

Does *Aphis asclepiadis* colony size
mediate *Formica rufa* and *Tapinoma*
sessile competition for mutualist aphids
at the Rocky Mountain region?

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Abstract

Mutualisms are the species interaction in which both mutualist partners benefit from the relationship. Most mutualisms involve multiple interacting species and associating with multiple mutualist partners can be beneficial if they provide complementary benefits. Yet, mutualist species may also compete with each other for access to partners, which can alter the benefits the partner receives. Ant-aphid mutualism is a food-for-protection in which aphids produce honeydew to feed ants while ants provide aphids protection against predators. Previous studies of the aphid *Aphis asclepiadis* and two species of ants, *Formica rufa* and *Tapinoma sessile*, show that both ant species provide mutualistic services to aphids. However, aphid colonies tended by *F. rufa* are five-fold larger than colonies that tended by *T. sessile*, and the mechanisms driving this pattern are unclear. In this study, we tested whether aphid colonies tended by *F. rufa* are larger than those tended by *T. sessile* because (1) *F. rufa* is a better mutualist or (2) *F. rufa* competitively excludes *T. sessile* from tending large aphid colonies. We experimentally established large and small colonies of the aphid *Aphis asclepiadis* on the host plant *Ligusticum porteri* in three experimental ant manipulation treatments: open to all ants, *F. rufa* excluded, and all ants excluded. We recorded the number of aphids on the plants and the number and species of ants tending the aphids every three days for three weeks (21 days). Our results did not provide support for either of the two hypotheses. We found that *F. rufa* was not significantly more likely to select large aphid colonies to tend, and *T. sessile* was not significantly more likely to tend aphid colonies when *F. rufa* was excluded. We also found that the benefit *F. rufa* provided for the aphid colonies were more variable across the aphid colony life cycle compared to the benefits provided by *T. sessile*, and the overall benefit the aphid colonies received when they tended by *F. rufa* and *T. sessile* were not significantly different. By studying these interactions, we are one step closer to understand mutualism in multi-species context.

Introduction

Mutualisms are interactions in which two species cooperate with each other to bring benefit for both. Most mutualisms involve both benefits and costs for interacting species (Holland et al., 2002). Furthermore, under different biotic and abiotic conditions (e.g. predator, nutrition, climate, etc.), these benefits and costs can fluctuate (Bronstein, 1994). For example, many plants share part of its photosynthate for mycorrhizal fungi to receive phosphorus from the fungi. However, when extra phosphorus is added into the soil, plants reduce or even eliminate their mycorrhizal fungi to conserve their resources. This suggest that species only willing to pay the cost for mutualism when they are in the need of benefits from their mutualist partners (Bronstein, 1994). However, these interactions can change under the presence of the third mutualist. Yet, how these interactions will change remains unknown.

Historically, mutualisms have typically been studied as species-specific interactions, but most mutualisms instead involve interactions between multiple species. Species may benefit from associating with multiple mutualist partners if those partners provide complementary benefits (Batstone et al., 2018). On the other hand, some species can only maximize the benefit from mutualism if they interact with only one “best” mutualist partner, and some partners may receive mutualistic services but provide little benefit in return. However, competition among

mutualist partners could reduce the benefits that species receive from their partners (Stanton, 2003). Multispecies mutualisms can show very complex ecological dynamics and it is important to understand these complexes to have better understand mutualism interactions.

Ants commonly engage in food-for-protection mutualisms with hemipterans (e.g. aphids) that produce sugar-rich liquids (i.e. honeydew) that ants consume (Styrsky and Eubanks, 2007). In exchange, ants protect aphids from predators including ladybug larvae, wasps, lacewing larvae, syrphid fly larvae, and mites. These interactions could greatly increase the growth of ants and aphid populations while greatly impacting the plant host fitness and the health of the plant community (Styrsky and Eubanks, 2007). Because ant-aphid mutualisms commonly involve interactions between multiple species of ants and aphids (Mooney et al., 2016), these interactions present an ideal system for studying the effects of mutualist diversity on partner fitness.

Previous work done by Annika Nelson near the Rocky Mountain Biological Laboratory shows that two species of mutualist ants, *Formica rufa* and *Tapinoma sessile*, both provide demographic benefits to the aphid *Aphis asclepiadis*. However, naturally occurring aphid colonies tended by *F. rufa* are on average five-fold larger than those tended by *T. sessile* (Nelson et al., unpublished data). Previous data also shows that *F. rufa* is more aggressive and competitively dominant (wins 76% of staged aggression tests) than *T. sessile* (wins 13% of staged aggression tests) (Nelson & Mooney, unpublished data). However, it is unclear whether the aphid colonies tended by *F. rufa* are larger than those tended by *T. sessile* because (1) *F. rufa* is a better mutualist that causes colonies to grow larger or (2) because *F. rufa* competitively excludes *T. sessile* from tending larger, more rewarding aphid colonies. In this study, we experimentally established small and large colonies of the aphid *Aphis asclepiadis* and manipulated the presence of ants to evaluate the mechanisms driving this pattern. Specifically, we tested two hypotheses:

1. We hypothesized that *F. rufa* tends larger aphid colonies than *T. sessile* because it competitively excludes *T. sessile* from tending larger, more rewarding aphid colonies. We predicted that for small and large aphid colonies experimentally established, *F. rufa* will primarily tend large aphid colonies while *T. sessile* will primarily tend small colonies when the plants are opened to all ants. We also predicted that when we experimentally exclude *F. rufa* but not *T. sessile* from small and large aphid colonies, *T. sessile* will tend both small and large aphid colonies in the absence of *F. rufa*.
2. We hypothesized that *F. rufa* tends larger aphid colonies than *T. sessile* because it is a better mutualist and causes *A. asclepiadis* colonies to grow at faster rates. We predicted that for small and large aphid colonies experimentally established on droughted and well-watered plants, aphid colonies tended by *F. rufa* would grow faster than those tended by *T. sessile* regardless of aphid colony starting size or host plant quality.

Method

Site study

We conducted our study in an open meadow near Rocky Mountain Biological Laboratory in Gunnison County, Colorado, USA. The site was on roadside meadow near the Snodgrass trailhead in A. Nelson's approved research sites (-106.963;38.926). (figure 1)

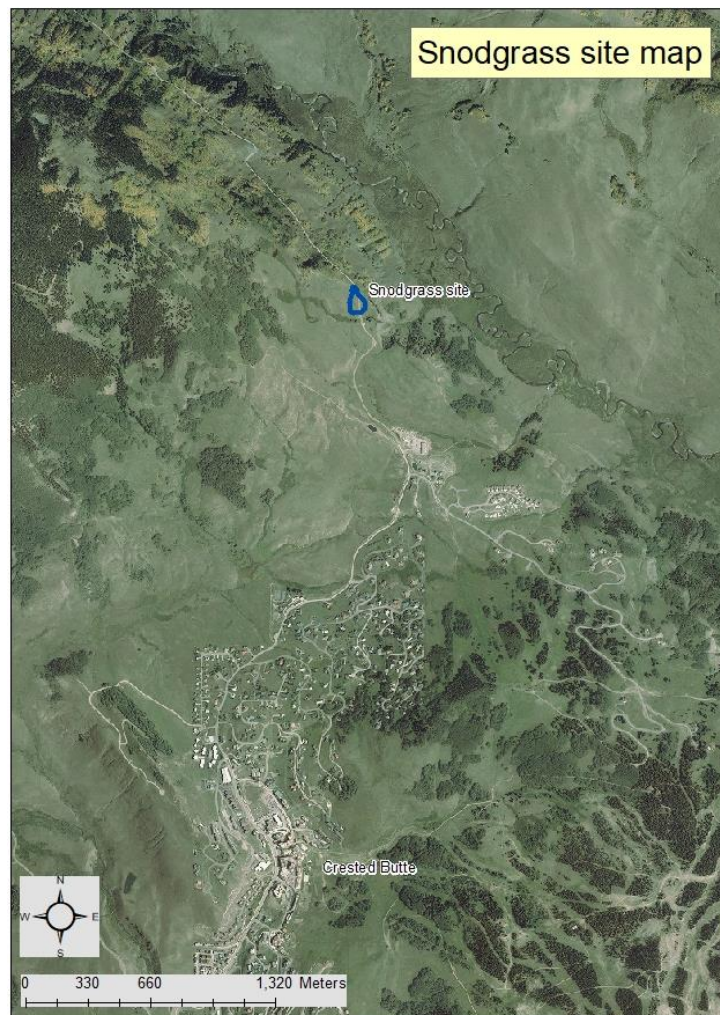


Figure 1: Snodgrass site

Natural history

The aphid *Aphis asclepiadis* (Aphididae) feeds on the flowering stalk and leaf nodes of the plant *Ligusticum porteri* (Apiaceae) (Addicott, 1978a, 1981). *Ligusticum porteri* is an herbaceous plant that is commonly found in open upland meadows throughout the Rocky Mountain region (Langenheim, 1962). The aphids, like many hemipterans, feed on the plant and produce secretion consists of carbohydrate, sugar, amino acid, and water to attract and feed ants (Styrsky and Eubanks, 2006). In return, ants protect *A. asclepiadis* from their natural predators such as ladybird beetles (Coccinellidae), syrphid fly larvae (Syrphidae), Lygus bugs (Miridae) and red spider mites (Acaridae). There are many ants tending *A. asclepiadis* including *Tapinoma sessile*, *Formica rufa* group and *Formica fusca* (Addicott, 1979). However, around the Rocky Mountain region, *F. rufa* and *T. sessile* are the most two common aphid mutualistic partners. *F. rufa* is categorized as extirpators, or dominant, species that will aggressively defend the food resources that belong to them but is very slow in finding resources (Cerdá et al., 2013). On the other hand, *T. sessile* is more subdominant ant that is smaller and less aggressive but are fast in finding food resources (Buczkowski, 2010).

Established aphid colonies

To evaluate our two hypotheses, we experimentally established small (40 aphids $\pm$ 25 aphids) and large (260 aphids $\pm$ 88 aphids) *A. asclepiadis* colonies on *Ligusticum porteri* host plants in multiple ant exclusion treatments. Aphid colonies were established on host plants with similar height, inflorescence number, phenology, and environmental condition. We used mesh bags to wrap around the aphid colonies for the first 3 days in order to promote colony establishment and excluded aphid predators when the colonies was newly established. There were six treatments total to test both of our hypotheses: (1) Small aphid colonies, open to all ants (n =20), (2) Large aphid colonies, open to all ants (n =20), (3) Small aphid colonies, *F. rufa* excluded (n =20), (4) Large aphid colonies, *F. rufa* excluded (n =20), (5) Small aphid colonies, all ants excluded (n=12), and (6) Large aphid colonies, all ants excluded (n=13) (Table 1). For treatments with ants excluded, we applied tape coated with sticky-paste barrier (Tanglefoot Company, Grand Rapids, Michigan, USA) onto the plant stem to stop the ants from accessing aphid colonies. For treatments that exclude *F. rufa* but not *T. sessile*, we did the same thing but put toothpick under the tape to create gaps large enough to *T. sessile* go through. We recorded the number of aphids on the plants and the number and species of ants tending the aphids every three days for three weeks (21 days). We calculated the aphid colony per capita grow rate using the equation $r = \ln(N_t / N_0) / t$, where N_t and N_0 are the final and initial population sizes (respectively) and t is the number of days since the colonies is open to ants (12 days)

Statistical analysis

To test whether *F. rufa* is more likely to select large aphid colonies to tend, we used a generalized linear model with a binomial distribution, with *F. rufa* presence as the dependent variable and aphid colony size as the independent variable. To test whether *F. rufa* competitively excludes *T. sessile* from large aphid colonies, we used a generalized linear model with a binomial distribution, with the presence of *T. sessile* as the dependent variable and aphid colony size, *F. rufa* exclusion treatment, and their interaction as independent variables. To test whether ants enhance aphid colony per capita growth rates, we used a linear model with aphid colony per capita growth as the dependent variable and the presence of ants, aphid colony size, and their interaction as independent variables. Finally, to test whether *F. rufa* is a better mutualist than *T. sessile*, we used a linear model with aphid colony per capita growth as the dependent variable and ant species (*F. rufa* or *T. sessile*), aphid colony size, and their interaction as independent variables.

Treatment	Aphid colonies size	<i>F.rufa</i>	<i>T.sessile</i>	Number of plants
1	Small	Open	Open	20
2	Large	Open	Open	20
3	Small	Exclude	Open	20
4	Large	Exclude	Open	20
5	Small	Exclude	Exclude	12
6	Large	Exclude	Exclude	13

Table 1: Experimental aphid colonies treatments.

Results

Is F. rufa a better competitor than T. sessile for aphid colonies?

The proportion of small aphid colonies tended by *F. rufa* (30%) did not significantly differ from the proportion of big colonies tended by *F. rufa* (20%) ($\chi^2 = 0.536$, $p = 0.464$) (figure 2), indicating that *F. rufa* does not preferentially choose to tend large aphid colonies. We also found that the presence of *T. sessile* at aphid colonies did not depend on aphid colony size ($\chi^2 = 0.254$, $p = 0.614$), whether *F. rufa* was excluded ($\chi^2 = 1.011$, $p = 0.315$), or the interaction between aphid colony size and *F. rufa* exclusion ($\chi^2 = 1.170$, $p = 0.279$), indicating that *F. rufa* does not competitively exclude *T. sessile* from tending aphid colonies. However, on average *T. sessile* tended 15% of big open aphid colonies, 35% of big *F.rufa* exclusion aphid colonies, and 30% of both open small and *F. rufa* small exclusion aphid colonies (figure 3).

Do ants increase aphid colony per capita growth rates?

Overall, aphid colony per capita growth rate depended on a significant interaction between the presence of ants (for *F. rufa* and *T. sessile* combined) and aphid colony size ($\chi^2 = 3.915$, $p = 0.047$), with ants providing benefits to small but not large aphid colonies. There was also a significant main effect of size ($\chi^2 = 18.496$, $p < 0.001$), where small aphid colonies grew

faster than large colonies, but there was no significant main effect of ant presence on aphid colony growth rate ($\chi^2 = 1.939$, $p = 0.163$) (figure 4).

Is F. rufa a better mutualist than T. sessile for aphids?

There was a marginally significant interaction between the species of tending ant (*F. rufa* versus *T. sessile*) and aphid colony size on aphid colony per capita growth rate ($\chi^2 = 3.308$, $p = 0.069$), with the different in grow rate of small and big aphid colonies tended by *F. rufa* was significantly larger than small and big aphid colonies tended by *T. sessile*. There was also a significant main effect of colony size ($\chi^2 = 21.866$, $p < 0.001$), with small aphid colonies growing faster than large colonies. However, there was no significant effect of ant species on aphid colony per capita growth ($\chi^2 = 0.107$, $p = 0.743$) (figure 5).

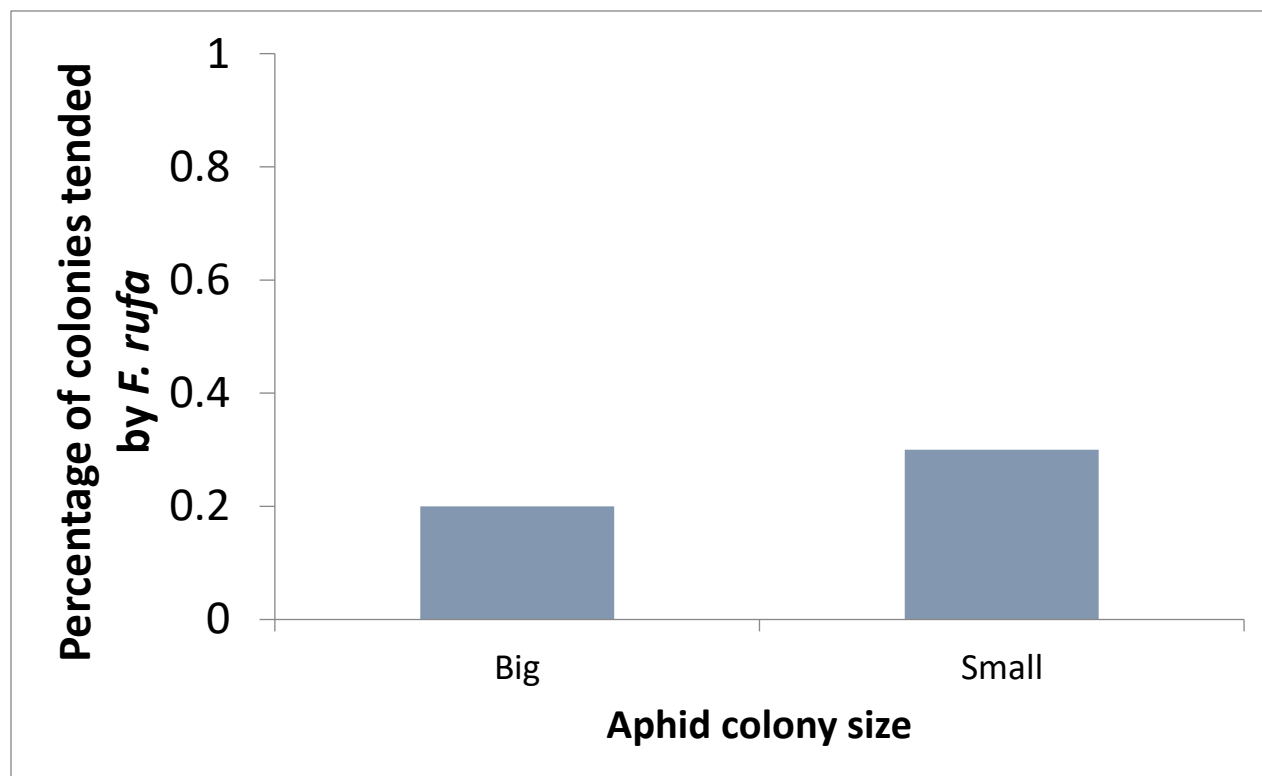


Figure 2: Percent aphid colonies tended by *F. rufa* base on size

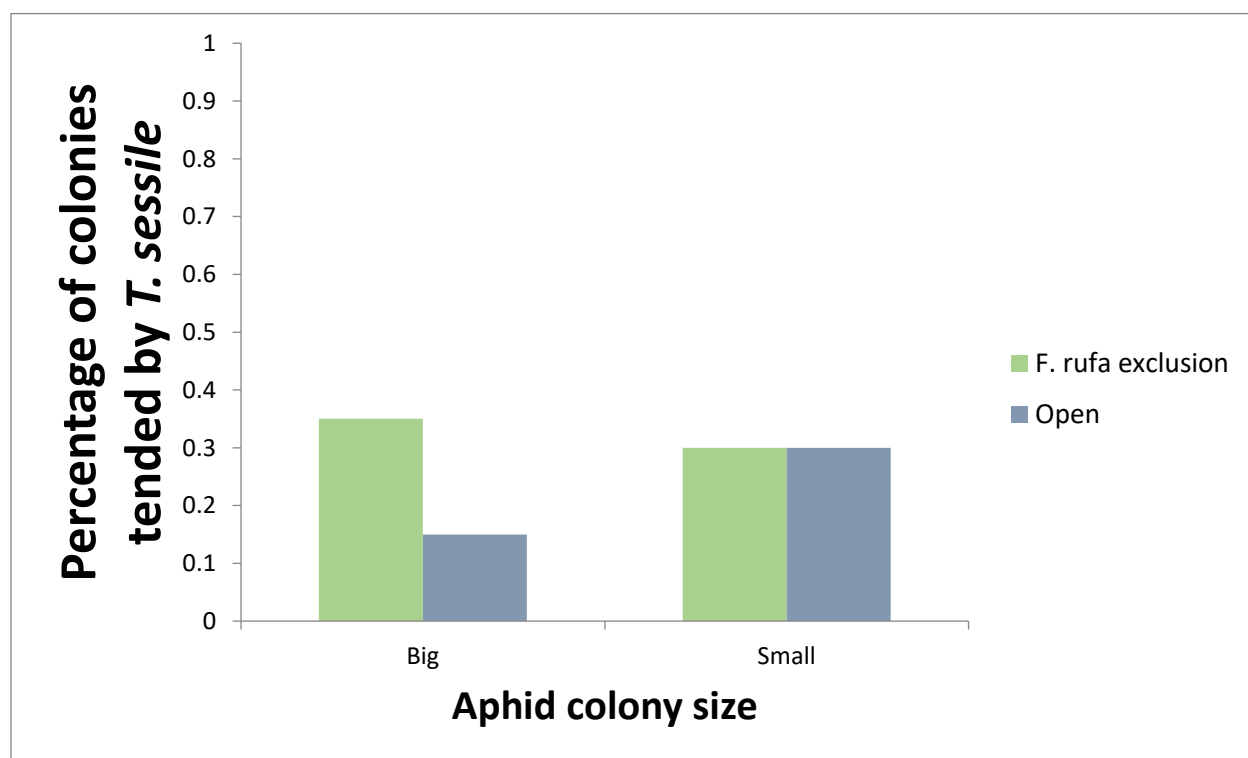


Figure 3: Percent aphid colonies tended by *T. sessile* base on size

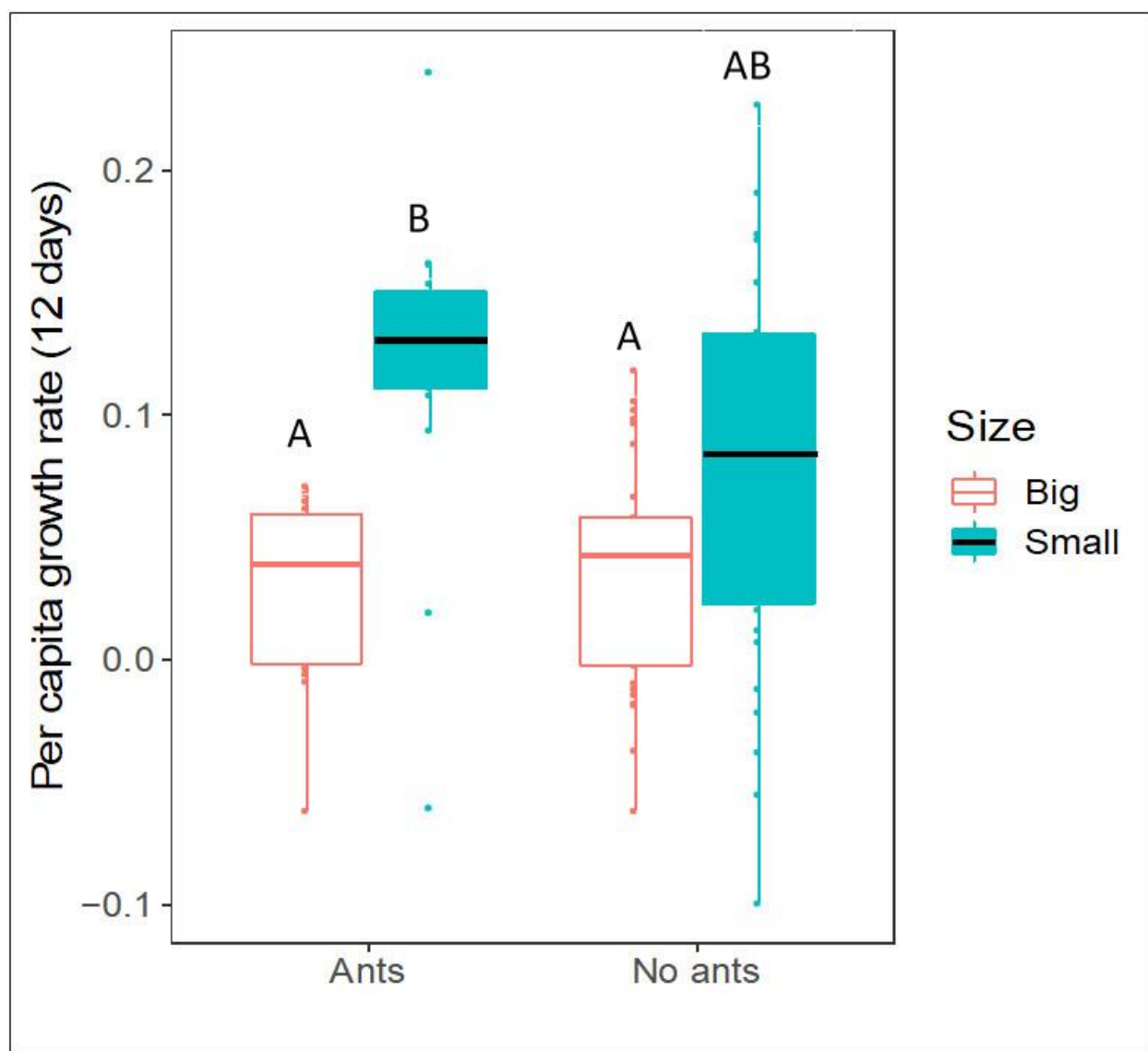


Figure 4: Percent capita growth rate of big and small aphid colonies under where there was no ants and when there was ant

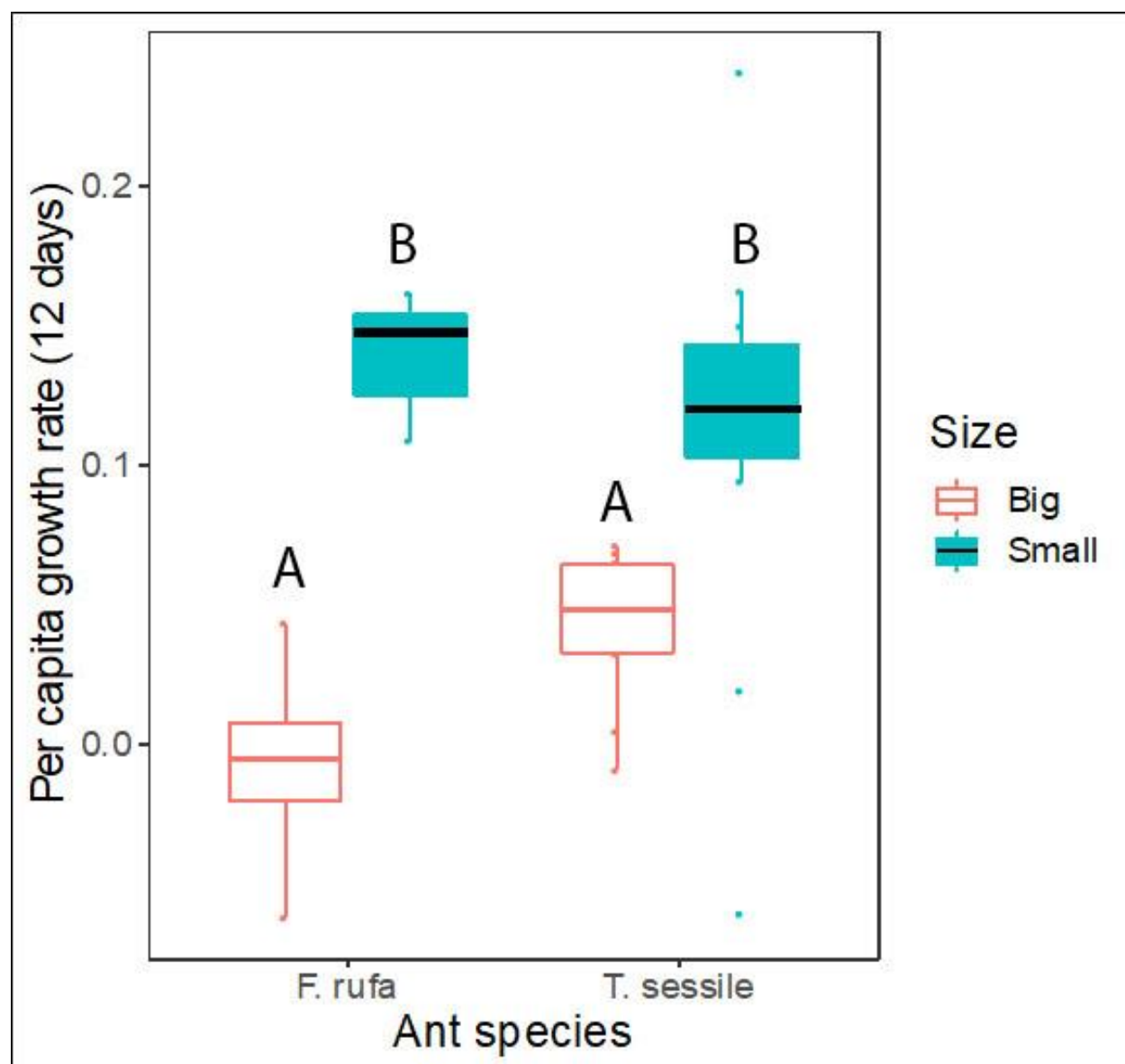


Figure 5: Percent capita growth rate of big and small aphid colonies tended by *F. rufa* and *T. sessile*.

Discussion

Aphid colonies tended by *F. rufa* are on average five-fold larger than those tended by *T. sessile* (Nelson et al., unpublished data). To determine which mechanisms drive this pattern, we tested two non-mutually exclusive hypotheses: (1) whether *F. rufa* competitively excludes *T. sessile* from tending large aphid colonies, or (2) whether *F. rufa* is a better mutualistic partner than *T. sessile* and causes aphid colonies to grow larger. However, we did not find conclusive evidence in support of either hypothesis. We had predicted that if *F. rufa* competitively excludes *T. sessile* from tending large aphid colonies, *F. rufa* would tend more large than small aphid colonies that were open to all ants, and *T. sessile* would tend more aphid colonies when *F. rufa* was excluded. However, we found that *F. rufa* was not significantly more likely to tend large aphid colonies, and *T. sessile* was not significantly more likely to tend aphid colonies when *F. rufa* was excluded. Moreover, we had predicted that if *F. rufa* is a better mutualist than *T. sessile*, aphid colonies that were tended by *F. rufa* would have a faster per capita growth rate than aphid colonies tended by *T. sessile*. However, we found that although the benefit *F. rufa* provided for the aphid colonies were more variable along the colonies' life cycle compare to those tended by *T. sessile*, the overall benefit that the aphid colonies received when they tended by *F. rufa* and *T. sessile* were not significantly different. The small colonies seemed to receive more benefit compare to the big colonies. Thus, additional studies are needed to determine why aphid colonies tended by *F. rufa* are five-fold larger than those tended by *T. sessile*.

Aphid colonies tended by *F. rufa* larger than colonies tended by *T. sessile* was not because *F. rufa* preferentially choose larger aphid colonies to tend since there was no significant difference in the number of small aphid colonies tended by *F. rufa* and the number of large aphid colonies tended by *F. rufa*. This may because *F. rufa* is a slow resource discover species, and it will tend and defend whichever aphid colonies they found (Cerdá et al. 2013; Nelson A, unpublised data). The presence of *T. sessile* at aphid colonies also did not depend on aphid colony size, *F. rufa* exclusion, or the interaction between size and *F. rufa* exclusion. This result was very surprising since *F. rufa* is a more competitively dominant ant that has previously shown suppress the presence of *T. sessile*, the subdominant ants, on aphid colonies (Cerdá et al. 2013, Buczkowski 2010). However, again, the rate at which *F. rufa* discover resource is also much slower than *T. sessile*, which means those aphid colonies where *F. rufa* never found have a high chance to be discovered by *T. sessile*. Also, even though there was no significant evidence to show that of *F. rufa* exclude *T. sessile* from tending aphid in our experiment, there was a pattern that there were more aphid colonies tended by *T. sessile* when *F. rufa* was excluded. We think that this was caused because not many established colonies were discovered by ants (30% small and 20% big aphid colonies discovered by *F. rufa*; 30% small and 25% big aphid colonies discovered by *T. sessile*, so the number of colonies tending by ants and colonies not tending by ants were not ideal. With a larger sample size, it is possible that these differences would be significant.

When we assessed how ants affect aphid colony growth rate, aphid colony per capita growth rate depended significantly on the interaction between the presence of ants and aphid colony size. The difference in the growth rate of small and big colonies when ant presence is

significantly bigger than no ant presence. Therefore, ants seemed to provide benefit to small aphid colonies but not large colonies. It had been documented that ants provide benefit to the aphid, and the exclusion of ants will significantly increase extinction rate of aphid colonies (Hosseini et al., 2017; Yao et al., 2000). Yet, the presence of ant seems to demographically benefit the aphid colonies when it was in its early stage. This is because smaller aphid colonies may easier to look after and defend than larger aphid colonies. In smaller aphid colony, each ant tending the colonies looks after less aphid than in bigger aphid colony. With less number of aphids to take care of, ants could provide more attention and protection to the aphids from their predators. There was also a significant difference in the grow rate of small and large aphid colonies. Smaller colonies seem to grow significantly faster than bigger colonies. This maybe because bigger aphid colonies attract more predators (Turchin and Kareiva, 1989). So, while the number of aphids that can reproduce is higher in bigger in large aphid colonies, the death rate also increases. However, overall, there were not a significant difference in benefit that aphid colonies tended by ants receive compare with those not tended by ants. This may be because not many established colonies were discovered by ants, so the number of colonies tending by ants and colonies not tending by ants were not ideal. Also, when we excluded ants with Tanglefoot, we also excluded red spider mites (*Tetranychus urticae*), one of the common aphid predators at the Rocky Mountain region (Sandrow, 2012). This may increase the growth rate of aphid colonies since one of its predators is also excluded with the ants.

The effect of *F. rufa* and *T. sessile* on aphid growth rate were also analyzed. There was a marginally significant interaction between ant species and aphid colonies size on aphid colonies per capita grow rate. Which mean there were more variation in the benefit small aphid colonies received compare to large aphid colonies when tended by *F. rufa* than the benefit *T. sessile* provided for the aphid colonies. Between *F. rufa* and *T. sessile*, *F. rufa* was proved to be the more aggressive one (Cerdá et al., 2013). Even though there were no significant different, *F. rufa* seems to provide more benefit to early staged aphid colonies than *T. sessile*. This could be because *F. rufa* is the more aggressive ant species that is better in defending its resources compare to other ants, and smaller aphid colonies are easier to keep track than bigger aphid colonies. With the combination of both, in protecting smaller aphid colonies, *F. rufa* performed a better job than *T. sessile*. This could be one possible explanation why colonies tended by *F. rufa* are bigger than colonies tended by *T. sessile*. *F. rufa* may be a better mutualist for small aphid colonies, which could cause them to grow larger at a faster rate. Yet, there were no different in the benefit both ants provide for the aphid. This may be because even though *F. rufa* is a more aggressive ant that is better at attacking predators, they usually have less individual tending aphid while *T. sessile* have more foragers tending at their aphid colonies (Fellers, 1987). Having less ants tending means that each individual has to look after a wider area, which could reduce their ability to protect every single aphid. On the other hand, *T. sessile*, with more ants tending on their colonies could be more efficient in finding aphid predators even though they are less effective in attacking compare to *F. rufa* since they primary use chemical defend rather than mechanical defend (Fellers, 1987). Therefore, the benefit that aphid colonies receive from *F. rufa* and *T. sessile* were not significantly different.

While studying species-specific mutualism can give information about how two species interact, it could not give prediction on how a third species can affect the interaction and other interesting ecological patterns. This is because species may differ in their effectiveness as mutualist partners and because species may compete for access to mutualists, it is important to study mutualisms in a multi-species context to understand these interactions at a larger view. Although aphid colonies tended by *F. rufa* are larger than those tended by *T. sessile*, it is unclear whether this pattern occurs because *F. rufa* is a better mutualist or because *F. rufa* competitively excludes *T. sessile*. Future studies should look more at the number of aphid colonies tended by *T. sessile* in open and *F. rufa* exclusion treatment in a larger time scale. Also, to determine the mechanisms driving this pattern, future studies should look into the how much benefits that each ant species can provide for the aphid colonies, and what is the maximum number of aphid that each individual of each ant species can efficiently protect.

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