**~~2020 Protocol~~**

~~Flower + Pollen provision collection~~

~~Materials~~

| * ~~Falcon tubes (50mL) x 20~~ | * ~~gloves~~ |
| --- | --- |
| * ~~Scissors~~ | * ~~ethanol+wipes~~ |
| * ~~cooler~~ |  |

~~Flowers~~

~~At each site pool 10 flowers per tree in a 50 mL falcon tube. Wipe scissors with ethanol after cutting. Store in cooler until lab processing.~~

~~In the lab, add 3-5 mL of buffer (MgCl2) to each tube and vortex to fully submerge flowers. Sonicate flowers for 10-15 minutes. Plate 100ul on LAM plates (3) and spread with shaker beads and incubate at 27C. Centrifuge (5 min) the remaining liquid and discard supernatant. Store pellet in freezer.~~

~~Pollen Provisions~~

~~For each collection, mark the nesting tube with the site number and nest number. Store tubes in cooler until lab processing. In the lab, dissect paper tubes and place each pollen provision in microcentrifuge tubes. Weigh samples and take out 100 mg for plating. Suspend 100 mg in buffer and serially dilute. Plate dilutions on LAM plates and incubate at 27C~~

2021 Collection Protocol

Sites

* 3 Managed orchards
* 3 unmanaged orchards

Sampling scheme (Monthly)

| May | June | July | August | September | October |
| --- | --- | --- | --- | --- | --- |
| Osmia, floral nectar | Leafcutter, floral nectar | Bee crop collections, floral nectar |  | Bee crop collections, floral nectar |  |

Sampling scheme (Weekly)

| Sunday | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday |
| --- | --- | --- | --- | --- | --- | --- |
| (Plant Diversity assessment) | Flowers -  Managed Orchards | Pollen Provisions –  Managed Sites | Culturing Day/ Media Day | Flowers –  Unmanaged Sites | Pollen Provision –  Unmanaged Sites | Culturing Day/ Media Day |
| (Plant Diversity assessment) | Flowers -Managed Orchards | Pollen Provisions –  Managed Sites | Culturing Day/ Media Day | Flowers – Unmanaged Sites | Pollen Provision –  Unmanaged Sites | Culturing Day/ Media Day |

Sampling will take place 3-4 times for two week time-spans during the growing season. During each collection period, sample types will be collected once a week for two weeks. Sample types include pollen provisions, floral nectar from apple trees and wildflowers, and bee crop contents.

**Flower Collection and Processing**

Flowers collected at field sites will be composed of apple flowers and wildflowers. Wild flowers will be collected at two separate dates from the 5 most abundant species per site. Five individual apple trees will be sampled from by collecting and pooling 10 flowers from each tree. Species will be identified using iNaturalist. At each site, flowers will be pooled from the same species in a 50 mL falcon tube. Scissors will be wiped with ethanol after cutting and stored in cooler until lab processing.

Wildflowers and apple flowers will have different processing methods. In the lab, add 3-5 mL of buffer (MgCl2) to each tube and vortex to fully submerge wildflowers. Wildflowers will be sonicated for 10-15 minutes. Plate 100ul on LB or TSA plates with antifungal and spread with shaker beads and incubate at 27C. Centrifuge (5 min) the remaining liquid and discard supernatant. Store pellet at -80C. Apple flowers will undergo nectary washing with MgCl2 buffer and 100 ul will be set aside for plating. Floral nectar washes will be plated on LB or TSA and screened on semi selective media to identify *Acinetobacter spp*.

**Pollen Provision Collection and Processing**

Solitary bee pollen provisions will be collected from mason bee boxes once a week for two weeks. When collected, each tube will be marked with the date and field site name. Tubes will be stored in cooler until lab processing. In the lab, dissect paper tubes and place each pollen provision in microcentrifuge tubes or cryotubes. Samples will be weighed and 100 mg will be set aside for plating. For plating, 100 mg of pollen provision will be suspended in buffer and serially diluted. Dilutions will be plated on LB or TSA with antifungal and screened on semi selective media to identify *Acinetobacter spp*. Remainder of the pollen provision will be stored at -80C.

**Bee crop Collection and Processing**

Bee crop contents will be collected by capturing foraging bees with an insect net. Bee will be transferred to collection bottles and labeled with collection number and date. Images will be taken of bee species and identified using iNaturalist. Bees will be placed in a cooler until taken to the lab for processing. Bees will be placed in fridge for 5 minutes to immobilize them and will be placed head-first into microcentrifuge tubes. Bees will then be spun for 1 minute at 5,000g. Bees will be removed and then euthanized in -20C freezer. Crop contents will be serially diluted and plated on TSA or LB and isolated will be screened for *Acinetobacter* identification. The remainder will be stored in at -80C once pelleted.