, cups can be emptied every few days. If cups are to be emptied periodically, then it is helpful to use disposable plastic cups for traps, where a trap consists of a removable cup nested within a cup that remains in the soil when traps are emptied.

A trap is sometimes covered with a cover board – a square piece of wood, plastic, or metal that is propped up with rocks or woodchips over the cup so that they allow arthropods to pass underneath but do not allow rain to fall in.

Specimens can be placed in a jar with 70% alcohol (ethanol or isopropyl rubbing alcohol) for long term storage. For long term preservation, samples can be dried and pinned using standard insect mounting practices.

Steps

1. Complete site description form for each location.
2. Choose your study location(s). Traps will laid out in a 3 cup by 3 cup grid, with five meter spacing (fig 1).
3. Dig holes and place cups in ground, being careful that the lip of the cup is at the soil surface.
4. Fill cup 1/4 full with soapy water.
5. Cover trap with slightly elevated board (fig 2).
6. Mark sites with flags.
7. Return to site after sampling period and run trap contents through a strainer.
8. Place specimens in 70% alcohol for transport and storage.
9. Separate invertebrates into morphospecies using whatever physical characteristics you would like. Count the number of *morphospecies* and the number of individuals in each category. Compare your results to the other plots/areas you chose.
10. Graph your results.

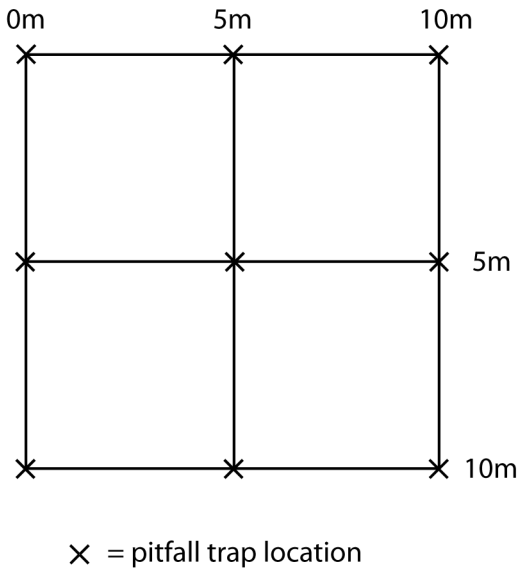
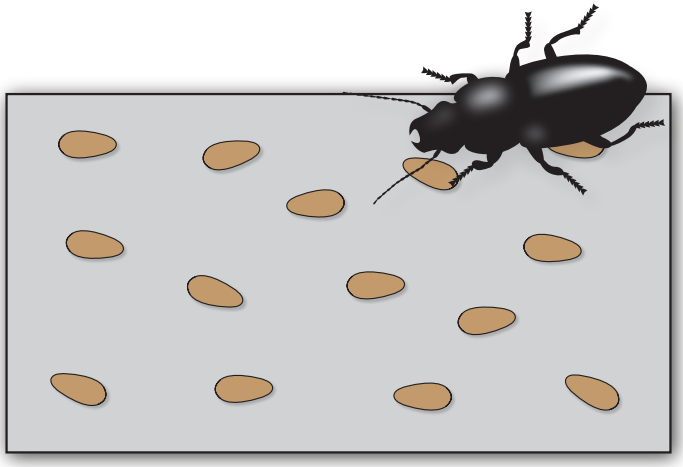


Figure 1. Suggested sampling locations for pitfall traps

Field Instructions: Estimating Weed Seed Predation by Ground Invertebrates



Purpose

To investigate weed seed control by ground invertebrates at a given site.

Example Question

How does weed seed predation by ground arthropods vary across potential bioenergy crops (corn fields, soybean fields, canola fields, managed grassland, restored prairie)?

Safety warning: Bee sting hazard. Anyone who is allergic to stings should take appropriate precautions. Students will not handle live animals, but fieldwork exposes them to sting potential.

Materials

- 8 1/2 x 11 sheets coarse sandpaper (1.5 sheets per site)
- White glue
- 1/2 in wire mesh (18 x 5 inch rectangle for each of 9 cards) other options may work
- Wire snips
- Small plates or scrap wood (multiples of 9)
- Weed seeds (foxtail, ragweed, pigweed or sesame seeds)

General notes on procedure

If you are interested in weed seed predation by ground invertebrates, then you must make small cages to keep birds and mammals away from the cards. A cage is made by taking a piece of 1/2 inch wire mesh, cutting it into a rectangle that is 18 by 5 inches, and curling the mesh into a cylinder. The cylindrical cage is placed around the seed card and pushed into the soil approximately 1 inch. Then a lid (small plastic plate or piece of scrap wood) is placed over the top of the cage and weighted with a rock to keep birds and mammals out. See photograph in figure 2.

Seed cards can be left in the field for 2 to 10 days. Short sampling periods can lead to low seed removal and long sampling periods can led to weather damaged cards and all seeds being removed across all habitats. Ideally, you would like the sampling period to be long enough for 20 to 80% of the seeds to be removed.

Steps

1. Gather weed seeds
2. Cut 9 weed cards per site. Six seed cards can be made by cutting the sandpaper into 4 1/4 by 3 1/2 inch pieces.
3. Cut the cages.
4. Count out 50 seeds per card. Put white glue in the center of the card and press the seeds into the wet glue so that the bottom of the seed is well covered but the top of the seed is clear of glue.
5. When the glue is dry, brush the seeds lightly to force any loose ones off the card. Make a note of how many seeds fall off so you know your starting number.
6. Choose your study location(s) near the center of each field of interest.
7. Complete site description form for each location.
8. Set-up seeds cards in a grid 3 cards x 3 cards, with 5 meter spacing inbeween. (fig 1).
9. Place a protective cage, with plate top, over each card (fig. 2).
10. Return after sampling period and collect cards and cages.
11. Count remaining seeds on cards and calculate seed predations rates. Seed predation rate is the number of seeds eaten, divided by the number of seeds at the beginning, divided by the number of days the card was in the field.

$$\text{seed predation rate} = \frac{\left(\frac{\text{\# of seeds eaten}}{\text{initial \# of seeds}}\right)}{\text{\# of days the card was in the field}}$$

12. Average the seed predation rate at the 9 sites and compare seed predation rates across habitats.

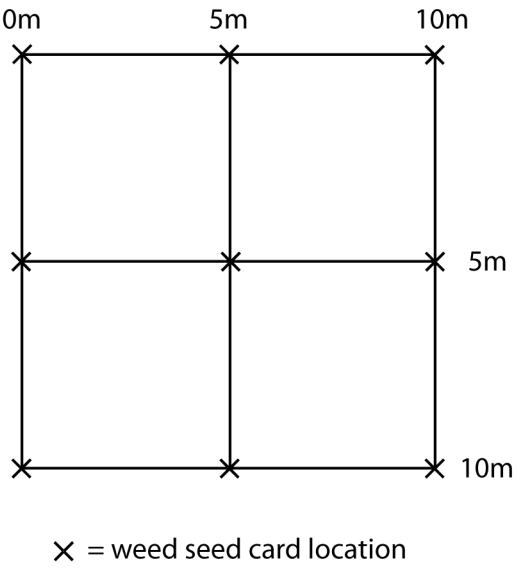


Figure 1. Suggested sampling locations for estimating weed seed predation

Figure 2. Weed seed predation cage set-up

Site Description

Observer's name _____

Site name _____ Date of observation_____

Address or latitude/longitude _____

Time of observation _____

Habitat description:

Type of vegetation _____ # species _____

Annual or perennial or mixed? _____

Vegetation height _____

Size of habitat _____

Soil cover *None* *sparse vegetation* *dense vegetation* *other* _____

Soil moisture *Dry* *Average* *Saturated* *other* _____

Soil temperature _____

Site use history _____

Land management description (burned, tilled, fertilizer use, etc) _____

_____

Adjacent land use (wooded, grassland, agricultural, urban, etc) _____

_____

Weather

Air temperature _____ Daily high/low _____

Cloud cover? *None* *mostly sunny* *mostly cloudy* *complete cover*

Wind speed _____

Precipitation at time of sampling _____

Precipitation in last 24 hours _____

Other relevant observations