

The Effect of Ant Tending on the Fitness of Aphids During and After Colony
Establishment

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Abstract:

Mutualisms are mutually exploitative relationships in which the benefits to both partners outweigh the costs. Mutualisms are expected to exist in a fragile balance, and these interactions may vary, both across their geographic range and temporally.

Valeriana edulis, a dioecious perennial, is host to an ant-aphid interaction that previous work has found to be deleterious to established colonies of the aphid, *Aphis valerianae*. Ant exclusion experiments and observations were performed on artificially established colonies at two sites to determine if the effect of ants was different for new or established colonies, or if it varied spatially or by plant sex. Contrary to our hypothesis, we found that ants had a negative effect on both new and established aphid colonies at both sites, and that there was no density-dependant effect on per capita aphid growth. Aphids performed better on female plants at one site, but there was no sex bias at the other site. Observational studies showed aphids preferred to colonize female plants at one site, but had no sex bias at the other.

Introduction:

Interactions between ant and aphids range from obligate or facilitative mutualisms to outright exploitation (Billick et al., 2007; Stadler and Dixon, 2005). Individually, each of these organisms is of great ecological and economic importance: Ants are one of the most abundant insects (Talbot, 1975), and aphids can be serious pests on wild and crop plants. Mutualistic relationships between these two arthropods have the potential to significantly affect ecosystems: ants have important effects on the ecosystems they inhabit, and aphids can have significant negative impact on their host plants (Stryksy and Eubanks, 2007).

Mutualisms are positive (+/+) interactions. They appear simple, but under closer inspection they are much more complicated than they seem. They are not altruistic, rather they are mutual exploitation: interactions in which both partners receive a net benefit (Bronstein, 1994). It should be expected that mutualisms are fragile: higher fitness might be achieved outside the mutualism or by exploiting the other partner (Sachs and Simms, 2006). The outcome of interactions between species can vary spatially or temporally. A good example of this is yucca moths, which pollinate yucca plants. However, their larvae also eat yucca seeds. In certain areas, this interaction is a mutualism, in others, it is parasitism (Thompson, 1988). Similar patterns have been found in ant-aphid interactions. (Billick et al., 2007, Stadler and Dixon, 2005)

Ant-aphid mutualisms are a type of protection mutualism, in which protection is provided in return for a service. More specifically, aphids feed on the phloem of plants and produce honeydew, a modified sugary byproduct. Ants then feed on honeydew, and in exchange protect the aphids from predators (Way, 1963). This system provides an easy source of carbohydrates for ants at minimal costs. However, ant attendance is costly for the aphids: despite the protection benefits, ants can hinder the reproductive success of aphids (Telegaar et al., 2011; Petry et al., in review); may increase parasitism by parasitoid wasps (Telegaar et al., 2011; Völkl, 1992); and promote costly behavior, such as the modification of honeydew, and increased rate of honeydew production which is not optimal for digestion (Yao et al., 2000; Stadler and Dixon, 2005).

Thus, the effect of ants on aphids is the net balance of costs and benefits. The net effect is generally positive. It is expected that if the net effect were negative, the aphids would escape the exploitive relationship. There is some evidence that this is true: ant-

aphid mutualisms are highly labile. Aphids are capable of leaving the mutualism when they become costly (Shingleton and Stern, 2002). Systems in which this does not appear to hold, (Petry et al, in review) are therefore of particular interest for studying the evolution of mutualisms and their ecological consequences. *Valeriana edulis* is a long-lived, dioecious perennial, which is host to an ant-aphid interaction between several species of ant (*Formica* sp. and *Tapinoma sessile*) and the aphid *Aphis valerianae*. Previous work on this system has demonstrated that the costs of ant attendance to *A. valerianae* outweigh the benefits for established aphid populations; though the ants protect aphids, reducing predation, they also cause a significant decrease in population growth (Petry et al., in review).

As seen in other potentially mutualistic systems, species interactions may be context dependent in this system (Bronstein, 1994), and thus provide a refuge allowing the persistence of ant attendance. Due to differences in allocation of herbivore defense between plant sexes (Cornelissen and Stiling, 2005), there may be different costs and benefits of ant attendance on male and female *Valeriana edulis*. Alternatively, the interaction may be mutually beneficial in certain locations, but not others (Thompson, 2005). Perhaps the outcome is temporally variable: that is, the interaction is mutualistic at a certain point in the aphid colony's existence, but not others. This is a particularly appealing explanation, as it would provide a benefit to all aphids in this system, rather than just a few in specific locations. During the initial colonization, one or more winged aphids land on a plant. The winged aphid proceeds to produce clones that colonize the plant. The emerging colony is extremely vulnerable to predation during these initial stages of colonization, the loss of a single aphid greatly decreases the colony's chances of

survival (Cushman and Whitham, 1989; Stadler and Dixon, 2005). In contrast, established colonies vary in size from 15-100 adults; the loss of a single aphid from an establish colony is not disastrous. A density dependant effect of ant attendance has been observed in membracids, (Billick and Tunkel, 2003; Cushman and Whitham, 1989) so it seems likely that a similar effect could be found in the *V. edulis* system.

Our experiment attempted to answer several questions. Do ants provide a fitness benefit that aid aphids during the establishment of new colonies? Is there any spatial variation in the effect of ants on aphid fitness? Does plant sex have any effect on the interaction outcome?

Methods:

Study Site:

Two test sites were utilized in this project in the vicinity of Crested Butte, Colorado: one near Brush Creek, the other near the Judd Falls trailhead. The Brush Creek site is east of the town of Crested Butte. The elevation of this site is 2780 meters. The Judd Falls site is approximately 1 km NW of Gothic, CO, and at approximately 2900 meters of elevation. Both sites were montane meadows dominated by herbaceous flowering perennials (Langenheim, 1962). Judd Falls had noticeably more grassy species, while Brush Creek had several woody shrubs. The same experiment was conducted at both sites. The experiment was started earlier at the Brush Creek site, due to the earlier start of the growing season there. The experiment was set up at the Brush Creek site during the weeks of July 3rd and 10th 2011; data collection began on the 13th. The experiment was set up at the Judd falls site on the week of July 17th; data collection began on the 26th.

Experimental Design:

At each site, 16 male and 16 female *Valeriana edulis*, grouped spatially into sixteen same-sex pairs, were selected using the following criteria: presence of ants, at least two flower stalks, and close proximity to other *V. edulis* (to control for spatial variation and ant abundance). Two inflorescence stalks from each experimental plant were selected for use. At the Judd falls site, the inflorescence stalks were also wrapped with wire to prevent herbivory by deer. At Brush Creek this was done during the second week of observations. Concurrently in each field, the inflorescence stalks of several *V. edulis* hosting aphid colonies were isolated with mesh bags to prevent predation. These plants were ‘source plants’ which were later used to stock the experimental plants.

Plants from each pair were assigned at random to either the ‘established’ or ‘unestablished’ treatment group. On each of the experimental plants, ants were excluded from tending aphids on the “exclusion” inflorescence stalk with a Tanglefoot barrier (Tanglefoot Company, Grand Rapids, Michigan). A parafilm (SPI Supplies, West Chester, Pennsylvania) barrier was utilized to protect the plant from the Tanglefoot. The experimental plants were stocked with aphids from the source plants. Each pair of *V. edulis* utilized a single source plant to control for genotypic variation among the aphids.

Initially, twenty aphids were placed on the two inflorescence stalks of the ‘established’ treatment group to mimic an established aphid colony. Experimental plants were stocked with a fixed density of aphids: a single aphid was placed on the two inflorescence stalks of the ‘unestablished’ treatment group, and twenty aphids were placed on the inflorescence stalks of the ‘established’ treatment group. Due to variation in aphid acceptance of new host plants, the plants were restocked, but still varied in initial

density in the 'established' treatment group. There was an initial density of 7-20 adults at Brush Creek and 9-18 adults at Judd Falls. (Mean: 15.3 adults at Brush Creek, 12.7 at Judd Falls. Mean total aphids [nymphs+adults]: 25.9 and 25.8, respectively)

Observations of aphid population growth, natural enemy abundance, and ant activity, were made of each treatment stalk every two days over three weeks: at Brush Creek from July 20th to August 4th; at Judd Falls from July 26th to August 9th. At each site, establishment data was also recorded. Several plants, approximately equal number male and female, were marked at each site and monitored regularly for aphid establishment to determine the effect of plant sex on aphid establishment.

Statistical Analysis:

Per capita aphid population growth was calculated as $\ln(x_2) - \ln(x_1)$, where x_1 is the initial density and x_2 is the final density. An ANOVA was used to analyze the effect of ant attendance, plant sex and aphid density and their interaction on per capita growth. A correlation was used to analyze the effect of the number of ants and predators observed on per capita aphid growth. For the establishment data, a randomization analysis of variance was used to determine the effect of plant sex on aphid establishment.

Results:

A model was created for each site. Insignificant interactions were removed from the full model, resulting in a model with presence of ants, plant sex, aphid density, and a blocking term as factors for both sites. The blocking term was not significant at either site ($p > 0.3519$).

At Brush Creek, only the presence of ants had a significant effect on aphid population growth ($F=11.067$ $p=0.0016$, Figure 1); the presence of ants had a negative

effect on per capita population growth. Plant sex was marginally significant ($F=2.927$ $p=0.0980$), with aphids performing better on female plants. Aphid density had no significant effect on per-capita aphid population growth ($F=1.426$ $p=0.2564$, Figure 2).

Analysis of the Judd Falls data showed that the presence of ants had a significant negative effect on aphid population growth ($F=7.058$ $p=0.0077$, Figure 3). Plant sex ($F=1.918$ $p=0.1548$) and aphid density ($F=0.141$ $p=0.6967$, Figure 4) had no significant effect on per-capita aphid population growth.

Analysis of the establishment data from Brush Creek demonstrated that plant sex had a marginally significant effect on aphid establishment ($F=3.085$ $p=0.0774$, Figure 5), with aphids preferring female plants. At Judd Falls, plant sex had no significant effect ($F=1.8645$, $p=0.1427$, Figure 6).

Correlation tests on the effect of number of predators and ants observed indicated that the number of predators had a marginally significant negative effect on aphid population growth at Brush Creek ($t=-1.8075$, $p=0.0756$). At Judd Falls, the number of ants had a marginally significant negative effect on aphid population growth ($t=2.0065$, $p=0.0538$). Ants did not have a significant effect on predator numbers at either site ($p>0.78$).

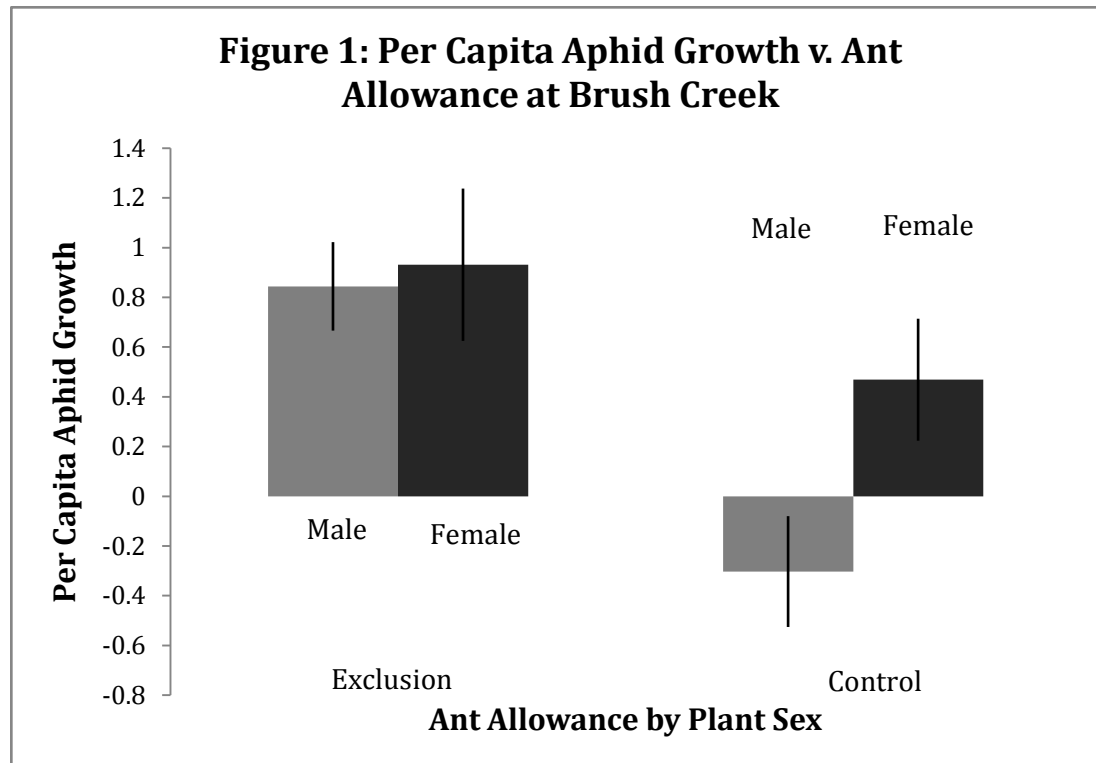


Figure 1: Per Capita Aphid Growth [$\ln(x_2) - \ln(x_1)$] by ant exclusion and inclusion (control) on male and female plants at Brush Creek.

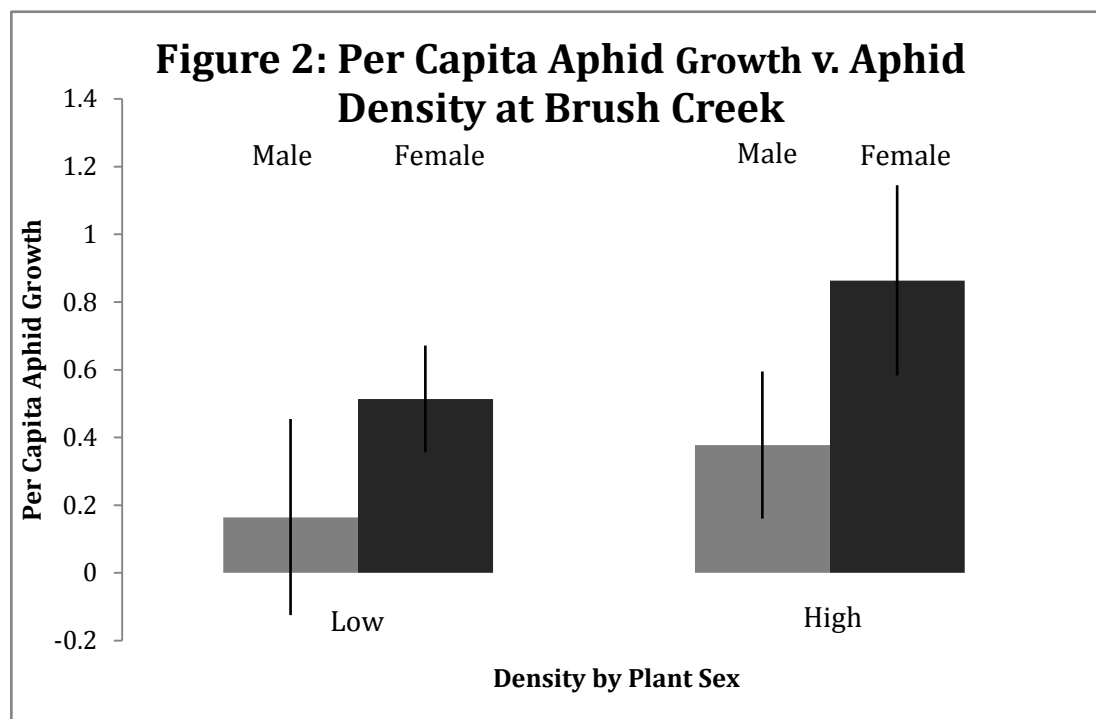


Figure 2: Per Capita Aphid Growth by initial aphid density (high or low) on male and female plants at Brush Creek.

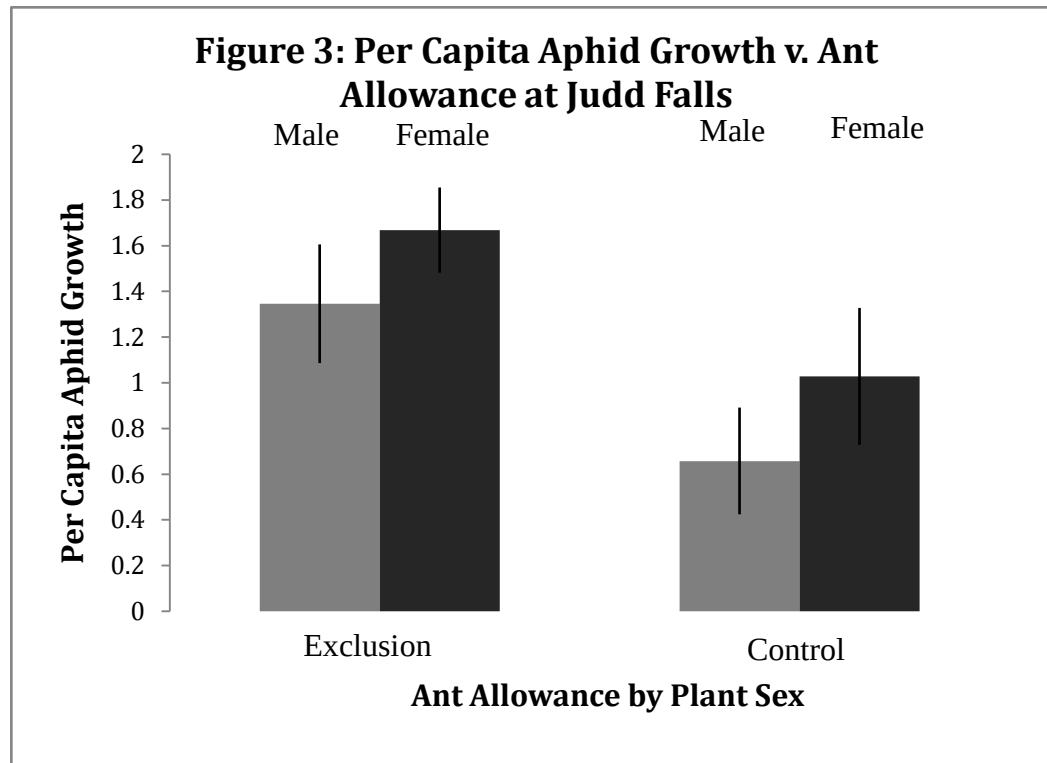


Figure 3: Per Capita Aphid Growth by ant exclusion and inclusion on male and female plants at Judd Falls.

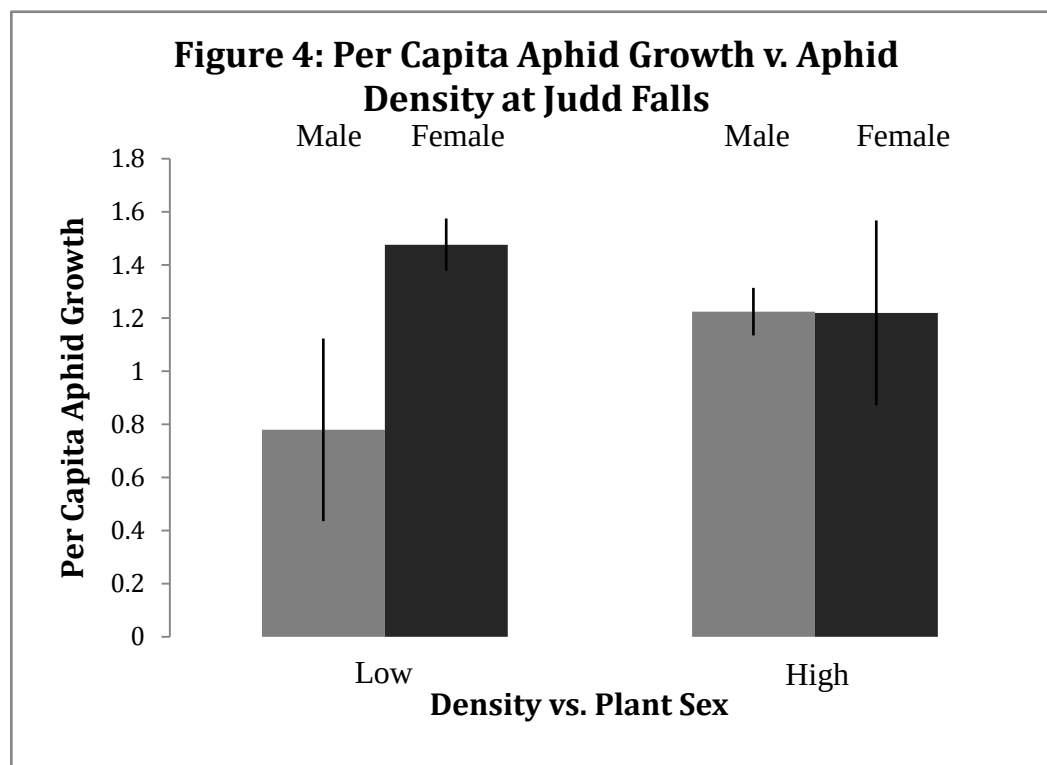


Figure 4: Per Capita Aphid Growth by initial aphid density on male and female plants at Judd Falls.

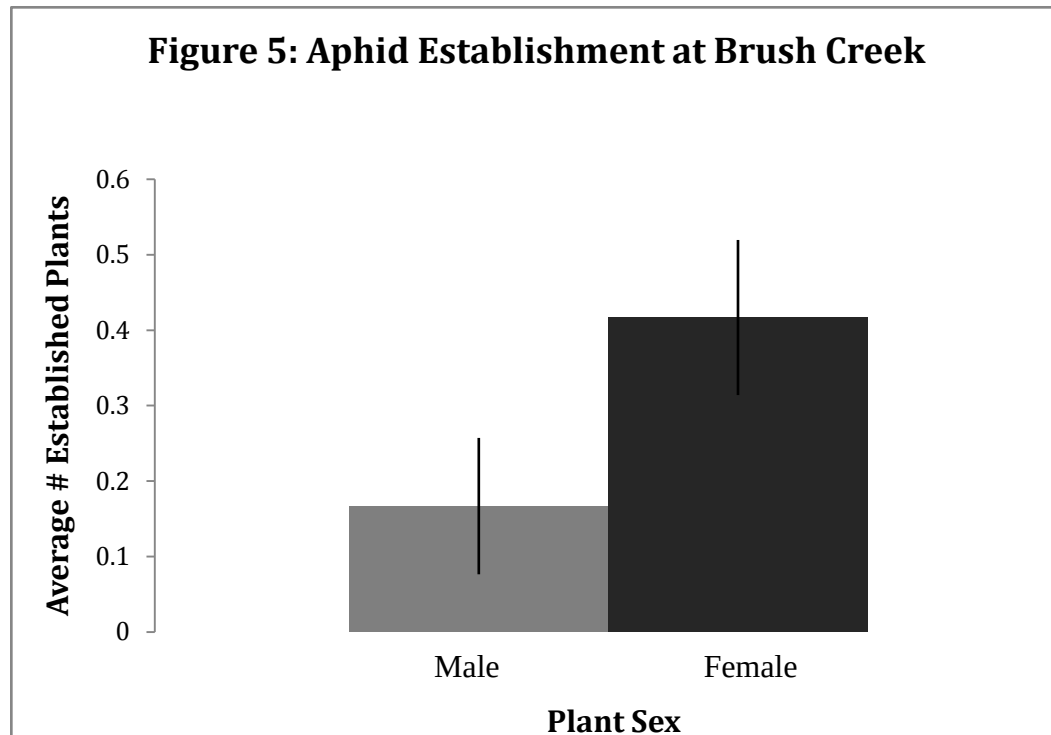


Figure 5: Aphid establishment by plant sex at Brush Creek.

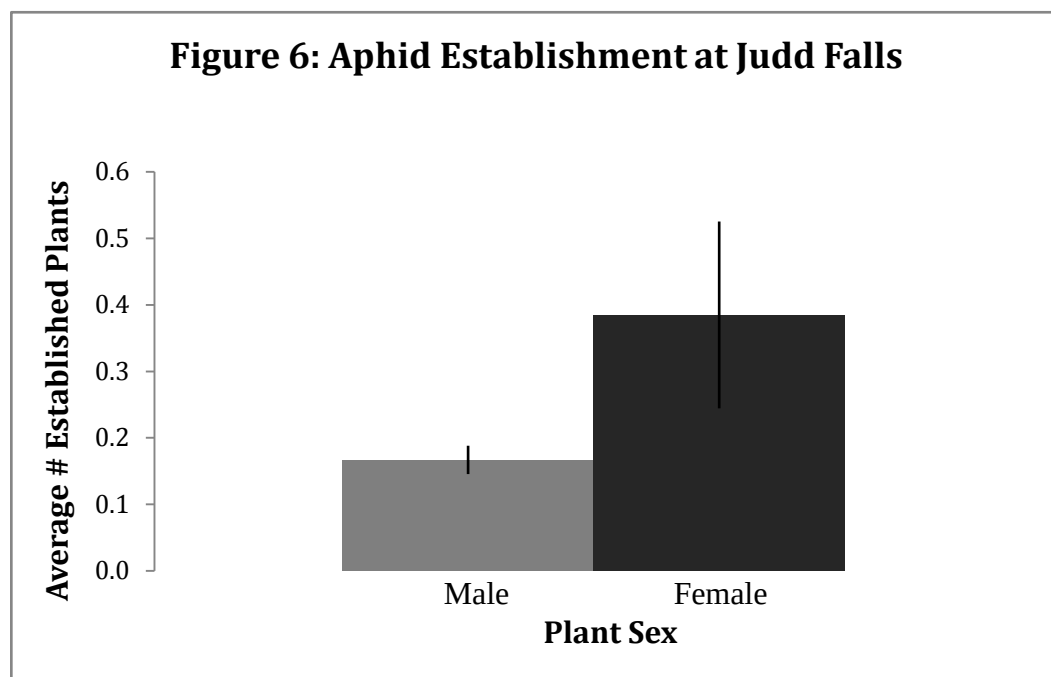


Figure 6: Aphid establishment by plant sex at Judd Falls.

Discussion:

Our results demonstrate that presence of ants has a significant negative effect on aphid population, regardless of initial aphid density, plant sex has marginal to no effect, and aphid density has no effect on per-capita aphid population growth. This lends partial support to the results of previous studies in the *Valeriana edulis* system. Previously, Lopez and Mooney determined that ants had a negative affect on aphid population growth at the Judd Falls site (Lopez and Mooney, 2010). It has been show in laboratory studies with no predators and field studies during low predation years that ants have a negative effect on aphids (Stadler and Dixon, 1998; Cushman and Whitham, 1989), and it is generally agreed that the main benefit to aphids is anti-predator (Stadler and Dixon, 2005; Herbert and Horn, 2008). Very few predators were observed at the Judd Falls site, and the presence of ants did not have any effect on the number of predators. This suggests that low predator numbers and the failure of ants to protect aphids are responsible for the negative effect of ants: the costs to the aphids outweigh the benefits.

It should be noted that ants, although present, were rarely observed on the experimental plants at Brush Creek, and that initial data analysis showed no significant effect of ants on aphid growth during the first two weeks of data collection. A sharp drop in aphid numbers on the ant inclusion plants was noted the third week. This suggests a change in ant behavior, perhaps a shift to predation, or the arrival of a new, unknown predator.

In a meta-study Cornelissen and Stiling (2005) found a tendency towards male-biased herbivory (though they noted cases of female-biased and un-biased herbivory), due to different resource allocation between plant sexes. Perry and Mooney (2009)

discovered that the sex of a plant has no effect on aphid predation or survival, however, they also found female-biased herbivory. This seems somewhat consistent with our results at Judd Falls. However at Brush Creek, aphids performed better on female plants. It is unknown why this is the case; perhaps it is due to differing herbivore defense between the sexes, or greater resource allocation to the inflorescence stalk for seed production.

Aphids had a preference for female plants during colony establishment at Brush Creek. This substantiates the findings of Perry and Mooney (2009), who found that aphids colonized female plants more often, although they found a stronger correlation. There was no significant bias at Judd Falls, where Perry and Mooney previously worked, suggesting temporal variation in aphid colonization.

In 2010, Lopez and Mooney found that per capita population growth was affected by population density, which they postulated was due to a combination of intra-specific competition and predator recruitment. However, our study found that population density had no significant effect on population growth. Lopez and Mooney's initial aphid densities varied from 20-1990 aphids. This suggests that at concentrations lower than twenty aphids, aphid density has no effect on population growth. This is expected: at these densities, a single inflorescence stalk is an abundant resource, and therefore we would expect to see little intra-specific competition for resources.

Conclusion:

Our hypothesis that ants would provide an initial fitness benefit to establishing aphid colonies was unsupported. Likewise, there was no spatial or geographic variance in the effect of ants. Plant sex did not have a significant effect on the ant-aphid interaction

outcome, supporting previous results. However, in observational establishment studies, aphids preferred female plants, suggesting that plant sex is a factor. Given our results, it appears that aphids are in an exploitative relationship in the *Valeriana edulis* system. Further research is required to determine if this is the case, as well as the exact effect of plant sex on this interaction.

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