

Ant Behavioral Responses to Aphids Colonizing *Ligusticum porteri*

Cale Kenamer

ABSTRACT

Ant-aphid mutualisms are keystone interactions that, if altered, have the potential to cause cascading, multitrophic effects on local arthropod and plant communities. In our study system, the flowering stalks of *Ligusticum. porteri* (Apiaceae) are colonized by the aphid *Aphis asclepiadis*, which relies on a protection mutualism with ants. We examined the mediating role of phenology in the host plant *L. porteri* on mutualistic ant behavior in the subalpine zone of the Rocky Mountains in Colorado, USA. To accomplish this, we utilized three tiers of observational data to infer the role of host plant phenology on ant abundance and behavior. First, we compiled six years of census data taken over June through August from 2017-2022 to determine ant abundance during pre-flowering, flowering, and post-flowering phenological stages. In 2022, we recorded focused observations of tending behavior in ants, adding to the census data collected in previous years. Lastly, we used an ethogram to examine effect of advanced, post-flowering phenology in aphid-colonized host plants on a range of 26 different ant behaviors divided among 6 behavioral categories. Ant abundance increased during the flowering stages of *L. porteri*. Additionally, ant tending behavior also increased during the flowering stages. Finally, during the post flowering stages (fruit initiation and maturation), ants spent the highest proportion of their time on communication, exploration, and grooming behaviors. Increased time spent during these stages on non-tending behaviors such as grooming suggests that ants may budget less time for aphid tending as host plant phenology advances, reducing protective benefits conferred by ants during this time. Taken together, these results suggest that, in years where early season snowmelt leads to advanced phenology of *L. porteri*, early season aphid colonies arriving in June will encounter host plant conditions that are less conducive to attracting the ants needed to protect them.

INTRODUCTION

Mutualisms between species provide a net benefit to each partner that cannot similarly be achieved on its own (Bronstein 1994). Although each partner must receive a net benefit to participate, the cost-benefit balance is often unique for each participant species (Bronstein 1994). For example, specialized ant-plant associations between myrmecophytic plant species and the ants that defend them are context dependent, and in nutrient-stressed or shady conditions the net benefit of defense conveyed to the plants is reduced while costs are increased, inducing a physiological response that reduces extrafloral nectar production. The balance of costs versus benefits within mutualisms makes them particularly susceptible to disruption via climate-induced environmental change, possibly leading to extinction of one partner, dissolution, and switching to alternative partners (Kiers et al. 2010). Recent examples show climate change associated disruptions of pollination (Miller-Struttmann et al. 2015), defensive symbiosis (Higashi et al. 2020) and likely many other mutualisms (Kiers et al. 2010).

Mutualisms between ants (Family Formicidae) and aphids (Family Aphididae) are often considered a keystone mutualism because of their outsized impact on local arthropod and plant communities (Eubanks and Styrsky 2006; Zhang et al. 2020). In these associations, ants protect aphids from natural enemies, and in return, consume a sugar-rich exudate from aphids known as honeydew (Eubanks and Styrsky 2006). In a recent review, Blanchard et al. (2019) detailed four major traits driving the aphid-ant mutualism that may be disrupted by elevated temperatures or carbon dioxide concentrations. One mechanism is effects on aphid behavior, specifically changes to the composition and excretion rate of honeydew (Blanchard et al. 2019). These honeydew traits can induce specific changes to ant behaviors (Detrain et al. 2016). However, ant-aphid mutualisms do not take place in isolation, and climate change is impacting the host plants on which these interactions occur (Mooney et al. 2021; Pringle et al. 2013). Specifically, climate change is accelerating the phenology of many plants, creating cascading effects within local insect communities reliant on them, including mutualists (Boullis et al. 2015; Rosenblatt and Schmitz 2016). However, the mediating role of host plant phenology on behavioral exchanges in the ant-aphid mutualism is understudied (Blanchard et al. 2019). Host plant phenology impacts ant protective behavior in other mutualisms (e.g. Ekka and Rastogi 2019).

The objective of this project was to quantify the behavioral repertoire for ants tending *Aphis asclepiadis* colonies on *Ligusticum porteri* (Apiaceae) at a variety of phenological stages. *L. porteri* flowering stalks are colonized by the aphid *Aphis asclepiadis* beginning in late June (Addicott 1981; Lagos-Kutz et al. 2016). Obligate-facultative mutualisms form between *A. asclepiadis* and several local species of ants soon after colonization of *L. porteri* (Mooney et al. 2016). Aphid abundance is sustained through this mutualism (Mooney et al. 2016), and aphid abundance is reduced in years with early snowmelt (Mooney et al. 2021). Years of early snowmelt are also associated with accelerated host plant flowering phenology (Iler et al. 2013). However, the timing of aphid colonization is independent of both host plant phenology and snowmelt (Mooney et al. 2019). Therefore, aphids can occur on host plants at wide range of phenological stages (Figure 1). Besides collecting honeydew, ants are also frequent floral visitors on *L. porteri*, where they consume nectar. Past research has explored interplay between plant-available carbohydrate sources such as nectar and aphid honeydew on ant behavior (e.g. Katayama et al. 2013). My research extended from this past work to examine how shifts in host plant flowering phenology may alter ant behavior towards aphid colonies. This provided insight into behaviorally mediated impacts of climate change in this system.

SPECIFIC METHODOLOGIES

Long-Term Monitoring

I continued monitoring of 20 host plant populations along a 300m elevation gradient. This gradient included 10 sites in and around Gothic townsite (Figure 2), and 10 non-Gothic sites in the surrounding area (Figure 2, 3). In June, we randomly selected 10 flowering plants along a 30m transect in each population. Continuing this work, I censused all arthropods on host plant flowering stalks each week from June-August. I also scored the flowering phenology of each host plant during weekly censuses; each plant received a numerical score from 0-8 for its terminal and primary inflorescences (umbels). This data collection effort added a sixth year of observations (2017-2022) to the existing dataset. In addition to long-term monitoring data, we also censused ant behavior in 2022. Total counts of ants on plants were divided into three categories of behaviors: ants foraging on flowers, ants tending aphids, or ants present elsewhere on the flowering stalk.

Experimental Aphid Colonies

The elevation gradient provided host plants of varying phenological stages. We experimentally created colonies of *Aphis asclepiadis* using the same methods described in Mooney et al. (2021). In brief, this included adding five field-collected aphids to replicate plants in a subset of the sites described above. We collected these aphids from the within the Gothic study sites. Each study site (4 in total) included four replicates of experimental *A. asclepiadis* colonies (N=16). Additionally, in the ‘Sun 5’ site, we took observations from three additional *L. porteri* plants that already hosted *A. asclepiadis* colonies. Aphids were introduced using a fine-haired brush and allowed to walk off the brush and into *L. porteri* umbels. All experimental colonies were allowed three days to establish. To do this, flying predators were excluded by enclosing the test plants in organza bags. Additionally, ants were excluded by wrapping stems with tape coated in an insect barrier (Tanglefoot, Scott’s Corporation, Marysville, OH). After this three-day establishment period, organza bags and insect barriers were removed from all plants.

Observations of Ant Behavior

Quantitative behavioral data of ants on host plants was recorded using a repertoire adapted from previously published ant ethograms (Wilson 1984; Brandão 1978; Sempo and Detrain 2003; Santos et al. 2005) and modified to fit expected and known field behaviors of worker ants within our study system (Table 1). This repertoire included six behavioral categories consisting of 26 total behaviors. The sampling sites were a subset of those described in the Long-Term Monitoring section above. To quantify ant behavioral responses, we used the focal animal sampling method (Bateson and Martin 2021; Dawkins 2007). We tracked the behaviors of randomly selected individual ants. Data from these individuals (including start and stop times of behaviors) were recorded continuously over a length of ten minutes. Monitoring took place over a total of nine days From July 26 to August 3 2022.

Data Analysis

To measure relatedness between *L. porteri* phenology and ant abundance, significance testing was conducted in R using the 'drop1()' function to obtain test statistics (likelihood ratio test) and p-value for the model. Our model was constructed in 'lmerTest' package using the 'glmer()' function with ants as the response variable and the number of flowering stalks as X1 and stage as X2. Data points were jittered to avoid overplotting. Focused behavioral observations from 2022 were analyzed using a binomial regression model with the proportion of ants tending out of the total counted across the three different behaviors as the response variable. The 'glmer()' function in the 'lmerTest' package was used, taking into account random effect of population for the nesting of observations within populations. Significance testing was conducted with the 'drop1()' function and the likelihood ratio test.

Data was analyzed using a generalized linear mixed effects model. From the focal sampling, we developed time budgets for ants across the range of observed behaviors, which were our response variables (Y). Time allocated to a behavior was defined as the mean proportion of observed time spent on a given behavior. If a behavior was observed during minutes 1-10, it was scored as "yes" for the corresponding minute during which it occurred. Our explanatory variable was host plant phenological stage (X). To account for non-independence of observations within populations, our statistical model also included a random effect of population. This was performed using the 'lmerTest' package in R to construct models and obtain test statistics and p-values (Kuznetsova et al. 2017). All of the above analyses were performed in R statistical Software Versions 2022.7.1.554 (R Core Team, 2022).

RESULTS

Long-Term Monitoring and Focused Aphid Tending Observations

The long-term monitoring data (2017-2022) revealed associations between ant abundance and flowering phenology. Overall, ants were most abundant on *L. porteri* plants during their flowering stages (4-6) compared the pre-flowering (0-3) and post-flowering stages (7-8), and this was true regardless of whether the plants were colonized by aphids. For host plants with ants but without aphids, ants were more abundant on plants with higher numbers of flowering stalks, but this overall pattern was also modified by host plant flowering stage ($N=2,094$, L.R.T. = 33.113, $P < 0.001$). Specifically, ants were more abundant per flowering stalk on plants at the flowering stage versus either the pre-flowering or post-flowering stages (Figure 4). On plants with aphids ($N = 697$), we found that the numbers of ants generally increased on host plants with larger aphid colonies. However, this pattern of ant recruitment was also modified by host plant phenology (L.R.T. = 144.109, $P < 0.001$). For these data, the number of ants counted on flowering stalks colonized by aphids was greatest when colonies occurred on host plants at the flowering stage (Figure 5).

In focused behavioral observations of ants in 2022, we observed similar patterns as in the long term monitoring data. In total, we counted 884 ants showing tending behavior, 289 ants foraging on flowers, and 1,998 ants foraging elsewhere on the plant. For these data, we observed that the number of ants showing tending behavior increased with aphid colony size (L.R.T. = 10,659, $P < 0.001$). However, this pattern was also modified by host plant phenological stage (L.R.T. = 7.633, $P = 0.022$). Specifically, counts of ants showing tending behavior at a given colony size was greatest for colonies on host plants at the flowering stage (Figure 8).

Behavioral Observations

The proportion of time spent per behavioral category for ants that were observed on focal plants during post-flowering phenological stages 7-8 (Figure 9; fruit initiation and fruit maturation). These ants budgeted the highest proportion of their time toward communication, exploration, and grooming behaviors. Ants engaged in exploration behaviors more frequently in stages 7.5-8 than in stage 7 (Figure 10(A); $\chi^2 = 3.0774$, $df = 1$, $p = 0.079386$). Grooming behavior was observed exclusively at stage 8 (figure 10(B); $\chi^2 = 3.5913$, $df = 1$, $p = 0.05808$). Likewise, immobility behavior was more likely to occur during phenological stages 7.5-8 than stage 7, with most observations occurring during stage 8 (Figure 10(C); $\chi^2 = 3.6506$, $df = 1$, $p = 0.056050$).

DISCUSSION

In order for a mutualism to persist, benefits to each participant must exceed the costs expended to receive them. Changing climate is altering cost-benefit analyses across a wide range of insect mutualisms, sometimes reinforcing them and sometimes leading to breakdown (Vidal et al. 2021). Understanding how changing climate is affecting the abiotic and biotic factors that influence these mutualisms is critical to developing predictions regarding how, if at all, they may shift over time. While previous studies have examined the mediating effects of climate change on insect mutualisms (Vidal et al. 2021), little research has been dedicated to examining the influence of host plant phenology in these relationships. Prior research on ant-aphid mutualisms has centered around the effects of elevated temperature and carbon dioxide levels without examining the mediating role of host plants (Blanchard et al. 2019).

Our long-term observational data (2017-2022), focused ant observations (2022), and ant ethogram observations (2022) all support the conclusion that the phenological stage of *L. porteri* plays a mediating role in the behavior of ants. In the long-term observational data, these effects were borne out through increased abundance of ants per flowering stalk found on host plants at the flowering stage versus the pre-flowering and post-flowering stages. Abundance can be used as a measure of ant behavior because it reflects a space use decision by ants to occupy an *L. porteri* plant rather than another part of their environment. Our observation supports the inference that ants are consuming floral nectar during that time, an important alternative source of carbohydrates to honeydew. Ants are generally regarded as nectar thieves, in that they consume nectar without effectively transporting pollen among plants (Levan and Holway, 2015). In addition, ant foraging on flowers often reduces pollinator visitation, further reducing seed set by plants (Rostás and Tautz, 2010). Given the focus on their roles as flower larcenists, we have few past studies with which we can compare our results.

This pattern of increased relative ant abundance was similarly observed on host plants that were colonized by aphids during the flowering stages versus other phenological stages. This further supports the idea that flowering *L. porteri* plants have a higher capacity to attract and retain ant populations than plants in the pre-flowering and post-flowering stages. One possible mechanism is that ant foraging on flowers increases the likelihood that aphid colonies will be discovered by ants. Honeydew foraging ants are likely to show so-called ‘trunk trail’ foraging patterns, where pheromone-mediated trails direct ants towards stable food sources (Quinet et al. 1997). The trunk trail system creates resource fidelity, such that a single ant forager may forage on a single plant or aphid colony for its entire lifetime (McIver and Yandell, 1998). In this way, foragers recruited to flowers may remain a stable source of mutualists to aphid colonies. Future research could experimentally manipulate ant access to floral nectar to determine if this enhances ant recruitment.

Focused behavioral observations in 2022 – tending, foraging, and other – allowed for further extrapolation of previously collected ant abundance data. Interestingly, as aphid colony size increased on flowering *L. porteri* host plants, ants exhibited a sharp increase in tending behavior relative to when similar increases occurred on pre-flowering and post-flowering plants. This is consistent with our long-term abundance observations and also indicates that ants are more likely to engage tending behavior during the flowering stages as aphid colony size increases. This result suggests that plant-available nectar from flowers may enhance ant tending behavior towards aphids. Past studies in other systems have focused on how plant-available nectar may compete with aphids for ant services as mutualists (e.g., Levan and Holway, 2015).

In addition to tending, ethogram observations taken during 2022 provided insight into how *L. porteri* mediates a range of other ant behaviors as it progresses into the post-flowering stages. Specifically, as phenology advanced through stages 7-8, ants increased the proportion of time spent performing non-tending behaviors like grooming and immobility. This reduction in tending behaviors negatively affects the ant-aphid mutualism by reducing the benefits conveyed to *A. asclepiadis* colonies that are reliant on ant tending. From this, it can be inferred that the mutualistic benefit conferred by ants to aphids is likely reduced as *L. porteri* flowers develop into fruit. Alteration of behavioral interactions between aphids and ants is a key mechanism by which climate change will impact ant-aphid mutualisms (Blanchard et al. 2019). For example, Barton and Ives (2014) found that elevated temperatures reduced ant aggression towards predators of the aphid *Rhopalosiphum maidis*. To the best of our knowledge, how host plant phenology may mediate ant behavior as mutualists has not been addressed by previous research. Such effects could be widespread, given the many plant taxa

showing accelerated flowering phenology due to climate change (Rafferty et al. 2017) and the many species of aphids that colonize inflorescences (Kund and Dixon 1995).

Importantly, *L. porteri* phenology is strongly correlated with snowmelt date (Mooney et al. 2021) but timing of first aphid colonization is not. Thus, during years of early snowmelt, most aphids colonizing host plants in June are likely arrive on plants at the post-flowering stages. Because the presence of ants and, by extension, their choice to tend versus not tend impacts the length of time *A. asclepiadis* colonies persist (Mooney et al. 2016), climate-driven shifts in phenology have the potential to shift the timeframe during which aphid colonies will remain viable. While previous research has reviewed the role of elevated temperature and atmospheric carbon dioxide in ant-aphid mutualisms (Blanchard et al. 2019), this is the first study to quantify the effect of *L. porteri* phenology on ant behavior.

Finally, our data demonstrates that ant tending behavior is negatively correlated with reduced aphid colony size. Earlier flowering times driven by changing climate alter the cost-benefit analysis for ants in these areas by reducing the benefit derived from participating in mutualisms with smaller, more predated upon aphid colonies. Because ant presence appears to mitigate the negative effect on colony growth rates of drought stressed plants with advanced phenology (Mooney et al. 2021), reduction in ant tending should further compromise the ability of *A. asclepiadis* to cope with climate-driven changes in *L. porteri* flowering times.

CONCLUSIONS

Our results suggest that climate-driven, temporal shift in the phenology of *L. porteri* plays a mediating role in the mutualism between *A. asclepiadis* and tending ants. Ant abundance on non-colonized and colonized *L. porteri* plants increases during the flowering stages relative to pre-flowering and post-flowering stages. Aphid tending behavior of ants also increases at a higher rate during flowering stages. Additionally, in post-flowering stages, ant behavior is concentrated among communication, exploration, and grooming categories. Thus, increased time budgeted toward non-tending behaviors such as grooming during later phenological stages indicates that ants may reduce the benefits they provide to *A. asclepiadis* colonies during this time.

In a broader context, the aphids that colonize host plants in June during early snowmelt years are likely to encounter plants that have advanced into the post-flowering phenological stages. Because ants appear to reduce tending behavior during those stages, these aphid colonies are likely to receive reduced benefits compared to years in which snowmelt occurs at a later date. This altered behavioral timing has the potential to disrupt the mutualism between *A. asclepiadis* colonies and the ants that protect them. If this is the case, changing climate may lead to cascading, multitrophic effects in the local arthropod and plant communities that interact with these organisms.

REFERENCES

- Addicott J (1981). Synonymy of *Aphis heraclella* Davis 1919 with *Aphis helianthi* Monell 1879 (Homoptera: Aphididae). *Can Entomol* 113:167–169.
- Barton, B. T., & Ives A. R. (2014). Direct and indirect effects of warming on aphids, their predators, and ant mutualists. *Ecology*, 95(6), 1479-1484.
- Bateson, M.; Martin, P. *Measuring Behaviour: An Introductory Guide*, 4th ed.; Cambridge University Press: Cambridge, UK, 2021.
- Blanchard S., Lognay G., Verheggen F., Detrain C. (2019). Today and tomorrow: impact of climate change on aphid biology and potential consequences on their mutualism with ants. *Physiological Entomology*, <https://doi.org/10.1111/phen.12275>.
- Boullis, A., Francis, F. & Verheggen, F. (2015)/ Climate change and tritrophic interactions: will modifications to greenhouse gas emissions increase the vulnerability of herbivorous insects to natural enemies? *Environmental Entomology*, 44, 277–286.
- Brandão, C.R.F. (1978). Division of labor within the worker caste of *Formica perpilosa* Wheeler (Hymenoptera: Formicidae). *Psyche* 85(2-3): 229-237.
- Bronstein J.L. (1994). Our current understanding of mutualism. *The Quarterly Review of Biology*, 69, 31–51.
- Ekka P., Rastogi N., (2019). A single lycaenid caterpillar gets an ant-constructed shelter and uninterrupted ant attendance. *Entomologia Experimentalis et Applicata*, 167(12) 1012-1019.
- Eubanks, M. D., & Styrsky, J. D. (2006). Ant-Hemipteran mutualisms: keystone interactions that alter food web dynamics and influence plant fitness. In *Trophic and guild in biological interactions control* (pp. 171-189). Springer, Dordrecht.
- Folgarait PJ, Davidson DW. (1994). Antiherbivore defenses of myrmecophytic *Cecropia* under different light regimes. *Oikos* 71:305–320.
- Folgarait PJ, Davidson DW. (1995). Myrmecophytic *Cecropia*: antiherbivore defenses under different nutrient treatments. *Oecologia* 104: 189–206.
- Heil M, Hilpert A, Fiala B, Linsenmair KE. (2001). Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia* 126:404–408.
- Higashi, C. H., Barton, B. T., & Oliver, K. M. (2020). Warmer nights offer no respite for a defensive mutualism. *Journal of Animal Ecology*, 89(8), 1895-1905.
- Iler, A. M., Inouye, D. W., Høye, T. T., Miller-Rushing, A. J., Burkle, L. A., & Johnston, E. B. (2013). Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate. *Global Change Biology*, 19(8), 2348-2359.
- Katayama, N., Hembry, D. H., Hojo, M. K., & Suzuki, N. (2013). Why do ants shift their foraging from extrafloral nectar to aphid honeydew?. *Ecological research*, 28(5), 919-926.
- Kiers T., Palmer T.M., Ives A.R. et al. (2010). Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters*, 13, 1459–1474.
- Kundu, R. and Dixon, A. F. G. (1995). Evolution of Complex Life Cycles in Aphids. *J. Anim. Ecol.* 64: 245.
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of statistical software*, 82, 1-26.
- Lagos-Kutz D, Favret C, Giordano R, Voegtlin D.J. (2016). The status of the members of the *Aphis asclepiadis* species group (Hemiptera:Aphididae) in the United States of America. *Ann Entomol Soc Am* 109:585–594. <https://doi.org/10.1093/aesa/saw020>.
- Levan, K. E. and Holway, D. A. (2015). Ant-aphid interactions increase ant floral visitation and reduce plant reproduction via decreased pollinator visitation. *Ecology* 96.

- McIver, J. D., & Yandell, K. (1998). Honeydew harvest in the western thatching ant (Hymenoptera: Formicidae). *American Entomologist*, 44(1), 30-35.
- Miller-Struttman, N. E., Geib, J. C., Franklin, J. D., Kevan, P. G., Holdo, R. M., Ebert-May, D., ... & Galen, C. (2015). Functional mismatch in a bumble bee pollination mutualism under climate change. *Science*, 349(6255), 1541-1544.
- Mooney E.H., Phillips J.S., Tillberg C.V. et al. (2016) Abiotic mediation of a mutualism drives herbivore distribution. *Ecol. Lett* 19:37-44. <https://doi.org/10.1111/ele.12540>.
- Mooney, E., Davidson, B., Den Uyl, J., Mullins, M., Medina, E., Nguyen, P., & Owens, J. (2019). Elevated temperatures alter an ant-aphid mutualism. *Entomologia Experimentalis et Applicata*, 167(10), 891-905.
- Mooney E.H., Mullins M., Uyl J., Trail S., Nguyen P., Owens J., et al. (2021). Early snowmelt reduces aphid abundance (*Aphis asclepiadis*) by creating water-stressed host plants (*Ligusticum porteri*) and altering interactions with ants. *Arthropod-Plant Interactions*. 15:33-46. <https://doi.org/10.1007/s11829-020-09793-2>.
- Mooney K.A., Pratt R.T., Singer M.S. (2012). The tri-trophic interactions hypothesis: interactive effects of host plant quality, diet breadth and natural enemies on herbivores. *PLoS One* 7:e34403. <https://doi.org/10.1371/journal.pone.0034403>.
- Mullins M., Uyl J.D., Cruz E., Trail S., Davidson B., Campbell D., Mooney E. (2020). Advanced phenology of intraguild predators shifts herbivore host plant preference and performance *Ecological Entomology* 45(5):1004-1014. <https://doi-org.libproxy.uccs.edu/10.1111/een.12878>.
- Pringle, E. G., Akçay, E., Raab, T. K., Dirzo, R., & Gordon, D. M. (2013). Water stress strengthens mutualism among ants, trees, and scale insects. *PLoS Biology*, 11(11), e1001705.
- Ray C., Rochefort, R., Ransom, Nesmith, J., Haultain S., Schaming, T., et al. (2021) Assessing trends and vulnerabilities in the mutualism between whitebark pine (*Pinus albicaulis*) and Clark's nutcracker (*Nucifraga columbiana*) in national parks of the Sierra-Cascade region. *PLoS ONE* 15(10): e0227161. <https://doi.org/10.1371/journal.pone.0227161>.
- Rafferty, N. E. and Nability, P. D. (2017). A Global test for phylogenetic signal in shifts in flowering time under climate change. *J. Ecol.* 105: 627-633.
- R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Robinson, A., Inouye, D.W., Ogilvie, J.E. et al. (2017). Multitrophic interactions mediate the effects of climate change on herbivore abundance. *Oecologia* 185, 181–190 <https://doi.org/10.1007/s00442-017-3934-0>.
- Rosenblatt, A.E. & Schmitz, O.J. (2016). Climate change, nutrition, and bottom-up and top-down food web processes. *Trends in Ecology and Evolution*, 31, 965–975.
- Rostás, M., & Tautz, J. (2010). Ants as pollinators of plants and the role of floral scents. In *All flesh is grass* (pp. 149-161). Springer, Dordrecht.
- Santos J.C., Yamamoto M., Oliveira F.R., Del Claro K. (2005). Behavioral Repertory of the Weaver Ant *Camponotus (Myrmobrachys) senex* (Hymenoptera: Formicidae). *Sociobiology* 45, 1-11.
- Sempo G., Detrain C. (2004). Between-species differences of behavioural repertoire of castes in the ant genus *Pheidole*: a methodological artefact? *Insectes Sociaux* 51(1) 48-54.
- Stamp Dawkins, M. *Observing Animal Behaviour: Design and Analysis of Quantitative Data*; Oxford University Press: Oxford, UK, 2007.
- Quinlet, Y., de Biseau, J. C., & Pasteels, J. M. (1997). Food recruitment as a component of trunk trail foraging behaviour of *Lasius fuliginosus* (Hymenoptera: Formicidae). *Behavioural Processes*, 40(1), 75-83.
- Vidal, M. C. et al. (2021). The variable effects of global change on insect mutualisms. *Curr. Opin. Insect Sci.* 47: 46–52

Wheeler, A.G. (2001). *Biology of the Plant Bugs (Hemiptera: Miridae): Pests, Predators, Opportunists*. Cornell University Press, Ithaca, New York.

Wilson E.O., (1984). The relation between caste ratios and division of labor in the ant genus *Pheidole* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 16:89-98.

Zhang, S., Zhang, Y. X., & Ma, K. M. (2020). Fine-scale variation of a keystone interaction: aphid-tending ants show stronger anti-herbivory effects on small leaves. *Arthropod-plant interactions*, 14(3), 357-361.

TABLES AND FIGURES

Figure 1(A): Aphid colony on a host plant at the pre-flowering stage	Figure 1(B): Aphid colony on a host plant at the flowering stage	Figure 1(C): Aphid colony on a host plant at the post-flowering stage
		

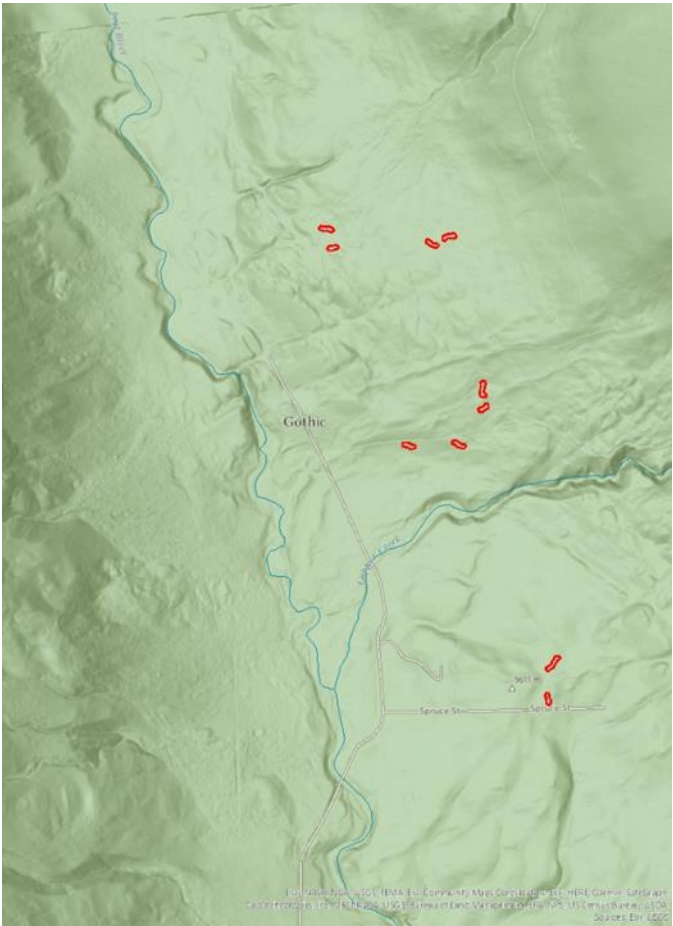


Figure 2 2022 Gothic Research Sites



Figure 3 2022 Non-Gothic Research Sites.

Behavioral category/behavioral act	Minute 1	Minute 2	Minute 3	Minute 4	Minute 5	Minute 6	Minute 7	Minute 8	Minute 9	Minute 10
Feeding										
Trophallaxis										
Predation on aphid										
Foraging on nectar										
Communication										
Antennation (Conspecific)										
Antennation (Interspecific - ant)										
Antennation (Interspecific - aphid)										
Defense										
Aggressive display										
Attacking (mandables + acid)										
Flicking formic acid										
Drumming										
Immobility										
Stopped on stalk										
Stopped on leaf										
Exploring										
Patrolling stalk										
Patrolling leaves										
Grooming										
Autogrooming 1st pair of legs										
Autogrooming antenna + 1st pair of legs										
Autogrooming 2nd pair of legs										
Autogrooming 3rd pair of legs + abdomen										
Autogrooming anus										
Allogrooming worker										
Antagonistic										
Attack against an individual										
Biting the head of another individual										
Biting another individual from behind										

Table 1 Ethogram of ant behaviors on *L. porteri* plants colonized by *A. asclepiadis*

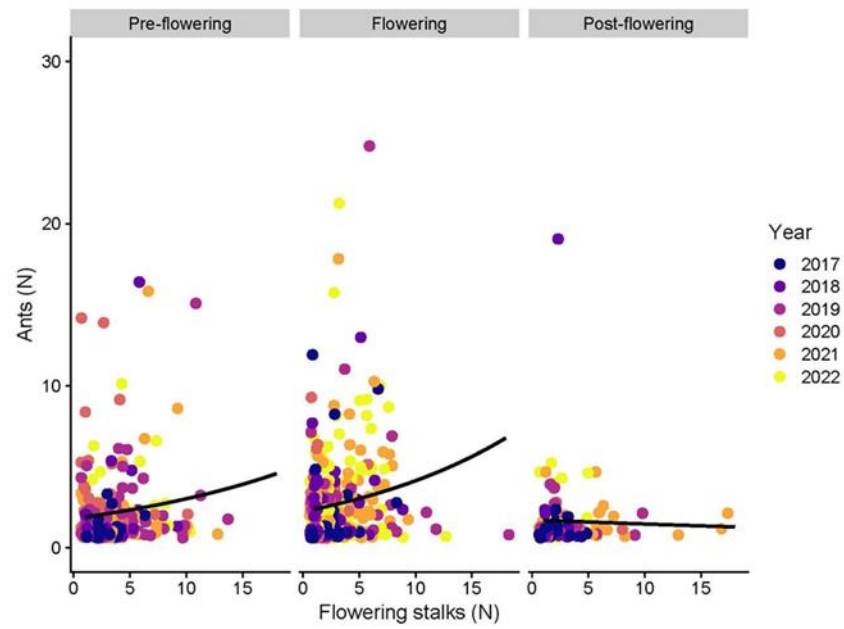


Figure 4 The number of ants counted/flowering stalk on plants without aphids monitored in 20 populations over 6 years (2017 – 2022). This data set has 697 observations and excludes observations with zero ants; points are jittered to avoid overplotting.

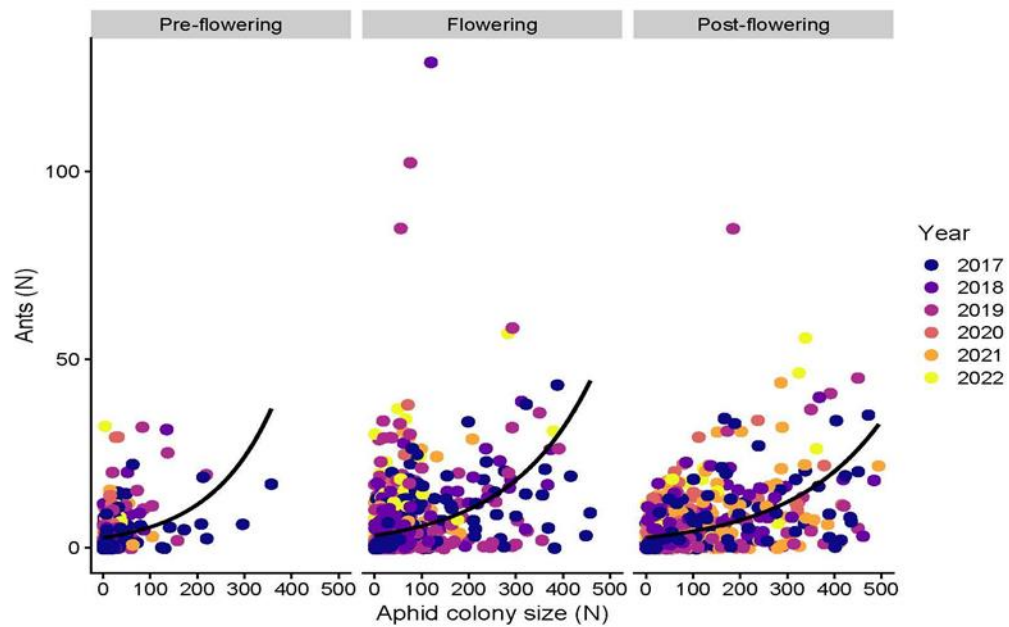


Figure 5 The number of ants counted on plants with aphids monitored in 20 populations over 6 years (2017-2022). This data set has 2,094 observations in total and excludes observations with zero ants; points are jittered to avoid overplotting.

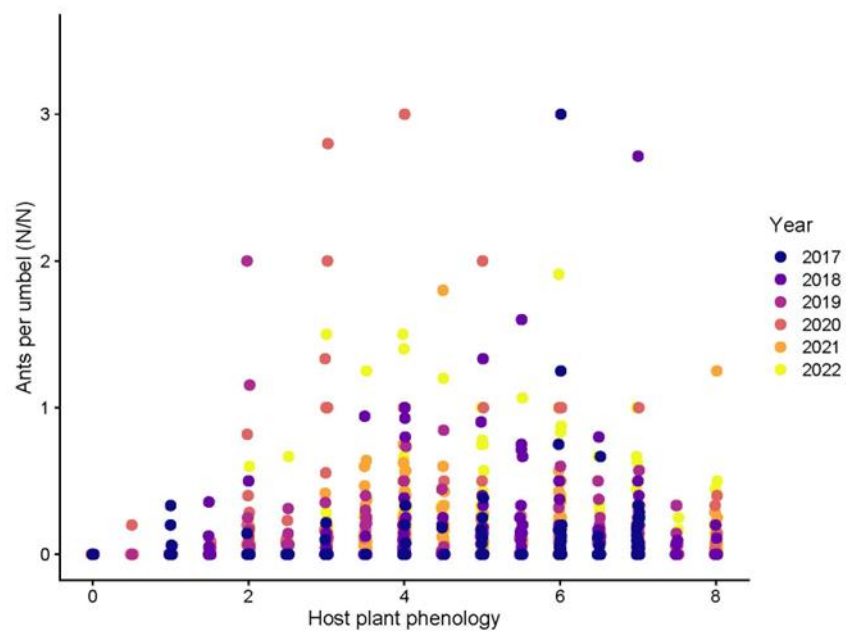


Figure 6 The number of ants counted per umbel on plants without aphids monitored in 20 populations over 6 years (2017-2022). This is a dataset of 8,001 observations and includes observations with zero ants.

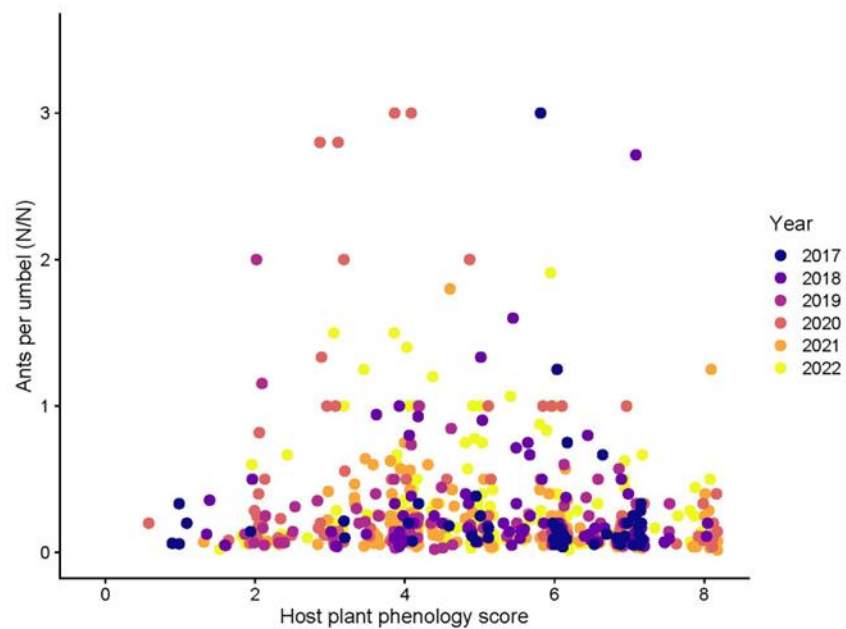


Figure 7 The number of ants counted per umbel on plants without aphids monitored in 20 populations over 6 years (2017 – 2022). This data set has 725 observations in total and excludes observations with zero ants; points are jittered to avoid overplotting.

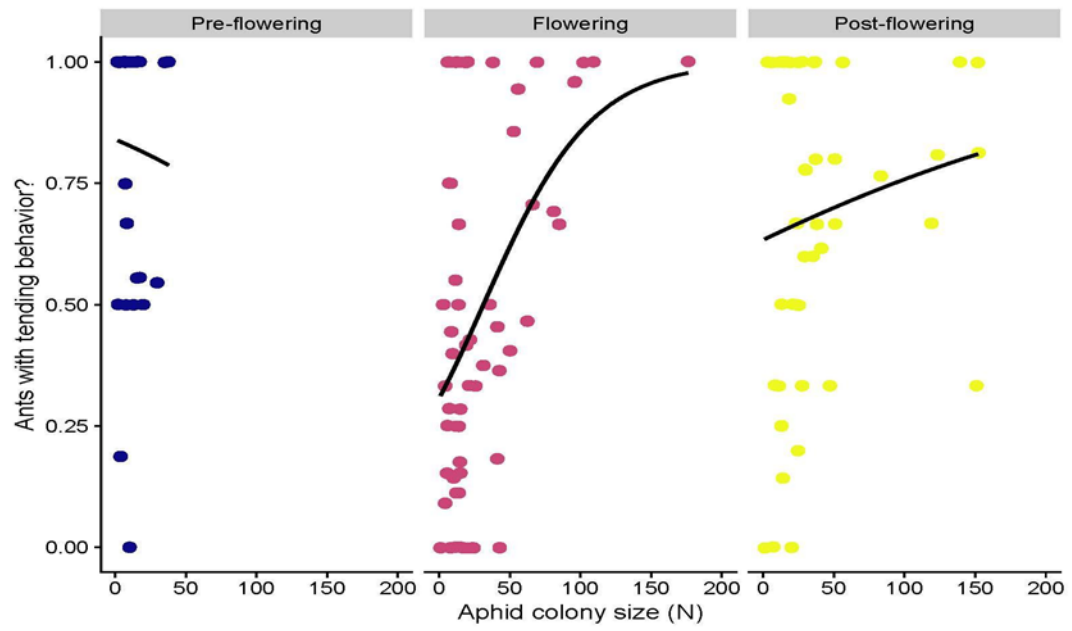


Figure 8 2022 Ant tending behavior across of a range of colony sizes on plants at different stages.

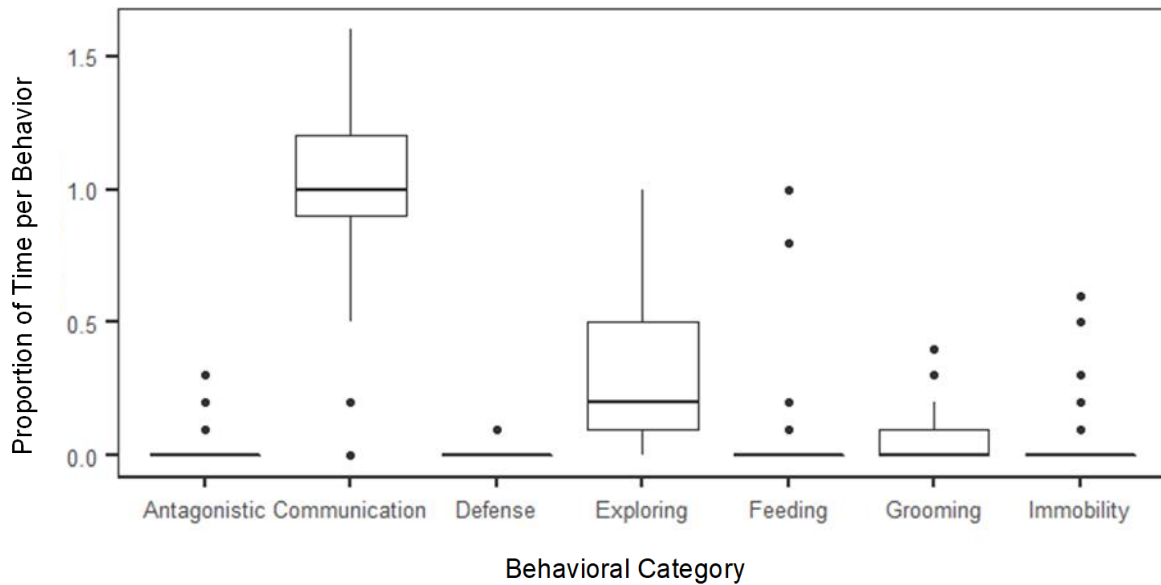


Figure 9 Ants spent the highest proportion of their time communicating, exploring, and grooming. Multiple behaviors were sometimes observed within the same minute, which is why some values are greater than 1.

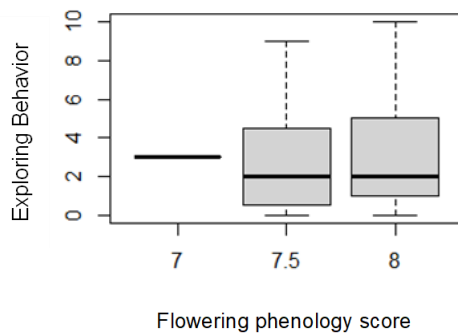


Figure 10(A) Ants were more likely to exhibit exploration behavior during phenological stages 7.5-8.

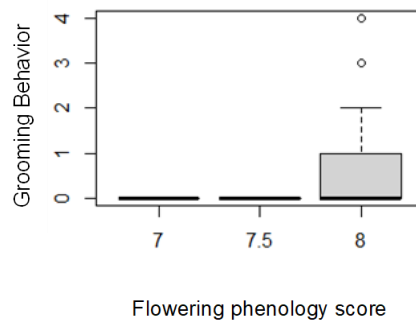


Figure 10(B) Ants exhibited grooming behavior exclusively during phenological stage 8.

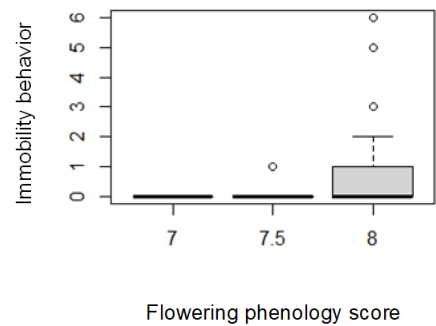


Figure 10(C) Ants exhibited immobility behaviors in more in phenological stages 7.5-8 than stage 7, with most observations occurring during stage 8.