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Version 1

Enhancements Cascades '24: Veg and Pollinator Survey V.1

Forked from a private protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for surveying florally enhanced and control stands within the timberland forests burned by the 2020 wildfire.

Questions

- 1
 1. Can native, flowering plants succeed (germinate and flower within 1-3 years after seeding) in burned slash piles with minimal site prep?
 2. Does plant composition affect pollinator visitation and plant coexistence? (i.e., restoration mixes designed to promote adaptive foraging result in plant communities that support greater pollinator diversity long term)
 3. Is there an effect of different site characteristics (slope, elevation, aspect) on seed mix success or pollinator response?
 4. Is there evidence of the dispersal of seeds in subsequent years?

Study area

- 2
 - Private harvested forest along the McKenzie Rv. burned by the Holiday farm fire in 2020 and subsequently salvage-logged.

Experimental Design

3 *Area of Study*

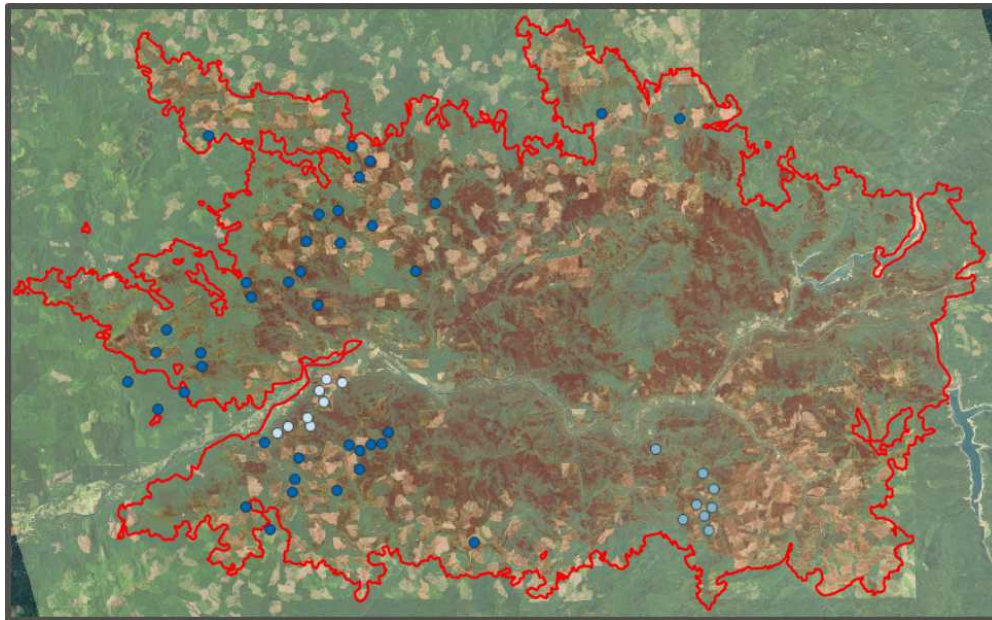


Figure 1: Map of Holiday Farm fire with all stands (NCASI heritage, enhanced, and control stands), we will be surveying a subset and not revisiting all NCASI heritage stands

We have three different Landowners for this study. Each vary by land management intensity, size, as well as landscape characteristics. Therefore when comparing the

efficacy of the enhancements across this landscape we need to consider a number of environmental and management practices. The section on safety practices is important

in maintaining our teams safety during survey, but also to maintain the trust/ability to continue partnering with these landowners.

4 **Experimental Timeline**

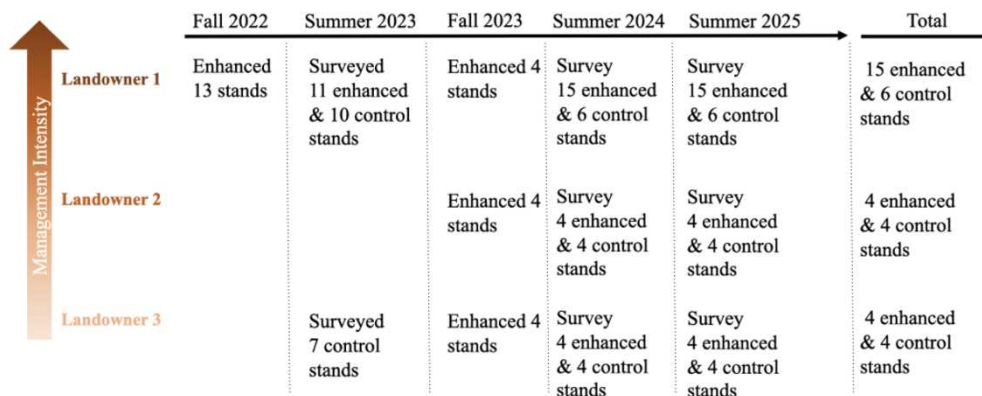
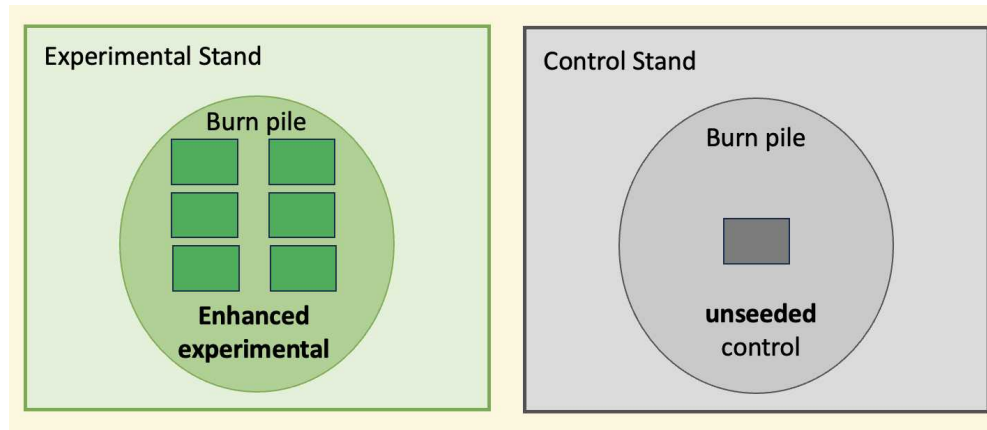


Figure 2: M-BACI Design, timeline of experimental set up and surveys

We employ a multiple before-after-control-impact (M-BACI) study design, as outlined in Figure 2. We seeded in Fall of 2022 and Fall of 2023 following the same protocol. We seeded six different mixes of native forb species in 2 m x 2 m plots within the burn piles of a stand. We define a stand as a homogenous area of forest or land that was previously forested defined on current structure (e.g., uniform salvage logging) or previous tree age or size. We selected study sites with stands >500 m apart, which We will assume stands were independent acknowledging some larger bee species can travel beyond that distance (Kendall et al. 2022). We selected control stands (those stands that received uniform management treatment and paired elevation) which were unenhanced

5 **Experimental Set Up: Burn Piles**



•**Within Enhanced stands,** we seeded six different mixes of native forb species in 2m x 2m plots within the

Figure 3: Burn pile experimental set ups. Six seeded plots in an enhanced stand, one unseeded plot in the control stands.

burn piles

•**Within Control,** we set up a 2m x 2m plot for monitoring

6 **Experimental Set Up: Seed Mixes**

The plant species are separated into six different species mixes, with differing degrees of specialist vs. generalist attractant plant species.

Contents of mixes (visualized in Figure 5 below):

- Mix 1 (all generalist): *Clarkia amoena* var. *lindleyi*, *Clarkia purpurea* ssp. *Quadrivulnera*, *Gilia capitata*, *Achillea millefolium*, *Eriophyllum lanatum*, *Phacelia heterophylla*, *Potentilla glandulosa*, *Potentilla gracilis* var. *gracilis*
- Mix 2 (all specialists): *Collinsia grandiflora*, *Collomia grandiflora*, *Madiagracilis*, *Microsteris gracilis*, *Geum macrophyllum*, *Grindelia integrifolia*, *Lupinus latifolius*, *Prunella vulgaris* var. *lanceolata*
- Mix 3 (generalist and specialist): *Clarkia amoena* var. *lindleyi*, *Gilia capitata*, *Achillea millefolium*, *Eriophyllum lanatum*, *Collinsia grandiflora*, *Collomia grandiflora*, *Grindelia integrifolia*, *Lupinus latifolius*
- Mix 4 (generalist and specialist): *Clarkia amoena* var. *lindleyi*, *Clarkia purpurea* ssp. *Quadrivulnera*, *Achillea millefolium*, *Eriophyllum lanatum*, *Madiagracilis*, *Microsteris gracilis*, *Geum macrophyllum*, *Prunella vulgaris* var. *lanceolata*
- Mix 5 (generalist and specialist): *Clarkia purpurea* ssp. *Quadrivulnera*, *Phacelia heterophylla*, *Potentilla glandulosa*, *Potentilla gracilis* var. *gracilis*, *Collinsia grandiflora*, *Collomia grandiflora*, *Grindelia integrifolia*, *Lupinus latifolius*



- Mix 6 (generalist and specialist)
: Gilia capitata, Phacelia heterophylla, Potentilla glandulosa, Potentilla gracilis var. gracilis, Madia gracilis, Microsteris gracilis, Geum macrophyllum, Prunella vulgaris var. lanceolata

Figure 4: 16 species that are included in all of the burn piles, each mix contains a subset of 8 species

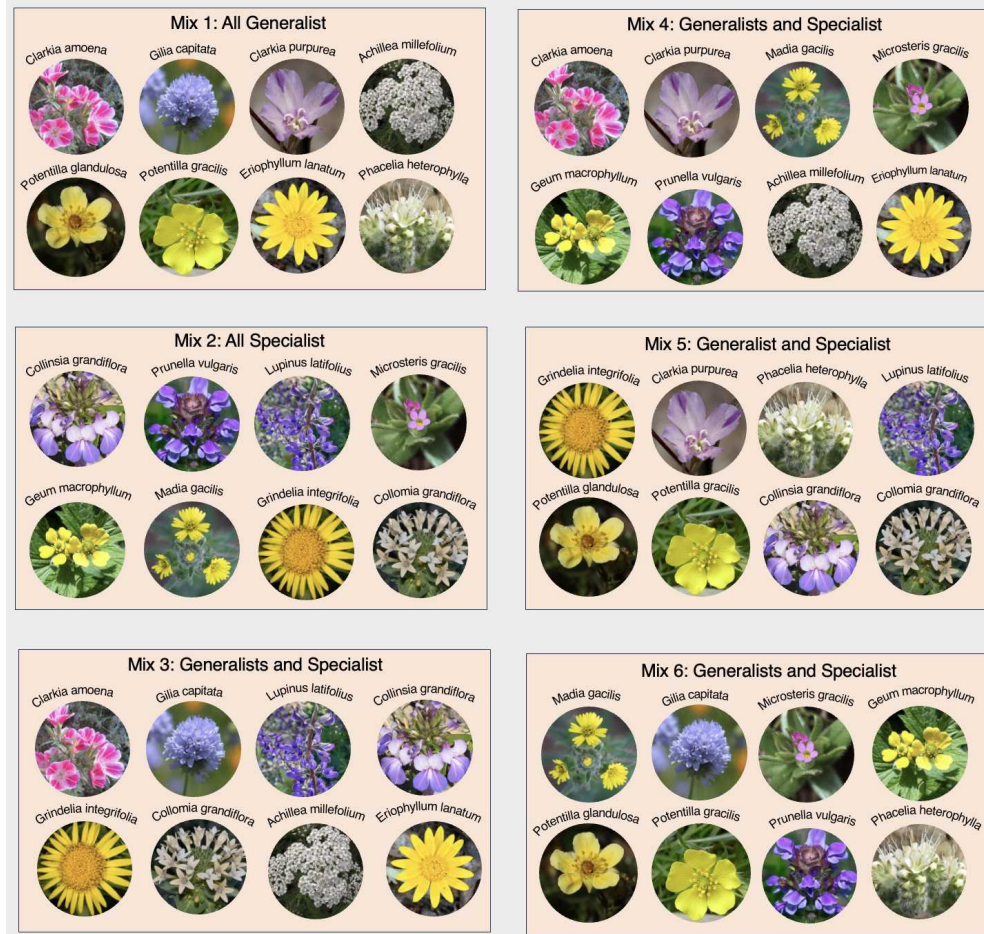


Figure 5: Each seed mix is made up of differing levels of specialization

6.1 Experimental Set Up: Single Species Plots

Clarkia amoena var. lindleyi	CLAAMO	Green, white
Clarkia purpurea ssp. quadrivulnera	CLAPUR	Green, red
Gilia capitata	GILCAP	Green, black
Achillea millefolium	ACHMIL	Green, pink
Eriophyllum lanatum	ERILAN	Green, grey
Phacelia heterophylla	PHAHET	White, red
Potentilla glandulosa	POTGLA	White, black
Potentilla gracilis var. gracilis	POTGRA	White, pink
Collinsia grandiflora	COLLIGRA	White, grey
Collomia grandiflora	COLLOGRA	Red, black
Madia gracilis	MADGRA	Red, pink
Microsteris gracilis	MICGRA	Red, grey
Geum macrophyllum	GEUMAC	Black, pink
Lupinus latifolius	LUPLAT	Black, grey
Prunella vulgaris var. lanceolata	PRUVUL	Pink grey
Grindelia integrifolia	GRIINT	Green, white, red

Table 1: Single species plot codes and colors

7 **Experimental Set Up: Stand Level Surveys Site Set Ups**

To understand the effect of enhancements on plant-pollinator communities, we survey at 2 spatial scales: **the stand** and **enhancement plot**. For stand-level both in enhanced and control stands, we establish three survey areas within the stand (each featuring two 32 m long transects) and a single 32 m long road transect (See Figure 6). We survey the six

2 m x 2 m enhancement plots in enhancement stands and one 2 m x 2 m plot in the seeded burn pile in control stands (See Figure 3).

Surveys include a **pollinator net walk**, **vegetation census**, and **passive sampling**.

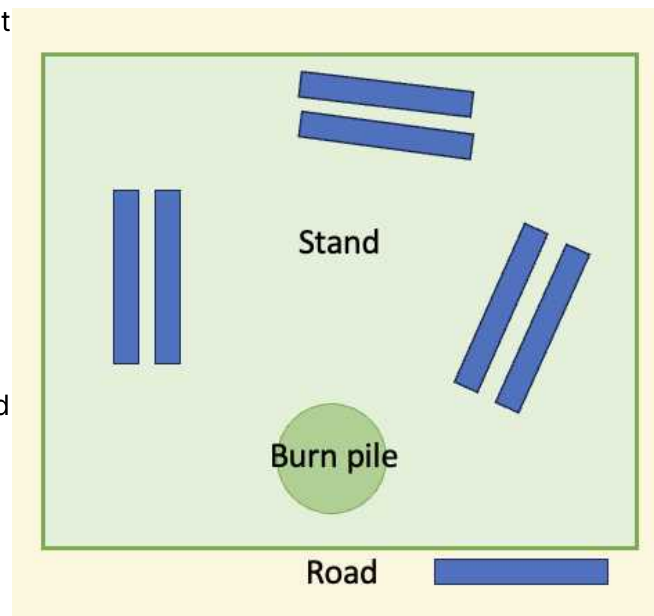


Figure 6: Stand level Survey Site Set Ups, each blue rectangle is an individual 32 m transect

Gear/materials check list

- 8 Personal sampling gear:
 - Phone (updated, synced, and charged)
 - net
 - stopwatch
 - insulated fanny pack
 - ice pack (frozen)
 - butterfly box + butterfly envelopes
 - kestral (one member of a team)
 - 2x thin sharpie(s)
 - Plant guide
 - small ziplock bags

Vegetation Surveys:

9 **Purpose of Vegetation Surveys**

The purpose of these vegetation surveys is to census the actively blooming plant species which bees may visit. We census by species, recording **number of individual** and **average number of blooms per individual**

We will be finding all blooming forbs, shrubs, and trees that are rooted in each plot.

Blooming is defined as having visible pollen-bearing structures, excluding wind-pollinated plants such as grasses and conifers.

10 **Vegetation Survey: Stand Level**

Each site we survey assigned a different type and subtype (See Figure 7)

Stand sites are numbered **1, 2, and 3** with each 32m transect within those sites being labeled **A or B**

Road sites are numbered **7** and since there is only one 32m transect within the site it is labeled **R**

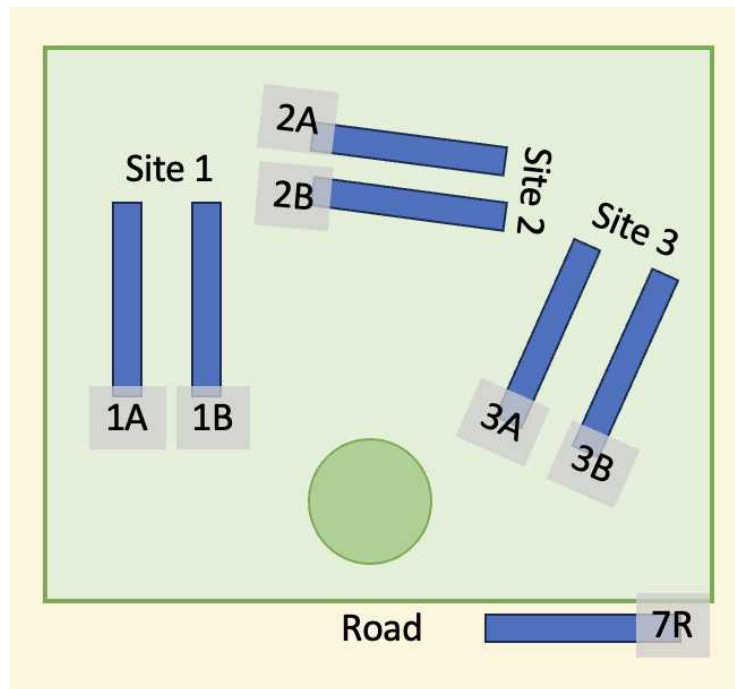


Figure 7, labeling scheme of each stand level transect.

Yes

9. Number of individuals

10. Average bloom for individual

- 1 m x 1 m quadrat every 4 m along each transect along the **left side** of the transect
Placed at: **0, 4, 8, 12, 16, 20, 24, 28**
- Open VegBloom form:
 1. fill out time
 2. observer,
 3. stand (ex. 500),
 4. site (ex. 3)
 5. transect (ex. A)
 6. subplot (ex. 0)
 7. If the plot has no blooming flowers, record FlowerPres = No.
 8. If blooms are present, record FlowerPres =

11 **Vegetation Survey: Plot Level**

This will be the same protocol for the one unseeded plot within control stand

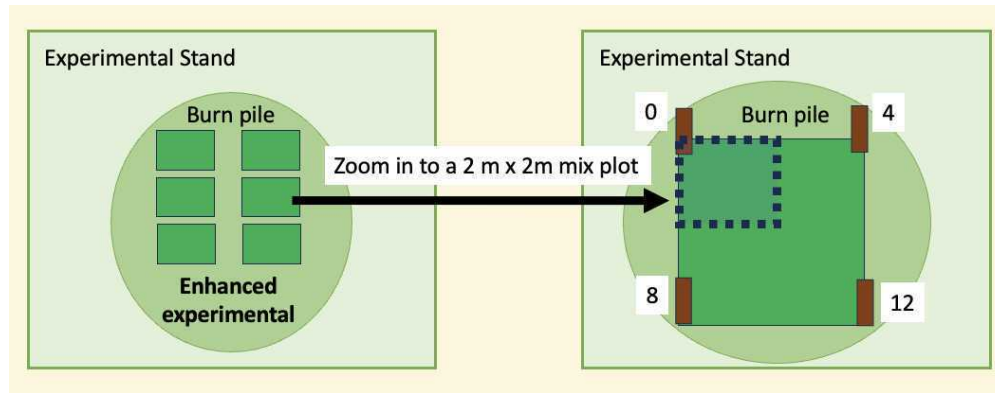


Figure 8: Vegetation survey of mix plots. The 2m x 2m plot is broken into four 1m x 1m subplots where all the blooming Vegetation is surveyed

- A 1 m x 1 m will be placed on every corner of the 2m x 2m plot.
 1. The top left corner being "0",
 2. top right being "4",
 3. bottom left "8", and
 4. bottom right is "12" (see figure 8)
- 5. If the mix plot is broken up, find the quarter of the mix plot labeled as "0", "4", "8", "12".

For each subplot:

- Open VegBloom form:
 1. fill out time
 2. observer,
 3. stand (ex. 500),
 4. **site is always 6**
 5. transect is the mix number, written on stake (ex. Mix2)
 6. subplot (ex. 0)
 7. If the plot has no blooming flowers, record FlowerPres = No.
 8. If blooms are present, record FlowerPres = Yes
 9. Number of individuals
 10. Average bloom for individuals

12 **Tips for Plant ID:**

plant guide made specifically for this area
 Ask fellow field techs!
 Oregon wildflower app
 inaturalist seek

Pollinator Surveys:

13 **Purpose of Pollinator Surveys**

The purpose of these surveys is to get a census of all insects pollinating the flowers that are located near/on each transect or plot.

COLLECT: You should collect any pollinating insect touching a flower's reproductive parts within the plot. This can include bees, flies, butterflies, and wasps (sometimes beetles). When in doubt, catch it.

IGNORE: You should ignore any insects that you know do not pollinate (just sitting in the flower), hemipterans, grasshoppers, ants, ladybugs, spiders, etc. Do not collect an insect if it is just sitting on the petals or leaves --only if it seems to be foraging and actively engaging in pollination.

14 **Conditions of a Pollinator Survey**

- you can only net when the temperature is between 18-32C (65-90F) and the windspeed is below 2.5 m/s.
 - **If conditions are out of this range** then mark down that the stand never received a pollinator surveyed and return at the soonest possible date
1. Fill out Data_Conditions START form with survey type, locality, transect, and weather conditions.
 2. Using your Kestrel and light meter, record temperature (Celsius), wind speed (m/s), and light.

15 **Net Survey Protocol**

1. Put on insulated fanny pack with ice pack and vial box with empty vials.
2. Put on gloves and sterilize by spraying with 10% bleach, making handwashing motions for 30 seconds, spraying with 70% ethanol enough that the bleach residue is removed, and repeating motions until ethanol has evaporated. Repeat if a pollen-covered bee or flower is touched directly or if hands are otherwise contaminated.
3. Sterilize net bag by spraying with 10% bleach, work in for 30 seconds, spraying with 70% ethanol, and letting ethanol evaporate. Repeat if visible pollen is smeared in the net, or if you sweep net a plant with lots of blooms (e.g., Ceanothus). Some plants have white pollen that is difficult to see, so when in doubt, re-sterilize.
4. Start your timer or stopwatch and begin looking for bees on both sides of the transect. Net only insects that are actively visiting flowers. Stop the timer when you confirm a bug in your net.
5. Collect specimen in a vial and/or take photos of specimen. Take photos of lepidopterans, queen bumblebees, and *B. occidentalis*. See below for Specimen protocol.
6. Start timer again after you have put the bee in a vial and are ready to begin searching for a new specimen (so you are not counting the time to get the bee out of the net, into a vial, or labeled in the sampling time). Avoid crushing/decapitating plants.

7. Survey for 10 active minutes. At the end of 10 minutes, fill out a Data_Conditions STOP form. Record weather, light, wind, and temperature if conditions changed significantly over the net survey period.
8. Fill out an InsectCollect form. InsectCollect forms should be filled out at least once per transect, maximally twice.

16 Specimens:

1. When an insect is confirmed to be trapped in your net, stop your timer.
 2. Grab the next empty vial in your vial box; open it and collect the insect in your net. Try to avoid closing the vial cap on the insect and causing damage to the specimen.
 3. On the vial, write in fine-tip sharpie: Date/time, Specimen ID number, and the 6-digit plant code the insect was caught on.
 4. If specimen was collected on an unknown plant, fill out an UnkPlant form and write the unknown plant ID on the vial. If the unknown plant is identified later that day/hitch, write the 6-letter plant code on a slip of Write-in-the-rain paper and place in vial with the specimen.
- **COLLECT:** You should collect any pollinating insect that touches the reproductive parts of a flower along the transect. This can include bees, flies, and wasps (sometimes beetles).

Bombus Queens:

- If a bumblebee queen is caught (~2-4x the size of a worker), collect it into a 6-dram glass vial. Take close photographs of the queen (chill in fanny pack if needed) similar to the following.



Bombus occidentalis:

- If an occidentalis is netted during a survey, collect in a glass vial and take photos similar to queens. Do not take any bee suspected to be or identified as *B. occidentalis*
- Enter the field Occidentalis? = Yes in the InsectCollect form

Butterflies and Moths:



- Attempt to take pictures of the lepidopteran on flowers, or net and take pictures in the net. We do not collect butterflies and moths, but can attempt to identify them by photo after the fact.

Unknown Plants:

- If an insect is collected on a plant that you can't ID immediately, fill out an UnknownPlant form along with labeled pictures similar to Veg surveys. Write the unknown plant ID (ie CM-Unk2) on the vial. If the unknown plant is identified later that day/hitch, write the 6-letter plant code on a slip of Write-in-the-rain paper and place in vial with the specimen.
- If the plant cannot be identified before the end of hitch, unknown plants will be identified as far as possible during scheduled QAQC office days.

Other Notes:

- **IGNORE:** You should ignore any insects that you know do not pollinate (just sitting in the flower), hemipterans, grasshoppers, ants, ladybugs, spiders, etc. Do not collect an insect if it is just sitting on the petals or leaves --only if it seems to be foraging and actively engaging in pollination.
- **HONEY BEES:** Only catch one honeybee per plant on each transect. If there is more than one present, spend a moment counting the number of honey bees on that plant. Label the vial +4HB
- If you get a particularly pollen-covered insect that smears pollen all over your net, spray the bag with bleach (destroys pollen), wait 30 seconds, then ethanol (helps evaporation). Wave it around to dry it.
- When sampling multiple transects, use a brightly colored spacer vial to mark between net walks.
- double check after net walks that the vial numbers match the ranges entered into InsectCollect form. near the plot. Start the stopwatch (it should countdown). You will stop the stopwatch every time you catch an insect, and start it again after you have put the insect in a vial and are ready to begin searching for a new specimen (so you are not counting any time to get the insect out of the net, into a vial, or labeled in the sampling time). **Avoid crushing/decapitating plants.**
- **COLLECT:** You should collect any pollinating insect touching a flower's reproductive parts within the plot. This can include bees, flies, butterflies, and wasps (sometimes beetles). When in doubt, catch it.
- **BUTTERFLIES AND MOTHS:** Collect into the envelopes located in the butterfly box.

17

1. At the truck, transfer vials (in order for each person) to the cryo vial boxes. Keep cryo vial boxes in ziplocs in cooler with dry ice.
2. Label the cryo box with: Site, date, number range from that collection period (i.e., W53, S245 & S246, 220620, #4567-4800).



3. Note when dry ice is getting low, and check minimally once per day.
4. If dry ice runs out and specimens were not kept frozen, write "Thawed" on the cryo box. Keep thawed specimens separate from unthawed specimens.

18 InsectCollect Form:

1. Fill out InsectCollect form with Time, Locality, Transect, and Survey type.
2. Record the range of specimens collected in the form
3. For specimens not collected, ie queens, butterflies, occidentalis, fill out another InsectCollect form with Time, Locality, Survey type, enter Status = Photo, and assign the next range of VialIDs after the specimens collected. Enter the number of photos and the camera the photos are located on in the form.

Analysis

- 19
- Assume canopy closure in 6-10 years after sapling planting in year 1, need plants to flower within 1-3 years
 - Model: Number of germinated seedlings or flowers produced ~ treatment (* or +) plot characteristics (slope etc.) + Block (stand)
 - Model: pollinator community visitation (frequency, diversity of visitors, other interaction metrics) ~ treatment (+ or *) plot characteristics (slope etc.) + Block (stand)
 - Treatment = "generalist" or "specialist" or "mixed"
 - Stand-level model of pollinator communities: Model: pollinator community (abundance, diversity) ~ enhanced/not enhanced (+ or *) plot characteristics (slope etc.) + Block (stand)
 - Block = random effect accounting for variation between slash pile0 not accounted for by fixed effects of plot characteristics