Herbivory-Induced Effects on Pollinator Communities: Consequences for Plant Reproduction

Pollination is a key process for ecosystem function, but little is known about how trophic interactions affect pollination services at the community level. Most flowering plants and pollinators in a community are connected – any one pollinator species interacts with multiple plant species; from the amalgamation of their interactions a mutualistic network emerges. The structure of the network makes it possible for disturbances originating at a single point to cascade through the rest of network.

In a pollination network, herbivory to a plant can affect the way it interacts with its pollinators, and in turn this can cause changes in how pollinators interact with other plant species in the community. Most flowering plants require pollen from the same species to reproduce, thus, "floral fidelity", or the degree to which a pollinator visits the same flowering species, is essential. Most pollinator species visit numerous plant species, but individuals specialize on single plant species. Floral fidelity is driven by the attractive cues displayed by plants, such as nectar and pollen quality, and floral display size, color, and scent. Those cues, however, are negatively affected by stresses to the plant, such as herbivory. Hence, floral changes induced by herbivory could reduce the degree of floral fidelity in its pollinators, thereby reducing the quality of pollination services to the damaged plants, and the plants they share pollinators with. In this study I sought to understand how damage to a focal plant species affects pollination services to other plant species in a community. Specifically, **I hypothesized that herbivory on a key plant species would reduce pollinator floral fidelity and that those behavioral changes would reduce the deposition of conspecific pollen to neighboring plant species.**

This summer, I collected preliminary data to assess whether herbivory affects community pollination via disruptions in plant-pollinator interactions. We created 2 pairs of 10x10m plots in a natural milkweed-dominated (*Asclepias syriaca*) community at the Graves Farm Wildlife Sanctuary in Williamsburg, MA. In each pair, one plot was treated to simulate herbivory and one plot was maintained as a control. We simulated herbivory in milkweeds by snipping half of each leaf in every milkweed within the plot boundaries and spraying a small amount of the stress signaling hormone, jasmonic acid. A week after the manipulations, we collected floral visitors and open flowers from three undamaged plant species (*Vicia cracca, Galium sp.* and *Clinopodium vulgare*) within the plots. Field collections were carried out over one week. We swabbed floral visitors with fuchsin jelly to remove all pollen from their bodies and mounted the jelly onto slides to assess pollen loads. Additionally, we removed and stored pollinia (i.e. milkweed pollen) and pollen baskets from the bee pollinators for further analyses. We removed the stigmas from the flowers and mounted them onto slides using fuchsin jelly. To measure seed production in one undamaged species, we isolated pollinated cow vetch (*V. cracca*) flowers and allowed them to mature into fruits.

Our preliminary results indicate that herbivory to a single dominant plant species has community wide effects on pollination services. Following damage to milkweeds, we observed a drastic change in the composition of pollinators visiting other flowering plant species (Fig. 1). For example, the three most common pollinators (*Bombus griseocollis*, *Apis mellifera* and *Xylocopa spp.*) in the control plots made up smaller proportions of the pollinator community in the damage plots. Additionally, in plots with herbivory, we observed marked differences in pollinator behavior (Fig 2). Herbivory reduced milkweed pollination, as pollinators transported less of its pollen (Fig. 2, left panel). In terms of community-wide pollination, however, the results are more abstruse. Limitations in our data prevent us from determining whether overall floral fidelity increased or decreased in damaged plots, but analyses of individual pollinator species suggest that the effects of herbivory on floral fidelity vary by species. For instance, we observed no changes in honeybee (*A. mellifera*) behavior (data not shown), but *B. griseocollis* unexpectedly carry more milkweed pollinia and have higher floral fidelity in damage plots (Fig. 2, center and right panels).

Similarly, our preliminary results indicate that herbivory to the dominant plant species has negative effects on the reproduction of neighboring plants. Remarkably, we observed reductions in conspecific pollen deposition on three neighboring plant species (Fig. 3). In cow vetch (*V. cracca*), reduced pollen deposition correlated with a notable reduction in seeds per fruit in damaged plots (Fig. 3; Fig 4). These observations provide compelling evidence corroborating our hypothesis that herbivory alters pollinator behavior and reduces plant reproduction on the community level. **Negative effects induced by herbivory ripple through the community to reduce reproduction in undamaged plant species. The reductions in pollination highlight the dramatic role that herbivory can have in a community through indirect effects. From our preliminary study, we can infer that the fate of non-dominant plant species is tied to that of dominant species**.

Future work will focus on bolstering our preliminary results by repeating the experiment with more plots to elucidate the immediate causes of the behavior changes observed here. During the summer of 2019, we will expand our research by sampling the pollinator community over an entire milkweed flowering period using 4 plot pairs. This will enable us to make inferences on the behavior of multiple pollinator species and the overall pollinator community. The following summer, we will investigate which floral trait changes are induced by herbivory, and which changes drive behavioral changes in pollinators.

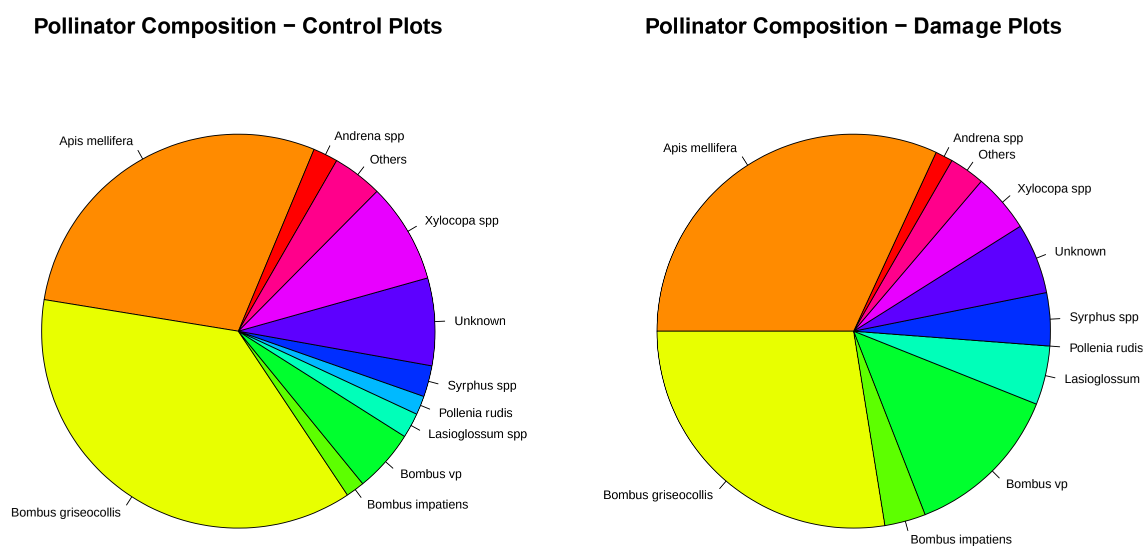

Figure 1: Pollinator community composition. Pie chart depicting differences in pollinator community composition in control (left panel) and damage plots (right panel). Goodness-of-fit test significant at $P<0.05$.

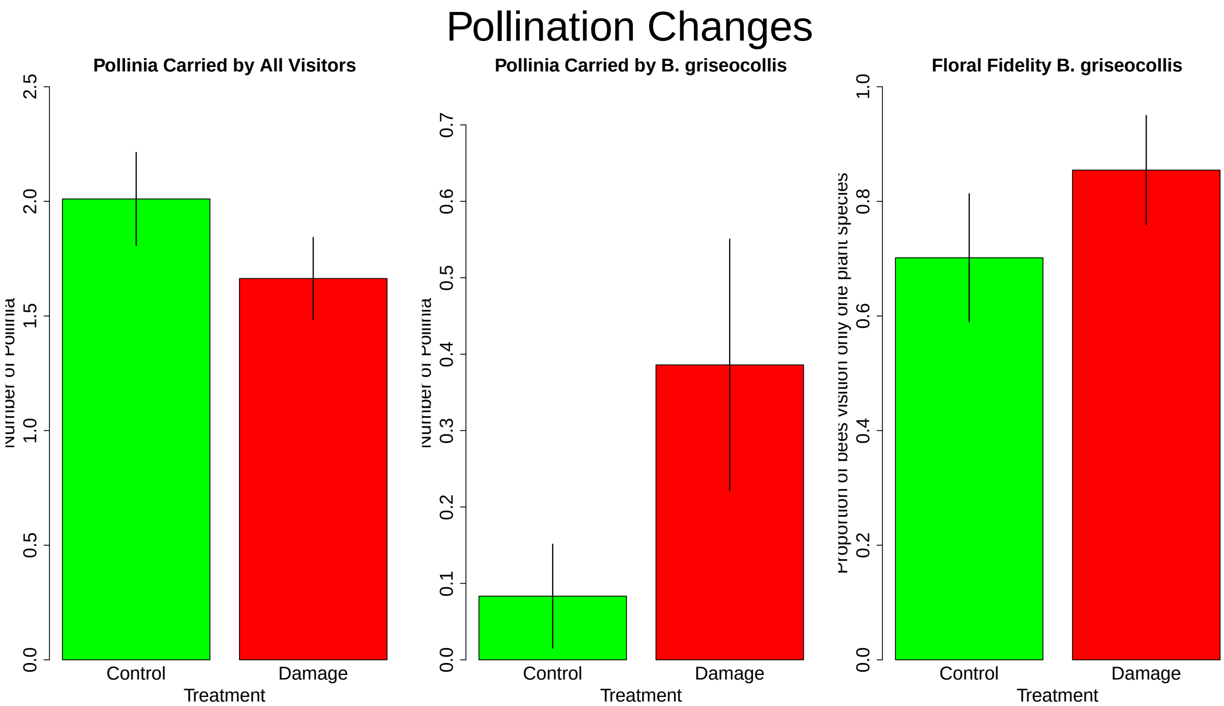

Figure 2. Pollination changes following herbivory. Bar graphs depict differences in plant-pollinator interactions between control (green) and damage (red) plot communities. First panel shows a reduction (~15%) in the amount of milkweed pollinia carried by pollinators in the damage plots. Second panel reveals a fourfold increase in pollinia carried by *B. griseocollis* individuals in damage plots when compared to individuals in control plots. The third panel shows an increase (~25%) in the floral fidelity of *B. griseocollis* in response to milkweed damage. Bar graphs represent means (±2SE's); all results are statistically significant ($P<0.05$).

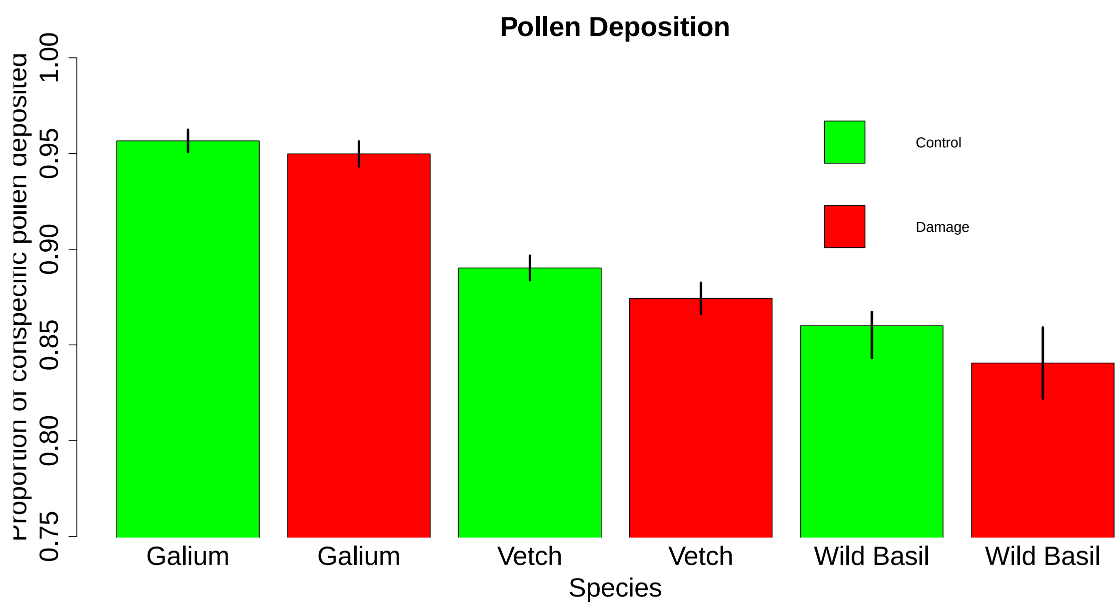

Figure 3: Conspecific pollen deposition on undamaged flowering species. Plot shows reductions in the proportion of conspecific pollen deposited on three neighboring plant species within damage (damage) plots

when compared to control (green) plots. Bar graphs represent means (±2SE's); all pair-wise results are statistically significant (*P*<0.05).

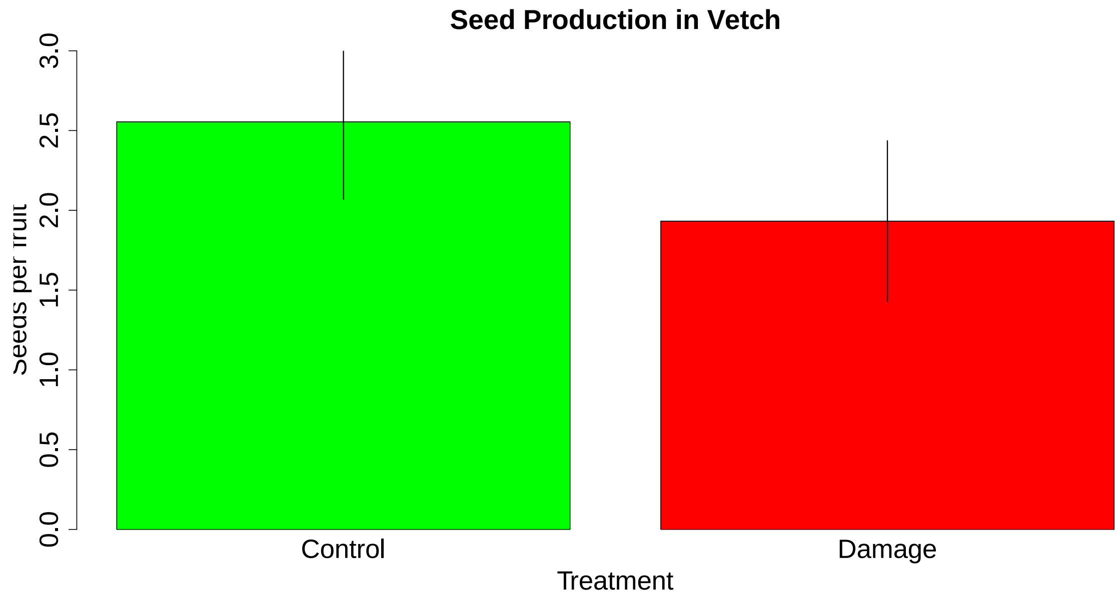

Figure 4: Seed production in cow vetch. Plot shows ~25% reduced seed per fruit produced in cow vetch in damage (red) plots when compared against seed production in control (green) plots. Bar graphs represent means (±2SE's); result is marginally significant (*P* = 0.09).