

“What’s For Lunch: Deciphering Ant Omnivory on Lupine”

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Abstract

True omnivores are animals which feed on both plant and prey sources, allowing them to effect multiple trophic levels within a food web. Ants are well-known omnivores and many participate in honeydew mutualisms with aphids. The western thatching ant, *Formica obscuripe*, tends the mutualist aphid *Aphis lupine* and will directly feed on the stems and inflorescences of the aphids' host plant, lupine, *Lupinus* spp. To understand the net effect of the thatching ant on lupine I looked at the ants effects on herbivores, aphids, and predators on the lupine, along with direct ant damage to the plant. I used a 2X2 factorial experiment crossing the observational factor, lupine near or far from ants with the manipulative factor, the presence or absence of aphids. Close proximity to thatching ants was associated with a significantly greater abundance of aphids, all ant species combined, the number of lupine flowers with chewing damage at the beginning of the experiment, the abundance of both other phloem feeding and chewing herbivores, and the average number of mature seeds and seed weight per inflorescence. Ants were also associated with significantly decreased aphid predator abundance on lupine. A structural equation model revealed that the net effect of thatching ants on lupine is positive, with the number of mature lupine seeds increasing in the presence of ants, and with the ants direct effect on the lupine having the greatest overall effect on the plant. Possible explanations for this outcome include differences in microclimate between observation locations. Further studies should be conducted to determine if microhabitat or other unknown factors are responsible for the positive correlation between proximity to thatching ants and lupine fitness.

Key Word

The western thatching ant, *Formica obscuripes*, structural equation model, ant omnivory, *Lupinus* spp., aphid manipulation, *Aphis lupine*, honeydew mutualism

Introduction

True omnivores are animals which feed on both plant and prey sources (Coll and Guershon 2002). Because omnivores feed on different trophic levels they have a variety of both direct and indirect effects at multiple trophic levels within food webs (Coll and Guershon 2002). While exclusion studies can evaluate the net effect of an omnivore's varied interactions, understanding the strengths of the individual interactions contributing to the net effect can be more difficult, but is necessary for identifying the interactions most important for determining the outcome of the net effect (Coll and Guershon 2002).

Ants are widely recognized as omnivores because they consume food resources from multiple trophic levels, which can directly and indirectly affect primary producers (Figure 1). Some ants directly consume plant parts, particularly in ant/plant mutualisms (Coll and Guershon 2002), but ants can affect plants through many indirect pathways. Some ants participate in a mutualism with herbivorous aphids while also feeding directly on both herbivores and arthropod predators (Mooney and Tillberg 2005). In this mutualism the phloem feeding aphids produce a sugary waste product called honeydew which the ants eat, and in return the ants provide protection from predators (Styrsky and Eubanks 2006). This type of mutualism is frequently referred to as a food-for-service mutualism (Abbot 2008). Though aphid feeding is detrimental to the plant, the majority of studies have found that the mutualistic interaction between the aphids and ants is beneficial to the host plant because the ants eat and harass other herbivores which are more harmful to the host plant (Styrsky and Eubanks 2006).

Ants that subsist on a carbohydrate rich and amino acid poor main diet, such as ants that feed on extra floral nectars (EFN's) or honeydew, can have increased foraging preferences towards sources of nitrogen (McIver and Yandell 1998; Cook and Davidson 2005; Ness et. al 2009). For example ants which feed on carbohydrate rich and nitrogen poor EFN's show an

increase in foraging effort towards nitrogen rich foods versus ants of the same species that do not have EFN's available (Ness et. al 2009). In Almont, Colorado, the western thatching ant, *Formica obscuripes* (Hymenoptera: Formicidae), tends the mutualist aphid *Aphis lupine* (Hemiptera: Aphididae) and will directly feed on the stems and inflorescences of the aphids' host plant, lupine (*Lupinus* spp.) (Figure 2). In this case the thatching ant might be utilizing lupine, which is a nitrogen fixing plant, as a dietary source of nitrogen in its carbohydrate rich diet.

In this study I asked two main questions: what is the net effect of the omnivorous ants on the lupine, and which interactions between the ants, the aphids, the herbivores and the plant are most important for this net effect. Specifically, I looked at the ants' effects on the lupine through the four path ways: direct ant herbivory, the ants' mutualism with aphids, their effect on other phloem feeding herbivores and their effect on chewing herbivores (Figure 1). I tested the effects of omnivorous ants on lupine by using a combination of experimentation and structural equation modeling to discern the relative strengths of each of these interactions as well as the net effect of the ants on the fitness of lupine.

Methods

To understand the net effect of omnivorous ants on lupine, I used a 2X2 factorial experiment with an observational factor, lupine near or far from western thatching ants, *Formica obscuripes* (Hymenoptera: Formicidae), crossed with a manipulative factor, the presence or absence of aphids, *Aphis lupini* (Hemiptera: Aphididae). I identified 30 pairs of lupine, *Lupinus* spp., with at least one early stage inflorescence, totaling 60 experimental plants. I located fifteen pairs of plants within four meters of a thatch ant mound and fifteen pairs of plants were located at least eight meters away from thatch ant mounds. I manipulated the aphids by manually transferring 50 aphids to one randomly designated lupine within each pair of plants. Surveys

were conducted for the initial number of aphids transferred to each plant within the aphid treatment group within 48 hours of transferring the aphids. For plants to which aphids did not successfully transfer the first time, I reapplied aphids to the plant and secured a non-woven bag around the site of aphid transfer to exclude predators. After 24 hours I removed the bag and conducted an initial survey of the number of aphids successfully transferred to the plant. I then surveyed each lupine and recorded the amount of plant damage and the number of inflorescences present on each. Additionally, I surveyed each plant weekly for three weeks and recorded the number of aphids, ants, plant damage, non-aphid herbivores, and predators present. I recorded several types of plant damage indicative of thatch ant activity. Specifically, I recorded the number of holes in flowers, inflorescences removed, holes in stems, and stems completely removed. I collected the seeds of each plant by securing a non-woven “breather” bag over each inflorescence after it stopped flowering, and then counted and weighed the resulting seeds.

I used generalized linear models (GLMs) to quantify the effects being near or far to thatch ants and the presence or absence of aphids on the abundance of total herbivores, phloem feeding herbivores, chewing herbivores, aphids, ants of all species, thatch ants, predators, and amount of damage to the lupines. I looked at lupine damage indicative of that ant activity; specifically, the starting number of flowers damaged by ants, the starting number of inflorescences chewed off, the number of holes chewed into stems, the starting number of stems chewed off, the ending number of inflorescences chewed off, the ending number of holes in stems, and the ending number of stems chewed off. I also used the GLM to analyze the effect of being near or far from thatch ants and the presence or absence of aphids on the number and weight of seeds produced. For plant damage assessed at the beginning of the experiment, only the effect of being near or far to thatch ants was used to analyze the data.

I used GLM's with different residual distributions depending on the distribution of variable under analysis. For data with a normal distribution before or after being natural log transformed, I used a Gaussian distribution. For data which were not normally distributed because low abundances of count data were collected, I used a GLM with a Poisson distribution. If the resulting Poisson model had a greater null deviance than the number of degrees of freedom, I used a GLM with a Quasipoisson distribution to analyze the data. GLM's were conducted in R version 2.11.0, with $P \leq 0.05$ considered significant and $P < 0.10$ considered marginally significant. I also used a structural equations model (SEM) to quantify the net effect and the relative direct and indirect effects within the net interaction between the ants and lupine using the program AMOS 5.0.1 (Figure 1).

Results

Thatching ants (*Formica obscuripes*) were associated with a significant increase in the abundance of ants present from all species (GLM:Gaussian, $t = -9.29$, $p < .001$ (Table 1, Figure 3)). This observed increase in total ant species in the presence of thatching ants indicates that my observational treatment near or far to thatching ants did correlate with an increase and decrease in abundances of thatching ants, respectively. Thatching ants were also correlated with an increase in the number of herbivores present (GLM:Gaussian, $t = -5.02$, $p < 0.001$ (Table 1, Figure 4)). Specifically, thatching ants were associated with significantly increased numbers of phloem feeding herbivores (GLM:Gaussian, $t = -3.64$, $p < 0.001$ (Table 1, Figure 5) and with a marginally significant change in the abundance of chewing herbivores (GLM:Gaussian, $t = -1.997$, $p = 0.051$ (Table 1, Figure 6)). Thatching ants were also associated with a significant increase in aphid abundance (GLM:Quasipoisson, $t = -2.78$, $p = 0.008$ (Table 1, Figure 7)). However, thatching ants were associated with significantly decreased numbers of predators

present on lupine (GLM:Gaussian, $t=2.98$, $p=0.004$ (Table 1, Figure 8)). Aphids (*Aphi lupini*) were associated with significantly increased numbers of herbivores (GLM:Gaussian, $t= -3.152$, $p=0.003$ (Table 1, Figure 4)) as well as phloem feeding herbivores (GLM:Gaussian $t=-3.769$ $p<0.001$ (Table 1, Figure 5)). The effect of aphids on the abundance of ants from all species, non-thatching ants, and predators was not significant, nor was the interaction between the ant and aphid treatments for any of the independent variables.

Thatching ants were associated with significantly increased numbers of flowers with chewing damage at the start of the experiment (GLM:Quasipoisson, $t=-2.88$, $p=0.006$ (Table 1, Figure 9)). However, thatching ants were not associated with a significant increase in the starting number of inflorescences cut, holes in stems, or stems cut (Table 1). Likewise, close proximity to thatching ants did not significantly affect the ending number of inflorescences cut, holes in stems, or number of stems cut. No plant damage variables were significantly affected by the presence of aphids or the interaction between the ant and aphid treatments.

Close proximity to thatching ants was correlated with a significantly higher total number of seeds produced by lupine (GLM:Gaussian, $t= -4.30$, $p= <0.001$ (Table 1) as well as the total number of mature seeds produced by lupine (GLM: quasipoisson, $t=-2.145$, $p= 0.037$ (Table 1, Figure 10)). Thatching ants were also associated with significantly increased total seed weight (GLM:Gaussian, $t=-3.734$, $p<0.001$ (Table 1)), total weight of mature seeds (GLM:Gaussian $t=-3.705$, $p<0.001$ (Table 1)), average seed weight for all seeds (GLM:Gaussian, $t=-5.736$, $p <0.001$ (Table 1) and average weight of mature seeds (GLM:Gaussian, $t=-5.168$, $p<0.001$ (Table 1)). In all cases seed number and weight were determined by dividing total seed number or weight for a given plant by the starting number of inflorescences on that plant. No seed set variables were

significantly affected by the presence of aphids or the interaction between the ant and aphid treatments.

For the structural equation model we used a Chi-square test to determine model fit. A non-significant p-value means that the model fits the data well. Our Chi-squared test resulted in a value of $X^2 = 6.189$ with 3 degrees of freedom and a probability level of 0.103 for $N = 58$ plants. The net effect of the SEM was that thatching ants positively affect the number of seeds produced by the lupine with thatching ants exerting a standardized total effect of 0.166 on the number of mature lupine seeds produced (Figure 11, Table 2). The significant paths were the effect of the thatching ants on the aphids ($P < 0.001$, Table 3), the effect of the aphids on the number of mature lupine seeds produced ($P = 0.021$, Table 3), and the effect of the thatch ants on the number of mature lupine seeds produced ($p = 0.01$, Table 3). The effect of the ants on chewing herbivores and phloem feeding herbivores was insignificant as was the effect of chewing and phloem feeding herbivores on the total number of mature lupine seeds produced (Table 3). The path which was most important for determining the net effect on the number of mature lupine seeds produced was that of the thatch ants directly on the seeds (Table 3).

Discussion

Omnivores can have multiple effects on primary producers through both direct and indirect pathways, as exemplified by the western thatching ant. For example, I found that close proximity to thatching ants was correlated with an increase in aphid abundance, indicating that the thatching ants and the aphids are undergoing a mutualism in which the aphids benefit from the presence of thatching ants. These aphids, in turn, exhibit a significant negative effect on the lupine. The thatching ants therefore have an indirect negative effect on the lupine in addition to their direct positive effect on the aphids. The omnivorous thatching ant also exhibits a direct

negative effect on the lupine by consuming flowers, as shown by the correlation between close proximity to thatching ants and a significant increase in the number of lupine flowers which had chewing damage at the beginning of the experiment. An additional indirect negative effect of the thatching ant on the lupine occurs through its interaction with phloem feeding and chewing herbivores. I found that close proximity to thatching ants correlated with an increase in the abundance of phloem feeding and chewing herbivores, indicating that the thatching ants were not exhibiting aggression towards these herbivores. In fact, the observed negative correlation between thatching ants and arthropod predators seems to be indirectly benefiting both phloem feeding and chewing herbivores, presumably by creating an environment with a lower predator abundance.

The observed positive effect of thatching ants on both phloem feeding and chewing herbivores is in contrast to previous studies which showed a decrease in chewing herbivore abundance in the presence of mutualistic ants (Styrsky and Eubanks 2006). However, this lack of aggression may be explained if the ants are obtaining more nitrogen in their diet relative to other mutualistic ants. Ness et al. (2009) have linked the carbohydrate/amino acid ratio of an ant's diet to its level of aggression. Specifically, their study found that ants which were provided with carbohydrate rich food sources spent more time foraging for nitrogen sources and showed more aggression towards herbivores than those which were provided with carbohydrate low and/or nitrogen rich food sources (Ness et. al 2009). Therefore, the observed positive correlation between herbivore abundance and proximity to thatching ants may be a result of an additional nitrogen source in the thatching ants' diet, for example: their consumption of lupine. This could lower the ants' level of aggression towards herbivores. Additionally, the thatch ants' aggression towards arthropod predators, combined with their lack of aggression towards herbivores, may be

indirectly benefiting herbivores, resulting in the observed increase in both phloem feeding and chewing herbivores in the presence of thatching ants.

Although this experiment indicated that close proximity to thatching ants is correlated with an increase in the abundance of chewing herbivores, phloem feeding herbivores, aphid and flower damage, close proximity to thatching ants was actually shown to increase both total seed set and total seed weight of lupine. It is possible that this result is an artifact of my thatching ant treatment being observational rather than manipulative. Lupine which were over eight meters from any thatching ant mound were most frequently located in aspen groves around my study site, while lupine which were within four meters of a thatching ant mound were frequently in open fields. This difference in location could have resulted in lupine located far from thatching ants being exposed to less sunlight, poorer soil conditions, and a difference in pollinator community or number. All or any of these factors could have resulted in the outcome of close proximity to thatching ants being associated with an increase in seed set and seed weight. In order to separate the effect of microhabitat from ant treatment, a second experiment which uses a manipulative factor for ants rather than an observational factor should be conducted in the future.

Ants are important omnivores that have a variety of direct and indirect effects on primary producers. This web of interactions can make predicting the ultimate effect of ants, such as the western thatching ant, on a given plant very difficult. For example, although this experiment showed only negative direct and indirect effects of the thatching ants on lupine for all of the interaction pathways observed, the overall effect of the ants on the lupine was positive. Additional studies of omnivores should take into account the multiple pathways through which an omnivore interacts with a primary producer in order to discern which interaction pathway is most important for that omnivore's net effect on a plant.

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Tables

				Ants		Aphids		Ants X Aphids	
Variable	Test	Test Stat	Trans.	Stat	p-value	stat	p-value	stat	p-value

number of predators	GLM:Gaussian	t	ln(+1)	3.021	0.004	- 1.096	0.278	- 0.915	0.364
number of herbivores	GLM:Gaussian	t	ln(+1)	5.064	<0.001	- 3.152	0.003	0.443	0.659
phloem feeding herbivores	GLM:Gaussian	t	ln(+1)	3.644	<0.001	- 3.769	<0.001	1.297	0.200
number of chewing herbivores	GLM: Gaussian	t	ln(+1)	1.997	0.051	0.188	0.851	- 0.797	0.429
number of aphids	GLM: Gaussian	t	ln(+1)	2.779	0.008	NA	NA	NA	NA
number of ants (all)	GLM:Gaussian	t	ln(+1)	9.287	<0.001	- 1.329	0.189	1.070	0.290
number of ants (not F. obscuripes)	GLM:quasipoisson	t	0.000	1.254	0.215	0.029	0.977	- 0.779	0.439
number starting flowers chewed	GLM:quasipoisson	t	0.000	2.880	0.006	NA	NA	NA	NA
number starting inf. Cut	GLM:binomial	z	0.000	0.307	0.759	NA	NA	NA	NA
number starting holes in stems	GLM:binomial	z	0.000	0.003	0.997	NA	NA	NA	NA
number starting stems cut	GLM:binomial	z	0.000	0.059	0.953	NA	NA	NA	NA
number ending inf. Cut	GLM:binomial	z	0.000	1.797	0.072	0.500	0.617	0.318	0.750
number ending holes in stems	GLM:binomial	z	0.000	0.006	0.995	0.007	0.994	0.006	0.995
number ending stems cut	GLM:binomial	z	0.000	1.344	0.179	0.372	0.710	0.372	0.710
total number of seeds produced	GLM:Gaussian	t	ln(+1)	4.295	<0.001	- 0.293	0.771	- 0.750	0.456
mature seeds produced	GLM:quasipoisson	t	0.000	2.145	0.037	- 0.600	0.551	- 0.855	0.397
total weight of seeds produced	GLM:Gaussian	t	Z, ln(+1)	3.739	<0.001	- 0.593	0.556	- 0.969	0.337
weight mature seeds produced	GLM:Gaussian	t	Z, ln(+1)	3.705	<0.001	- 0.625	0.535	- 0.963	0.340
average weight all seeds	GLM:Gaussian	t	Z, ln(+1)	5.736	<0.001	- 0.027	0.978	- 1.050	0.298
average weight mature seeds	GLM:Gaussian	t	Z, ln(+1)	5.168	<0.001	- 0.135	0.893	- 0.676	0.502

Table 1: Results of statistical tests. All statistical tests performed were GLM's with the distribution specified in the table. Significant P values are bolded.

	change in total ants	change in total phloem feeders	change in total aphids	change in total chewing herbivores
total phloem feeders	0.122 (0.151)	0(0)	0(0)	0(0)
total aphids	20.49 (0.890)	0(0)	0(0)	0(0)
total chewing herbivores	0.072(0.154)	0(0)	0(0)	0(0)

total mature seeds	0.04 (0.166)	0.022(0.073)	-0.007(-0.661)	0.017(0.033)
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Table 2: matrices for total net effects of the Ant-Effect SEM with standardization by their standard deviation in parenthesis

Variables	Ant-Effect SEM			
Independent	Dependent	Estimate	SE	P
total chewing herbivores	total ants	0.072 (0.154)	0.061	0.239
total aphids	total ants	20.49 (.890)	1.472	***
total phloem feeding herbivores	total ants	0.122 (0.151)	0.106	0.25
mature seeds/inflorescence	total aphids	-0.007 (-0.661)	0.003	0.021
mature seeds/inflorescence	total phloem feeders	0.022 (0.073)	0.038	0.564
mature seeds/inflorescence	total ants	0.179 (0.738)	0.069	0.01
mature seeds/inflorescence	total chewing herbivores	0.017 (0.033)	0.066	0.795

Table 3: matrices for regression weights of the Ant-Effect SEM with standardization by their standard deviation in parenthesis

Figures

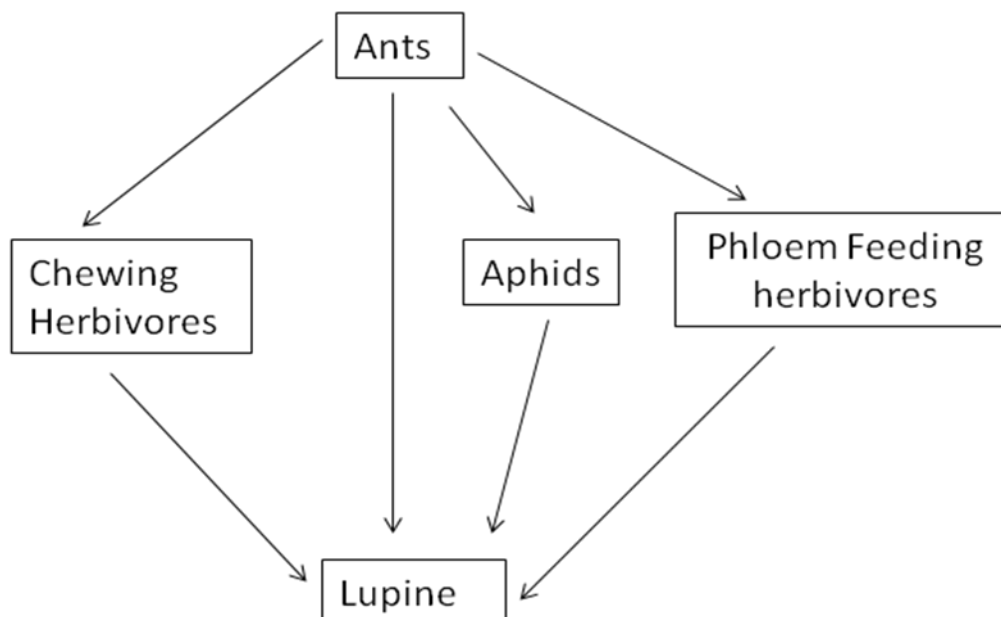


Figure 1. Direct and indirect interactions between ants, *Formica obscuripes*, and lupine, *Lupinus* spp. This structure will be used in a confirmatory structural equation model (SEM) to test for relative interaction strengths.



Figure 2: *Formica obscuripes* biting into the back of a flower. Picture courtesy of Josh Grinath.

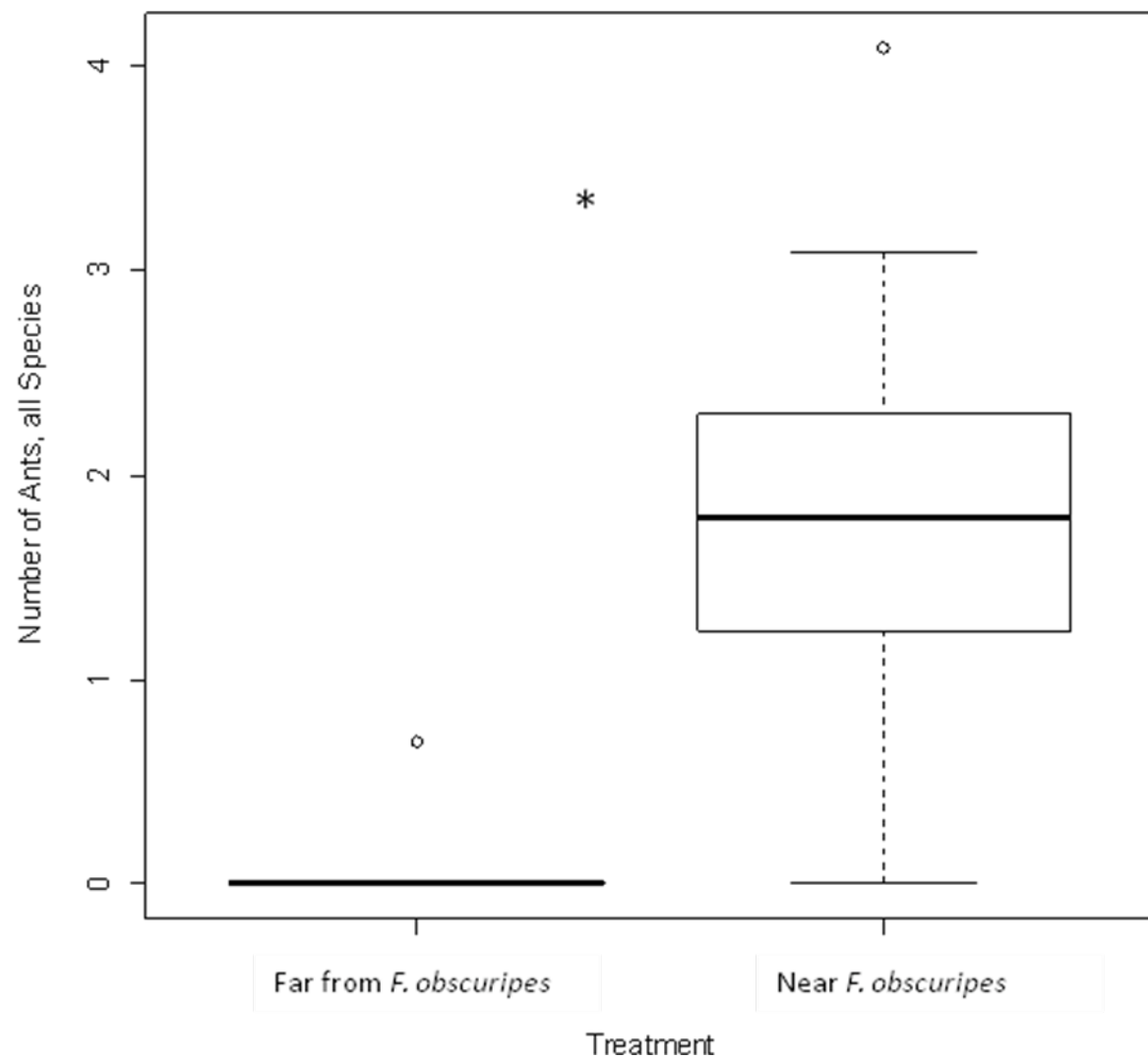


Figure 3. Thatching ants were associated with significantly increased number of ants present from all species (GLM:Gaussian, $t=-9.29$, $p<.001$).

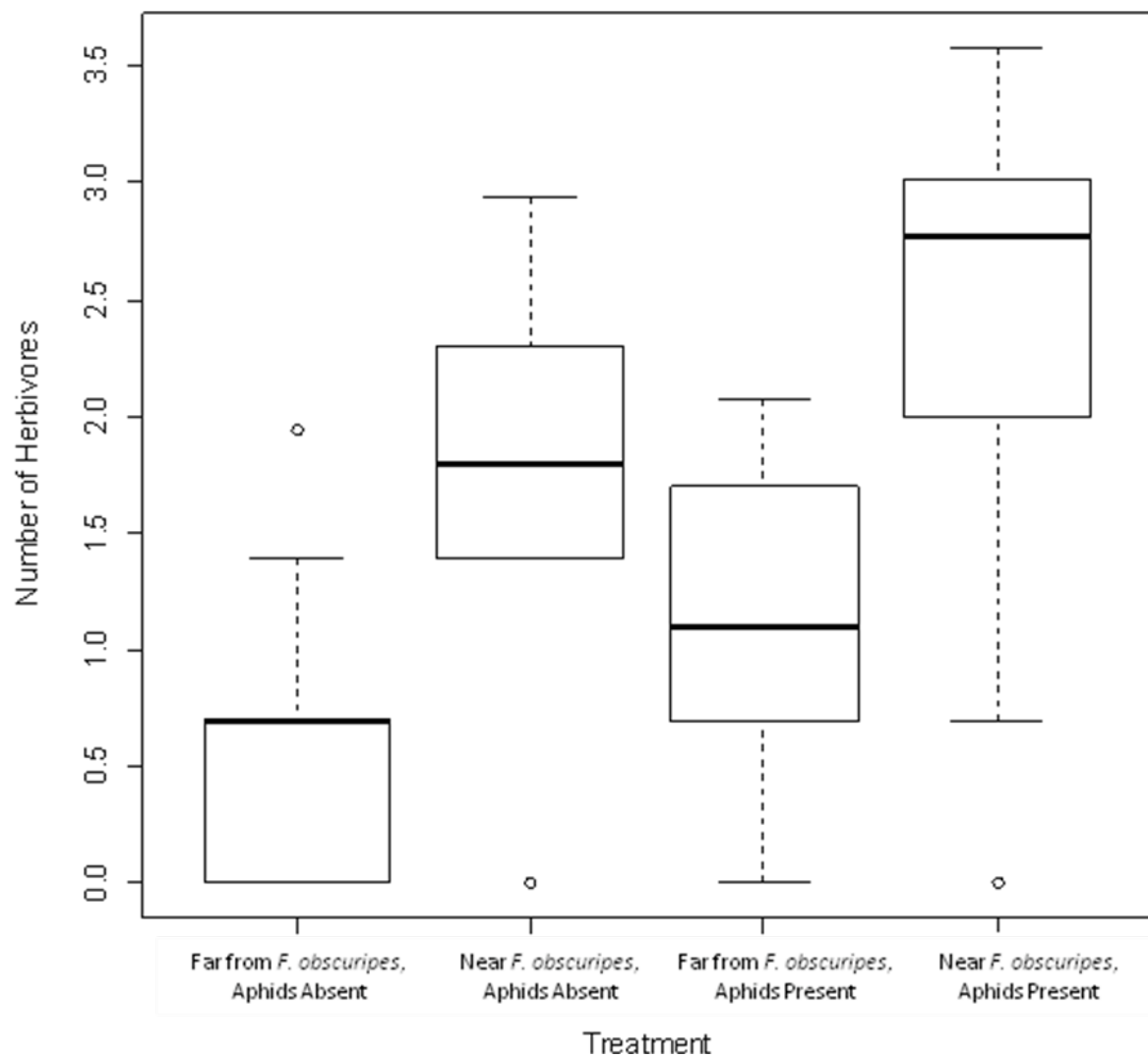


Figure 4: Ants (*Formica obscuripes*) were associated with significantly increased number of herbivores (GLM:Gaussian, $t = -5.02$, $p < 0.001$) The presence of aphids (*Aphis lupini*) significantly increased the number of herbivores present (GLM:Gaussian, $t = -3.22$, $p = 0.002$)

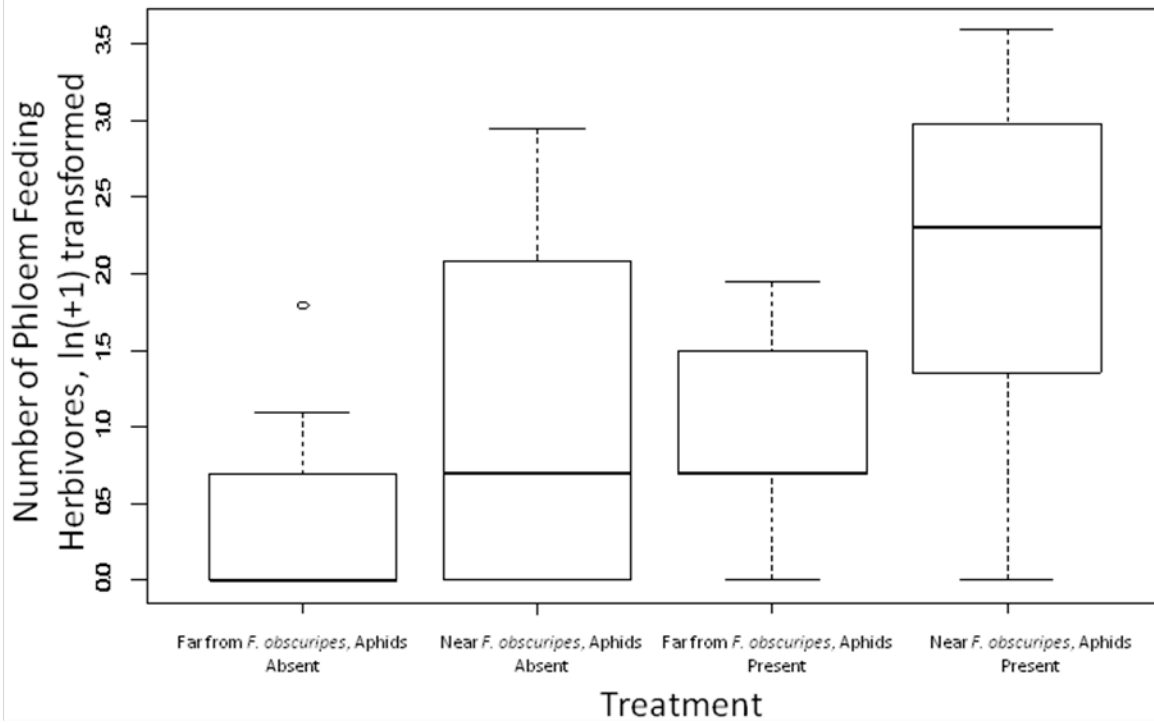


Figure 5: Thatching ants were associated with significantly increased numbers of phloem feeding herbivores (GLM:Gaussian, $t=-3.64$, $p<0.001$). The presence of aphids (*Aphi lupini*) significantly increased the number of phloem feeding herbivores present (GLM:Gaussian, $t=-3.644$, $p<0.001$)

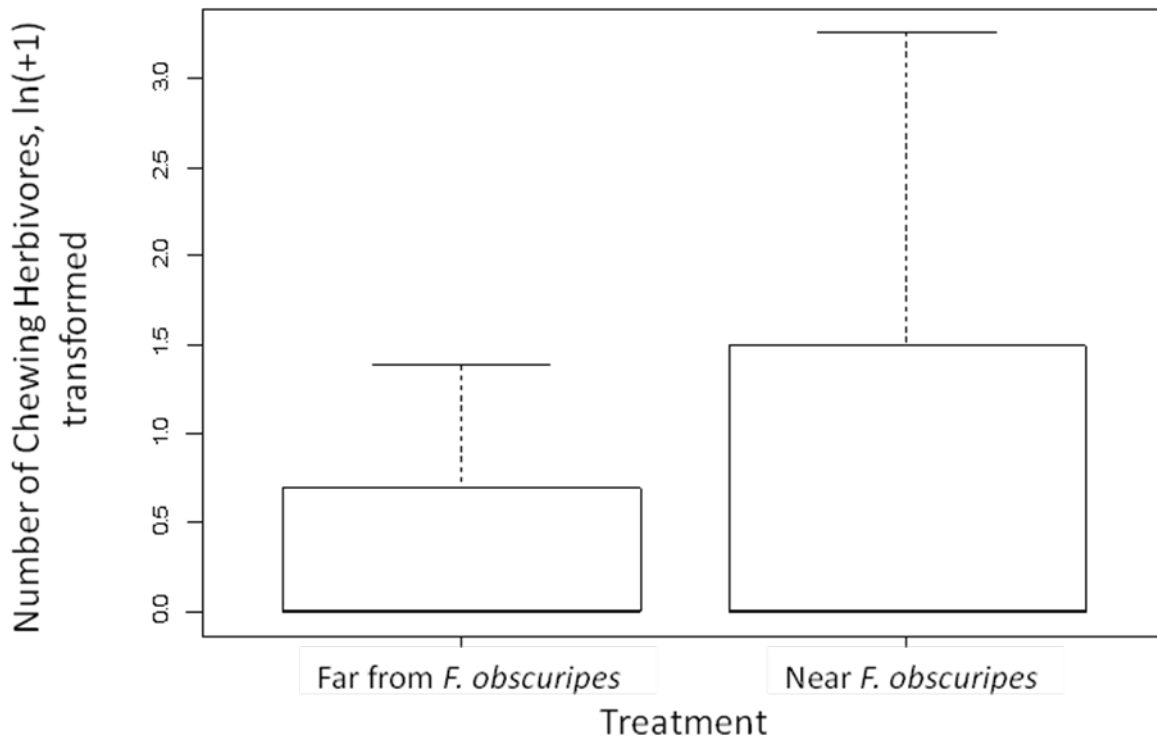


Figure 6: Thatching ants were not associated with a significant change in the number of chewing herbivores present (GLM:Gaussian, $t=-1.997$, $p=0.0508$).

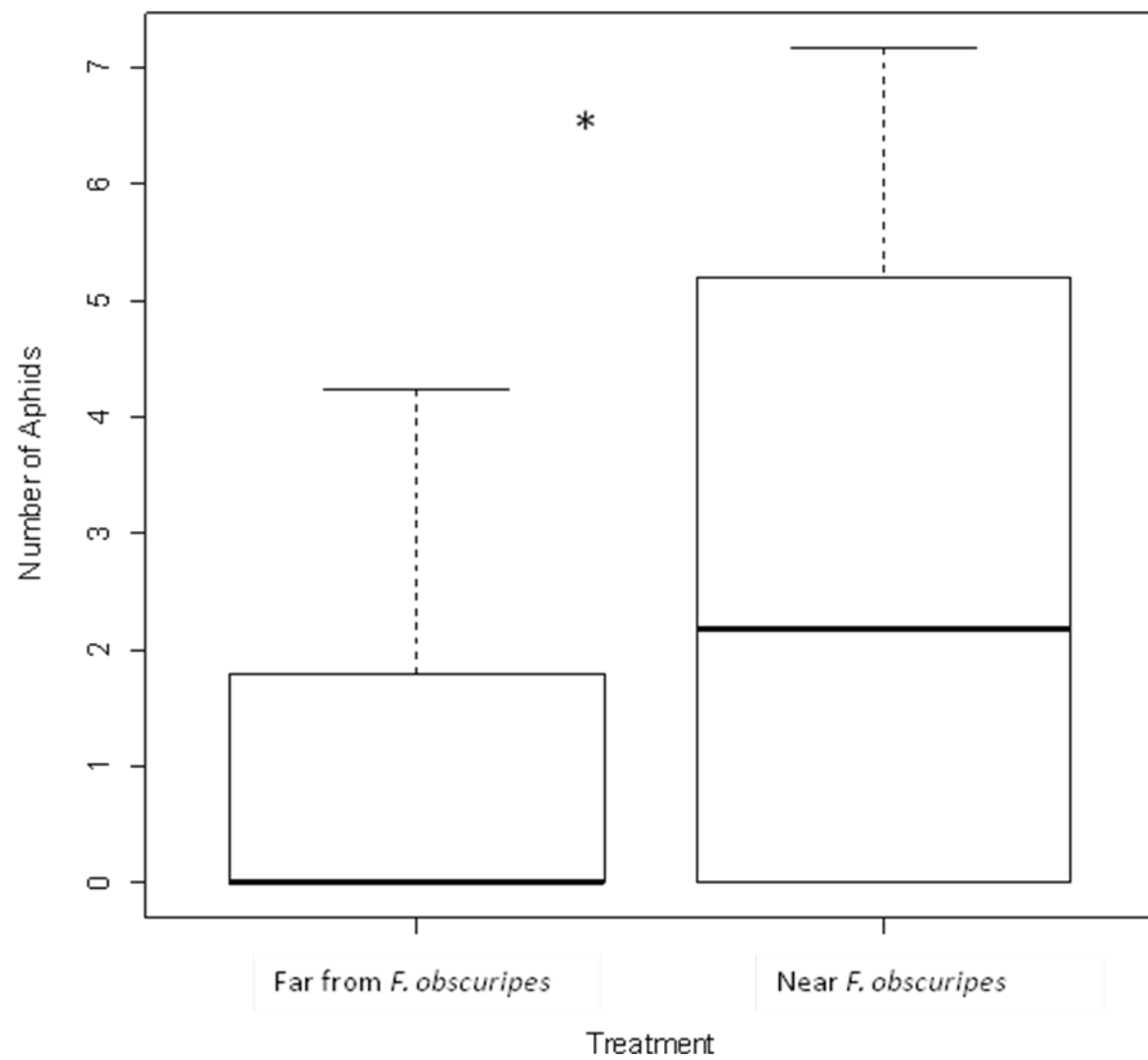


Figure 7: Thatching ants were associated with significantly increased numbers of aphids (GLM:quasipoisson, $t=-2.78$, $p=0.008$).

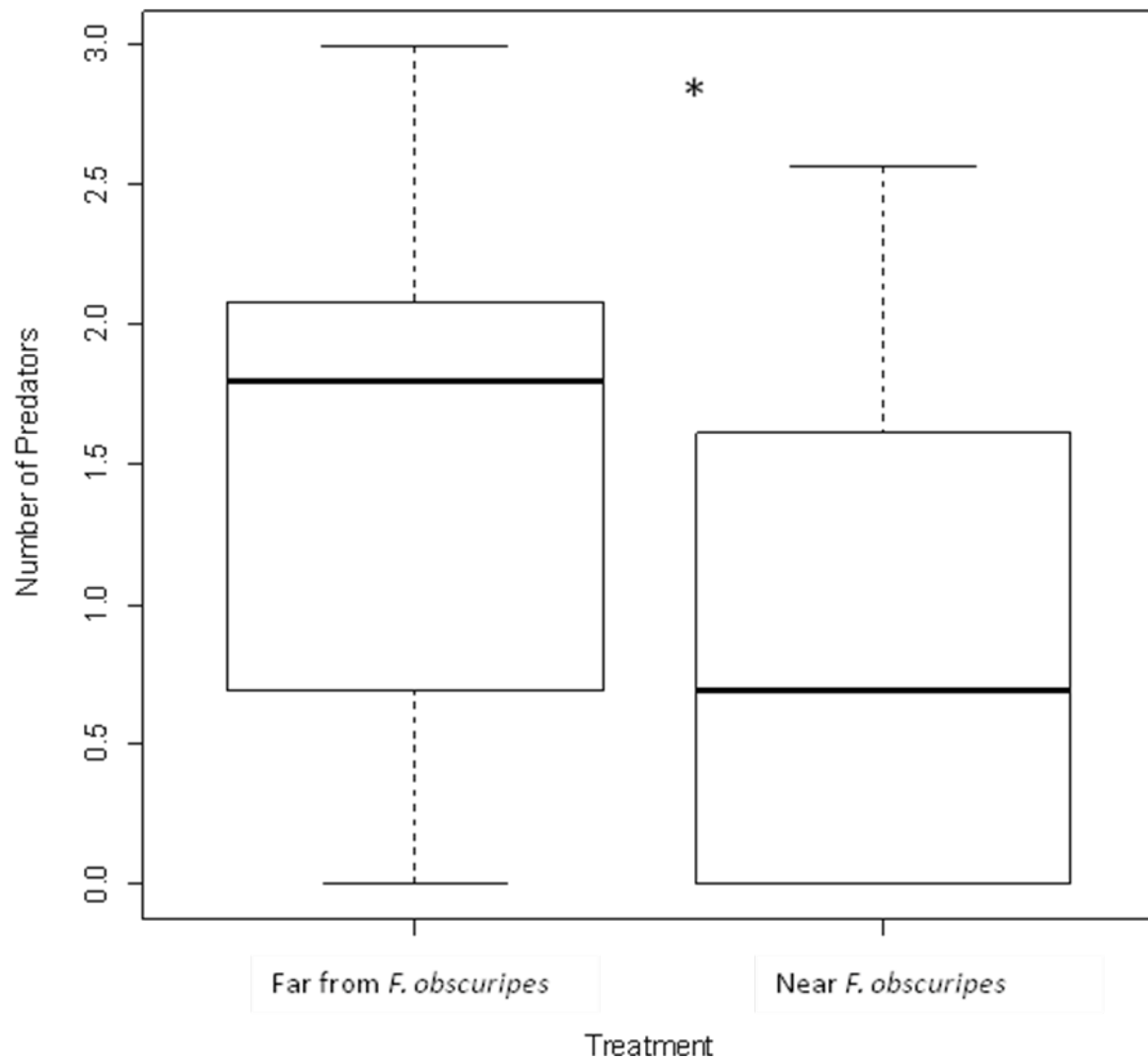


Figure 8: Thatching ants were associated with significantly decreased numbers of predators present on lupine (GLM:Gaussian, $t=2.98$, $p=0.004$).

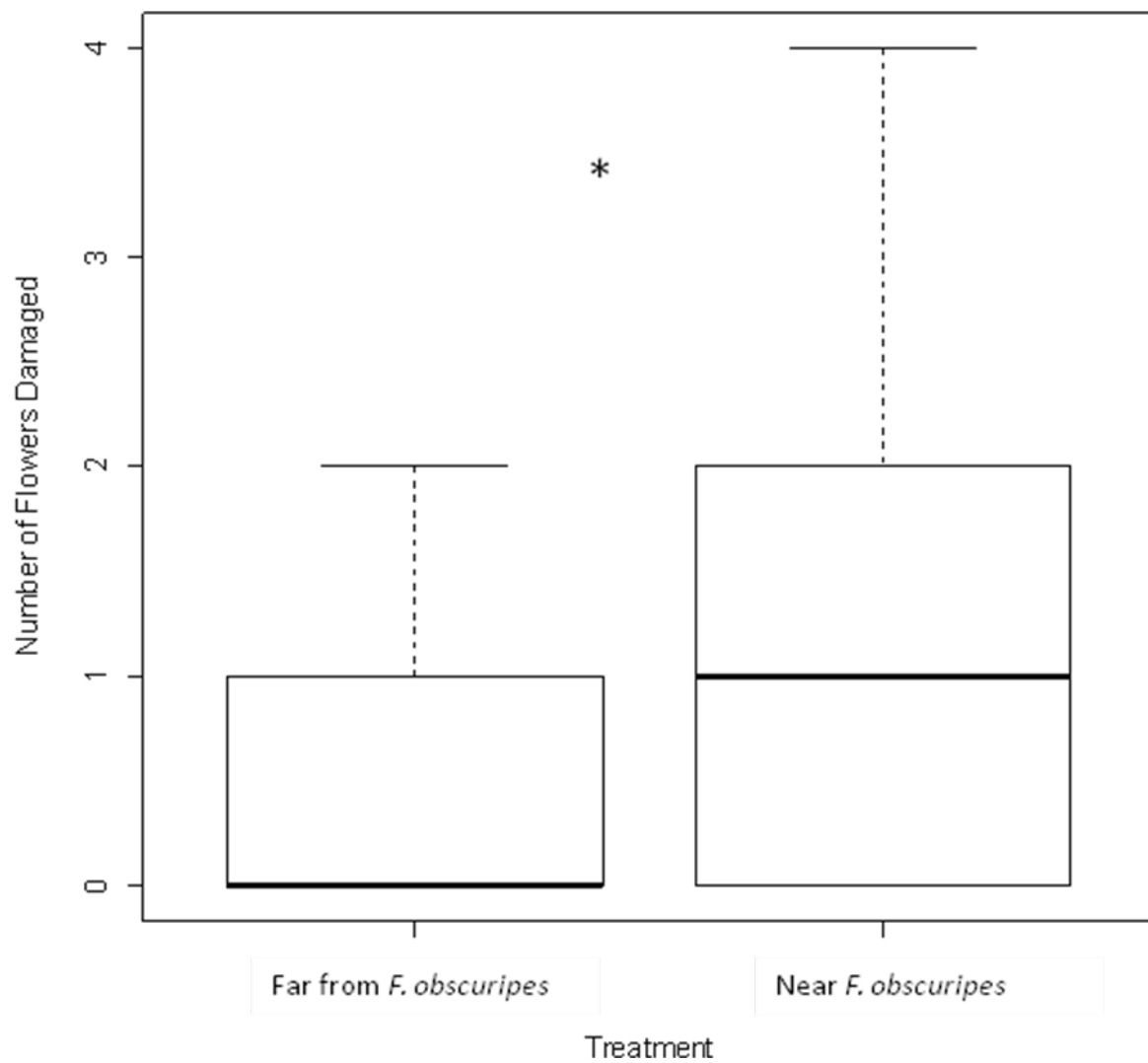


Figure 9: Thatching ants were associated with significantly increased numbers of flowers with chewing damage at the start of the experiment (GLM:quasipoisson, $t=-2.88$, $p=0.006$).

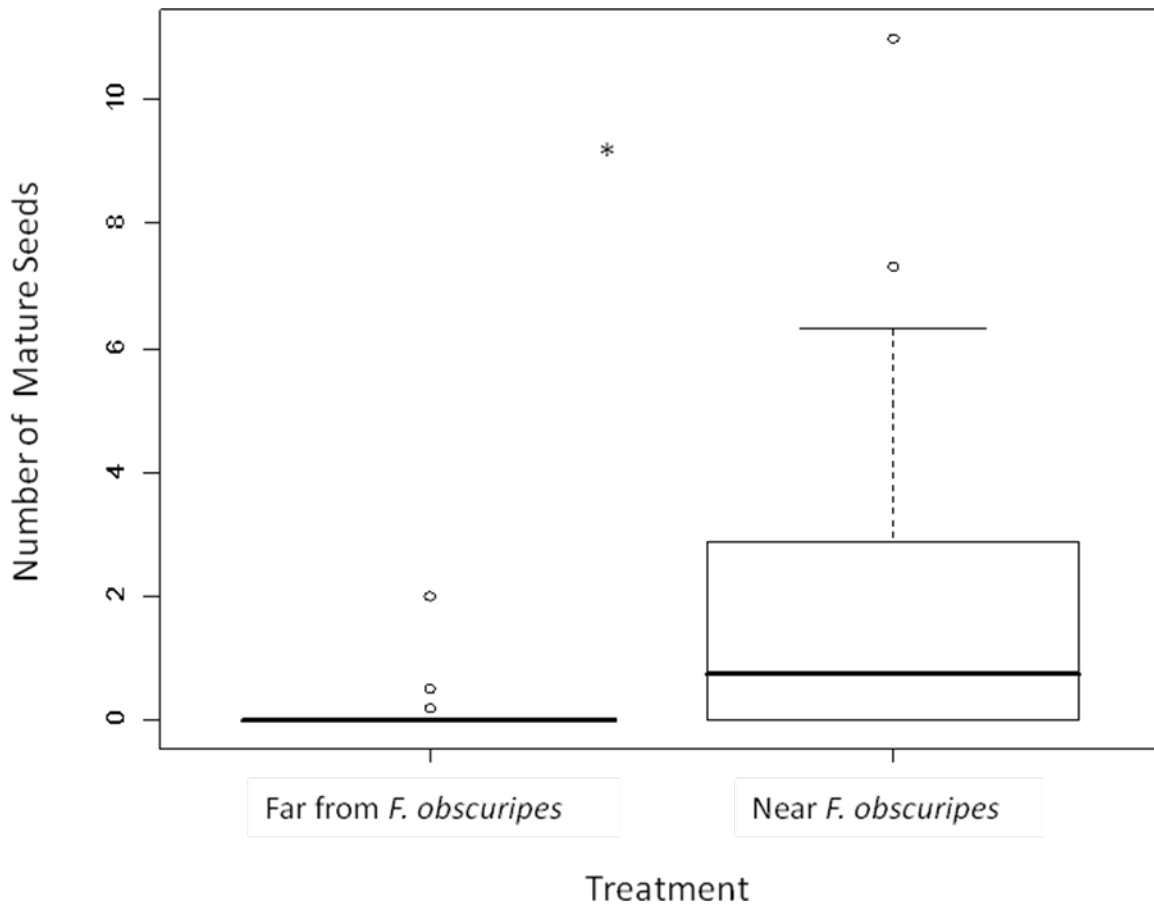


Figure 10: Thatching ants were associated with significantly increased numbers of mature seeds produced by lupine (GLM: quasipoisson, $t=-2.145$, $p=0.037$).

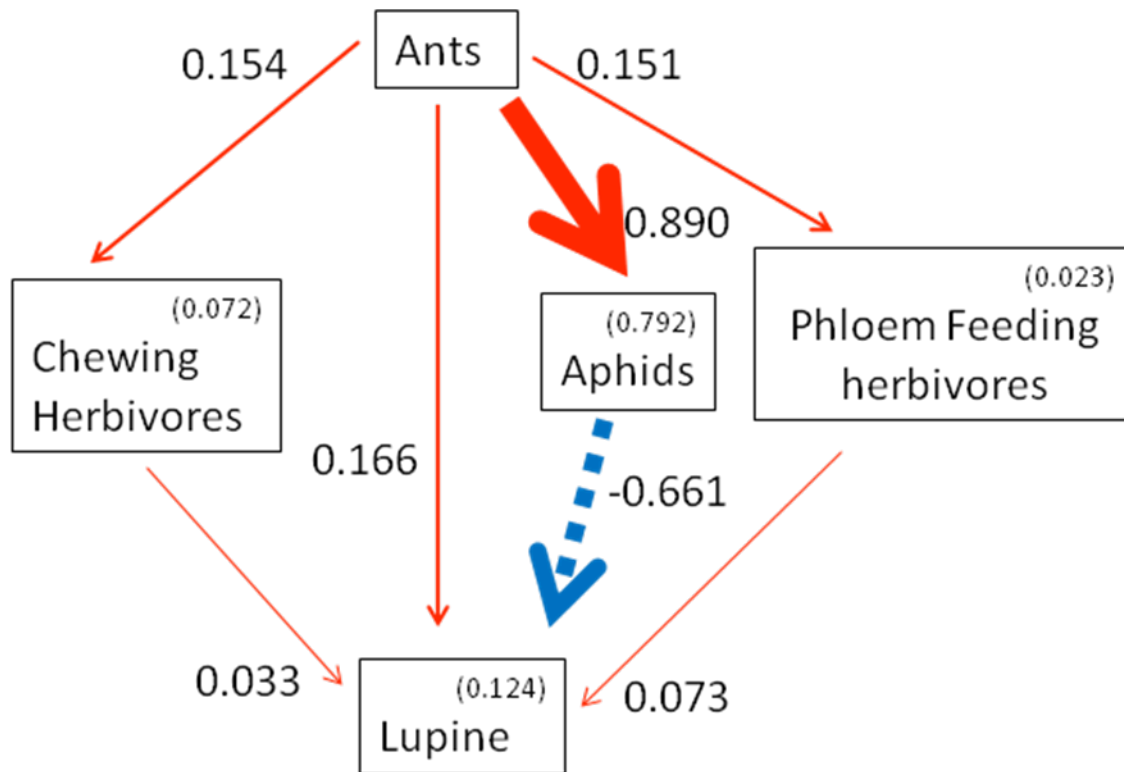


Figure 11: Diagram of SEM results. Red solid lines represent positive interactions. Blue dashed lines represent negative interactions. R^2 values are given in the upper right hand corner of each box. Arrow width is proportional to the standardized total effects of each interaction, which are also written adjacent to the arrow representing that interaction.