Almond Field Protocol 2018 - FFAR/Bee Better Survey

Lauren Ponisio

Abstract

Citation: Lauren Ponisio Almond Field Protocol 2018 - FFAR/Bee Better Survey. **protocols.io**
dx.doi.org/10.17504/protocols.io.rwwd7fe
Published: 23 Jul 2018

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.

UV sterilize each nest: Place each nest in UV sterilizer (holes up) and sterilize for 15 minutes with holes facing up into the UV. Then rotate nest and UV sterilize again. Try to UV sterilize nests only a few days or less before field work, rather than doing it weeks in advance, then place them back in the box they come in (essential to keep them away from other dead bee bodies).

Pre-Season Preparation

Step 4.

Prepare nests:

Place one liner into each nesting hole.

Label each nest with an abbreviated site name. Use sharpie to write on the side of the nest and on the roof. Use sharpie to write A-G along the top row of the holes. Write 1-14 down one side of the nest along the column of the holes. Now each hole has a label that can be written on vials as we collect bees, pollen, and larvae collected from that nest. Examples: If bee may came from a site called Airway, it will have AW

written on the side and a tube can be from AW G7.

Pre-Season Preparation

Step 5.

Osmia management:

Gather your 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 two layers of a strong aluminum or steel reinforced tape.

Obtain Osmia from Jim Watts. Put 10 male and 10 female cocoons in individual vials as controls (label with date and "Osmia control" and put a C on the cap). Immediately put these controls into a ziploc bag labeled "Osmia controls" and store in dry shipper or -80 freezer. Sort bees into cardboard 'jewelry boxes' with 110 males and 110 females in each box (male cocoons are smaller than females) If Jim specifies different ratios, follow Jim's instructions. Store Osmia into refrigerator any time they are not being worked on.

Prior to deployment, Osmia must be incubated for 5 days at 75F. DO NOT incubate for less than 5 days, or the Osmia will not emerge properly! Start incubation 5 days prior to planned deployment (if the Osmia get incubated for an extra day or two, they will be fine).

Pre-Season Preparation

Step 6.

Site selection:

Establish list of sites working with farm managers (discuss with Hillary Sardinas and see notes on farmer contacts). A 'Bee Better' site must have some form of native habitat adjacent to the almond orchard. Set up three transects along the edges of the orchard. For a 'Bee Better' site, make sure that all transects are bordered by hedgerow. Space these three transects out as much as possible.

Field Preparation

Step 7.

Each person should have the following field supplies prepared prior to starting a day in the field:

- 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
- Sharpies
- Weather data sheets
- Net (wipe handle with ethanol before each round, or any time that you accidentally touch net with skin)

Field lead should also have the following supplies:

- GPS with working batteries

- Falcon tubes
- Transect tape
- Flagging tape/pin flags
- Dry shipper/dry ice cooler

Field Preparation

Step 8.

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.

Nest Installation

Step 9.

Supplies: rope, wire, wire cutter, zip-ties, scissors, bee nests, pheromone spray, Osmia (inside jewelry boxes, in portable incubator), gloves, ethanol, bleach, duct tape, sharpies, plastic cups, water, trowel, printed protocols, “Osmia installation and removal datasheet”

Nest Installation

Step 10.

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). Find a spot for each nest on one of these three transects. Try to space the nests out as much as possible so that they are away from each other as well as honey bee boxes (the goal is to not have the Osmia from different nests mix). Label each nest with the site initials on at least one side as well as the front. Also label each nest with a chronological number based on the number of nests on the site (ex. WS Nest 1, WS Nest 2, WS Nest 3, WS Nest 4, etc.) Double check that each nest has labels “A-H” on the top (columns) and “1-13” on the sides (rows). (Note: Some nests have 12 rows and some have 13. This is fine.)

Nest Installation

Step 11.

Select your tree. Think about where the bees won’t get dust on them and where they will get either South or East facing exposure (so, if facing south/east means the nest is facing a road, try to place the nest a few rows in so that dust from the road will not get onto the nest). Affix the nest to the tree using a combination of the supplies available to you (rope, wire, zip-ties, scissors). Flag the tree by tying flagging tape to both the trunk and a branch.

Nest Installation

Step 12.

Place 1 emergence box on top of the Binderboard (nestled under the roof), or attach a tube to the nest using duct tape, depending on the nest type. Remove tape that covers the emergence box holes. Each emergence box has the correct ratios of males to females already.

Nest Installation

Step 13.

Spray 5 spritzes of the pheromone spray on the nesting block in an effort to completely cover the nest with pheromones. GPS the location of the nest.

Nest Installation

Step 14.

If the site does not have mud present, make mud for the bees by mixing dirt from the site and water in 2-3 plastic cups per nest. Partially bury these cups in the hole that you dig to collect dirt, and make sure that the mud is very wet (since some of the water will evaporate over time).

Nest Installation

Step 15.

Fill out data collection sheet for the site.

Wild Bee Collection

Step 16.

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 17.

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 18.

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

Wild Bee Collection

Step 19.

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 20.

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. For Bee Better Sites, split time between hedgerow and almond rows by alternating your 30-minute rounds (ex. Round 1 in almond, Round 2 in hedgerow, Round 3 in almond, etc.). Do not do all of the almond collection at one time and then all of the hedgerow collection afterwards.

Wild Bee Collection

Step 21.

Search your transect wild bees (not honey bees and not *Osmia*) 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 22.

Label each vial with the following:

Site code (if caught in the almond row) or Site Code-HR (if caught in the hedgerow)
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, and HR if caught in the hedgerow

Wild Bee Collection

Step 23.
Once your 30 minutes of active sampling are completed, record the weather and windspeed with your Kestral.

Wild Bee Collection

Step 24.
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.

Apis Collection

Step 25.
Supplies: gloves, ethanol, bleach, sharpies, dry shipper/dry ice coolers, Ziplocs screw-cap tubes, printed protocols and datasheet, weather data slips, Kestrel, stopwatch, insect net

Apis Collection

Step 26.
Put on gloves, sterilize gloves and forceps.

Apis Collection

Step 27.
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. (This only needs to happen once per site for bee collection, so if collecting Apis and wild bees on the same day, only one control is necessary).

Apis Collection

Step 28.
Take weather data at the beginning and end of Apis sampling period (only one person needs to do this for the entire sampling period).

Apis Collection

Step 29.

Collect 10 Apis from each transect (total of 30 Apis per site). At Bee Better sites, collect 5 from almond trees and 5 from the hedgerow. From control sites, collect all 10 from almond trees. This collection does not need to be timed or follow any specific protocol – just collect the first 10 Apis that you can.

Apis Collection

Step 30.

Label each vial with the following:

Site (if caught in the almond rows) or Site-HR (if caught in the hedgerow)
Transect
Apis
Plant collected from (for HR)
Date
Collector Initials

Example: WS1, Apis, 2/18/18, KCT (for collection in almond row); CB-HR, Apis, Manzanita, 2/16/18, KCT (for collection in hedgerow)

Label the cap with the site initials and HR if collected in hedgerow

Apis Collection

Step 31.

Collect all three transects' Apis in a single Ziploc bag labeled with the site, date, and "Apis." Put bag in the dry shipper.

Flower Collection

Step 32.

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

Flower Collection

Step 33.

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

Flower Collection

Step 34.

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 35.

Randomly select 5 trees in each transect. Collect 8 flowers from each tree and put all of the flowers from one tree into the same vial (so you should end up with five vials per transect – one per tree, each containing 8 flowers). Flowers should be collected from different parts of the tree, and should include all parts of the flower (petals, stamen, stigma, etc.).

Flower Collection

Step 36.

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 37.

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 38.

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

Flower Collection

Step 39.

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.

Osmia/Nest Collection

Step 40.

Supplies: wire cutter, gloves, ethanol, bleach, lighters, forceps, sharpies, dry shipper/dry ice cooler, ziplocs, screw-cap tubes, Osmia datasheet

Osmia/Nest Collection

Step 41.

Upon arrival to site, put on gloves, sterilize gloves and sterilize foreceps.

Osmia/Nest Collection

Step 42.

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 a “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.

All controls should go into 1 ziplock bag for the day (makes it easier to sort them later in the lab). Put a paper label inside of that ziplock bag that says “Controls.” Keep this bag available through the day and put in the dry shipper at the end of the day.

Osmia/Nest Collection

Step 43.

Wait at the nest and collect bees. The easiest way to do this is to wait until a bee arrives. She will fly into her hole. Place the rim of an empty vial against the hole and she will back up into it. Close top or screw top on. If the bee gets scared and stays in the nest, or if youre losing patience, you can pull the nesting liner out and use foreceps and just nab her. However, this will likely require also extracting the tube contents (pollen, larvae, mud) in the field, so please have a work surface like the lid of your box.

Between collecting bees, sterilize hands and foreceps. Try not to touch your face or skin.

Osmia/Nest Collection

Step 44.

Label each vial with the following:

Site

Nest Number

Labeling code for that hole

Date

Collector initials

Example: WS Nest 1, H8, 3/1/18, KCT

Osmia/Nest Collection

Step 45.

After you have collected all of the bees (usually takes 1.5 hours), place all bee vials from one nest into a ziploc bag, labeled with site, date, "Osmia," and nest number. Use separate ziplocs for each nest at a site. When all nests have been collected, place ziplocs inside dry shipper. If you have bees waiting inside their vials and they are freaking out and you feel bad but don't want to put in your ziplock into the shipper until the end of the site visit, you can place the vials on dry ice and they will die immediately and more humanely.

Osmia/Nest Collection

Step 46.

Untie the nest. Carry the nest in an upright gentle fashion and place inside of the car. You will need to

collect larvae, pollen, and mud from each nesting hole later. At this stage, the larvae are very sensitive and the pollen can squish them, so do not tip nests over, keep faces slightly tipped upwards as you carry nests. Nests can sit on their backs (with the tubes facing upward) if necessary while transporting them to the lab

Osmia/Nest Collection

Step 47.

Between field sites, throw away your gloves and use a new pair. Resterilize gloves and equipment at the new site.

Processing Nests

Step 48.

Supplies: gloves, ethanol, bleach, lighters, forceps, sharpies, dry shipper/dry ice cooler (or lab -80 if done in Kearney), ziplocs, screw-cap vials, paper towels, trash bags, datasheet

Processing Nests

Step 49.

Upon arrival to lab, sterilize work surface with bleach and ethanol. Put on gloves and sterilize gloves and forceps with bleach and ethanol. Flame sterilize forceps. You will re-sterilize forceps (bleach, ethanol, flame sterilization) between touching each component of the nests (so after touching each pollen provision, larvae, and mud wall), and re-sterilize gloves after each tube. Replace gloves between each site.

Processing Nests

Step 50.

Select a nest and loosen the binding as necessary (using a screwdriver to remove the roofs if necessary, and cutting zip ties as necessary).

Processing Nests

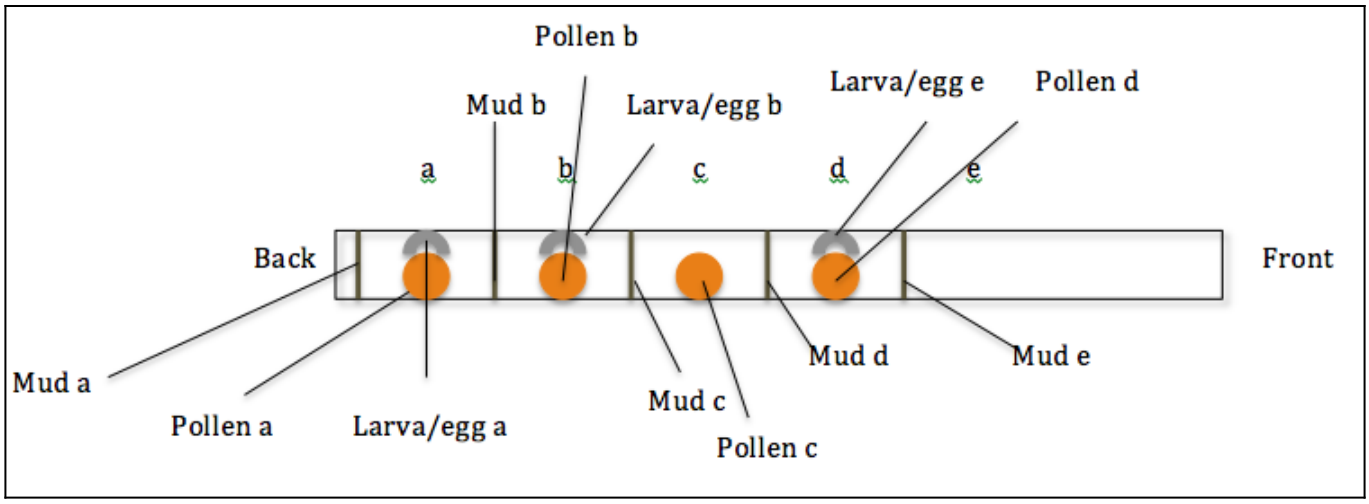
Step 51.

Look at your Osmia installation/removal datasheet (under “bees collected”) to see which holes should have larvae and pollen. For each bee collected, carefully pull out liner inside the nesting block using forceps. Carefully slice or unwind the tube to expose nesting chambers.

Processing Nests

Step 52.

Label the chambers from back to front, where the furthest back (oldest) chamber is labeled with a lower-case a, and then subsequent chambers moving forward in the nest are labeled with subsequent letters. Associate the mud wall at the back of the chamber with that letter. See example picture:



Processing Nests

Step 53.

Note: Each cell gets its own letter. If a cell is missing a component (for instance, there is no larva in cell c above), do not reassign the letter to the next cell (for instance, the larva letters skip “larva c” and go straight to “larva d” in cell d). The mud in the back is mud a. If there is no mud in the back, just skip mud a, do not reassign it to the mud at the front of the cell. If the tube ends in mud (like in the example), assign the mud to the next letter, even if there is no pollen or larva to go with that cell (following the example of the picture above, where cell e gets started but only has a mud wall).

Processing Nests

Step 54.

Note: Be very careful to label from back to front, and not from front to back. Even if you start unwinding the tube from the front, you need the furthest back (oldest) cell to be cell a.

Processing Nests

Step 55.

Using your sterile forceps, separate each component of each cell into a separate vial. Sterilize forceps between components of the cell (so between mud, larvae, and pollen). Each vial will only have one thing in it (so for instance, one vial will have the mud wall from cell a, one vial will have the pollen ball from cell a, and one vial will have the egg/larva from cell a). In the example above, you would need 12 vials (mud a, pollen a, larva a, mud b, pollen b, larva b, mud c, pollen c, mud d, pollen d, larva d, mud e).

Processing Nests

Step 56.

Label each vial with the following:

Site

Nest Number

Tube Code

Cell Letter

Component
Collector initials
Date processed

Example: RB Nest 1, G7a pollen, KCT, 2/28/18. Note that you should label with the date processed, not the date the nest was picked up.

Processing Nests

Step 57.

Note: the larvae are extremely fragile and often pop when you touch them, so use extreme caution when handling with forceps. Always use flat forceps, not pointed forceps, and be very gentle.

Processing Nests

Step 58.

Once all of the tubes for bees collected have been collected, check each of the other tubes for contents. Gently pull out each tube starting from the top left (A1) and look into it to check for contents. There will often be some tubes filled even if you did not collect a bee from that tube. Be very careful to keep track of what hole you are pulling a tube out of while checking for contents. Dispose of any empty tubes.

Processing Nests

Step 59.

Pulling these nests apart is going to make a mess. Between each liner, keep your tabletop clean of debris. Between nesting blocks, sterilize your tabletop surface with bleach and then ethanol.

Processing Nests

Step 60.

After each nest is fully processed, put all of the vials from that nest into a Ziploc bag that is labeled with site, nest number, date, and “Nest Contents.” Place this bag into the dry shipper.