Ants (Hymenoptera: Formicidae) and Trophobiont Leafhopper Nymphs (Hemiptera: Cicadellidae) Become more Abundant in Shaded Conditions: Implications for Mutualism

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Source: Florida Entomologist, 97(4) : 1378-1385
Published By: Florida Entomological Society
URL: https://doi.org/10.1653/024.097.0412
ANTS (HYMENOPTERA: FORMICIDAE) AND TROPHOBIONT LEAFHOPPER NYMPHS (HEMIPTERA: CICADELLIDAE) BECOME MORE ABUNDANT IN SHADED CONDITIONS: IMPLICATIONS FOR MUTUALISM

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ABSTRACT
Abiotic factors have dramatic effects on herbivore insect populations. However, little is known about the effects of the abiotic factor of shade on the mutualism between ants and Hemiptera (aphids, scales, mealy bugs, whiteflies, treehoppers, leafhoppers). The trophobiont leafhopper Dalbulus quinquenotatus DeLong & Nault (Hemiptera: Cicadellidae) is obligatorily tended by the ant Brachymyrmex obscurior Forel (Hymenoptera: Formicidae) on the basal leaves of gamagrass Tripsacum dactyloides L. (Poales: Poaceae). To better understand the effect of abiotic factors on this relationship, we conducted a comparative experiment to investigate the abundance of ants and leafhoppers (nymphs and adults) under shaded and full-sunlight conditions. We observed mutualism between D. quinquenotatus and B. obscurior on both shaded and non-shaded gamagrasses. Nevertheless, the greatest number of B. obscurior were found tending the greatest number of D. quinquenotatus on shaded T. dactyloides. Most of these leafhoppers were nymphs. Nymphs were observed in clusters on the basal leaves. On the other hand, ant-tended D. quinquenotatus adults were observed at similar frequencies on shaded and non-shaded gamagrasses. The adults were also seen on the basal leaves and produced honeydew, but were more mobile than the nymphs. Our results highlight the importance of abiotic factors in mutualism as well as the complex interaction between shaded plants, immature leafhoppers, and tending ants.

Key Words: shade, Dalbulus, tending ants, immature Hemiptera

RESUMEN
Los factores abióticos tienen un efecto significativo en las poblaciones de insectos herbívoros. Sin embargo, poco se sabe sobre el efecto del factor abiótico sombra en el mutualismo entre hormigas y hemípteros (áfidos, coccidos, pseudococcidos, aleyrodidos, membráceos, cicadélidos). El cicadélido trofobionte Dalbulus quinquenotatus DeLong & Nault (Hemíptera: Cicadellidae) es atendido obligatoriamente por la hormiga Brachymyrmex obscurior Forel (Hymenoptera: Formicidae) en las hojas basales de la gramínea Tripsacum dactyloides L. (Poales: Poaceae). Para conocer mejor el efecto de los factores abióticos en esta relación efectuamos un experimento comparativo con el objetivo de determinar la abundancia de las hormigas y las chicharritas (ninfa y adultos) bajo condiciones de sombra y sol. Encontramos que el mutualismo entre D. quinquenotatus y B. obscurior existe en T. dactyloides en condiciones de sombra y sol. Sin embargo, la mayor abundancia de B. obscurior existió al presentarse la mayor abundancia de D. quinquenotatus en T. dactyloides que recibió sombra. La mayoría de los cicadélidos fueron ninfas. Estas estaban en forma agregada en las hojas basales de T. dactyloides. Por otro lado, los adultos de D. quinquenotatus atendidos por las hormigas fueron observados en una abundancia similar en plantas con y sin sombra. Estos adultos se encontraron también en las hojas basales y produjeron mielcilla, pero fueron más móviles que las ninfas. Nuestros resultados resaltan la importancia de los factores abióticos en el mutualismo, así como la interacción entre plantas bajo la sombra, cicadélidos en estado inmaduro y hormigas.

Palabras Clave: Sombra, Dalbulus, hormigas, hemípteros inmaduros

Most ants depend on sugar secretions as a food resource, and these secretions are produced in great quantity in shaded habitats (Perfecto et al. 1996; Vandermeer et al. 2002; de la Mora et al. 2013) by herbivorous insects, including trophobiont hemipterans (Moya-Raygoza & Larsen 2008). Honeydew is a sugary excretion of carbohydrates, amino acids, and water that attracts ants to establish a mutualistic association. The hemipterans offer food in the form of honeydew,
and the ants provide protection to the hemipterans against predators and parasitoids (Way 1963; Buckley 1987; Blüthgen et al. 2006; Gibb & Cunningham 2009; Zhang et al. 1012; Zhang et al. 2015). Ants are abundant when the population of hemipterans is large because of the increased quantity of honeydew (Katayama & Suzuki 2010). With increased production of honeydew resulting in increased visits by tending ants (Bronstein 1994; Itiokia & Inoue 1996), honeydew is the base on which ant-hemipteran mutualism is built (Wiss 2006; Styrsky & Eubanks 2007; Detrain et al. 2010).

To our knowledge, no study to date has investigated whether abundance of ants is associated with abundance of immature (nymph) or mature (adult) stages of trophobiont leafhoppers on shaded host plants. We hypothesized that shade would affect the intensity of mutualism by increasing the number of leafhopper nymphs and tending ants on gamagrass Tripsacum dactyloides L. (Poales: Poaceae). Immature leafhoppers are the keystone in the association with ants in shaded habitats, because of the great food resources offered to ants. Wood (1982) found nymphs of the treehopper Enchenopa binotata Say (Membracidae) in great aggregation with abundant tending ants, and Cushman & Whitham (1989) present evidence that large aggregations of nymphs benefit more from ant tending than small aggregations. Neither study, however, investigated the effect of shade. In the present study, we focus in the hemipteran trophobiont leafhopper Dalbulus quinquenotatus DeLong & Nault (Hemiptera: Cicadellidae), which occurs in obligatory mutualism with the ant Brachymyrmex obscurior Forel (Hymenoptera: Formicidae). This leafhopper-ant mutualism takes place on the basal leaves of the gamagrass host plant (Tripsacum spp.) (Nault et al. 1983; Larsen et al. 1991; Moya-Raygoza & Nault 2000) in shaded and sunny habitats. Dalbulus quinquenotatus is sedentary and gregarious, and produces large quantities of honeydew. This honeydew is a food resource for ants, which in return protect D. quinquenotatus from natural enemies (Moya-Raygoza & Nault 2000; Larsen et al. 2001). The objective in this study was to examine the abundance of ants and leafhoppers (nymphs and adults) on shaded and unshaded T. dactyloides plants.

**MATERIALS AND METHODS**

This experiment, comparing the effects of shade and non-shade conditions, was conducted in Zapopan, state of Jalisco, Mexico (N 20° 44' -W 103') at 1,570 m elevation. Gamagrass (T. dactyloides) plants were obtained by rhizome propagation. Rhizomes were collected from natural Zapopan gamagrass populations and grown in pots (30 cm in diam x 33 cm height). The pots contained the same type of soil as that in which the gamagrass grows naturally in the field. We used 30 pots, divided into 2 treatments: 15 pots for the shade treatment and 15 pots for the non-shade treatment. Each pot contained green young plants that had recently emerged from the rhizomes. The plants were the same age and had either 2 or 3 stems. Each pot was considered one replicate. The shade treatment group included a total of 34 stems and the non-shade treatment group included a total of 32 stems. The pots with the plants were arranged in 2 circles, one for each treatment. For the shade treatment, a mesh was installed that produced 45% shade. The mesh was 3 m high and covered the 15 pots in the shaded treatment; no shade was installed in the non-shaded treatment. The distance between treatments was 20 m (Fig. 1). First we installed the mesh, and then the 30 pots divided into 2 treatments; 3 days later D. quinquenotatus adults were deposited on the T. dactyloides plants.

*Dalbulus quinquenotatus* adults were collected with an aspirator from a *T. dactyloides* field population located at Zapopan. The collected adults were gender separated in the lab and deposited on the basal leaves of the gamagrass plants reared in the pots. Twenty adults (10 females and 10 males) of *D. quinquenotatus* were deposited in each pot. Each pot was covered with a screen for 2 weeks to allow adults to oviposit and become established on the basal leaves. After the 2 weeks, the screens were removed and one additional pot per treatment, containing a nest of *B. obscurior* of similar size, was installed in the center of each circle. Similar abundance of ants between the 2 nests was evident when ants were connected to the leafhoppers as shown in Fig. 5. The distance from the nest to each pot was 1 m, so that the ants had to traverse the same distance to reach leafhoppers in all treatment. After 2 weeks, nymphs were present. *Dalbulus quinquenotatus* development time from egg to adult is about 34 days at 24 °C. Nymphs begin hatching on day 12 and nymphal abundance peaks on day 16 (Moya-Raygoza 2004). No arthropod predators or parasitoids such spiders, hemipterans or dryinids (Hymenoptera: Dryinidae) that may attack *D. quinquenotatus* were observed during the experiment. Also, no plant with extrafloral nectaries that may attract ants was present in the experimental area.

After 2 weeks, when the nymphs appeared, counting of nymphs, adults, and ants on each stem from the 2 treatments commenced. The counting of all the insects was conducted without disturbing them, between 10:00 and 13:00 hours, every 7 days. To avoid variation the counting was conducted randomly on each collection date. The time in observing each plant was between 2 to several minutes and it was facilitated because...
mutualism was given only on the basal leaves of the gamagrass on a leaf segment no longer than 5 cm. The experiment was conducted between 11 Oct and 29 Nov 2006. The mean daily high temperature was 25.56 °C (SE = 0.31). Plants were watered every 4 days. Repeated-measures analysis of variance (ANOVA) on transformed ($r^2$) numbers of ants and nymph and adult leafhoppers was used to compare these quantities between the 2 treatments over time. All statistical analysis was performed using SPSS software (SPSS 11.5 for Windows, SPSS, Chicago, IL). 

RESULTS

The leafhopper *D. quinquenotatus* and the ant *B. obscurior* occurred in both shaded and unshaded treatments. However, significantly more leafhoppers were tended by *B. obscurior* on the shaded *T. dactyloides* over the course of 49 days (repeated-measures ANOVA; $F = 3.00; df = 1,7; P = 0.004$) (Fig. 2). The difference in the numbers of leafhoppers tended appeared to be related to the abundance of nymphs. We found significantly more nymphs, tended by *B. obscurior*, in the shaded treatment than in the unshaded treatment (repeated-measures ANOVA; $F = 2.73; df = 1,7; P = 0.01$) (Fig. 3). Nymphs emerged after placement of adults in the covered pots during the first 2 weeks; before introducing the ants. These nymphs had soft bodies and produced honeydew after the first stadium. The number of ant-tended *D. quinquenotatus* adults, however, was similar in shaded and non-shaded treatments (repeated-measures ANOVA; $F = 0.81; df = 1,7; P = 0.58$) (Fig. 4). These adults produced honeydew and were more mobile than the nymphs. In both treatments, more adults were found between days 35 and 49, after all of the nymphs had completed their development.

The numbers of ants also differed between the 2 treatments. Significantly more ants were found tending *D. quinquenotatus* on the basal leaves of shaded *T. dactyloides* plants, throughout the 49 days (repeated-measures ANOVA; $F = 2.67; df = 1,7; P = 0.01$) (Fig. 5). The largest number of ants and nymphs in the shaded treatment occurred during the first 35 days, before all the nymphs became adults (Figs. 3 and 5).

DISCUSSION

We found significantly larger numbers of the leafhopper *D. quinquenotatus* on the shaded *T. dactyloides* plants, tended by significantly greater numbers of *B. obscurior*, compared with *T. dactyloides* plants without shade. Similar results were reported by Moya-Raygoza & Larsen (2008), who found large numbers of *D. quinquenotatus* and *B. obscurior* on gamagrass plants exposed to 50% shade in artificially constructed habitats. In addition, this positive effect of shade in the mutualism between ants and *D. quinquenotatus* was observed in nature, i.e., increased numbers of ants and leafhoppers were found in shelters built by ants in wild gamagrass (Moya-Raygoza & Larsen 2008). Grasses under shade have advantages in terms of water and nitrogen that in turn support...
herbivorous insects. For example, shaded conditions result in stomatal closure, reduced transpiration, and decreased water loss in *T. dactyloides* (Fay & Knapp 1995). In grasses, such as *Axonopus compressus* (Sw.) P. Beauv and *Pennisetum clandestinum* Hochst. ex Chiov., shade increases their nutritive value for herbivores through an increase in nitrogen content (Samarakoon et al. 1990). Therefore, shaded grasses are superior food resources for phloem-feeding hemipterans such as leafhoppers, because they depend entirely on a liquid diet and nitrogen from the host plant for survival and reproduction.

Many trophobiont hemipterans produce copious excretions of honeydew to attract tending ants. Positive density-dependent ant-hemipteran mutualisms, such as we observed, have been associated with honeydew production of several trophobiont hemipterans. Cushman & Whitham (1989) observed that the population of the ant *Formica altipetens* Wheeler increased along with increased abundance of the membracid *Publilia modesta* Uhler, because large aggregations of the hemipteran, produce more honeydew and thus attracted more ants. Katayama & Suzuki (2003) found that the number of *Tetramorium caespitum* L. and *Lasius niger* L. ants increased as the density of the aphid *Aphis craccivora* Koch (Aphididae) increased, mainly because of copious honeydew production. Similarly, Fischer et al. (2001) found that the aphid *Metopeurum fuscoviride* Stroyan is preferred by *L. niger* ants for its abundant production of honeydew. Finally, Itioka & Inoue (1996) reported that the abundance of *L. niger* increases with the abundance of the scale insect *Ceroplastes rubens* Maskell (Coccidae).

Positive density-dependence has been reported in hemipteran nymphs and their tending ants. Wood (1982) found large aggregations of the treehopper nymphs *E. binotata* with large aggregations of tending ants. Cushman & Whitham (1989) provide evidence that large nymphal aggregations benefit more from ant tending than small nymphal aggregations, and Fischer et al. (2002) found that the abundance of *L. niger* ants is correlated with the amount of honeydew produced by third and fourth instar *M. fuscoviride* nymphs.

As mentioned before, to our knowledge no study has investigated the role of shade in the

Fig. 2. Mean (± SE) number of total *Dalbulus quinquenotatus* leafhoppers per stem on unshaded and shaded *Tripsacum dactyloides*. Day 1 is when nymphs first appeared.
Fig. 3. Mean (± SE) number of *Dalbulus quinquenotatus* leafhopper nymphs per stem on unshaded and shaded *Tripsacum dactyloides*. Day 1 is when nymphs first appeared.

Fig. 4. Mean (± SE) number of *Dalbulus quinquenotatus* leafhopper adults per stem on unshaded and shaded *Tripsacum dactyloides*. Day 1 is when nymphs first appeared.
abundance of immature trophobiont hemipterans and their tending ants. We found that large numbers of leafhopper nymphs were associated with large numbers of ants on shaded *T. dactyloides*, when compared with plants exposed to full sunlight. The greater abundance of nymphs might be related in part to the increased water and nitrogen content of *T. dactyloides* in the shaded treatment. This would be in accordance with the findings of Ross et al. (1991) that the leafhopper *Cameocephala floridana* Ball increases in abundance and body size when feeding on shaded grasses with high nitrogen content. Future studies are needed to demonstrate the effect of water and nitrogen on the abundance in nymphs of *D. quinquenotatus*.

After *D. quinquenotatus* eggs hatch, nymphs appear and start to feed on the basal leaves of *T. dactyloides*. In shaded gamagrass, nymphs cluster on the leaves, and the removal of honeydew by ants reduces the death of nymphs by suffocation from accumulated honeydew and the formation of sooty mold. Young trophobiont hemipterans can become asphyxiated by the sticky liquid and molds if ants are absent or sparse (Wiss 2006). Moreover, nymphs in large aggregations with consequently large populations of ants are better protected in shaded gamagrass. These nymphs have soft bodies susceptible to predation if they are not protected by ants. In natural conditions, *D. quinquenotatus* nymphs live in shelters made by *Solenopsis geminata* (F.) (Formicidae) in shaded habitats that protect the nymphs from dry- inund wasp parasitoids (Moya-Raygoza & Larsen 2008). Cushman & Whitham (1989) investigated the tending ant *Formica altipetens* Wheeler and the membracid *P. modesta*, and found that only nymphs, and not adults, benefit from ant tending, because nymphs are susceptible to predation by spiders, whereas adults are more agile and capable of escaping from predation. Nymphs of *D. quinquenotatus* have little mobility. Therefore, they are better protected when ants are abundant.

The great abundance of leafhopper nymphs in the shaded habitat is the driving force that determines ant abundance and, in turn, mutualism. The hemipterans that produce honeydew are subject to selection pressure because they produce the resources that attract ants (Yao et al. 2000). Styrsky & Eubanks (2007) and Detrain et al. (2010) concluded that hemipteran trophobionts are the keystone on which mutualism is built. In our case, an abiotic factor, shade, enhances that mutualism. Wiss (2006) argues that hemipteran trophobiont populations, with their honeydew that is rich in sugars and amino acids, are targets for selection and this affects the ecological interactions of these organisms. A large number of trophobiont hemipterans dramatically change the
structure of the arthropod community because tending ants alter the abundance and distribution of predators, parasitoids, and herbivorous insects (Styrsky & Eubanks 2007).

The effect of abiotic factors on herbivore insect populations has been studied for many years (Masters et al. 1998). However, this is one of the first studies to evaluate the effect of abiotic factors on mutualism between hemipteran trophobionts and ants. Abiotic factors affecting mutualism between ants and plants bearing extrafloral nectaries, such as temperature and precipitation, have received little attention in spite of the dramatic effects such factors can have on that mutualism (Rico-Gray et al. 2012). Extrafloral nectaries and trophobiont hemipterans are analogous food resources because both produce secretions specifically developed to be utilized by ants. Future studies examining the role of abiotic factors affecting these 2 food resources consumed by ants, which generally co-occur in the same community, would help to further characterize these mutualistic relationships.

ACKNOWLEDGMENTS

We thank Claudia S. Copeland (Carpe Diem Biomedical Writing and Editing) for editing the manuscript and Victor Rico-Gray for the comments and suggestions to the manuscript. Temperature information was provided by the Observatorio Meteorológico (Universidad de Guadalajara).

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