|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sunday | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday |
|  | 1 | 2 | 3 | 4 | 5 | 6 |
|  |  |  |  |  |  |  |
| 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|  |  |  |  |  |  |  |
| 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|  |  | Bumble bees arrive | Build microcolonies (~18)  For days 2-4 of experiment |  |  | TRAIN HB  Build microcolonies (~6)  For days 5-7 of experiment |
| 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|  |  | DAY 1: INFECT PLANTS  Build microcolonies (~9)  For days 8 & 9 | REPEAT DAY 1 | DAY 2 | DAY 3 | DAY 4 |
| 28 | 29 | 30 | 31 | 1 | 2 | 3 |
| DAY 5 | DAY 6 | DAY 7 | DAY 8 | DAY 9 | DAY 10 | DAY 11 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

**AUGUST 2016**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **EXPERIMENT** | **DAY** | **TENT 1** Honey bee  (HB+ plant ID) | **TENT 2** Control  Bombus + Plant | **TENT 3** Treatment  Bombus +Plant |
| 1. Acute/Plant Differences | Day 1  (8/24) | HB + RC |  |  |
|  |  |
|  |  |
| Day 2  (8/25) | HB + BFT | B1C + RC | B4T + RC |
| B2C + RC | B5T + RC |
| B3C + RC | B6T + RC |
| Day 3  (8/26) | HB + WC | B1C + BFT | B4T + BFT |
| B2C + BFT | B5T + BFT |
| B3C + BFT | B6T + BFT |
| 2. Chronic  Exposure | Day 4  (8/27) | HB + WC | B1C + WC | B4T + WC |
| B2C + WC | B5T + WC |
| B3C + WC | B6T + WC |
| Day 5  (8/28) | HB + WC | B1C + WC | B4T + WC |
| B2C + WC | B5T + WC |
| B3C + WC | B6T + WC |
| Day 6  (8/29) | HB + WC | B1C + WC | B4T + WC |
| B2C + WC | B5T + WC |
| B3C + WC | B6T + WC |
| 3. Diversity | Day 7  (8/30) | HB + Mix | B1C + WC | B4T + WC |
| B2C + WC | B4T + WC |
| B3C + WC | B6T + WC |
| Day 8  (8/31) |  | B1C + Mix | B4T + Mix |
| B2C + Mix | B5T + Mix |
| B3T + Mix | B6T + Mix |
| 4. Co-mingle | Day 9  (9/1) |  | BBColony1C +WC | HB+ BBColony4T +WC |
| Day 10  (9/2) |  | BBColony2C+WC | HB+ BBColony5T +WC |
| Day 11  (9/3) |  | BBColony3C +WC | HB+ BBColony6T +WC |

Key For Schedule:

HB= honey bee (used two 5-frame nucs)

B1= Bombus colony, subscript: enclosure #

RC=Red Clover

WC= White Clover

BFT= Birds Foot trefoil.

Experiments 1-3:

Acute/Plant Differences: Plants were brought from greenhouse, watered, and placed in honey bee tent. Honey bees allowed to forage for 9 hours. The next day, the plants were distributed between the 3 enclosures in the treatment tent. Plants that were not foraged on by honey bees were placed in the 3 enclosures in the control tent. All inflorescence numbers were standardized: 13-20 inflorescences for white and red clover. ~30-40 inflorescences for birds foot trefoil. Diversity experiment had the following #: Bumble bee microcolonies of 12 bumble bees were allowed to forage on the flowers for 6 hours. After 6 hours, all bees were collected. Bumble bees were brought back to lab and transferred to a clean container with sucrose only and held in growth chambers for 7 days to allow for the onset of vial infection and to clear any pollen from their guts. After 7 days, the bumble bees were individually transferred to 1.5 ml vials and placed in -80.

Experiment 4. Honey bees and a full BioBest colony were placed in the treatment tent and allowed to forage on white clover for 7 hours. Control tent had a single Biobest colony only. This was repeated 3 times.

Prior to experiment:

Train honey bees for 3-4 days to allow bees to forage on potted plants (mixed). Give feeder after removing plants at end of day. Give pollen patty to hb.

After bombus have foraged, return to lab to growth chambers for 3 days to allow for viral replication

Chalk entrance to be able to tell which bees were actually foraging. Mark bees with paint before incubation period?

“Microcolonies were created and left for at least 1 week- given pollen at beginning (weigh) and 30% sucrose *ad libitum*- give brood?”

This experiment is not about behavior- It is mechanistic demonstration of transmission.

Co-mingle experiment:

Prep:

Shake more bees into HB colony to boost population.

Collect HB samples from the two colonies to get VL levels as a “before” experiment (doubles as an “after” VL for previous set of experiments)

SET UP:

Use plant storage tent for HB+Bombus comingle treatment

Use Control Tent for control (Bombus only)

Use entire BioBest colonies. Replicates are Biobest colonies.

For each day, provide one set of WC plants (2-3 pots per replicate).

Allow comingle treatment and control treatment to forage for 9 hours.

Pull colonies. Let incubate. Pull bumble bee samples to pollen starve before testing viral loads.