Ant Aphid Interactions on Pollination and Seed Production of Common Milkweed (*Asclepias syriaca*)

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Abstract. Interactions between ants and aphids have been studied extensively and provide an ecologically important example of a mutualistic relationship. Common Milkweed (*Asclepias syriaca*) is often host to the ant-aphid mutualism and studies suggest that this relationship discourages pollinators. Examining this mutualism and its effects on plant fitness and plant health could further understanding of its role at the community level. In our study, we measured the coloration of milkweed stems, height, number and size of the seed pods, and number of seeds in seed pods. Seed count and seed pod production was used as a measure of plant fitness. These measures were compared to the relative abundances of ants and aphids on Common Milkweed plants. Out of 47 measurements, our findings suggest that there is no significant effect of ant-aphid interactions on the seed pod production ($P=0.6560$), seed count ($P=0.2054$), and stem coloration ($P>0.6170$). Our results suggest ant-aphid interactions do not affect Common Milkweed fitness, and ants and aphids have no stem color preference. Our study offers information for future studies to look further into the effects of ant-aphid interactions on plant health, plant pollination, which might have long-term effects on plant fitness.

Pollinators are essential factors of biodiversity, serving a vital role to the ecosystem they live in (Bascompte and Jordano 2007). Plant-pollinator interactions are necessary not only to the organisms themselves, but for the survival and persistence of organisms in the entire ecosystem. However, over the past century, pollinators have been on the decline due to numerous factors including climate change and the spread of foreign organisms (Goulson et al. 2015). Pollinator decline is resulting in detrimental ecological impacts worldwide including the loss of biodiversity, ecosystem stability, and overall crop production (Potts et al. 2010). Research into the cause of pollinator decline and the implications of pollinator decline has been ongoing for decades, with many new discoveries every week (Lundin et al. 2017). Our study intends to look at the effects of ant-aphid interactions on pollinators and seed production with Common Milkweed (*Asclepias syriaca)* flowers.

Common Milkweed is a flowering plant native to northern Lower Peninsula Michigan. It is widespread in open fields, prairies, along roadsides, and powerlines. Common Milkweed also plays host to many species interactions and is pollinated by numerous species. Around 450 different insects are known to use Common Milkweed as a food source (Stevens 1992). The most commonly known milkweed herbivore is the Monarch Butterfly (*Danaus plexippus*) caterpillar. The Monarch Butterfly caterpillar has evolved to sequester the toxins milkweed plants produce (Taylor 2017). Common Milkweed produces cardenolides, which are cardiac toxins poisonous in large quantities, to avoid predation of its leaves and its nutrients. Many other insect species have evolved a similar resistance to the cardenolides of milkweed. Among these are many different species, ants and aphids are common on milkweed and they share a positive relationship with one another and are studied extensively (Styrsky et al. 2006). Their interaction is an example of a mutualism where both the ants and the aphids benefit. Aphids are small sap-sucking insects that extract sugars from the plant they live on and use these sugars as their food source. This makes the aphids harmful to the plants they infest and lower the plant's health (Müller-Schwarze 2009). One of the biggest predators of aphids are ladybugs, and aphids only have a few ways to defend themselves from these predators (Müller et al. 1999). One way is through their mutualistic relationship with ants. Ants herd aphids into different regions of the plant in order to better protect the aphids from predators. Ant presence reduces aphid predation by more than fifty percent (Mooney and Agrawal 2008). In return, the aphids provide the ant honeydew, a sugary substance that the aphids excrete as a byproduct of feeding on the plant's phloem sap (Stadler and Dixon 2005). The ants then use this honeydew as their food source in exchange for the protection of the aphids.

Many studies look into the effect aphids have on the plants they infest and most suggest that aphids affect the plant chemistry of the plants they infest. On milkweed specifically, Jacobus De Roode studied the effect aphids have on milkweed cardenolide production. Aphid presence on milkweed leaves is correlated with a decrease in overall cardenolide concentrations. Aphid presence also changes the structure of cardenolides produced by milkweed. The aphids alter the way milkweed produces its cardenolides by changing the chemical reactions within the plant, resulting in a different cardenolide product. Milkweed nectar also contains small amounts of these cardenolides, which pollinators later consume. These chemical changes in cardenolide concentrations within the plant and in the nectar might have an effect on pollinators of milkweed plants, which affects the reproductive cycle and seed production of these plants (De Roode et al. 2011).

All milkweed plants have a thick stem stretching from the roots to the top of the plant. This thick stem ranges in color from green to brown, with some of them having brown or green spots as well (Taylor 2017). Some flowering plants evolved to develop ant and/or aphid mimicry spots on their stems and leaves (Lev-Yadun et al. 2002). One genera of flowers exhibit dark spots and flecks along the stem that mimic the coloration and size of ants. Another genera of angiosperms develop dark anthers on their stems and leaf veins that are the same size, shape, and color of aphids. These anthers even sway in the wind to furthermore look like aphids. A third genera of plants use both mimicry patterns throughout its stems and leaf veins. This research into these different coloration patterns, hypothesized that these mimicry markings deter insect and other larger herbivores from eating the plant, increasing the plant's health and fitness. However, it is unknown whether these mimicry coloration patterns on flowering plants affects ant-aphid colonization. Aphids are known to negatively affect plant health, but they might be better for a plant than other larger herbivores (Stadler and Dixon 2005). Stem coloration may play an important role in ant and aphid abundances across a variety of plants, including Common Milkweed.

With the further decline of pollinators, ant-aphid interactions are being studied and their possible effects on pollinators. A study by Katherine LeVan in 2015, found that ant-aphid presence on cotton plants, *Gossypium hirsutum*, decreases pollinator visitation. When the abundance of only aphids or only ants increases, pollinator preference and seed production are unaffected, but when both ant and aphid abundances increases, pollinators visit these plants less and spend less time pollinating them. Later on, they also measured a decrease in seed pod production and seed count of these plants with higher abundances of ants and aphids. Their results suggest that the ant-aphid mutualism discourages pollination, which can be later measured with lower plant fitness (LeVan et al. 2015). The mutualism between fire ants and aphids on mungbean plants also revealed that the presence of this mutualism lowered the plant's output of flowering mung beans and plant seeds. Regardless of aphid presence, red imported fire ants (*Solenopsis invicta*) decrease plant yield, seed pod number, and number of seeds in each pod. The interaction of the ants with aphids also resulted in adverse effects on yield, seed production, and pod production (Wu et al. 2014). This shows that ants and aphids can affect crop yield and seed production by compromising the reproduction process of the plants.

Overall the studies above suggest that ants and aphids can impact plant health, which can later on affect plant fitness. In our study, we examined the effect of stem coloration on ant-aphid colonization and if this mutualism affects seed production of Common Milkweed. Our study was conducted in northern Michigan looking at the different levels of seed production between Common Milkweed plants with and without ants and/or aphids. We expected to observe higher ant-aphid populations on plants with spotted stems and milkweed plants with higher ant-aphid abundances to have less seed production than milkweed plants without.

METHODOLOGY

Study Sites

Our study included observation and seed pod collection of Common Milkweed plants around the University of Michigan Biological Station (UMBS) in Pellston, Michigan, United States. Sites included, the UMBS UV field (45.561253, -84.679538), Lake Kathleen (45.528628, -84.770799), near the UMBS beach on Douglas Lake (45.559661, -84.674519), and along Riggsville Rd. on UMBS property (45.557119, -84.676157). Each location had healthy, reproductive-age plants and had little tree coverage, allowing for high sun exposure. We specifically looked for milkweed plants closer to a treeline or water source for our study, as they were not wilting and had started their seed production process.

Measurements

We recorded data from 47 Common Milkweed plants, recording number of seed pods, maximum seed pod circumference and length, total seed count in seed pods, ant and aphid presence, plant height, and the coloration pattern of each plant. We grouped coloration patterns into five groups: brown, green, green with brown spots, brown with green spots, and 50/50 green/brown mixture. We categorized milkweed plants with ant-aphid presence as high ant-aphid presence or low ant-aphid presence. Plants with two or more leaves covered with aphids were classified as high ant-aphid presence and plants with only one leaf with aphids were classified as low ant-aphid presence. Milkweed plants that were wilting, dead, or had no seed pods were not included in our observations. The latitude and longitude of the plants was determined using the iPhone app GPSUtilities.

Seed Pod Collection

We collected the largest seed pod from 29 plants. We did not collect a seed pod from every plant to reduce the impact of our study on the future populations of Common Milkweed in our study sites. We collected a seed pod from a plant with an ant-aphid presence and another seed pod from the closest seed pod bearing plant without an ant-aphid presence. These pods were packaged into containers and labeled with an ID number, which allowed us to return each seed pod to its original location after the seeds were counted. We then used these 29 seed pods in a linear regression comparing seed pod circumference and number of seeds, to create a standard curve to estimate the number of seeds in the uncollected pods.

Analyses

For all of our data analysis and figures, we used RStudio with packages ggplot2 and MASS. We constructed boxplots comparing the number of seeds pods and the number of seeds within pods of milkweed plants without ant-aphids, with low levels of ant-aphids, and with high levels of ant-aphids. We created linear regression lines and general linear models between continuous variables like ant presence and number of seeds. For our general linear models, we used a poisson distribution to better analyze our non-normally distributed data. Analysis of variance tests (ANOVA) was used to look at relationships among aphid abundance and seed pod number and number of seeds, as well as ant abundance and milkweed stem coloration. We used a chi-square test of independence to analyze a relationship between aphid abundance and stem coloration.

RESULTS

A prediction model was created to predict the number of seeds in uncollected seed pods. Seed pod circumference was positively correlated with the number of seeds within the pods themselves ($F= 4.364$, $df= 28$, $P=0.04626$). We created this linear regression and used the regression line to estimate the number of seeds of 18 uncollected pods.

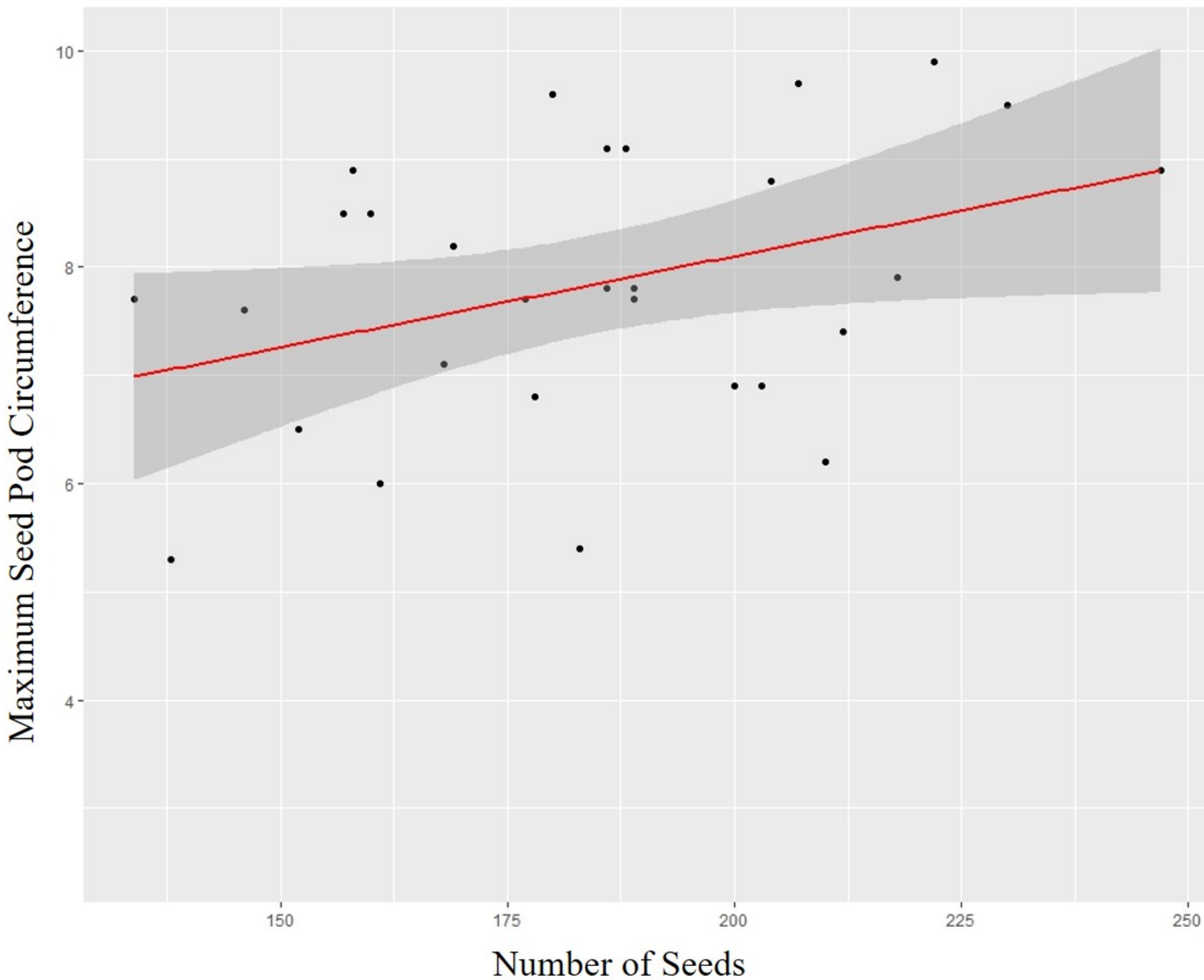

Figure 1: Maximum seed pod circumference vs. number of seeds. The red line is the regression line used to estimate number of seeds in uncollected pods (R^2= 0.2745, P=0.04626). The gray shaded area represents the 95% confidence interval of the linear regression.

The means for seed pod production of the three aphid abundances are as follows, 176.1, 193.3, and 179.2, for high abundance, low abundance, and none respectively. The means for total seed count of the three aphid abundances are as follows, 3, 3.66, and 3.36, for high, low, and none respectively. The number of seed pods did not show a relationship with levels of aphid presence on each plant (F= 0.361, df= 2, P= 0.699). When comparing the means between different aphid densities, a correlation was not observed (Tukey HSD tests, P>0.6920). No relationship was found between number of seeds within each pod (F= 1.624, df=2, P=0.209). An ANOVA test comparing the means within the different aphid levels and their effect on number of seeds, found no relationships (Tukey HSD tests, P> 0.1996).

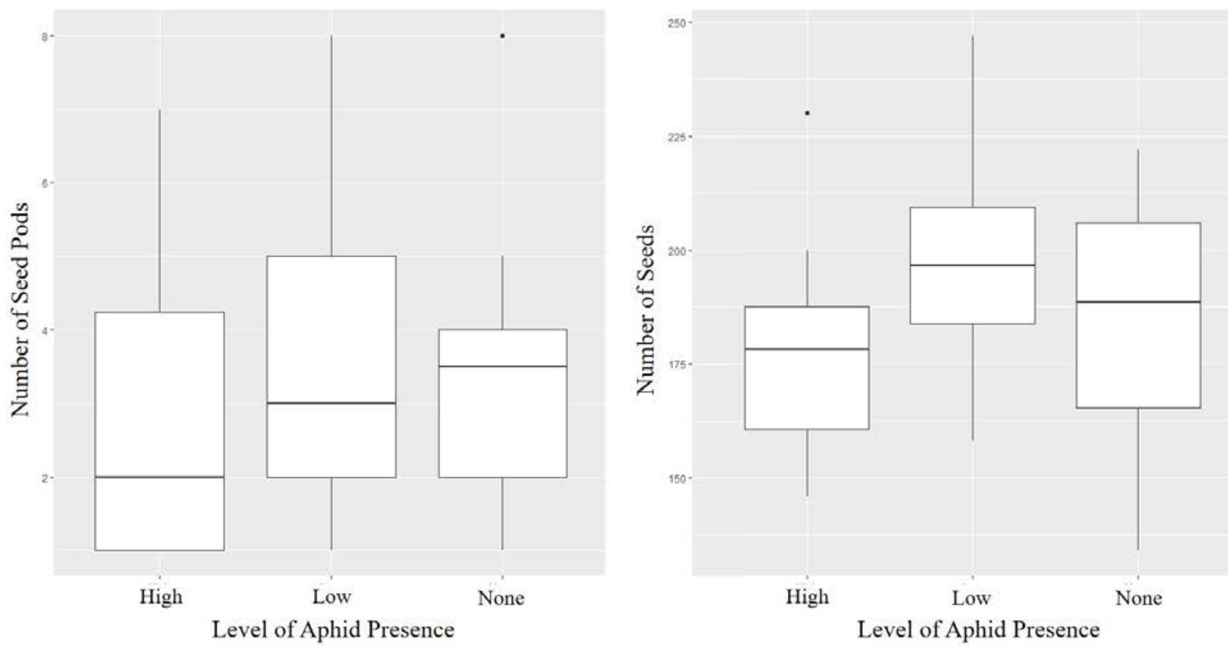

Figure 2: Number of seed pods vs. level of aphid presence (left) (P=0.699) and level of aphid presence vs. number of seeds (right) (P=0.209). Boxes indicate the first and third quartiles, whiskers indicate the range, and dots are outliers. Horizontal lines inside boxes indicate medians.

Seed pod production with ant abundance showed no correlation (P= 0.1760). Similarly, numbers of seeds within each pod did not correlate with ant abundance (P= 0.8737).

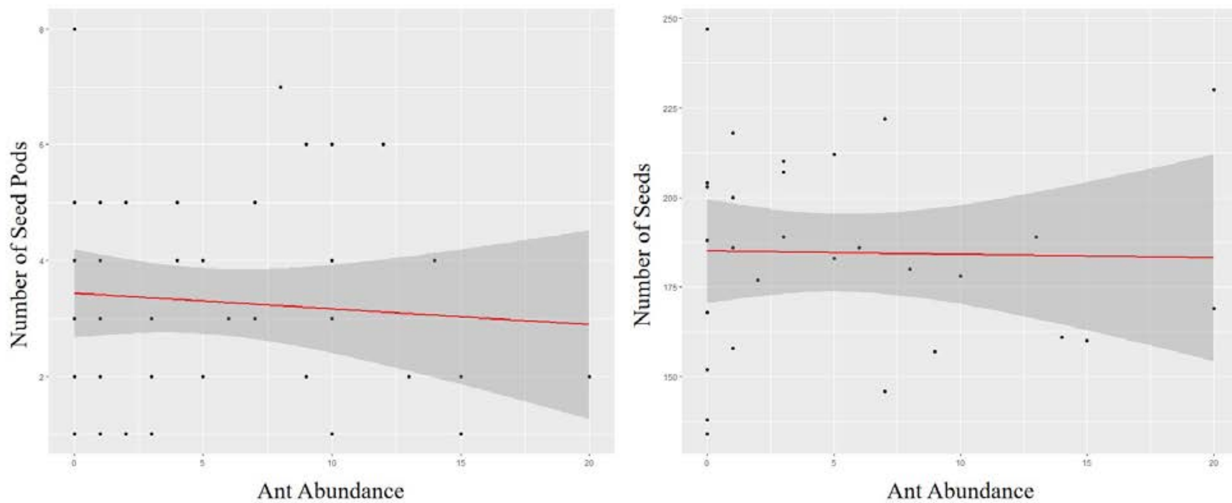

Figure 3: Ant abundance vs. number of seed pods (left) (R^2= 0.0158, P= 0.1760). Ant abundance vs. number of seeds (right) (R^2= 0.0366, P= 0.8737) The red lines are the regression lines and the gray shaded areas represent the 95% confidence interval of the linear regressions.

The relationship between seed pod production and levels of ant and aphid abundances were not significant (P > 0.6560). The relationship between seed counts and ant and aphid abundances had an insignificant relationship as well (P=0.2054).

Chi-squared analyses looking at aphid abundance and stem coloration expressed no correlation between the two (P > 0.7081). A one-way ANOVA analysis of variance suggested no relationship between ant abundance and stem coloration (P= 0.6170, Tukey HSD test, P>0.5191).

DISCUSSION

Before conducting our study, we hypothesized that Common Milkweed plants with ant-aphid interactions would have a lower seed count than milkweed plants without ant-aphid interactions. Our results suggest that ant-aphid interactions do not affect the seed production of Common Milkweed and that ants and aphids have no preference for specific stem coloration patterns.

Based off our results, the ant-aphid mutualism does not affect plant fitness. Fitness is defined as “the genetic contribution of an organism’s descendants to future generations” (Cain et al. 2013). This means that fitness is the measure of an organism’s ability to pass on its genetic information to the next generations. For Common Milkweed, a plant’s seed production is a good measure of plant fitness. In our study, we measured plant fitness in terms of seed pod production and seed count. Seed pod production did not differ between plants with or without ants or aphids. Seed count also did not vary betweens milkweed plants with or without the ant-aphid mutualism. It is known that aphids can reduce plant health of the plants they infest (Müller-Schwarze 2009), but here we see that the reproductive cycle or fitness of Common Milkweed was unaffected by plant health.

We can speculate at least two reasons why milkweed fitness might have been unaffected when milkweed health was reduced. First, the co-evolution between aphids and plants provides us a clear example of an evolutionary arms race. Plants are continuously evolving mechanisms to counteract aphids feeding, and aphids are also evolving to develop defenses against plant chemicals. Aphids have evolved to share a mutualistic relationship with ants to enhance their own fitness (Züst and Agrawal 2016) and plants are evolving to negate the negative effects of aphids extracting their phloem sap. Our results suggest that milkweed plants might have a current evolutionary advantage over aphids and have evolved to counteract aphid feeding mechanisms from harming plant fitness.

Secondly, Common Milkweed plays host to a variety of herbivores and insects, but the ants-aphid mutualism might be less harmful to plant health than other insect herbivores. For example, Monarch Butterfly caterpillars exclusively eat and mature on milkweed plants. These caterpillars eat many of the Milkweed leaves, reducing the plant's health by reducing the surface area available for photosynthesis (De Roode et al. 2011). With aphids, only phloem sap is extracted and plant surface area is unaffected (Züst and Agrawal 2016), possibly resulting in higher health than a plant infested with a Monarch caterpillar. More importantly, when the ant-aphid mutualism occurs on plants, the abundances of other insect herbivores, such as the Monarch caterpillar, are greatly reduced (Müller-Schwarze 2009). In our study we did not measure plant health, but there might be a better trade-off for plants to have ant-aphid interactions over Monarch Butterfly interactions. This hypothesis might be supported by decreased cardenolide production when milkweed is infested with ants and aphids (De Roode et al. 2011). Decreased cardenolide production might allow for more aphids to colonize and survive on milkweed plants, allowing for the ant-aphid mutualism to prevail.

The results of our study do not agree with other studies looking at the effect of ant-aphid interactions on seed production. Other studies used mungbean plants and cotton plants, both angiosperms, but they do not exhibit the same plant defenses milkweed plants use. Common Milkweed is a much different flowering plant than others and more heavily relies on pollinators (Eldredge 2015), which might confound our study system. Milkweed also plays host to many more insects and organisms than other plants do (Stevens 1992), which might change the effect ant-aphid interactions have with milkweed pollinators. These factors make our study different than previous studies. The differences between our results and previous findings might be due to the different species of plants used or it could be due to differences in methodology. In our study, we used Milkweed plants all around the University of Michigan's Biological Station and not plants grown and kept inside greenhouses. This might contribute to our insignificant test results as ant and aphid presences were not as well controlled in our study system compared to greenhouses used in other studies. The LeVan study on cotton plants and the Wu study on mung bean plants were conducted over multiple years and measured plant fitness and health over a much longer time frame. Our study was conducted over a few weeks and measured only plant fitness. If we were able to measure plant health in addition to plant fitness, our results and conclusions may differ.

In addition to these new aspects to look into, our methodology could be refined in future studies. We did not measure seed pod production over the entire season, we only recorded the number of seed pods growing when we harvested or measured the plant. This could have confounded our data as milkweed seed pods might have grown after we visited the plant. Likewise, we only harvested our plant's seed pods whenever we found them and did not wait for the seed pods to reach maturity. Milkweed seed pods might grow more seeds until the time the seed pod bursts and disperses the seeds. We were under the assumption that seeds within seed pods only grow bigger as the seed pod matures and do not grow more seeds. This assumption might have led us to have confounding variables within our data, resulting in faulty results. In future studies, we suggest that the effects of ant-aphid abundances on seed production and pollination, should be studied in a controlled environment. Using plants grown in a greenhouse would allow for many confounding variables to be eliminated and for better data collection methods. Furthermore, conducting an observational study looking at the differences in pollination between plants with and without ant-aphid interactions would help us further understand the effect ants and aphids have on pollinators. LeVan's study suggests that the ant-aphid mutualism decreases pollinator visitation, but it has yet to be replicated on a different study system like Common Milkweed.

It is important to look into these future studies for a number of reasons. Ant-aphid interactions provide a greater understanding of the role of milkweed in the community. Studying the ant-aphid mutualism on seed production and pollination helps predict the effects on pollinator populations and their effects within ecosystems. Common Milkweed holds an important function within its ecosystem by providing a food source for multiple genera of pollinators. Globally pollinators are on the decline, with entire populations going extinct, but pollinators are not just essential components of entire ecosystems, but they are crucial to maintaining biodiversity worldwide.

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REFERENCES

Bascompte, J., and P. Jordano. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. Annual Review of Ecology Evolution and Systematics 38:567–593.

Cain, M. L., W. D. Bowman, S. D. Hacker. Ecology. 2014. Sinauer Associates.

De Roode, J. C., R. M. Rarick, A. J. Mongue, N. M. Gerardo, and M. D. Hunter. 2011. Aphids indirectly increase virulence and transmission potential of a monarch butterfly parasite by reducing defensive chemistry of a shared food plant. Ecology Letters. 14:453-461.

Eldredge, E. P. 2015. Plant Materials Milkweed pollination biology. USDA NRCS Technical Note:1–7.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957.

Levan, K. E., and D. A. Holway. 2015. Ant-aphid interactions increase ant floral visitation and reduce plant reproduction via decreased pollinator visitation. Ecology 96:1620–1630.

Lev-Yadun, S., and M. Inbar. 2002. Defensive ant, aphid and caterpillar mimicry in plants? Biological Journal of the Linnean Society 77:393–398.

Lundin, O., G. P. Svensson, M. C. Larsson, G. Birgersson, V. Hederström, Å. Lankinen, O. Anderbrant, and M. Rundlöf. 2017. The role of pollinators, pests and different yield components for organic and conventional white clover seed yields. Field Crops Research 210:1–8.

Mooney, K., and A. Agrawal. 2008. Plant genotype shapes ant-aphid interactions: implications for community structure and indirect plant defense. The American Naturalist 171:E195–E205.

Muller, C. B., I.C.T. Adriaanse, R. Belshaw and H.C.J. Godfray. 1999. The structure of an aphid-parasitoid community. Journal of Animal Ecology. 68:346-370.

Müller-Schwarze, D. 2009. Hands-On Chemical Ecology:3–6.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010.

Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

Stadler, B., and A. F. G. Dixon. 2005. Ecology and evolution of aphid-ant interactions. Annual Review of Ecology, Evolution, and Systematics 36:345–372.

Stevens, M. 1992. Plant guide: *Asclepias syriaca*. USDA NRCS:1–3.

Styrsky, J. D., and M. D. Eubanks. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. Proceedings. Biological sciences / The Royal Society 274:151–64.

Taylor, D. 2017. Plant of the week common milkweed (Asclepias syriaca L.):1–2.

Wu, D., L. Zeng, Y. Lu, and Y. Xu. 2014. Effects of *Solenopsis invicta* (Hymenoptera: Formicidae) and its interaction with aphids on the seed productions of mungbean and rapeseed plants. Journal of Economic Entomology 107:1758–1764.