Plant Transmission Experiment Methods:

Experiment Overview:

To test for viral transmission between bee species through the use of shared floral resources, we conducted a series of experiments where we allowed infected honey bees to forage on arrays of flowering plants within a screened enclosure and later transferred these plants to two separate enclosures where non-infected bumble bee micro colonies were allowed to forage. All bees and flowers were tested after each experiment. The experiment consisted of 4 parts where we explored how viral transmission between bee species through the use of shared floral resources is influenced by: 1) plant species, 2) plant diversity, 3) multiple exposures to infected plant, and lastly, 4) if direct contact or co-mingling is necessary for viral transmission by allowing bumble bees and honey bees to forage at the same time on the same plants.

Setup and Pre-screening

On May 16, 2016, seeds of red clover (*Trifolium pratense*), white clover (*Trifolium repens*), and birdsfoot trefoil (*Lotus corniculatus*) were broadcasted in 8 in. diameter, 6.5 in. deep plastic pots to achieve ~100 seeds per pot. Miracle Grow Potting mix was used. To prevent visitation by any non-experimental insects, plants were maintained in a greenhouse until the start of the experiment. To encourage flowering, the white clover and red clover plants were trimmed once and twice, respectively and grow lights were used to maintain 14 hours of sunlight. To ensure virus-free plants at the start of the experiment, haphazard composite samples of each plant species were collected and tested for DWV using RT-qPCR protocols.

From each of two five-frame honey bee colonies, a composite sample of 50 bees were collected and tested for deformed wing virus using RT-qPCR to ensure infection. We received 7 bumble bee colonies from a commercial supplier. To ensure these bees were not infected with DWV, we pollen starved 10 bees from each colony for 72 hours and tested each bee using RT-qPCR. Bees were pollen starved to rid their guts of pollen that may have contained inactive virus particles. All colonies were negative for deformed wing virus. From the larger colonies, we created microcolonies of 12 adult bees. Microcolonies were fed 30% sucrose solution *ad libitum* and allowed to acclimate for up to 5 days in a growth chamber maintained at 26 C and 52-55% RH. New microcolonies were made every three days to ensure each microcolony used in the experiment was about the same age.

Three 10 x 10 x 10 (height?) foot screened enclosures with tarp bottoms were set up and assigned a treatment: honey bee tent, bumble bee control tent, or bumble bee treatment. One additional tent was used as a plant holding area to keep unwanted insects from visiting the plants during the experiment. To restrict bumble bees to a smaller foraging area, three hoop houses were set up in each of the two bumble bee control and treatment tents. Hoop houses (3 x 4 x 2.5 ft) were constructed of white row cover fabric stretched and stapled over two pieces of arching PVC tubes that were screwed to a wooden frame.

Experimental Design:

On each day of the experiment, plants were transported from the greenhouse to the plant holding tent and watered. Inflorescences were counted to ensure a standard range across replicates and treatments. Honey bee colonies were placed in the honey bee tent 24 hours prior to the experiment to acclimate. To infect the flowering plants, we placed plants within the screened enclosure with the two honey bee nucleus colonies and allowed bee foragers to visit the flowers. After 9 hours, we transferred plants to a holding tent area for 15 hours to allow for nectar regeneration. We were confident that deposited viral particles would remain infective on plants for this time period due to previous findings by Singh et al., who found that virus particles on pollen grains can remained infective for 6 months in ambient conditions. After the nectar regeneration period, we transferred honey bee visited plants to the treatment bumble bee tent and distributed these among the three hoop houses (CITE). For the control bumble bee tent, we transferred clean flowering plants from the greenhouse directly into each of three hoop houses within the control bumble bee tent. To prepare the bumble bee micro colonies for the experiment, feeders were removed at least one hour before the start of the experiment. We placed each micro colony container consisting of 12 workers inside a cardboard box fitted with a plastic tube that allowed access to the outside of the box. We assigned each prepared micro colony box to either the bumble bee control or treatment tent and placed one into each of the 6 hoop houses containing either a control plant or honey bee visited plant. Each micro colony was allowed to forage within their hoop house enclosures for 6 hours. We ensured floral visitation of both the honey bees and bumble bees through observations and video footage. After six hours, we collected all inflorescence heads and bumble bees. Inflorescences were stored at -80°C. Bumble bee micro colonies were placed into new containers and fed 30% sucrose *ad libitum* for one week in a growth chamber. If bumble bees were exposed to infective virus during the experiment, this one week ‘incubation’ period allowed for the onset of viral infection. Bees were not fed pollen during this one week period to clean their guts of pollen that could have inactive virus particles, resulting in a false positive result during the viral assays. After one week, bees were collected and stored in -80°C.

To test if plant species influences the transmission of DWV between bee species, the experiment was conducted a total of three times using white clover, red clover, and birdsfoot trefoil. We standardized the number of inflorescences used in each replicate: 15-20 white clover inflorescences, 13-15 red clover inflorescences, and 31-40 birdsfoot trefoil inflorescences. Since birdsfoot trefoil inflorescences contain at least half the number of florets as red clover and white clover, we used about twice as many infloresences. To test if plant diversity affects virus transmission, the experiment was repeated and bees were allowed to forage on floral arrays containing all three plant species. Each diversity array consisted of 7-8 white clover inflorescences, 6 red clover inflorescences, and 15-21 birdsfoot trefoil inflorescences. To test if multiple exposures to infected plants is necessary for viral transmission, the experiment was repeated using white clover. Each white clover array consisted of 15-20 inflorescences. Six bumble bee micro colonies were either assigned the treatment group or control group and subjected to three foraging events over three days. Each day, micro colonies set up in the hoops houses were allowed to forage on white clover plants. The treatment group received clover that was foraged on by infected honey bees the day before. The control group received ‘clean’ plants. Plants were allowed to regenerate nectar between honey bee and bumble bee foraging bouts as in the other experiments. A new white clover plant was used each day. After three exposure events, bumble bees were collected, transferred to new containers, provided with 30% sucrose ad libitum, and ‘incubated’ for one week as in the previous experiments and stored at -80°C. Flowers were collected each day of the multiple exposure experiment and stored at -80°C.

To test if direct exposure, or co-mingling, on flowers is necessary for transmission of DWV between bee species, we used full bumble bee colonies each containing 75-100 workers and white clover arrays consisting of 41-47 inflorescences. We placed two honey bee nucleus colonies, a single bumble bee colony, and pots of white clover plants into a 10 x 10 x 10ft tent enclosure. For the control, we placed a single bumble bee colony with plants into a separate 10 x 10 x 10 ft tent enclosure. We allowed all bees to forage on the plants for a total of 7 hours. We observed normal floral visitation by both bee species. After 7 hours, we collected all bumble bees and placed them back into their boxes and brought back to the lab’s growth chambers. This was repeated three times over the course of three days using the same honey bee colonies but different bumble bee colonies. The bumble bee colonies were fed pollen and 30% sucrose *ad libitum* for three weeks in growth chambers to encourage the spread of DWV throughout the colony. After three weeks, we made pollen-starved micro colonies consisting of 12 bees. After a one-week pollen starvation period, all bees were collected and stored at -80°C.

Lab Methods:

Stats: