

Effects of Host Plant Quality and Ant Tending for Treehopper *Publilia concava*

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ABSTRACT Few studies have evaluated the relative importance of bottom-up regulation for the population dynamics of mutualism. To address this, we tested the hypothesis that host plant quality affects the strength of the mutualism between *Publilia concava* (Say) (Hemiptera: Membracidae) treehoppers feeding on *Solidago altissima* L. and ants. *P. concava* is a phloem-feeding treehopper that excretes a sugary waste product called honeydew. Ants collect this honeydew as a food resource, and treehoppers benefit both directly (e.g., feeding facilitation) and indirectly (e.g., protection from predators). We evaluated the effect of host plant quality on both the direct and indirect effects of this mutualism by using a factorial design that manipulated N–P–K fertilizer level and ant presence. For the experiment that focused on the direct effects of ant tending, both body size and survivorship were monitored. For the experiment that included the indirect effects of ant tending, only survivorship was monitored. Both host plant quality and ant tending increased the performance (survivorship and size) of *Publilia* treehoppers. However, we find no support for the hypothesis that host plant quality influences the strength of the mutualism in this system—there was no significant interaction between ant tending and fertilization for any measure of treehopper performance considered. We suggest that this result is explained by the independence of per capita tending levels with both host plant quality and treehopper density in this experiment.

KEY WORDS ants, Membracidae, mutualism, *Solidago altissima*

Recent studies of mutualism have emphasized the context dependence or conditionality of these interactions, and ant–homopteran mutualisms have provided a model system for these studies. In ant–homopteran mutualisms, ants protect homopterans and facilitate feeding in exchange for honeydew (the sugary excretion of homopterans) (Buckley 1987). Ant–homopteran mutualisms are a type of host–visitor mutualism, characterized by a host that provides a resource reward to a visitor in exchange for a variety of potential services (Thompson 1982). Other examples of host–visitor mutualism include seed dispersal, pollination, and other ant–protection mutualisms.

Conditionality resulting from top-down forces in ant–protection mutualisms is relatively well established. For example, previous studies have shown that predator protection is a major component of the benefit received by homopterans from ant tending (Morales 2000b), that the among-year variation in benefit to homopterans from ant tending is correlated with the density of predators for a given year (Cushman and Whitham 1989), and that the effectiveness of ant protection is a function of ant density (Itioka and Inoue 1996) and species (Del-Claro and Oliveira 2000).

Less is known about conditionality in ant–protection mutualisms resulting from bottom-up forces (but see Dyer and Letourneau 1999). In general, conditionality in host–visitor mutualisms can result from variation in either the effectiveness or density of mutualist partners. A number of studies from ant–protection systems have shown that the magnitude of host benefit varies with the density of visitors (Breton and Addicott 1992a; Flatt and Weisser 2000; Morales 2000a,b). Some of the factors that influence the level of ant density in these systems include the quality and quantity of reward production (Völkl et al. 1999; Fischer et al. 2001), host density (Breton and Addicott 1992a; Morales 2000a,b), and the spatial distribution of hosts (Cushman and Whitham 1991, Morales 2000a). Thus, one important way that bottom-up factors may regulate host–visitor mutualisms is by influencing the attractiveness of hosts to visitors (Cushman 1991). Because host plant quality can mediate the quality of host rewards (Gardener and Gillman 2001), and because mutualist hosts can alter the quality of their host plants (Kay et al. 2004), there is a strong conceptual basis for understanding the relative importance of bottom-up factors in these systems.

Studies that have examined the response of ants to variation in the quality of homopteran honeydew (Völkl et al. 1999) and artificial nectar (Blüthgen and

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Fiedler 2004) are generally consistent with the hypothesis of bottom-up regulation. However, studies that have tested the host plant mediation hypothesis directly have provided mixed support (Baylis and Pierce 1991, Breton and Addicott 1992b, Wimp and Whitham 2001, Stadler et al. 2002, Billick et al. 2005, Quental et al. 2005). Some of the most consistent results come from studies of ant-lycaenid systems showing increased ant-tending rates for caterpillars feeding on high-quality hosts plants. Even for these studies, however, strong evidence that this increase in ant tending translates to an increase in survivorship is lacking (Baylis and Pierce 1991, Billick et al. 2005). Although the hypothesis of host plant mediation of host-visitor mutualism is attractive, studies are needed to evaluate the importance or frequency of this mechanism of conditionality relative to other factors that can affect the outcome of mutualism.

Materials and Methods

Here, we report results from a study exploring the impact of host plant quality on the mutualism between ants and *Publilia concava* Say (Hemiptera: Membracidae) treehoppers. Previous studies have shown that *Publilia* spp. benefit from ant tending both indirectly by protection from predators (Bristow 1984; Cushman and Whitham 1989, 1991; Morales 2000b) and directly (Morales 2000b), such as may occur through feeding facilitation and increased body size (Banks and Nixon 1958, El-Ziady 1960, Takeda et al. 1982, Bristow 1984, Stadler and Dixon 1999, Flatt and Weisser 2000). Although predator protection may be the most obvious mechanism of benefit in ant-homopteran mutualisms (Buckley 1987), there are several reasons to isolate direct from indirect benefits when evaluating the role of host plant quality in this mutualism. First, a recent study of the closely related species *P. modesta* shows that when ant tended, treehoppers can cause systemic reductions in the nitrogen content of their host plants (Kay et al. 2004). Consequently, increased host plant quality may serve to mediate the top-down effect of treehoppers in this system. More generally, isolating direct from indirect benefits will allow greater insight into the potential underlying mechanisms in this mutualism. In this study, we evaluated the effect of host plant quality for both the direct and total (direct + indirect) benefits of ant tending by excluding predators in 1 of 2 yr of this study.

Study System. *P. concava* is a univoltine subsocial insect typically found on the host plant *Solidago altissima* L. (tall goldenrod, a perennial herb) and tended by ants in the genera *Myrmica* and *Formica* for populations located in northwestern Massachusetts. Our study site was a field located in Hopkins Memorial Forest, Williamstown, MA (42° 43' 34" N, 73° 13' 25" W).

P. concava adults overwinter in the leaf litter and emerge in early spring to mate. Females produce one or two broods within a variable time period but usually not exceeding 1 mo between broods. Two broods from a given female can be found on one or

two host plants (Bristow 1983). We define an aggregation as all treehoppers on a given host plant, and aggregation size can vary from a few to >1,000 (Morales 2000b). Additional life history details of *P. concava* can be found in Morales (2000b) and Zink (2003).

Like other homopterans, *P. concava* is a phloem-feeding insect that filters large amounts of plant sap to meet its nitrogen requirements. Recent work indicates that potassium and phosphorous may be equally important components of insect growth (Stern and Elser 2002). Because we were interested in testing for a significant effect of host plant quality rather than nitrogen limitation per se, we manipulated host plant quality by using the slow-release fertilizer Osmocote (8.2% NH₄, 5.8% NH₃, 14% P₂O₅, and 14% K₂O; The Scotts Company, Maryville, OH).

Experimental Design. Goldenrod rhizomes were collected from a location bordering a railway near our study site (≈1.5 km) in late April and early May. Plants were collected from this site because the soil was rocky, facilitating the removal and separation of rhizomes. Rhizomes were separated into sections of ≈20 cm with at least one developing shoot and planted in 7.25-liter pots with a 4:1 mixture of potting soil (Agway, no fertilizer added; Gro Max, Hudson, NY) and Perlite (Schultz, St. Louis, MO). Half of the plants were provided with 17.5 g of Osmocote.

Gravid treehopper females were identified by locating male-female pairs (males typically guard females after mating; M.A.M., personal observation). Pairs were collected from a nearby site (≈2 km) with a high density of treehoppers to facilitate collections. Pairs were collected in late May and confined to potted plants (two pairs per pot) by using mesh netting over wire tomato cages. Oviposition subsequently occurred on host plants, and nymphs hatched beginning in early July.

In 2002, we evaluated the importance of host plant quality focusing on the direct benefits of ants to treehoppers. Forty-two *Myrmica* spp. colonies within a 35 by 10-m area were located by baiting; 14 colonies were randomly chosen for use in this study ($\bar{x} \pm SE$ nearest-neighbor distance = 3.6 ± 0.6). Of these 14 colonies, 13 colonies were later identified as *Myrmica fracticornis* Forel, and one colony was identified as *Myrmica latifrons* Starcke (= *M. emeryana*). Despite locating plants adjacent to *Myrmica* colonies, *Myrmica* tenders were replaced on some plants by *Formica* species in the "fusca" and "pallidefulva" groups, especially toward the end of the experiment. Because turnover was unrelated to fertilization ($\chi^2_{df=1} = 0.225$, $P = 0.635$), and because excluding plants that had been replaced by *Formica* tenders did not affect our qualitative conclusions, these plants were included in analyses.

Potted plants ($n = 56$) were transferred to the study site on 23 June. Four goldenrod plants were arranged in a square array around each ant colony ($n = 14$) at a distance of 0.5 m from the main colony entrance. Arrays consisted of one plant from each treatment; ants and fertilizer, no ants and fertilizer, ants and no

fertilizer, and neither ants nor fertilizer, randomly arranged around colonies. Pots were buried so that the level of soil in the pot was at the same height as the ground outside the pot. The extra rim of the pot was removed to allow easy ant access to ants—ant tending was observed within ≈ 1 d of transferring pots. Note that the density of treehopper-occupied plants used in this experimental design is consistent with that of natural populations of treehoppers tended by *Myrmica* spp. ants in this region (e.g., $\approx 67\%$ of colonies tend two or fewer aggregations; M.A.M., unpublished). Because of an error in the experimental setup, two fertilized plants were replaced with unfertilized plants, resulting in an unbalanced design.

We excluded ants using Tangle-Trap® (Tanglefoot, Grand Rapids, MI) applied to the base of exclusion plants beginning 8 July, at which time we began censuses. We censused treehopper nymphs and adults every 2 to 3 wk for a total of four times throughout the summer, ending 1 September. Ant censuses were timed to coincide with the period immediately before and after treehopper censuses.

We excluded predators (primarily coccinellid beetle adults and larvae, syrphid fly larvae, and salticid spiders in the area of the study site) from all treatment plants throughout the study with mesh-netting bags. Bags were draped over a supporting stake and cinched near the bottom with a draw cord. For the ant-excluded treatments, bags were cinched above the Tangle-Trap. For the ant-tended treatments, a small opening was left at the base of the bags to provide ants with access to treehoppers. To maintain the effectiveness of ant treatments and to monitor predator exclusions, we checked plants approximately daily. We clipped vegetation that was surrounding the plants to prevent ant invasions and watered plants as needed.

In 2005, we repeated the experiment without excluding predators to evaluate the effects of host plant quality on the total benefits of ants to treehopper. The experimental design was similar to that used in 2002 but without excluding predators. Because there was no effect of ant species in the 2002 experiment (see above), no attempt was made to control for the identity of ant-tending species in 2005. Also, to focus on survivorship (rather than differential fecundity), experimental manipulations did not begin until the majority of treehopper nymphs had hatched resulting in a total of only three censuses.

Finally, for the 2002 experiment, treehoppers were confined to their host plant for overwintering by sealing mesh bags (used for predator exclusions during the summer) with duct tape around the base of pots in late September. For the untended treatments, the Tangle-Trap was covered with duct tape to prevent trapping treehoppers. Emergence from overwintering was observed by 10 May 2003 (about a week earlier than natural populations, matching the difference in goldenrod phenology for potted plants) and a final census of adult abundance was taken on 4 June 2003.

Data Analysis. To confirm that fertilization treatments significantly affected host plant quality, we collected and had leaves analyzed for percentage of

nitrogen (University of Nebraska Lab, ECS 4010 CN analyzer, Costech, Valencia, CA) from each of the 56 experimental plants. Additionally, we collected leaves from 26 control plants located within our study site. Half of these “nontreatment” leaves were from plants with treehoppers and half were from plants without treehoppers. Leaves were collected on 13 August 2002 from the top third of each plant but below developing inflorescences (all plants ultimately flowered).

A subsample of adult treehoppers ($n = 276$) was collected 20 and 25 September 2002 from all plants with treehoppers. Approximately 10% or a maximum of 10 treehoppers from each plant was taken. Treehoppers were sexed and subsequently measured for length and width by using a dissecting scope (SZX-9, Olympus, Melville, NY) attached to an imaging system (model 4912 camera, Cohu, San Diego, CA; LG-3 framegrabber, Scion Corporation, Frederick, MD; and software, Scion Corporation 2000). Treehoppers were weighed to the nearest 0.01 mg by using a semi-analytical balance (AX205DR, Mettler-Toledo, Columbus, OH). Length, width, (measured at the longest and widest point), and weight were combined to a single measure of treehopper size before analysis by using principal components analysis (PCA).

Summer survivorship data were analyzed using mixed effects models with the package nlme (Pinheiro and Bates 2000) in the statistical environment R (R Development Core Team 2004). Mixed effects models provide a flexible alternative to analyses such as repeated measures or split-plot (i.e., block) designs by allowing random effects to specify a grouping structure for the data, whereas the fixed effects specify the treatments or variables of interest (Pinheiro and Bates 2000, Saavedra and Douglass 2002). Mixed effects models can effectively handle unbalanced designs (as in the current experiment) and allow the variance-covariance structure to be modeled explicitly (Pinheiro and Bates 2000).

The statistical models used for inference were selected using Akaike's An Information Criterion (AIC) (Burnham and Anderson 1998). The simplest, or “base” model, was based on the assumption of equal variances and compound sphericity for the repeated measures analyses. These assumptions were tested by comparing the base model to one with separate variance estimates or to one that specified an autoregressive error structure for the repeated measures designs. The more complex model was used if it had a $\Delta AIC \geq 3$ (Burnham and Anderson 1998). Statistical inferences of treehopper survivorship are therefore based on mixed effect models that included separate variances for each treatment combination, and for the 2002 experiments only, autocorrelated within group errors for the repeated measures analyses of treehopper density. For the analysis of treehopper size, the base model (i.e., equal variances) had a lower AIC score and therefore was used for making statistical inferences.

Analysis of overwintering survivorship was based on the natural log of the ratio of spring population density in 2003 (plus 1 to account for zero values) and the

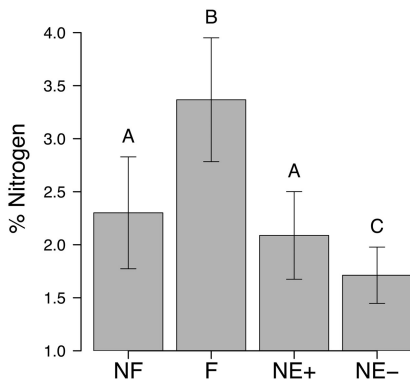


Fig. 1. Percentage of nitrogen of leaf samples from fertilizer supplemented plants (F), control plants (NF), and nonexperimental plants (i.e., naturally occurring within the area of our study) with (NE+) or without (NE-) treehoppers. Error bars represent 0 ± 1 SD (SD is used to emphasize the range of variation among individual samples). The analysis of nitrogen content was based on a model with separate variance estimates (see Materials and Methods) and posthoc comparisons used pairwise *t*-tests with separate variance estimates. *P* values were adjusted for multiple comparisons using Bonferroni corrections. Treatments with the same letter are not significantly different from each other.

population density for the final summer census in 2002 discounted by the number of treehoppers sampled for the size analysis.

In all analyses, colony was modeled as a random effect (i.e., a blocking variable). For the repeated measures analyses, colony and plant nested within colony were treated as random effects to account for the grouping structure of the data (Pinheiro and Bates 2000). Similarly, because multiple treehoppers were analyzed from a single plant, colony and plant nested within colony were treated as random effects for the analysis of size.

Results

Host Plant Quality. Fertilization with Osmocote significantly increased the percentage of nitrogen of supplemented plants relative to control plants in our experiment ($\bar{x} \pm \text{SE} = 3.36 \pm 0.11$ versus 2.3 ± 0.1 ; $F_{3,78} = 55.47$; $P < 0.0001$; Fig. 1). A comparison of the nonexperimental plants in the area of our study site showed that plants with treehoppers had significantly higher nitrogen content than plants without treehoppers (Fig. 1). Relative to nonexperimental plants with treehoppers, percentage of nitrogen was significantly higher for the supplemented but not control plants (Fig. 1). Thus, nitrogen manipulation in this study was equivalent to enrichment, although enrichment levels overlapped with naturally occurring variation in percentage of nitrogen (i.e., approximately half of the fertilized plants fell within the 99% CI of naturally occurring, nonexperimental plants; Fig. 1).

Treehopper Survivorship: Treatment Effects. For both the direct benefit (predators excluded) and full-benefit (predators not excluded) experiments, anal-

Table 1. Effects of ant tending and fertilization on measures of treehopper survivorship

Source of variation	df	<i>F</i>	<i>P</i>
Repeated measures analysis (2002)			
Ant	1, 39	2.14	0.152
Fertilization	1, 39	8.14	0.007
Ant \times fertilization	1, 39	0.44	0.513
Census	3, 156	31.04	<0.001
Ant \times census*	3, 156	3.84	0.011
Fertilization \times census*	3, 156	4.36	0.006
Ant \times fertilization \times census*	3, 156	0.16	0.923
Repeated measures analysis (2005)			
Ant	1, 39	2.38	0.131
Fertilization	1, 39	7.13	0.011
Ant \times fertilization	1, 39	0.04	0.833
Census	2, 104	50.06	<0.001
Ant \times census*	2, 104	14.72	<0.001
Fertilization \times census*	2, 104	2.4	0.096
Ant \times fertilization \times census*	2, 104	0.29	0.751
Overwinter survival (2002–2003)			
Ant	1, 37	1.05	0.312
Fertilization	1, 37	0.69	0.411
Ant \times fertilization	1, 37	0.17	0.682
Spring density (2003)			
Ant	1, 39	8.96	0.005
Fertilization	1, 39	21.47	<0.001
Ant \times fertilization	1, 39	0.04	0.837

* A significant treatment \times time interaction represents a treatment effect in repeated measures designs.

ysis of treehopper survivorship based on repeated measures analysis showed a significant positive effect of ant tending and fertilization status, but with no significant interaction between these treatments (Table 1; Figs. 2 and 3). Neither ant tending, fertilization, nor the interaction between these treatments had any effect on overwintering survival (Table 1; Fig. 2B).

Individual Performance. Before the size analysis, PCA was used to derive a single metric of treehopper size (Table 2). The first principal component explained 77.5% of the scaled variation in treehopper length, width, and weight, and corresponding factor scores were used as our metric of treehopper size. As with the survivorship data, both ant tending and fertilization significantly increased treehopper size, but with no significant interaction between these treatments (Table 3). In decreasing order of relative magnitude, sex of treehopper, fertilization, and ant tending all increased treehopper size (Fig. 4).

Treehopper Survivorship: Ant-Tending Level. To confirm that treehopper survivorship was a function of variation in ant-tending level, we fit the following model to the data from the direct-benefit experiment:

$$\ln \left(\frac{H_{t+1}}{H_t} \right) = r_f - q_f H_t + a \frac{R_t}{H_t}$$

where *H* defines treehopper (i.e., host) density, *r* defines the per capita growth rate (typically negative in this study), *q* defines the decrease in growth rate with increasing density (i.e., density dependence), *a* defines the magnitude of the ant-tending effect, *R* defines the recruitment response of ants (i.e., ant-tending level), and *t* indexes the census period. This

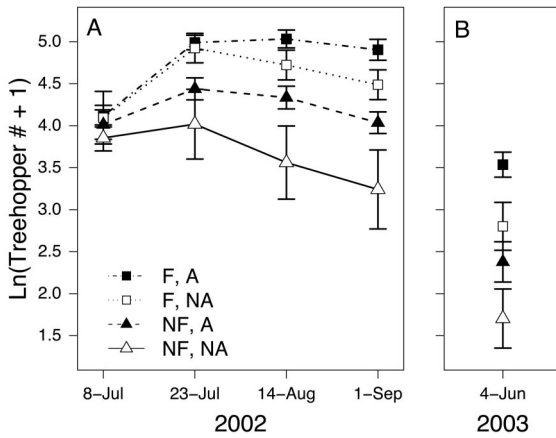


Fig. 2. Treehopper survivorship during the developmental period from early instar to adult before overwintering (A) and adult treehopper abundance after overwintering (B). Closed symbols denote ant-tending treatments, and open symbols denote ant-excluded treatments. Squares denote fertilizer treatments, and triangles denote control treatments. Additive comparisons ($\bar{x} \pm SE$) for treehopper density ($\ln + 1$) at the final census: ant tending versus excluded, 3 ± 0.17 versus 2.29 ± 0.24 . Fertilization treatment versus control, 3.17 ± 0.17 versus 2.04 ± 0.22 . Treatment means \pm SE of treehopper density ($\ln + 1$) at the final census: A-F, 3.54 ± 0.24 ; A-NF, 2.38 ± 0.15 ; NA-F, 2.8 ± 0.29 ; and NA-NF, 1.7 ± 0.35 .

model is analogous to that in Morales (2000a) but with a separate per capita growth rate and density-dependent term for each level of fertilization (f).

Analysis was based on a mixed effects model with the right-hand side of the equation above representing the fixed effects and plant nested within grid as the grouping variables. Ant-tending level was calculated

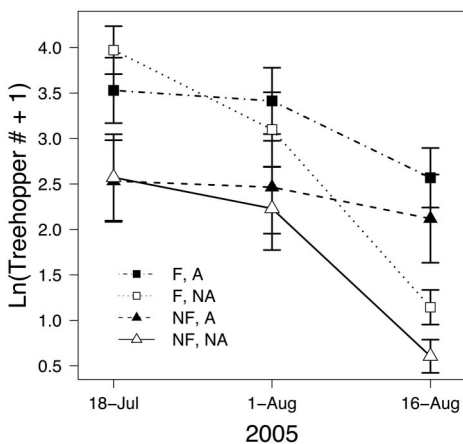


Fig. 3. Treehopper survivorship during the developmental period from early instar to adult before overwintering. Closed symbols denote ant-tending treatments, and open symbols denote ant-excluded treatments. Squares denote fertilizer treatments, and triangles denote control treatments.

Table 2. Correlation matrix for weight, length, and width and factor loadings for the first principal component

Variable	Wt (mg)	Length (mm)	Width (mm)
Wt (mg)	1	—	—
Length (mm)	0.53	1	—
Width (mm)	0.6	0.85	1
Factor loading	0.515	0.597	0.615
Mean \pm SE	2.32 ± 0.87	4.58 ± 0.2	2.04 ± 0.11

as the average number of ants per plant immediately before and after each treehopper census. Analysis was confined to the last three censuses because complete ant counts were available for the middle two censuses only. Additionally, to avoid adding an arbitrary constant to zero values, one replicate was excluded because it had declined to zero by the third census. Because density dependence was not significant (density + density \times fertilization | r + fertilization + per capita tending: $\chi^2_{df=2} = 2.06, P = 0.357$), it was excluded from the final model.

Results showed that treehopper survivorship increased with increasing per capita tending ($a = 0.85$; $F_{1,52} = 10.49$; $P = 0.002$; see Table 4 for full results). Similar results were obtained for an analysis restricted to ant-tended aggregations only ($a = 0.82$; $F_{1,26} = 4.89$; $P = 0.039$; see Table 4 for full results). Notably, the parameter estimates for the fitted model show substantial overlap for these two data sets (Table 4).

Ant Tending. A mixed effects model with plant nested within grid as the grouping variable was used to test the effect of fertilization treatment on ant-tending level by using treehopper density as a covariate (\ln - \ln transformed). Ant-tending level was calculated as described above. Results showed no effect of fertilization on ant-tending level for either the 2002 or 2005 experiment (fertilization + fertilization \times density | density + census. 2002: $\chi^2_{df=2} = 1.38, P = 0.5$; 2005: $\chi^2_{df=3} = 2.62, P = 0.27$).

Discussion

There is a substantial literature documenting the effect of host plant quality on insect performance, but relatively few studies that address the role of host plant quality for the species interactions of herbivorous insects (Awmack and Leather 2002). We report here a significant positive effect of both ant tending and fertilization on treehopper survivorship and size at maturity (Tables 1 and 3). However, we find no evidence of an interaction between ant tending and

Table 3. Effect of ant tending, fertilization, and sex of treehopper on first principal component of body size

Source of variation	df	F	P
Ant	1,36	7.17	0.011
Fertilization	1,36	16.27	<0.001
Ant \times fertilization	1,36	0.43	0.52
Sex	1,222	215.05	<0.001

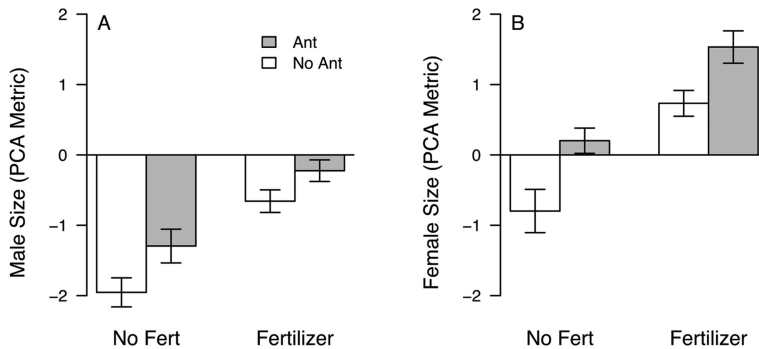


Fig. 4. Effect of fertilization treatment and ant tending on treehopper size for males (A) and females (B) as measured by the first principal component of weight, length, and width. Additive comparisons ($\bar{x} \pm SE$): ant tending versus excluded, 0.27 ± 0.12 versus -0.34 ± 0.13 ; fertilization treatment versus control, 0.33 ± 0.11 versus -0.7 ± 0.14 ; and males versus females, -0.71 ± 0.11 versus 0.64 ± 0.12 .

fertilization for any measure of treehopper performance that we considered. We conclude that host plant quality did not significantly affect the strength of the interaction between ants and *P. concava* treehoppers in this study.

Host Plant Mediation: Ant Tending. Cushman (1991) outlines two conditions that must be satisfied for host plant mediation of ant–protection mutualisms. First, host plant quality must affect ant-tending level or attentiveness and second, ant tending level or attentiveness must affect the degree of benefit for the ant-tended partner. Previous studies provide indirect support for both of these conditions. For example, several studies have shown that “high-quality” nectars increase ant recruitment (Lanza 1988, Blüthgen and Fiedler 2004). Other studies have shown that host plant identity or phenology can influence the composition of honeydew (Douglas 1993, Fischer and Shingleton 2001), and that this influence can translate into changes in the relative attractiveness of ant-tended homopterans (Fischer et al. 2001). Nevertheless, studies that have tested the host plant mediation hypothesis directly are largely consistent with the results of our study. Breton and Addicott (1992b) found a significant effect of host plant quality for the aphid *Aphis varians* on *Epilobium angustifolium*, but no evidence of host plant mediation (although a main effect of ant tending was not detected in that study). Wimp and Whitham (2001) found a significant positive effect of both ant tending and host plant identity on survivorship of the aphid *Chaitophorus populicola*

but with no significant interaction between these factors. Baylis and Pierce (1991) and Billick et al. (2005) both demonstrated a significant increase in ant tending for lycaenids feeding on high-quality hosts, but neither study reported a significant link between this increase in ant tending and lycaenid survivorship (although a trend toward increases survivorship was reported in the Baylis and Pierce study). Finally, Stadler et al. (2002) found significant evidence of host plant mediation for only one of four aphid species studied. In our study, we found that increasing ant-tending level increases benefit to treehoppers (Table 4) but that host plant quality had no effect on ant-tending level (Table 3). We suggest that the absence of host plant mediation in this study probably results from the independence of ant-tending level and host plant quality.

The independence of ant tending and host plant quality in this study could result from no change in the honeydew composition for treehoppers on high-quality plants (Quental et al. 2005) or to compensatory changes in the rate of honeydew excretion. Alternatively, ants may not respond to changes in the composition of honeydew. A recent study of sugar and amino acid preferences among 51 species of ants showed large variation in preference for specific amino acids (ranging from preference to relative aversion), although ants generally preferred sugar solutions with amino acids relative to those without (Blüthgen and Fiedler 2004).

Table 4. Effects of fertilization and per capita tending on treehopper survivorship (direct-effects experiment)

Source of variation	df	F	P	Mean	95% CI
Ant-tended + excluded treatment					
<i>r</i> (intercept)	1, 52	58.09	<0.001	-0.42	-0.54, -0.31
<i>f</i> (Δr , fertilizer treatment)	1, 40	12.69	0.001	0.22	0.09, 0.35
<i>a</i> (ants per treehopper)	1, 52	10.49	0.002	0.85	0.33, 1.38
Ant-tended treatment only					
<i>r</i> (intercept)	1, 26	20.46	<0.001	-0.43	-0.63, -0.24
<i>f</i> (Δr , fertilizer treatment)	1, 13	10.16	0.007	0.26	0.08, 0.43
<i>a</i> (ants per treehopper)	1, 26	4.89	<0.036	0.82	0.06, 1.58

Previous studies of this system have shown that benefit to treehoppers is density dependent and that this density-dependent benefit is driven by the recruitment pattern of ants (Morales 2000a,b). Note that ant tending in this study was independent of treehopper density (i.e., the 95% CI of the slope on a log-log scale spanned 1; 2002: 0.71–1.32; and 2005: 0.63–1.14; see Ant Tending in Results for a description of the statistical model). Correspondingly, treehoppers did not show density-dependent benefit ($\ln[N_{\text{final}}/N_{\text{start}}] = \text{Ant} \times N_{\text{start}} | \text{Ant} + N_{\text{start}}$; 2002: $F_{1, 51} < 0.01$; $P = 0.96$; and 2005: $F_{1, 43} < 0.1$; $P = 0.32$). Because fertilization increased treehopper density in this study, it is possible that we would have detected a significant interaction between the ant and fertilization treatments in the presence of density-dependent benefit.

Host Plant Quality. Although our results show that bottom-up factors were relatively unimportant in generating conditional outcomes in this study of an ant-treehopper mutualism, host plant quality may be a critical factor in promoting the persistence of this mutualism. In particular, in nitrogen-poor sites, it is possible that only ant-tended treehoppers on high-quality host plants would have survived. In fact, results from a fertilization study by Strauss (1987) suggest that this may be a common condition. Strauss (1987) found a large increase in the density of ants and treehoppers in plots that were fertilized. Results from our study suggest that the fertilization-associated increase in ant and treehopper density observed by Strauss (1987) reflected an inability of treehoppers to persist in low-nutrient environments rather than a qualitative change in the attractiveness of treehoppers to ants in fertilized plots.

Because the effects of ant tending and host plant quality are additive, the interaction between ants and *Publilia* treehoppers may be “obligate” in nutrient-poor environments and “facultative” in nutrient-rich environments. Note that because zero density represents a “veil” line, such a result might show up as a statistical interaction between ant and fertilizer treatments but would not indicate host plant mediation of mutualism per se. In general, it will be difficult to interpret statistical interactions in the context of species interaction coefficients for experiments where a substantial number of replicates have declined to zero. Regardless, these results argue against categorizing mutualisms as obligate or facultative and support the view that mutualism is especially important for species in marginal habitats. Even “facultative” mutualisms may represent an important conservation strategy for threatened or endangered species.

Individual Performance Measures. Although the results presented here argue against categorizing mutualisms as strictly obligate or facultative, an increasing number of studies is finding that ant-tended homopterans do separate along two lines based on the relative costs and benefits of ant tending (Stadler and Dixon 1999). In particular, homopterans that show a net benefit of ant tending for measures of performance such as body size and weight tend to depend more strongly on ant tending. In contrast, homopterans that

show a net cost of ant tending for measures of performance such as body size and weight tend to benefit from ant tending only under conditions of high predator abundance and in general rely less strongly on ant tending.

Stadler et al. (2002) tested for host plant mediation across four aphid species and found evidence for host plant mediation in the obligately tended species *Metopeurum fuscoviride* only. As for the aphid *M. fuscoviride*, *P. concava* shows a positive effect of ant tending on individual performance measures (Fig. 4; also see Bristow 1984 for an example from *P. reticulata*), and this and previous studies (Morales 2000b) suggest that *P. concava* benefits from ant tending even at experimentally reduced levels of predators. That we did not detect a positive effect of host plant quality on the interaction between ants and treehoppers in this study suggests that it may not be possible to generalize regarding the effect of host plant mediation of mutualism on the basis of observed costs or benefits of ant tending at the level of individual performance.

In conclusion, this study evaluates the effects of host plant quality on the mutualism between ants and treehoppers. We find a significant positive effect of both ants and host plant quality on treehopper performance, but no evidence that host plant quality mediates the mutualism. This study adds to a growing list showing that the strength of bottom-up effects in host–visitor mutualism is relatively weak compared with top-down effects. Although bottom effects almost certainly contribute to conditionality in mutualism, they may not be a primary factor in determining the dynamics of these interactions.

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