

Pepper Field Protocol 2018 - FFAR/Bee Better Survey

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Abstract

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Protocol

General Notes

Step 1.

Gloves: Wear gloves in the lab and in the field when handling nests, tubes, equipment. Sterilize your gloves, then avoid touching your face, clothes, and skin. Re-sterilize your gloves as needed. Wear gloves that fit. Change gloves often. To sterilize gloves, wet gloves with bleach thoroughly so that the entire surface of each glove makes contact with the bleach. Rub hands together and air dry. Wet gloves with ethanol, thoroughly, then allow to air dry.

General Notes

Step 2.

Handling vials: When opening any bag of vials: do not reach hands inside of the bag or ziplock, but rather pour out the vials onto a cleaned, sterilize surface (the inside of a UV hood, a sterilized tray, etc). Anytime you work with vials, do not touch the inside of each vial and make sure you do not contaminate vial insides by breathing into the vial or introducing foreign material. When collecting insects, do not talk while collecting and aim vial away from your mouth.

Pre-Season Preparation

Step 3.

Each person should have the following field supplies prepared: fanny pack for dry ice, 50 screw-top vials, spray bottle filled with ethanol, spray bottle filled with bleach + water (approx. 10% bleach), nitrile gloves (at least 5 pairs), Kestral with working batteries, stopwatch with working batteries, Ziploc bags for specimen storage, thin sharpie, weather data sheets, clean net

Field lead should also have the following supplies: GPS with working batteries, Falcon tubes, transect tape, flagging tape/pin flags, dry shipper or dry ice cooler

Net bags should be soaked in a solution of bleach and water for 5 minutes on any days off to ensure complete sterilization. Make sure to completely rinse net bags afterwards and allow to air-dry overnight. Net handles should also be wiped with the same bleach solution on days off as well.

Equipment

Step 4.

Bins with: pre-sterilized screw-top vials, spray bottle filled with ethanol, spray bottle filled with bleach (10%), nitrile gloves (at least 5 pairs), Ziploc bags with more ziplocs within, Kestral with batteries, Clipboard with weather data sheet, lighter

1 Insulated Fanny pack per person, each with: Forceps, Sharpie, Pencil, stopwatch,

Other equipment: 7 nets (pre-cleaned with bleach and rinsed), stock bottles of bleach (10%) and ethanol (75%), GPS Garmin, Transect tape (x2), flagging tape, duct tape, scissors, trash bag, dry ice in Styrofoam cooler, dry shipper, insect guns (nature bound), printed protocols, site locations, and field work check list, 25 ml falcon tubes

ur emergence boxes (either from the nesting blocks sent from Pollinator Paradise or from another source). Punch 1 hole in the side of the box (going through both bottom and top halves) and 1 hole on the top of the box (will only need to punch through the top). If boxes were obtained from Pollinator Paradise, the side hole might already be punched, but you will still need to add the top hole. Cover both holes with

Site Selection

Step 5.

Establish list of sites working with farm managers

Field Preparation

Step 6.

Net bags should be soaked in a solution of bleach and water for 5 minutes on any days off to ensure complete sterilization. Make sure to completely rinse net bags afterwards and allow to air dry overnight.

Net handles should be wiped with the same bleach and water solution on days off as well.

Transect Selection

Step 7.

Upon arrival to each site, find the 3 transects that mark out each site, or if these transects have not been established yet, establish transects (see protocol above). Make 3, 50m transects. We will sample within 10m from the edge of each transect

Wild Bee Collection

Step 8.

Supplies: gloves, ethanol, bleach, sharpies, dry shipper/dry ice cooler, Ziplocs, screw-top tubes, weather data slips, Kestral, stopwatch, insect net

Wild Bee Collection

Step 9.

Prior to collection, measure the temperature and windspeed using the Kestral as follows:

To turn the Kestral on and off, press and hold the center button.

Temperature: Stand in a sunny spot (not in the shade of a tree). Hold the Kestral away from your body, and shade the white temperature monitor with your hand (this blue and white coil heats up very quickly in direct sunlight and will read an artificially high temperature if it is not shaded). Use the left and right buttons to get to the temperature screen, without a little picture of wind. Make sure that the temperature is being recorded in degrees Celsius (if not, press the center and right buttons together to change units). Wait until the temperature stabilizes and then record that stable temperature. **Collection can only occur between 17C and 24C.** If your Kestral records a temperature higher or lower than this, do not begin sampling.

Wind: Stand facing the wind and hold the Kestral perpendicular to the wind, so that the propellor is spinning. Use the left and right buttons to get to the average windspeed screen (denoted by a picture of wind and the letters AVG). Make sure that the windspeed is being recorded in m/s (if not, press the center and right buttons together to change units). Wait until the windspeed stabilizes and then record that stable average windspeed. **Collection can only occur below 2.5 m/s.** If your Kestral records an average windspeed higher than this, do not begin sampling.

Also record the time at which you collect this information.

Wild Bee Collection

Step 10.

At the beginning of each collection round, please put on new nitrile gloves and sterilize with bleach and ethanol.

Wild Bee Collection

Step 11.

Collect your no-template control: Take one vial, label it with the date, site abbreviation, and the full word "control" on the vial with sharpie. On the vial cap, write the Site Initials and "C" (for control). Hold the vial open to the air for 30 seconds, then close the vial. Place the vial back inside the Ziploc bag.

Wild Bee Collection

Step 12.

Collection will occur in increments of 30 minutes of active sampling, which will be recorded with your stopwatch as follows:

1. Ensure your stopwatch is in countdown mode. If your stopwatch is not in countdown mode, use the blue button in the center (mode) to toggle through modes until you reach countdown.
2. Once in countdown mode, reset the stopwatch to read 30'00'00 (30 minutes and 00 seconds) by using the blue button on the left (split/reset). If hitting reset does not pull up 30'00'00, you can set the amount of time to countdown by pressing both the left (split/reset) and center (mode) at the same time and holding until the stopwatch resets to all 0's. Then, use the left (split/reset) button to toggle between numbers (format is H:MM'SS') and the right (start/stop) button to set the MM numbers to 30. Once set, press the center (mode) button to get out of edit mode.

3. Press the black button on the right (start/stop) to start and stop the countdown. You will start the countdown when you begin sampling and stop it every time you catch an insect with your net. Keep the timer stopped while you put the insect in a vial and sterilize your gloves. Then, press start again when you return to active sampling.

This collection will occur for a total of 3 active sampling hours per transect (total of 9 hours per site). This can be split up between days if necessary.

Wild Bee Collection

Step 13.

Search your transect for wild bees (including honey bees) that are visiting flowers. Please also collect any flies or wasps that you see visiting flowers. Once you have collected an insect, you will stop your times (so that you are only timing your active sampling time, not your processing time).

Wild Bee Collection

Step 14.

Label each vial with the following:

Site code

Transect number

Bee type (if known)

Plant collected from

Date

Collector initials

Example: WS2-HR, Anthophora, Purple Sage, 2/12/18, KCT

Label the top of the vial with the site initials

Wild Bee Collection

Step 15.

Once your 30 minutes of active sampling are completed, record the weather and windspeed with your Kestral.

Wild Bee Collection

Step 16.

When finished at a site, put all of the bees from all collectors into a Ziploc labeled with the site, date, and "wild bee." Put bag in the dry shipper.

Flower Collection

Step 17.

Supplies: gloves, ethanol, bleach, lighters, forceps, 15mL Falcon tubes, Ziplocs, sharpies, dry shipper/dry ice cooler

Flower Collection

Step 18.

Upon arrival to site, put on gloves, sterilize gloves, sterilize forceps

Flower Collection

Step 19.

Collect no template control into a falcon tube. Take one vial, label it with the date, site abbreviation, and full word "control" on the vial with a sharpie. Also put the site abbreviation and "C" on the cap. A separate control must be taken in a falcon tube EVEN if a wild bee or Apis control was taken on the same day in a microcentrifuge tube.

Flower Collection

Step 20.

We will collect three vials, one from each transect. Each vial has 5 flowers. For each vial, randomly select 5 plants in each transect. Collect 1 flowers from each plant and put all of the flowers from into the same vial. Flowers should include all parts of the flower (petals, stamen, stigma, etc.).

Flower Collection

Step 21.

Try not to damage to flower too much when putting it into the tube. If too many petals fall of outside of the tube, remove the flower and collect a new one. It is alright if the petals fall of while inside the tube, as long as all of the petals get included in collection.

Flower Collection

Step 22.

Label each vial with the following:

Site

Transect

Flowers + Round Number

Date

Collector Initials

Example: HC3, Flowers Round 2, 3/1/18, KCT

Flower Collection

Step 23.

Fully sterilize gloves and forceps between trees (bleach and ethanol gloves and forceps, flame sterilize forceps once ethanol has dried).

Flower Collection

Step 24.

Collect all three transects' flower tubes in a single Ziploc bag labeled with the site, date, and Flowers

Round #. Put bag in the dry shipper.

Flower Collection: To create a reference collection of pollen

Step 25.

Walk around the field site and follow the same protocol as above, but note that you may only be able to collect one flower per plant in a microcentrifuge tube
