

The effect of ants on membracid nymph size and instar

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RMBL
2007

Abstract

This project aimed to determine the mechanism of which treehopper nymphs grow in the presence of ants. Nymphs (*Publilia modesta*) are larger when their mutualist partner (*Formica obscuripes*) is present. There are two ways in which the nymphs can grow: (1) nymphs grow at the same rate, but are larger for a given stage of development, (2) nymphs grow faster (go through instars more quickly). This study investigates which of the two ways the nymphs are growing by manipulating the presence or absence of ants to the nymphs during development. Four samples of the nymphs were taken in four weeks. Stage of nymph development and size of nymph were determined and analyzed over time with respect to treatment group (ants or no ants). We found that the presence of ants causes the membracid nymphs to grow faster and that size was not affected. This implies that there is no extra cost to nymphs to grow more quickly, because they are able to preserve their size and still grow at a faster rate.

Keywords: treehopper, membracid, nymph, size, instar, *Publilia modesta*, *Formica obscuripes*, Almont Triangle, mutualism

Introduction

A mutualism between two organisms is an interspecies interaction that is beneficial for both organisms (Smith and Douglas, 1987). Mutualisms can change over the duration of the lifetime of the organisms, as well as across generations. These mutualisms can deteriorate evolutionarily via cheating organisms; whereby one of the organisms involved in the mutualism reaps the benefits of the mutualism, but provides less to their mutualistic partner over time (Ferriere et al., 2002). This suggests that mutualisms are not always necessarily obligatory, but are dependent on benefits gained for both organisms, and can change over time.

The stability of the ant-treehopper mutualism is dependant on appropriate benefits for both species. The ant *Formica obscuripes* and the membracid *Publilia modesta* (Uhler) have a mutualism in which the ant provides protection for the membracids from predation of spiders, lacewing, larvae and coccinellids (i.e. Cushman and Whitham, 1989; Morales, 2000), and the ants gain nutrients by feeding on the honeydew produced by the

membracids. The relationship between *F. obscuripes* and the membracid nymph is crucial to the survival of the nymphs because the nymphs (being much smaller and less mobile than adults) are more susceptible to predation than membracid adults (Cushman and Whitham, 1989). The presence of ants has a positive effect on the abundance and survivorship of membracid nymphs (Reithel and Billick, 2006; Billick and Tonkel, 2003; Billick, et al., 2001).

When two species exist in close proximity, there is often an element of developmental plasticity that can occur caused by this coexistence. This can be dependant on the presence or absence of the other organism (Pekarsky, et al. 2002). Pekarsky et al. (2002) found that mayfly size and time of maturation depended on the presence of the trout. Mayflies changed the timing of larval development depending on risk factors, such as predation by trout. The presence of a predator dictates degree of investment in defense and reproduction (Dahl and Peckarsky, 2003). These developmental plasticity findings show that organisms adjust morphology and metabolism in accordance to environmental factors. There is little research, however, about physiological adjustments due to a mutualism.

The implications of mutualism-induced morphology changes could have effects for the interaction between the two species and the physiology of the species involved. *F. obscuripes* protect the membracid nymphs from predation (Cushman & Whitham, 1989). The presence or absence of the ant, which protects the nymphs from predation, might affect the size or growth patterns (either overall faster, or per instar) of the membracid nymphs. The protection from the ants allows the nymphs to allocate their energy toward growth, instead of defense.

It is still unknown how the presence of ants affects the growth of the membracid. This study will investigate how the presence or absence of ants affects the growth patterns of membracid nymphs. Ant-tending has been found to shorten the time spent during development and therefore possibly increase growth rate of the membracid nymph (Bristow, 1984). The presence of *F. obscuripes* has been shown to increase the size of membracid nymphs (Abbot et al., unpublished data). However, from these findings, it is unclear as to whether the nymphs with ants present are bigger because they are at a later instar, or because they are bigger for a given instar. An increased size in most insects has been found to increase fitness, fecundity, success in overwintering, and decrease predation dangers (Hinton, 1981). There are two ways for an organism to get larger, and this study will investigate by which mechanism the membracid nymphs get larger with the presence of ants.

The first possible mechanism for growing larger involves faster growth rate, implying that the presence of ants aids the nymphs in growing faster. The second possible mechanism for growing larger is that the nymphs are growing at a slower rate, and are allocating their energy to being larger per instar. To test this question of why the membracid nymphs are larger in size with ants present, we will manipulate presence of ants and compare nymph size and instar with nymphs not exposed to ants.

Methods

Study Site and Organism

The study site for this experiment is located at Almont Triangle (elev. 2720 m), approximately 25 miles north of Gunnison, Colorado. Almont Triangle is composed of

meadows with relatively dry vegetation surrounded by aspen groves. Dominant plant species of the meadows include *Artemisia tridentata* (Sagebrush), *Chrysothamnus spp.* (rabbitbrush), grasses and herbaceous perennials, such as *Wyethia spp.* (mule's ear).

At Almont Triangle, the adult treehopper, *Publilia modesta* (Membracidae) is commonly found tended by the ant, *Formica obscuripes* on six different host plants where females oviposit: *Wyethia spp.*, *Chrysothamnus viscidiflorus*, *Erigeron sp.*, *Balsamorhiza sagittata*, *Helianthella quinquenervis*, and *Lupinus spp.* The two most common host plants are *Wyethia spp.* and *C. viscidiflorus*. The membracids feed on the phloem of the host plants. Even though the treehoppers prefer *Wyethia spp.* over *C. viscidiflorus*, the *Wyethia spp.* senesces earlier than *C. viscidiflorus* and decreases survivorship of the nymphs (Reithel and Billick, 2006). The host plant *C. viscidiflorus* was therefore chosen for the current study because they are more abundant and tend to senesce later than the other host plants.

P. modesta reproduce annually at Almont Triangle in June. The adult membracids mate in May and after mating the males die. The females oviposit on the stems of the plants and lay multiple egg clutches on different plants. The nymphs are born from late June to early July, throughout a three-week period. In July and August the nymphs aggregate, while the adult membracids stay with them during maturation. The nymphs develop through five instars and go through a molting process in between each instar. They become adults and by September they overwinter until the following May when mating occurs.

Formica obscuripes is an aggressive ant that feeds on the honeydew produced by treehoppers, aphids, and other insects that excrete the sucrose-rich honeydew. *F.*

obscuripes protect the treehopper from predation in return for the nutrients gained from the honeydew. These ants live in huge colonies that consist of about 40,000 ants per colony. These mounds are very abundant at Almont Triangle.

Methods and Materials

We initially selected 102 *C. viscidiflorus* ramets with the presence of both ants and membracid adults as host plants. However, we only used 88 in the experiment because some of the selected plants were dying. We tightly wrapped the base of each ramet in duct tape to control for any effect the tanglefoot that was also applied, might have on the quality of the plant. We applied tanglefoot, a sticky substance that traps ants from crawling up the stems of the plant, to half of the plants on the duct-taped portion of the stem. We applied the tanglefoot according to the treatment group (ant exclusion group). We then trimmed all surrounding vegetation for all the plants, using utility scissors, to prevent alternative ways for ants in the exclusion group to have access to the plants.

To control for any possible spatial differences within the experimental plot, we assigned plants to 11 clusters of eight plants each. Each cluster consisted of plants that were located in the same relative region. We randomly assigned each ramet a treatment group (presence or absence of ants) and the week of collection (1-4) using a die within the clusters of eight. Therefore, in each cluster, there were four ramets with the presence of ants and four without. We collected one plant from each treatment group, in each cluster, each week for four weeks to bring back to the laboratory for further analysis.

We collected all the ramets in freezer ziplock bags in the field and we put the bags of plants in the freezer about one hour after collection. We put the plants in the freezer to prevent nymph movement during the measurement and instar identification process.

Once frozen, we collected nymphs from each plant and placed each nymph in a Petri dish using a paintbrush and a pin. We used an ocular micrometer to measure the length (mm) of each nymph. We used a previously made identification chart of five nymph instars to identify the instar of each nymph (Erickson, unpublished data). We recorded length and instar of each nymph within each corresponding plant and treatment group.

Weekly surveys were conducted in the field in which the abundance of ants, membracid adults, and nymphs were counted for each individual plant. Plants were collected on July 5th, 10th, 18th, 24th after the surveys were conducted.

Statistical Analysis

We wanted to investigate the effects of treatment on nymph density, and then nymph density on nymph size and instar. We took averages of nymph size and instar and calculated number of nymphs per plant for density analyses. We did regression analyses to analyze these density questions.

We also used regression analysis to analyze the effects of instar on size. We used residual nymph sizes in this regression to eliminate the effects of instar on nymph size. Nymph residual size was determined by using the regression line (see Figure 3) of instar on size, and then the residual size (distance of a data point from the trend line) was calculated and used as a response variable. We then wanted to figure out the effects of

treatment on size and instar. We used an ANOVA to analyze these data (see Figures 3B and 4).

We nested the variable ‘plant’ within ‘treatment’ to account for variability among individual plants. We analyzed each week separately and averaged all weeks together in the figures. Week four data was excluded because there were no ‘no ant’ data for that week. Some plants were excluded from analysis if there were ants on No Ant plants, the plant had fallen over, or wrong identification of *Chrysothamnus spp.* All statistics were conducted using the JMP® program, version 5.1.

Results

Between 4 and 9 plants had nymphs present per treatment in each week (see Table I). To confirm the existence of the ant-treehopper mutualism in my experiment, the effects of the treatment on nymph density were analyzed. Figure 1 shows the number of nymphs in the Ant treatment (mean = 6.6, SD = 1.4), and the number of nymphs in the No Ant treatment (mean = 2.3, SD = 1.5). The number of nymphs increases when ants are present by almost three-fold. This was analyzed for individual weeks, and then we averaged all three weeks together (see Figure 1). A one-way analysis of variance (ANOVA) revealed a significant effect for all weeks, $F_{1,81} = 4.17$, $p = 0.04$, confirming previous results that ants increase nymph abundance.

From Figure 1, it is clear that nymph density is positively affected by the presence of ants, so we investigated the effects of nymph density on nymph size and instar (see Figures 2A, and 2B respectively). As the number of nymphs per plant increases, there is no effect of increased or decreased nymph size or instar. A regression revealed non-significant effects for the effects of nymph density on nymph size, $n = 48$, $p = 0.43$, and

for nymph density on nymph instar, $n = 48$, $p = 0.9$. This shows that nymph density is not a factor in determining the size or instar of membracid nymphs.

Figures 3A and 3B show that nymph size increases with nymph instar. A regression reveals that there is a significant relationship between instar and size, $p < 0.0001$. Figures 3A and 3B show that treatment (presence or absence of ants) had no effect on the relationship between instar and size. Figure 3A shows that nymph size per instar is not significantly effected by the presence of ants and Figure 3B shows that the residual nymph size, the size of the nymphs without the effects of instar on size, is unaffected by the treatment, $F_{1,377} = 1.6$, $p = 0.2$. The nymphs with ants present had a mean size of 1.49 mm, $SD = 0.33$, and nymphs without ants were a mean size of 1.48 mm, $SD = 0.36$.

Figure 4 shows that the nymphs with exposure to ants were found to be at a later instar averaged across all three weeks than those nymphs excluded from ants. Nymphs with ants present had a mean instar of 1.7, $SD = 0.89$, and nymphs without ants had a mean instar of 1.45, $SD = 0.66$. A one-way ANOVA reveled that there was a significant effect of ants on nymph instar ($F_{1,377} = 6.1$, $p = 0.01$), showing that the nymphs with ants grew at a faster rate.

Discussion

I started with two alternative hypotheses to explain why ants cause membracid nymphs to grow larger. The first hypothesis is that nymphs are larger when ants are present because they are bigger for a given instar, while growth rate remains constant with or without ants. The second hypothesis is that nymphs are bigger when ants are

present because they are growing at a faster rate and moving from instar to instar more quickly than those nymphs without ants.

The results from this experiment support the second hypothesis. Membracid nymphs grow more quickly when ants are present (Figure 4). Figures 3A and 3B indicate that the first hypothesis, that nymphs are bigger for a given instar, is not true, since there was no significant effect of ants on nymph size.

Since there was no effect of nymph size due to the treatment, but there were significant effects of treatment on instar, this indicates that the ants are facilitating extra benefits to the nymphs, with no cost. Previous work with aphids shows that ant-tending can be positive to aphid growth at low densities, but then becomes a negative effect at high density levels of aphids (Breton and Addicott, 1992). This experiment found no density effects on instar and size (see Figures 2A and 2B), which means that ant-tending positively effects the membracid nymph growth rate, but there is no apparent cost, as there is in aphids. This also implies that there is no apparent nymph competition since the number of nymphs present doesn't effect their size or stage of development.

These findings are interesting because they suggest that there is some physiological adjustment occurring in the membracid nymphs, causing them to grow at a faster rate when ants are present. Peckarsky et al. (2002) showed that metabolism adjustments occur in a predator-prey relationship, however, the findings in this study show the first indication of a morphological adjustment that occurs in individuals with the presence of a mutualism.

The benefit of being able to grow at a faster rate, but remain the same size for the nymphs is crucial, because it allows the nymphs to reach adulthood earlier in the summer,

and shortens the window of vulnerability to predation. These findings suggest that the mutualism between ants and membracids is fairly loose because the membracids do not have to make a large energy investment that might effect their density or size, but rather simply gain the benefit of growing more quickly when ants are present. Other fitness benefits of becoming an adult faster for the nymphs include, increased likelihood of surviving an early frost, and increased survivorship on host plants that tend to senesce earlier in the summer.

In the ant-treehopper mutualism, membracids are growing faster, more abundant, and have increased survivorship, which implies that ant-tending has a positive effect on the fitness of the membracid nymphs. When the ants are not present, it is possible that the membracids are expending more energy trying to escape predation instead of feeding and therefore growing faster.

Acknowledgements

I would like to thank Ian Billick for being my mentor and helping me with all the steps to make this research a possibility. I would also like to thank Dan Erickson and Josh Grinath for all the hours of field and lab work that contributed to this project. I would also like to thank Jennie Reithel, Laura Aldrich-Wolfe, and Ian Billick for editing help. I would also like to thank RMBL for allowing me to work at their lab, and the NSF REU program for funding.

References cited

- Abbot, P., Grinath, J., Brown, J., Erickson, D., Peeden, E., & Billick, I. (2007). Intraspecific variation in the stoichiometry of an ant-tended herbivore. Unpublished data.
- Billick, I., & Tonkel, K. (2003). The relative importance of spatial vs. temporal variability in generating a conditional mutualism. *Ecology*, 84, 289-295.
- Billick, I., Weidmann, & M., Reithel, J. (2001). The relationship between ant-tending and maternal care in the treehopper *Publilia modesta*. *Behavioral Ecology and sociobiology*, 51, 41-46.
- Breton, L. M., & Addicott, J. F. (1992). Density-dependent mutualism in an aphid-ant interaction. *Ecology*, 73, 2175-2180.
- Bristow, C. M. (1984). Differential benefits to two species of Homoptera on New York iron weed. *The Journal of Animal Ecology*, 53, 715-726.
- Cushman, J. H. & Witham, T. G. (1989). Conditional mutualism in a membracid-ant association: temporal, age-specific, and density-dependent effects. *Ecology* 70, 1040-1047.
- Dahl, J. & Peckarsky, B. L. (2003). Does living in streams with fish involve a cost of induced morphological defense? *Canadian Journal of Zoology*. 81, 1825-1828.
- Ferriere, R., Bronstein, J., Rinaldi, S., Law, R., & Gauduhon, M. (2002). Cheating and the evolutionary stability of mutualisms. *Biological Sciences*, 269, 773-780.
- Hinton, H. E. (1981). *Biology of Insect Eggs*. Pergamon, Oxford.
- MacArthur, R. H., & Pianka, E. R. (1966) On the optimal use of a patchy environment. *The American Naturalist* 100, 603-609.
- Morales, M. A. (2000). Mechanisms and density dependence of benefit in an ant-membracid mutualism. *Ecology* 81, 482-489.
- Pekarsky, B. L., McIntosh, A. R., Taylor, B. W., & Dahl, J. (2002). Predator chemicals induce changes in mayfly life history traits: a whole-stream manipulation. *Ecology* 83, 612-618.
- Reithel, J. S. & Billick I. (2006). Bottom-up mediation of an ant-membracid mutualism: effects from different host plants. *Evolutionary Ecology*, 20, 27-38.
- Smith, D. C. & Douglas A. E. (1987). *The biology of symbiosis*. Arnold, London.

Tables

Table I. Average nymph size and instar for all nymphs found, number of plants (successful treatment and nymphs present) used in data analysis. Nymph density (number of nymphs from plants in which treatment was successful: both plants with and without nymphs included). Sorted by treatment and week.

<i>Week 1</i>	Avg size (mm)	Avg instar	# plants	# nymphs/plant
Ant	1.4	1.87	8	12.1
No ant	1.59	1.6	4	2.3

<i>Week 2</i>	Avg size (mm)	Avg instar	# plants	# nymphs/plant
Ant	1.48	1.4	5	0.9
No ant	1.2	1.13	5	4.75

<i>Week 3</i>	Avg size (mm)	Avg instar	# plants	# nymphs/plant
Ant	1.57	2.19	9	5.36
No ant	1.24	1.3	4	0.9

<i>Week 4</i>	Avg size (mm)	Avg instar	# plants	# nymphs/plant
Ant	1.37	2.2	9	7.3
No ant	N/A	N/A	N/A	N/A

Figures

The effect of ants on nymph density

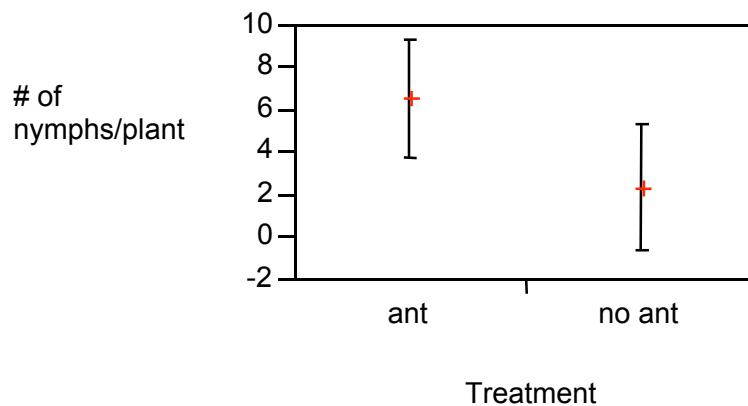


Figure 1. Across three weeks, when ants are present, nymph density significantly increases than when ants are not present.

The effect of nymph density on average nymph size

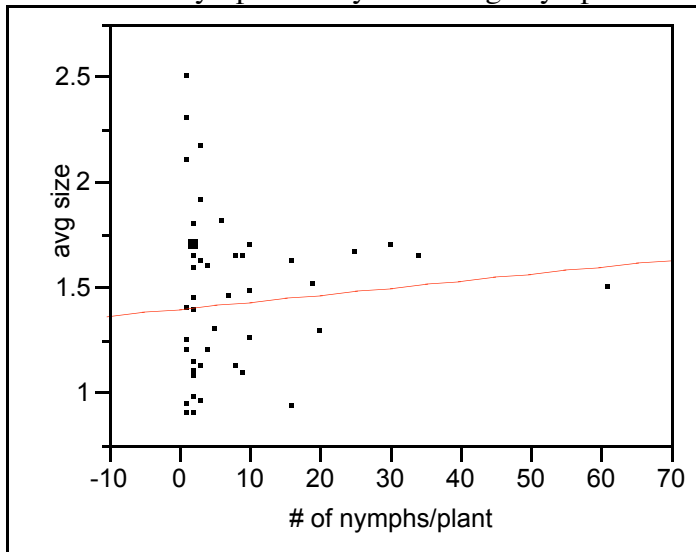


Figure 2A. There is no significant effect of nymph density on average nymph size for all weeks.

The effect of nymph density on average nymph instar

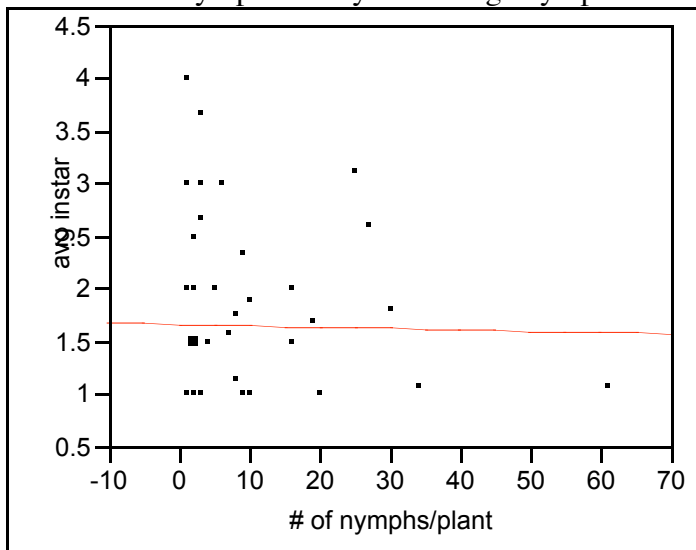


Figure 2B. There is no significant effect of nymph density on average nymph instar for all weeks.

The effect of nymph instar and presence or absence of ants on nymph size

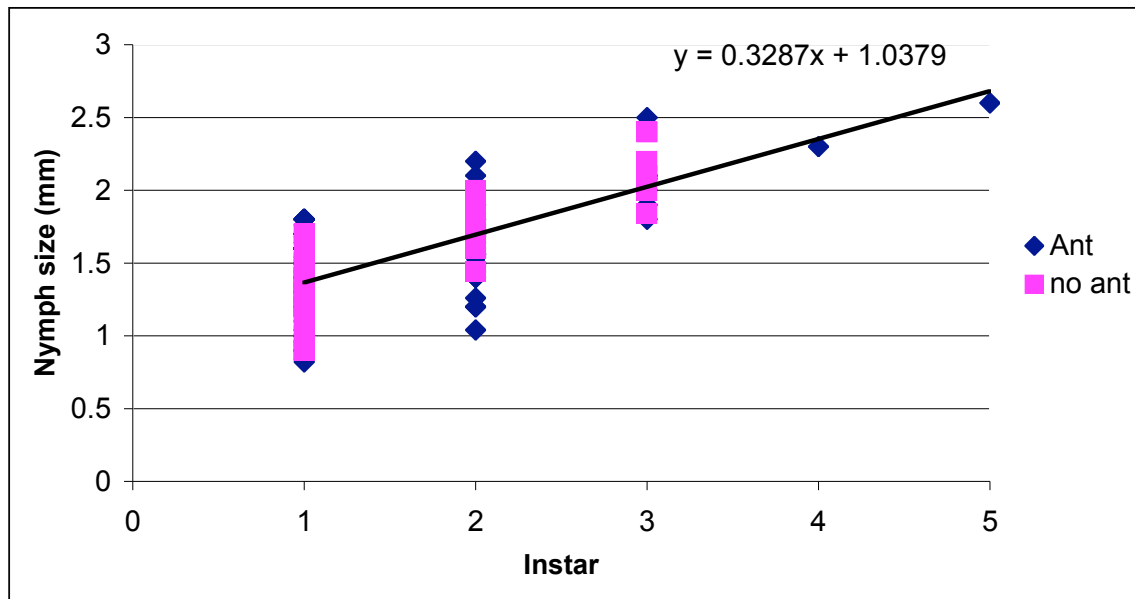


Figure 3A. Nymph size significantly increases with each instar. However, there is no significant difference in size/instar between treatment groups (ant and no ant). Data is from all weeks.

The effect of ants on residual nymph size

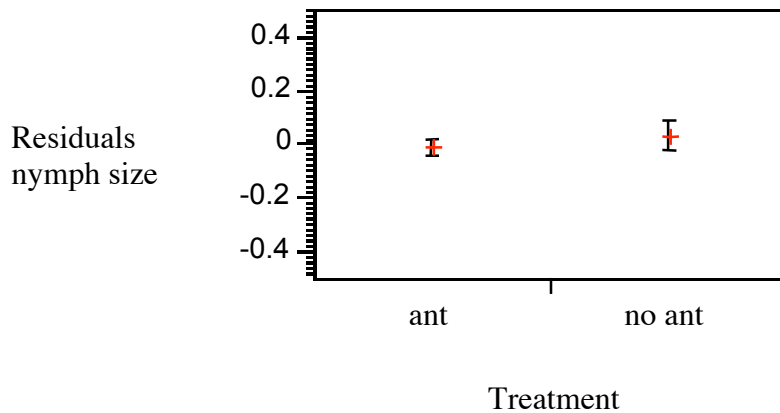


Figure 3B. For all three weeks, there is no significant effect of treatment on residual nymph size.

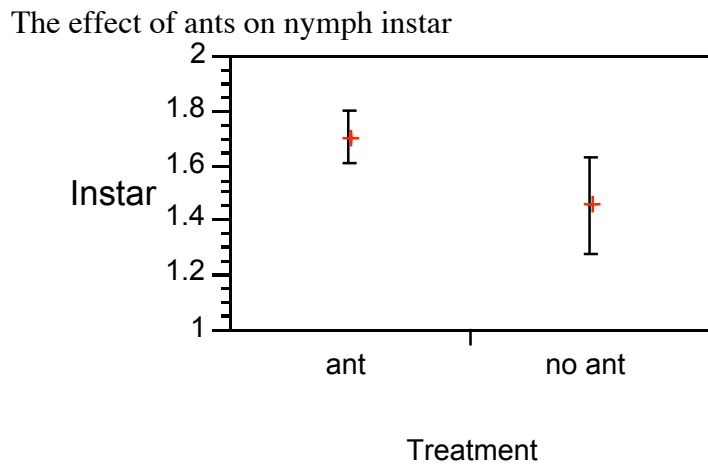


Figure 4. For all three weeks, when ants are present, nymphs are at a significantly later instar than when they are excluded from ants.