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and survivorship in an ant-treehopper
mutualism

THE TIMING OF THE ANT-EFFECT ON NYMPH SIZE AND SURVIVORSHIP IN AN ANT-TREEHOPPER MUTUALISM

ABSTRACT: This study surveyed the effect of removing ants at different times on survivorship and growth rates among different stages of treehopper nymphs. We excluded ants from different subsets of membracids every week and measured treehopper numbers and estimated nymph instars. We found ant-tending affects average treehopper size in the final two weeks of growth. Generally, ant exclusion earlier than the fourth instar decreases treehopper number and size. Furthermore, greater treehopper density increases treehopper size, but only when ants are always absent. Greater totals of new adults seen in earlier studies may be a direct product of the ant effect on treehopper growth rates.

KEYWORDS: treehopper, membracid, mutualism, ant-tending, *Publilia modesta*, *Formica obscuripes*, *Chrysothamnus viscidiflorus*, Almont Triangle.

INTRODUCTION

A mutualism is a shared strategy between two organisms where a cooperative net benefit to fitness is gained for each partner (Pierce *et al.* 2002). Optimal foraging theory suggests that organisms will maximize fitness through a minimal expenditure of energy for the optimal gain in resources (Krebs and Davies 1997). In order to maximize fitness, partners will compromise in energy expenditure when there are changes to benefits from a mutualism. The nature of an individual mutualism changes over an organism's lifetime, both in the organism's ability to expend energy, and or the benefit the organisms reap from the relationship. When faced with these fluctuations, a weak mutualism with a small positive difference between benefits and costs may have a higher chance of becoming a commensalism or parasitic relationship. Because they change over time, many mutualisms may not be obligatory, but rather, conditional. Determining the relative strength of mutualisms can reveal their evolutionary origins and the forces that create cooperative behavior.

In a treehopper-ant mutualism, the strength of the relationship depends on the benefits to fitness for each partner. In the case of the mutualism between the treehopper *Publilia modesta* and the ant *Formica obscuripes*, the ant benefits by feeding upon the ‘honeydew’ excretions of *Publilia modesta*, rarely if ever feeding upon the membracids themselves. In return, *F. obscuripes* increases survivorship in *P. modesta* by protecting them from the salticid spiders and coccinellid larvae (Reithel unpub. data) that prey upon the treehopper. *F. obscuripes* also might increase *P. modesta* fitness through indirect means, by allowing the treehoppers more time to feed and insert stylets (Morales 2000), because of the decrease in distracting predation.

What is unknown is how the impact of ants on treehopper survivorship and size changes over the course of a summer. *Formica obscuripes* tend developing *Publilia modesta* nymphs from late June to mid September, and thus the benefits and costs to each partner may change over time until the nymphs reach adulthood. Body size, number of treehoppers, and the production of honeydew change. Depending on the relationship between size and number of treehoppers, quantity of honeydew may go up or down. Quality of honeydew may change over time as well, possibly depending on the treehoppers’ need for ant protection. The need for protection in treehoppers may be determined by combination of increasing size, shell hardness, decreasing maternal care (Billick *et al.* 2001), as well as changing density of treehopper aggregations. In addition, ants closer to nests decrease in tending through the summer regardless of treehopper need for their protection (Crain 2005). Treehoppers may benefit more from ant-tending at different instars, surviving or growing more depending on their vulnerability to predators and the decrease in ant tending numbers. Ants may also have differential benefits from

treehoppers over time. Yet the degree to which the effect of ants on treehoppers and vice versa is time-sensitive has not been experimentally tested.

Understanding the effect of ant tending on treehopper size may help us interpret the finding that ant-tending reduces nitrogen content in treehopper bodies (Kay *et al.* 2004). Kay theorizes treehoppers in the presence of ants might be increasing nitrogen content in their honeydew at expense to body nitrogen, in order to greater attract ant tending or to deter predation. However, larger treehoppers may have smaller nitrogen content in their bodies (Billick unpub. data). If ant tending increases treehopper size, then Kay's theory regarding treehopper nitrogen manipulation is unfounded. Determining ant-effect on the size of treehoppers and aggregation size over time, and the effect of aggregation on body size separate from the ant-effect, might clarify the role of aggregation size and time in the three-way relationship of ants, nitrogen, and body size.

Do tending ants differentially affect treehoppers in different nymph instars, by increasing nymph size or number? Because ant tending is likely the highest when adults are present (Billick *et al.* 2001) and drops continuously from then on, and treehopper size and shell is weakest at hatching and increases through development, we hypothesize the maximum treehopper survivorship will likely be seen after the third instar, when size is large and ant-tending is still high. However, this does not account for density-dependent and size-dependent effects, which will be different depending on the size of each aggregation. Size of treehoppers may or may not be increased by ant tending. We tested our hypotheses by manipulating ant tending on different plants over time and measuring the effect on treehopper survivorship and size.

METHODS

We performed the experiment in the arid Almont Triangle region, 25 miles north of Gunnison, Colorado. Temperatures vary between 0 and 30 degrees Celsius during the three months of experimentation, June, July, and August. *Artemisia tridentata* Nutt. and *Chrysothamnus viscidiflorus* Nutt. (Hook.) are the main flora of the region. Respectively, big sagebrush and rabbitbrush are small woody perennials that dominate the land, but herbaceous plants such as *Wyethia spp.* (common name: mule's ear) and small grasses are present as well. Both *C. Viscidiflorus* and *Wyethia spp.* are in the *Asteraceae* family

The membracid treehopper *P. modesta* commonly oviposits into and feeds on both *Wyethia spp.* and *C. viscidiflorus*. *Publilia modesta* is common to the Western United States (Koppe and Yonke 1973). After the adult treehoppers overwinter at the base of these two plants, they mate in May, and oviposit their eggs into the stems of the plants from early May to late June. Females may lay multiple clutches of eggs on different plants. In both plants, the treehopper nymphs emerge from their eggs in late June and early July. They aggregate, and adults stay with nymphs during development until August. Both adults and nymphs feed on the phloem of their host plant, from the stem and ribs, ejecting the excretion onto the plant when not in the presence of ants, or beading it between their anal setae for tending ants to feed upon. The nymphs develop through five instars until they emerge as adults in mid August and early September. These adults will disperse in the fall and eventually move towards the base of their plants to overwinter until May.

Formica obscuripes is an aggressive ant that forages in many temperatures (Billick unpublished data). The ant species feeds on the sugary honeydew excreted from

the treehoppers, as well as from other honeydew-excreting insects such as aphids. They supplement this high sugar diet through predation on insects. *F. obscuripes* is the primary ant attendant of *Publilia modesta* at Almont Triangle, protecting the treehopper from predation (Reithel unpublished data) while feeding on their excretions. While there are other ant species, *F. obscuripes* most effectively protects treehoppers at the Almont Triangle. The ant is also the most abundant and at a high density, with nests varying in distance from each other from several to only a few meters apart.

Testing Temporal Variation in Ant Exclusion

We determined whether there was a critical time in ant-tending by manipulating the length of time ants were available to membracids. We conducted the experiment from late June through early September. During the weeks of June 26th to July 24th, we excluded ants from different subsets of membracids on plants on different weeks. On June 20th-22nd we selected 120 healthy *C. viscidiflorus* plants based on the presence of oviposition scars and adult treehoppers. We measured the height of each plant at the highest point of the entire bush, and the distance between the plant and the nearest ant colony. We duct-taped each plant at the base, and trimmed surrounding vegetation in order to keep ants from crossing onto plants on no-ant treatments. This served as a control for all treatments, to guarantee the duct tape and the trimming had the same effect on all plants and treehoppers. We randomly assigned individuals within each group of six plants to six treatments. On June 28th, we spread Tanglefoot® on duct tape at the base of plants in treatment #1, excluding ants from tending the treehoppers for the remainder of the five weeks. Without ant protection, spiders, flying predators, and predators already present on the plants were free to feed on the treehoppers. On July 5th, 12th, 19th, and 26th,

we excluded ants in the same manner for plants assigned treatments 2, 3, 4, and 5, respectively. Plants of treatment #6 never had ants excluded.

We counted tending ants, nymph aggregation size, number of new adults, and estimated nymph instar for every plant weekly. We counted the number of tending *Formica obscuripes* ants weekly starting June 27th until August 1st for every plant. We counted the number of nymphs present on each plant. We measured treehopper size on each plant using a previously prepared index card with nymphs of different discrete sizes glued to it. We collected these nymphs weekly from a *Wyethia* plant, sized using a 0.02 mm caliper, and then sorted these nymphs by discrete average size categories and distinctive traits, into the five instars. We selected average sized nymphs and glued them to the card as different categories. These sizes were truthed weekly with the calipers. Each week as nymphs grew, we made new cards with new nymphs to represent the different new instars. We used these cards to count the number of nymphs in each size category present on each plant.

We added acrylic dots on adult's pronotums with Painters Speedball® opaque paint markers once nymphs approached the fifth instar. Each week after this marking, we determined the number of new adults by counting adults without dots on each plant, and then marked these adults as well, to determine the new adults for the next week. We measured and counted all adults' sizes as well.

At the end of five weeks, we measured the height of plants again, as well as observed their health, and calculated change in plant size.

ANALYSES

We tested the effect of the week and treatment on the response variables: the number of nymphs, the percent nymphs of each size, the number of new adults, and number of tending ants. Repeated measures tested the variance and significance between the response variables of each week and the independent variable of the number of nymphs in the previous week. We also tested the effect of nymph number on development for ant-tended treatments and non-tended treatments using a repeated measures variable test. Average instar per treatment was calculated by multiplying the number of each successively larger nymph size by a successively higher number, and then averaging these numbers over total nymph number.

$$\frac{(\# \text{ instar 1} + 2(\# \text{ instar 2}) + 3(\# \text{ instar 3}) + 4(\# \text{ instar 4}) + 5(\# \text{ instar 5}))}{\text{total nymphs per week}}$$

Statistics were conducted in the JMP® program version 5.1.

RESULTS

The number of nymphs that had plants varied each week (Table 1). Treatment had no significant effect on the average number of nymphs surviving through the five weeks ($F_{4,94} = 0.022833$, $P=0.7092$). Nymphs decreased in number in all six treatments (Fig. 1A). In week five, treatments sorted into the predicted order, with the earlier treatments having fewer nymphs (Fig. 1B). Treatments 4 through 6 remained on average high in nymph number (about 6), while Treatments 1 through 3 had on average half as many ($F_{4,94} = 1.3739$, $P = 0.2489$).

Nymphs had five definite instar size groupings between 0.5 and 4 millimeters, and the means of the sizes of each instar were used to identify instar for every nymph (Table 2). Treatment significantly affected average instar size of nymphs ($F_{4,51} = 0.2386$, $P = 0.0252$). Removing ants from plants earlier in the summer decreased the average size of

treehopper nymphs on plants, most clearly seen between weeks 4 and 5 (Fig. 2). Except for treatment 1 rivaling the sizes of treatment 4 and 5 in week 5, earlier exclusions resulted in smaller nymphs on average. Except for treatment 1, again treatments 2 and 3 were grouped close together and resulted in smaller nymphs than treatments 4 through 6.

There was an interaction between treatment, total nymph number, and time ($F_{4,51} = 0.274677$, $P = 0.0133$). Total nymph numbers in treatment 1 significantly affected average nymph size in week five (Fig. 3, $R^2 = 0.633425$, $y = 0.0320531x + 1.8021618$, $P = 0.0181$). Larger aggregations of nymphs increased average nymph sizes. In contrast, treehopper sizes of treatment 5 and 6 were not affected in any week by total nymph number (Fig. 4A, week 4: $R^2 = 0.000181$, $y = -0.0002821x + 3.3264181$, $P = 0.9564$; Fig. 4B, week 5: $R^2 = 0.037774$, $y = 0.0041745x + 3.2601887$, $P = 0.3986$). Thus treehopper nymph size is density-dependent only when ants are not tending.

Table 1		
Week	# of plants with nymphs	# of plants without nymphs
1	81	39
2	74	46
3	78	42
4	70	50
5	65	55

Table 1 The number of plants with and without treehopper nymphs per week.

Table 2			
Size Class	Smallest Size (mm)	Largest Size (mm)	Mean Size (mm)
1	0.5	1.5	1.17 (wk 3)
2	1.5	2.25	1.94 (wk 3)
3	2.25	3.0	2.63 (wk 5)
4	3.0	3.75	3.3 (wk 5)
5	3.75	4.25	3.97 (wk 5)
Adult	4.25	5	-

Table 2 Estimated size ranges for treehopper nymphs, estimated to parallel the 5 different instars. Mean size was calculated weekly from sample treehopper nymphs of each size class (the week that the measurement was used is shown in parentheses).

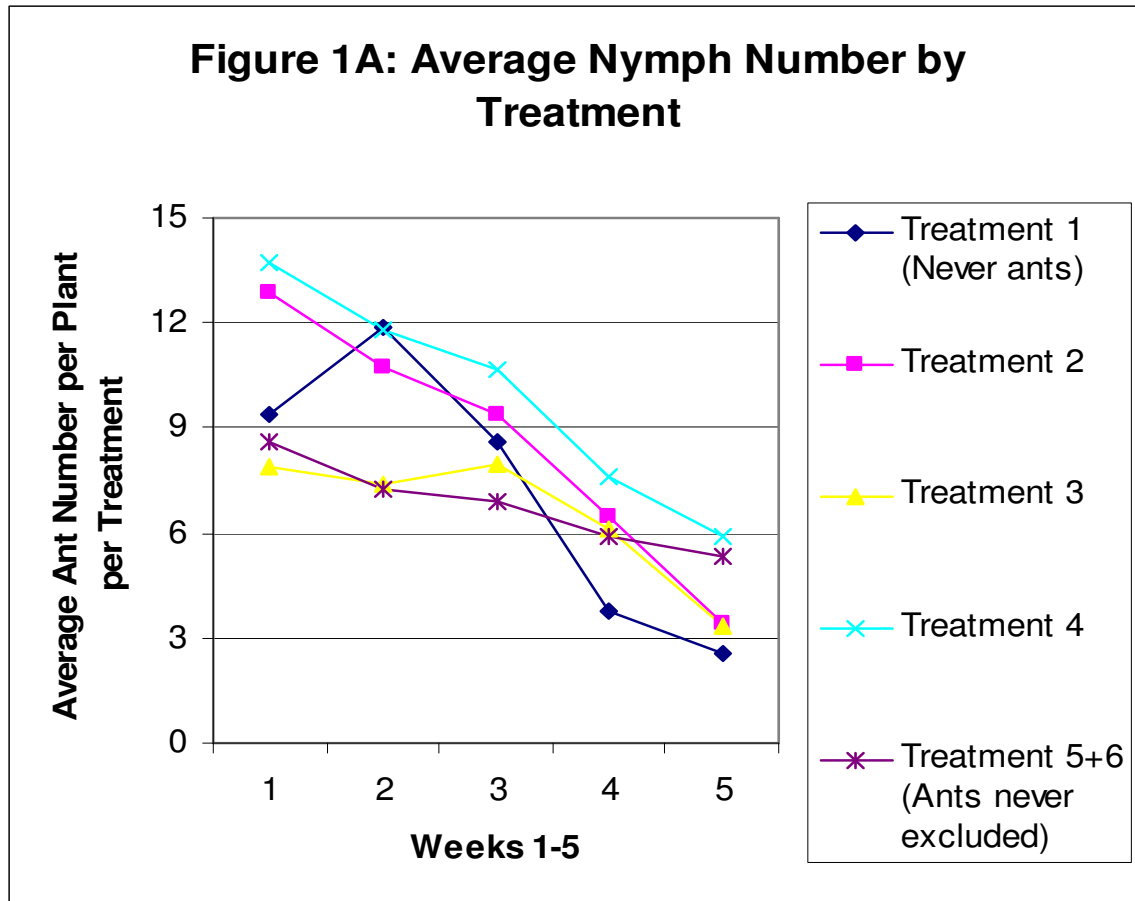


Figure 1A The effect of treatment upon average nymph survivorship.

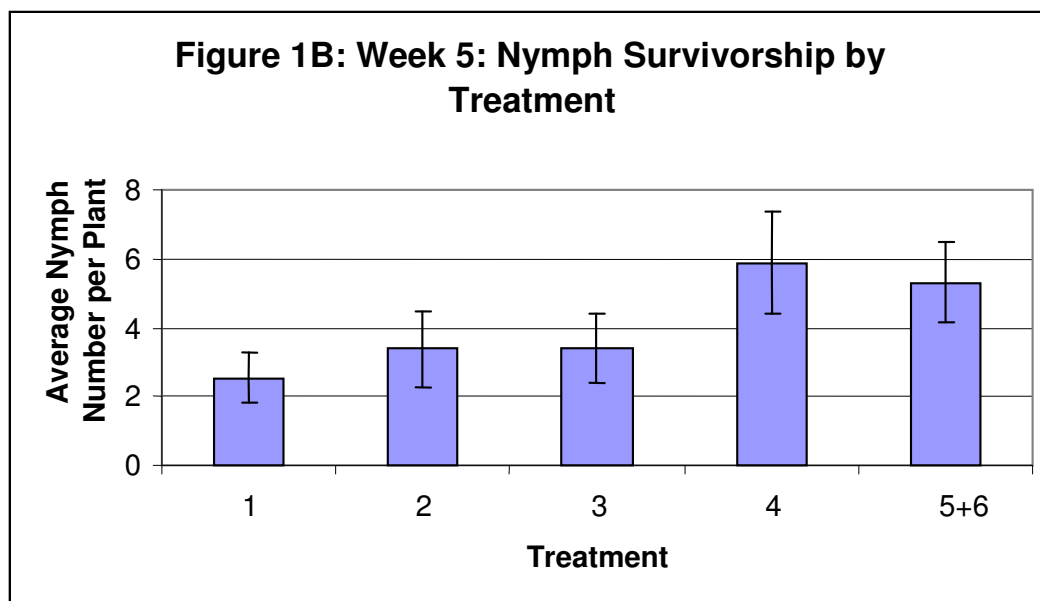


Figure 1B The effect of treatment on average nymph survivorship in week 5 alone.

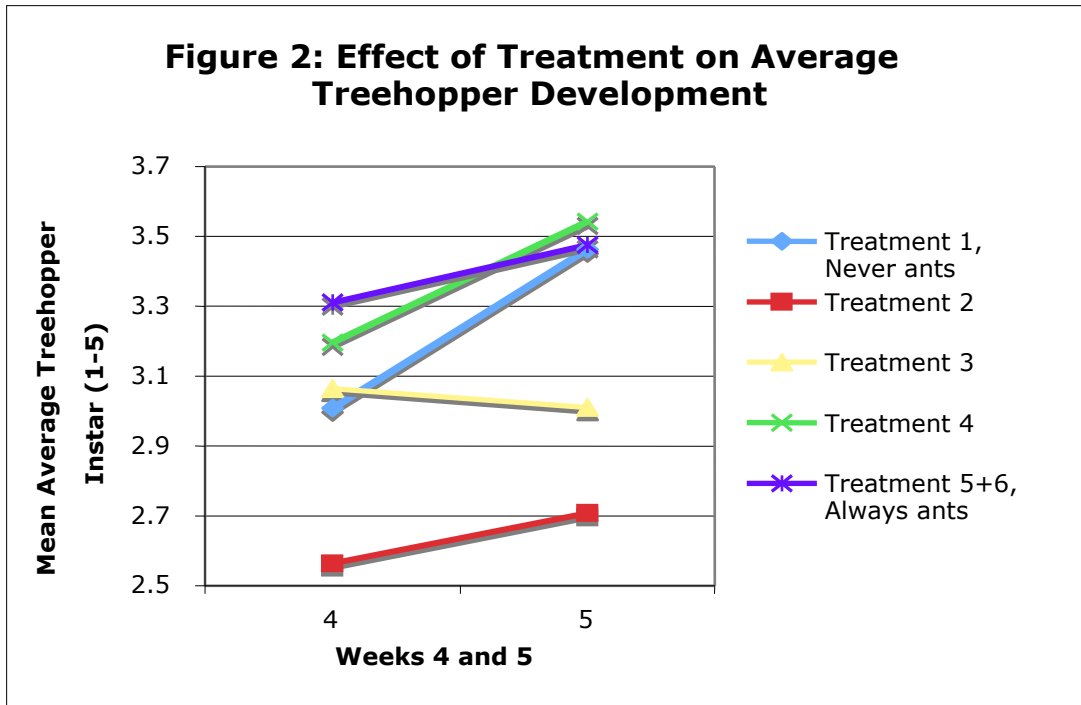


Figure 2 The effect of treatment upon average treehopper nymph development size.

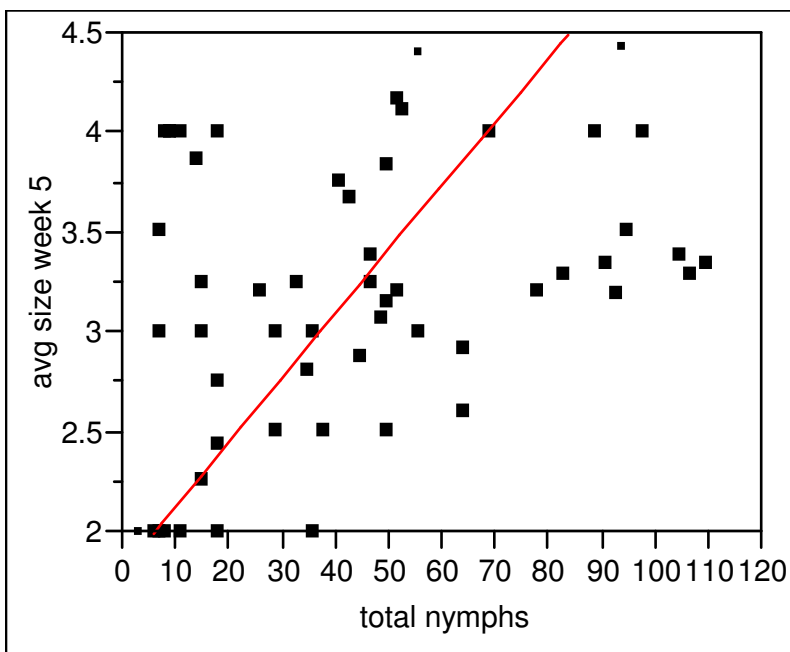


Figure 3 For treatment 1 (no ants) week 5, the effect of nymph number on average nymph size.

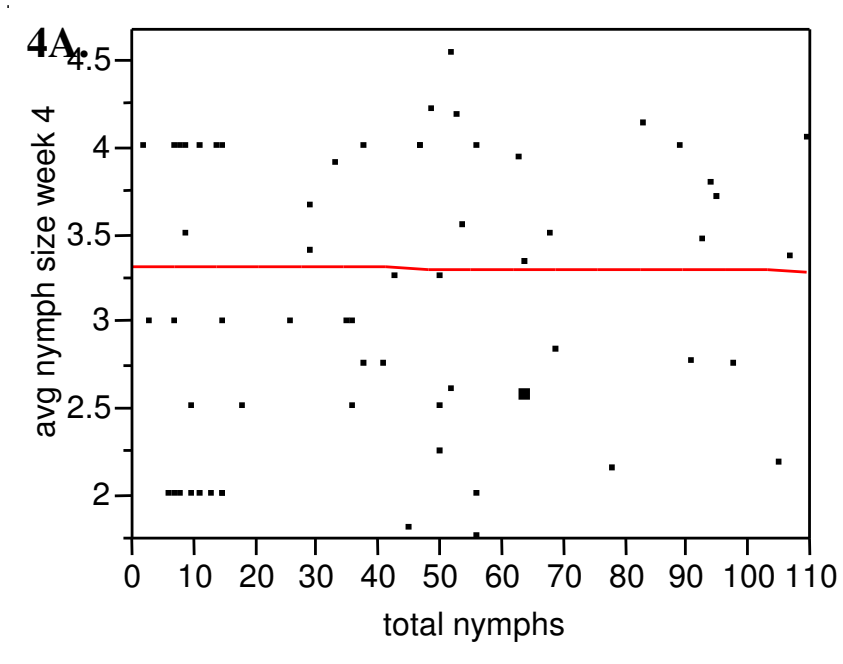


Figure 4A For treatments 5 and 6 (always ants), the effect of nymph number on average nymph size for week 4.

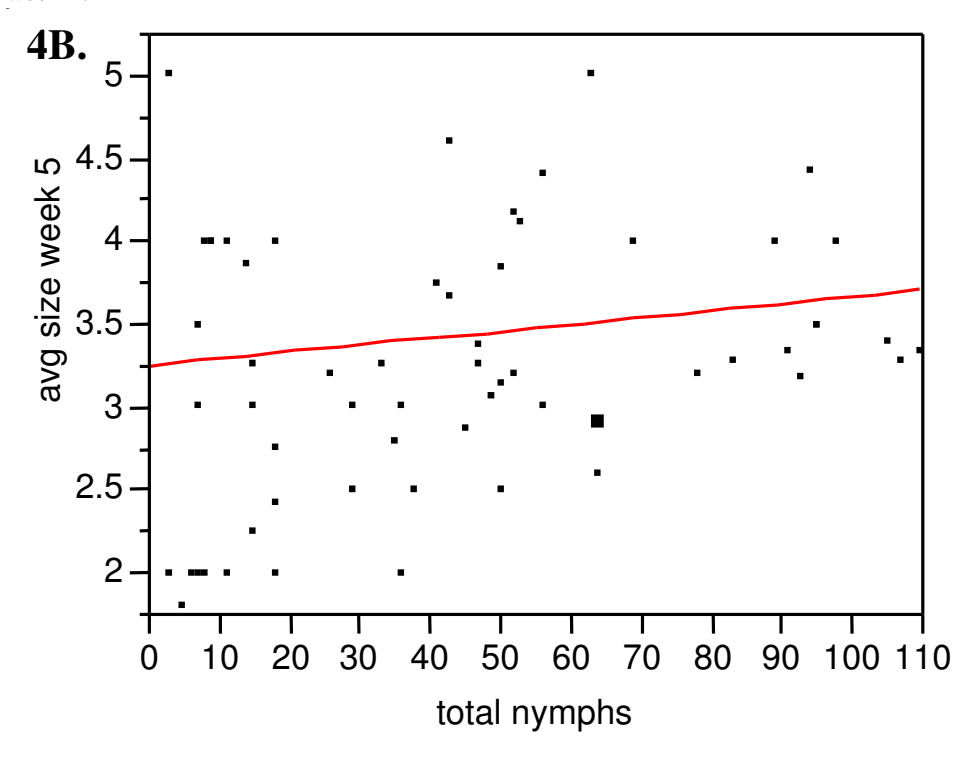


Figure 4B For treatments 5 and 6 (always ants), the effect of nymph number on average nymph size for week 5.

DISCUSSION

Although removing ant tending at different times throughout the summer did not have a visible effect on the number of nymphs present, this result does not necessarily mean ants did not have effect on the survivorship of nymphs. We suspected the effect of ants would only occur weeks after each treatment's ant exclusion, when predators might succeed in lowering nymph numbers by attrition. This lag effect may still have occurred, but taken much longer than expected. Ant tending has been proven to increase the final number of new treehopper adults on *Chrysothamnus viscidiflorus* (Billick *et al.* 2001, Billick and Tonkel 2003) and we saw this gradient in week five of the experiment (Fig. 1A). The earlier treatments had fewer nymphs, in the exact order predicted, with treatments 1 through 3 having fewer number of nymphs (Fig. 1B). This may be representative of a nymph mortality lag effect that only shows in week 5. In fact, this lag on the effect of removing ants at different times during the summer may only be detected when fewer nymphs reach the adult stage on non-tended plants.

Only one of my hypotheses was supported: ant exclusion likely had the greatest effect before the fourth treehopper instar had developed. Treatment 3 was applied in the second week of measurement, when treehoppers were mostly still in their second and third instars. In the later treatments, nymphs were given time to progress to larger average sizes. If the results of week five are taken into account, then treatments earlier than 4 had lower survivorship (Fig. 1B) suggesting the ant exclusion had the most effect on instars 1, 2, and 3.

While density may have been unaffected, except for treatment 1, excluding ants earlier decreased treehopper size (Fig. 3), unlike systems where aphids feed ants with

expense to their bodies, becoming smaller and even less fecund (Yao *et al.* 2003). Ants likely prevent predators from distracting treehoppers from feeding. Because treehoppers need time to insert their stylets, and avoiding predators means fleeing without feeding, predators may affect treehopper nutrient uptake, and thus decrease treehopper body size. Ants might prevent this sublethal predator effect.

Total nymph number also increases average treehopper size, but only in the absence of ants in week five (Fig. 4A and 4B). Individual treehopper's chances of being harassed by predators might decrease with more treehoppers present. Thus the total amount of time each surviving treehopper spends running instead of eating is lowered with a greater number of treehoppers, and body size might increase proportionately. When ants are present, predators are not, and thus this effect wouldn't be seen in treatment five (Fig. 4B). Because of a lag effect, treatment 1 might only be seen after four weeks, when lack of feeding has had a chance to take effect on the nymphs of less populated plants.

Ant exclusion may have the greatest effect on small treehoppers upon hatching, creating the effect where a few surviving large treehoppers skew average instar size in Treatment 1. If treatment 1 had many treehoppers in week 5 this would suggest that they would have large treehoppers (Fig 4A). However, treatment 1 had the fewest number of treehoppers of the six treatments (Fig 1B). This suggests the rarer plants with relatively large numbers of treehoppers in treatment 1 had relatively larger nymph sizes, but when compared to the other treatments, treatment 1 still had the fewest nymphs. If mortality is high, but body size large, this would suggest the remaining treehoppers are large, while the small treehoppers had perished earlier. If enough small treehoppers die quickly, then

average size would be higher among survivors. Treatments 2 and 3 might not have been affected by this because of increased numbers of nymphs in weeks two and three saving smaller treehoppers through a density-dependent effect.

The effect of ants on size may also explain the effect of ants on the number of new adult treehoppers. If ant tending increases treehopper size, and only treehoppers in fifth instars can switch over to adults, then a lag effect would kill off treehoppers not prepared to switch over to adult treehoppers, and fewer adults would appear. Also, if ants prevent sublethal distraction from feeding, perhaps nymphs without ant protection are less likely to build up the necessary reserves of energy needed to undergo the molt into the adult stage.

Finally, because ants affect treehopper instar size, ants cannot be said to coerce treehoppers into manipulating their nitrogen stoichiometry (Kay *et al.* 2004). If greater treehopper size decreases their nitrogen body levels, and ants affect treehopper size, then treehoppers do not necessarily manipulate nitrogen levels based on ant-tending, but based on the allometric size effect. This size effect is likely due to the high percent of nitrogen in the cuticle of treehoppers, which increases less compared to the increase in volume as treehoppers grow. If percent nitrogen is only surface area divided by volume, then larger treehoppers will consequently have a much smaller percentage of nitrogen.

The lack of proof for Kay's theory classifies the mutualism of *Formica obscuripes* and *Publilia modesta* as a behavioral mutualism, not physiological (Margulis 1993). *F. obscuripes* only have evolved non-predatorial behavior in regards to *P. modesta*, and these membracids do not necessarily manipulate their bodies to satisfy ant-tending. Ant and treehopper have a more or less constant positive effect upon each other,

but the two seem to have evolved little to link their relationship tightly beyond behavior. This information will help phylogenetic mapping by possibly arranging this mutualism more proximately on the evolutionary scale than other more derived ant-membracid mutualisms.

In summary, if ants increased survivorship, the effect could only be seen after a lag lasting almost to the onset of adulthood. However, ants altered treehopper size, with a density-dependent effect when ants were absent. This implies treehoppers do not manipulate nitrogen levels in their bodies, but ants affect membracid size, and this in turn affects nitrogen levels in *Publilia modesta*.

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