Does plant density and/or diversity influence transmission of viruses between bee species?

To explore the role of plants in viral transmission, we will collaborate with UMN researchers and utilize already established field. Under the direction of Marla Spivak, Ph.D. student Morgan Carr-Markell has designed and created large field plots to study the foraging preferences and recruiting behavior of honeybees. Eight 20 x 20 foot plots of different floral assemblages were created to explore honey bee preference for dense vs. dispersed plant assemblages as well as native vs. non native plantings. To examine plant density, four of the plots contain *Agastache foeniculum* (Anise hyssop); two of which are high density plots (6” spacing), and two are low density plots (36” spacing). To examine plant diversity, four plots with different plant assemblages were created: two plots with native plants: (*Agastache foeniculum* (anise hyssop), *Liatris aspera* (smooth blazing star), *Asclepias verticillata* (whorled milkweed), *Dalea purpurea* (purple prairie clover); and two plants are planted with non-native plants (*Trifolium pratense* (red clover), *Trifolium hybridum* (alsike clover), *Lotus corniculatus* (birds-foot trefoil), *Brassica Napus* (canola/rapeseed).

We will purchase XX bumble bee colonies from a commercial supplier and test each for DWV upon arrival using PCR. In the past, we have successfully acquired bumble bee colonies free of DWV and know it to be possible. To examine visitation rates and abundance/diversity of bee species visiting the plots, we will conduct daily visitation surveys in each of the plots XX days before the start of the experiment. We will allow plants to become naturally infected with viruses from foragers prior to the start of the experiment. To ensure we have infected foragers visiting the plots, we will net bees from each plot and test them for RNA viruses. Since several honey bee colonies are present on the premises and could be contributing to RNA virus deposition on the flowers, are will also collect bees directly from the colonies and test them for viruses. We will also collect and test a sample of flowers from each. To examine if plant density influences transmission of viruses between bee species, we will set up netted enclosures around each of the plots containing *A. foeniculum* at high (2) and low (2) densities. A bumble bee colony will be placed within each of the netted enclosures and allowed to forage for XX time. Bees will be brought back to the lab and ‘incubated’ in the growth chamber for 1 week after which they will be sacrificed and tested for DWV. We will repeat this for XX times for a total of XX replicates. We will compare the viral prevalence and viral load for bumble bee colonies foraging in dense vs. dispersed plots. To examine if plant diversity and/or assemblage influences transmission of viruses between bees, we set up netted enclosures around each of the 4 plots containing either native or non- native plant species. These four plots will serve as ‘high diversity’ plots. For ‘low diversity’ plots, we will use the high density plots containing *A. foeniculum*. We will place bumble bee colonies in each enclosure and allow them to forage. After XX (days/hours) we will transfer the colonies to the lab, incubate them for XX, sacrifice and test each for DWV. This will be repeated for a total of XX replicates. We will compare the prevalence of infection for each colony exposed to high vs. low diversity plots and also examine differences between our two ‘high diversity’ plots containing either native or non-native plant species.