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RESEARCH

Biology and Ecology of *Alchisme grossa* in a Cloud Forest of the Bolivian Yungas

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ABSTRACT. Treehoppers (Membracidae) exhibit different levels of sociality, from solitary to presocial. Although they are one of the best biological systems to study the evolution of maternal care in insects, information on the biology of species in this group is scarce. This work describes the biology and ecology of *Alchisme grossa* (Fairmaire) (Hemiptera: Membracidae) in a rain cloud forest of Bolivia. This subsocial membracid utilizes two host-plant species, *Brugmansia suaveolens* (Humb. & Bonpl. ex Wild) Bercht. & J.Presl and *Solanum ursinum* (Rusby) (both Solanaceae), the first one being used during the whole year and the second one almost exclusively during the wet season. The development of *A. grossa* from egg to adult occurred on the plant where eggs were laid. Maternal care was observed during the complete nymphal development, and involved behavioral traits such as food facilitation and antidepredatory defense. Life cycle was longer on *B. suaveolens* during the dry season and shorter on *S. ursinum* during the wet season. Mortality was similar on both host plants during the wet season but was lower on *B. suaveolens* during the dry season. The presence of a secondary female companion to the egg-guarding female individual and occasional iteropary is also reported.

Key Words: *Brugmansia suaveolens*, Hoplophorionini, maternal care, Solanum ursinum

Treehoppers (Hemiptera: Membracidae) are a highly diverse, cosmopolitan, sap-feeding group of insects. They range from monophagous, which use a single species of host plant, to polyphagous, which use plants from different families (Dietrich and Deitz 1991; Wood 1993a; Cryan et al. 2004; Morales and Beal 2006; Lin 2006, 2007). Diet breadth has been associated to latitudinal effects: tropical treehoppers tend to be oligophagous or polyphagous and temperate treehoppers tend to be monophagous (Wood 1984, Reithel and Campbell 2008). Various genera of membracids show host-specialization patterns in which a synchrony occurs between the insect's life cycle and host-plant phenology (Wood and Olmstead 1984, Wood 1993b, Sattman and Cocroft 2003).

Membracids are characterized by a pronotum that can show huge variation among species (Funkhouser 1917, Wood 1993a, Dietrich et al. 2001, Lin 2006). Among the roles suggested for this unique structure are crypsis (i.e., mimetism with the host plant or imitation of other insects) and defense (i.e., aposematism or mechanical barrier; Wood 1974, 1993a; Roy et al. 2007). Chemical and mechanical cues are the most important signals mediating intraspecific (i.e., courtship, aggregation) and interspecific (i.e., mutualism with ants) communication (Nault et al. 1974; Cocroft 2003, 2005). Oviposition habits in membracids vary from semelparous to iteroparous, depending in some cases on the presence of egg-guarding behavior in the species (Wood 1993a, Lin 2006).

Treehoppers include species that exhibit a solitary mode of life and also species exhibiting nymphal or adult aggregations with subsocial behaviors, i.e., continuous maternal care of progeny (Wood 1993a, Stegmann and Linsenmair 2002, Cryan et al. 2004, Lin 2006). The tribe Hoplophorionini, which includes extensively studied subsocial and presocial species (e.g., *Umbonia crassicornis* and *Platycotis vittata*), displays behavioral traits such as maternal care, an ancient trait in the tribe which can involve behaviors such as antidepredatory defense, egg-guarding, and nymphal feeding facilitation (Wood 1993a,b; Lin 2004, 2006). Few descriptive studies on the life history and ecology of these insects have been reported, particularly of members of the tribe

Hoplophorionini, thus limiting the advance of social studies in Hemiptera (McKamey and Deitz 1996, Lin 2007).

Alchisme grossa (Fairmaire) (Hemiptera: Membracidae) is a Hoplophorionini treehopper that exhibits a neotropical distribution in Central and South America (Dietrich and Deitz 1991, Wood 1993a, Lin 2004). In a cloud forest of the Bolivian Yungas, *A. grossa* utilizes *Brugmansia suaveolens* (Humb. & Bonpl. ex Wild) Bercht. & C. Presl (Solanaceae) and *Solanum ursinum* (Rusby) (Solanaceae) as host plants. We have characterized the life cycle of *A. grossa* on both host plants, the social patterns associated with maternal care, and host-plant use and preference during the year.

Methods

Study Site. Field work was performed at Incachaca (Cochabamba, Bolivia, 17°13′S to 65°49′W; 2,450 meters above sea level (m.a.s.l.)) within the Yungas biogeographical region, which is characterized by a wide altitudinal cline and ∼3,700 mm of rainfall per year concentrated from October to March (Navarro and Maldonado 2002). Although *A. grossa* was frequently found when walking within the native forest, for conveniency of access, the study site chosen was a small 90-yr-old pine forest (∼6 Ha) planted within the native forest; the understory vegetation is composed mostly of small native trees and bushes, among which the most abundant are *B. suaveolens* and *S. ursinum*, the host plants of *A. grossa*.

Life Cycle, Host-Plant Use and Behavior of *A. grossa*. *A. grossa* was monitored on both host plants during 1 yr (February 2011–February 2012), to describe, during the dry and wet seasons, its life cycle, host-plant use, and behavioral patterns. The pronotum of 100 egg-guarding females and 100 solitary females was marked with a permanent marker (Stabilo). This process was performed twice on *B. suaveolens* (dry and wet season) and only once on *S. ursinum* (wet season), corresponding to the observed presence of the insect on each host plant. A code was used based on dots, lines, and colors which characterized every single individual.

A. grossa constitutes social groups composed of an egg-guarding female and her clutch, named from now onward a "family." In total, 158 families of *A. grossa* on *B. suaveolens* (87 in the wet season and 71 in the dry season) and 30 families on *S. ursinum* (in the wet season) were monitored during their complete development, and the data were used to describe its life cycle. Families were observed every week during the whole development process to determine the developmental stage, number of individuals, and behavioral patterns. The duration of the first and second instars on *B. suaveolens* during the dry and wet seasons and on both host plants during the wet season was compared with two-way repeated measures Analysis of variance (ANOVA) followed by Holm–Sidak post hoc tests (Sokal and Rohlf 2012). Only these two instars were compared because almost 90% of mortality events occurred during these stages, and hence the number of surviving replicates was too small for trustworthy comparisons using later instars. The mortality of families was defined as the ratio between the number of families whose members had all died before reaching the third-instar stage over the total number of families studied on each host plant and season.

Host-Plant Characterization. All host-plant individuals in the study area were counted at one point during the wet season when both host plants were being used. Two indices were constructed to characterize host plants, one related to resource availability (plant volume approximated to a rectangular parallelepiped) and another related to resource quality (number of young leaves/number of mature leaves). The total number of families on each host-plant individual was also counted to characterize the pattern of host-plant use. These proxies were compared using one-way Analysis of variance (ANOVA) on ranks followed by post hoc Dunn's test (Sokal and Rohlf 2012).

Egg Clutch Recognition and Maternal Care. To characterize recognition and/or orientation patterns by females, 21 egg-guarding marked females on different plants of *B. suaveolens* were removed and relocated 60 cm below their original clutch on the main stem. The number of females which returned to their clutches was observed 90 min after relocation and then 7 d later. Results were compared with an exact binomial probability test (Sokal and Rohlf 2012).

To evaluate the importance of female presence on the survivorship of the offspring, 10 guarding females were manually removed from their respective families on *B. suaveolens*, five of which were at the egg-guarding stage and five at the first-instar stage. The clutches were observed 90 min after guarding female removal, 7 d later and 14 d later.

Female Defense Patterns. Seven morphospecies of predators (one reduviid, one coccinelid, one vespid wasp, and four arachnids), two of parasitic mites parasitizing eggs, nymphs, and adults, and one of an ichneumonid endoparasitoid parasitizing only nymphs were found. To characterize defense behavior of females, the most abundant predator (reduviid, $n = 9$) was collected, positioned individually over the midrib in the tip of *B. suaveolens* leaf, and confronted with an *A. grossa* female under two treatments: 1) egg-guarding female ($n = 5$) and 2) solitary female ($n = 4$). Behavioral responses of females were recorded from the moment the predator was positioned on the leaf until the moment it left it. Female behaviors considered were: to confront the predator (the female produced strong and fast kicking movements with its metathoracic legs, twisting body motions, or wing fanning and buzzing) and to escape from the predator (the female flies, jumps, or walks away from the leaf). Data were analyzed using Fisher's exact test to compare the frequency of escaping and confronting events in solitary and egg-guarding females (Sokal and Rohlf 2012).

Results

General Observations. Sex in adults was determined by observing the presence of an ovipositor in females and the frontal section of the pronotum (black in males and bright green in females). Mating events were observed in the principal stem of both host plants in sunny mornings during the wet season. In both host plants, the female oviposits on the midrib tissue of the underside of a leaf, covers the eggs with accessory gland secretion, and positions itself over the clutch; this grouping

constitutes a family. Normally, an individual host plant hosted several families, but in most cases, a single family was observed per leaf. Only in six cases (out of 158), two families were found on the same leaf on *B. suaveolens*. Approximately 1 wk before egg hatching, the female produced a series of feeding slits, i.e., small perforations on the midrib tissue around the clutch made by the female using her ovipositor, which facilitated feeding by first-instar nymphs (Lin 2006).

Life cycles on both host plants were constituted by an equal number of stages (egg, five nymphal instars, and adult). The five nymphal instars differed in size, color, and ornamentation (dorsal spines). Thus, first instars had a transparent pronotum and lacked dorsal spines; second instars had black stripes on the pronotum and dorsal spines were almost imperceptible; and third, fourth, and fifth instars had two green anterior dorsal spines and two black posterior spines, and a pronotum with black stripes. During the first three instars, nymphs remained aggregated within the leaf (usually on the underside midrib) where they were born. Fourth and fifth nymphal instars showed distinct patterns of dispersion depending on the host plant where they developed. Nymphs on *B. suaveolens* remained aggregated within the leaf until the end of the fifth instar. In *S. ursinum*, aggregation was observed only until the end of the third instar. Usually, dispersed nymphs formed mixed familiar aggregations on the principal stem with adults and nymphs of other families. The duration of the fourth and fifth nymphal instars on *S. ursinum* could still be estimated by periodically observing the remaining nymphs on the principal stem of the plant because the families on an individual plant were normally at different stages of development. In two of the six cases where two females oviposited on the same leaf on *B. suaveolens*, one of the two egg-guarding females abandoned the leaf and the remaining female guarded the two groups of nymphs which had become mixed within the leaf.

Mean total duration of the cycle on *B. suaveolens* varied depending on the season (dry season $= 113$ d and wet season $= 76$ d), while the mean duration on *S. ursinum* (wet season only) was 51 d. Duration of the first two nymphal instars showed differences between seasons and hosts ($F = 42.343$; df $= 2$; $P < 0.01$), being longer during the dry season on *B. suaveolens*, and shorter on *S. ursinum* during the wet season ($P < 0.05$; Table 1). Family mortality was 0.5 on *B. suaveolens* during the dry season, 0.83 on *B. suaveolens* during the wet season, and 0.86 on *S. ursinum* during the wet season.

In 45 of the 158 families monitored on *B. suaveolens*, a fifth-instar nymph (3 cases) or mature adults (42 cases, seven with males and 35 with females) were observed at some point of the development of the family accompanying the guarding female. Nymphs and males never accompanied the guarding female for more than 2 d, whereas females were seen accompanying the guarding female for periods of up to 1 wk. When the companions were adult females, offspring were at the egg stage in 18 of the 35 cases. In six of these 18 cases, the guarding female left the family and the female companion took her place over the egg clutch for a period no longer than 1 wk. Within this period, either the original guarding female came back to the family and the family continued normal development (four cases) or the original guarding female did not come back and the family died. In those cases where a companion female was observed next to a clutch of nymphs, it remained no more than 7 d.

Besides the previously described behaviors such as covering eggs with secretion, facilitating nymphal feeding through construction of feeding slits, and egg guarding, *A. grossa* mothers produced abdomen vibrations, suggesting communication as shown by other membracids (Cocroft 1999a,b, 2001, 2005; Cocroft and Rodríguez 2005).

At the end of 2011, two previously marked and monitored egg-guarding females were found performing a second oviposition event, suggesting that *A. grossa* could be a moderately or facultatively iteroparous species.

Host-Plant Use. In the forest where fieldwork was performed, *A. grossa* was found using *B. suaveolens* from April to September (dry season) and both host plants from October to March (wet season);

Table 1. Summary of the life cycle of *Alchisme grossa* on *B. suaveolens* during the dry season (April–September 2011) and on *B. suaveolens* and *S. ursinum* during the wet season (October 2011 to March 2012)

Stage	Instar duration on *B. suaveolens* (dry season)	Instar duration on *B. suaveolens* (wet season)	Instar duration on *S. ursinum* (wet season)
Egg	48 ± 7	31 ± 2	21 ± 7
First instar	19 ± 1	14 ± 1	6 ± 2
Second instar	18 ± 1	10 ± 1	6 ± 2
Third instar	12 ± 2	13 ± 1	9 ± 1
Fourth instar	14 ± 3	5 ± 1	6 ± 3
Fifth instar	2 ± 1	3 ± 0	3 ± 1
Total	113 ± 3	76 ± 1	51 ± 3

Duration of each stage is shown in days (mean $\pm$ SD).

nevertheless, individuals developing on one of these host plants rarely utilized the alternative host to feed or oviposite (CFP, unpublished data). The phenology of each host plant was characterized. A pattern of asynchronic continuous cycles of flowering and fructification during the year was observed with a mean of three events of flowering and fructification per plant. The development of each individual of *A. grossa* from egg to adult was completed on the host plant where they were born; nevertheless, the mechanism that triggers host-plant alternation during the wet season remains unknown.

Resource Quality and Host-Plant Use. The number of individuals of *S. ursinum* in the study area was around 10 times that of *B. suaveolens*. Although resource quality did not differ between host plants ($H = 2.127$, df $= 1$, $P = 0.145$), resource availability differed significantly between host plants ($H = 14.430$, df $= 1$, $P < 0.01$), being higher in *B. suaveolens*. The total number of families per plant on each of the two host plants differed significantly ($H = 8.921$, df $= 1$, $P < 0.05$), being higher on *B. suaveolens* ($P < 0.05$).

Maternal Care: Orientation and Predation Bioassays. Five of the 21 relocated egg-guarding females returned to their clutches within 90 min of being relocated, while a total of 15 had come back to their egg clutches 7 d later; this pattern was significantly different from that expected by chance (exact binomial probability, $P < 0.05$). Egg clutches to which the egg-guarding female did not return did not survive.

In the case of egg clutches where the egg-guarding female was manually removed, 80% of them suffered predation after 2 wk and the rest showed signs of desiccation; in the case of removal of the guarding female from clutches with first-instar nymphs, the nymphs apparently died of starvation after 1 or 2 d.

None of the egg-guarding females fled in the presence of a natural predator and all of them showed confronting behavior; none of the solitary females displayed confronting behavior: 78% of them escaped and the rest remained motionless. Both behaviors differed significantly between treatments ($P < 0.05$).

Discussion

Patterns of host-plant use and specialization in a single plant family, as now described for *A. grossa,* have also been reported for other membracids (Wood and Guttman 1983, Wood and Olmstead 1984). These patterns have been related to biogeographical features, whereby higher degrees of ecological specialization are found at higher elevations (Wood 1984) and within relatively constant landscapes (Futuyma and Moreno 1988), two conditions prevailing in our study site.

Host specialization has been argued to be driven in many cases by secondary metabolites of plants (Futuyma and Moreno 1988, Schoonhoven et al. 2005, Iason et al. 2012). Both *B. suaveolens* and *S. ursinum* are characterized by the presence of alkaloids, one the most studied type of compounds in relation with ecological interactions

(Roberts and Wink 1998, Eich 2008). Chemical traits could be a factor affecting the quality as hosts of *B. suaveolens* and *S. ursinum* and hence feeding preferences by *A. grossa*, as has been observed in other phytophagous insects (Sotka et al. 2003, Hori et al. 2011). Changes in the feeding preferences could explain variations in the patterns of use of both host plants. Nevertheless, the patterns of specialization observed can also be explained by historical restrictions such as phylogenetic constraints in species in which a particular specialization represent the retention of an ancestral state (Bernays and Chapman 1994, Futuyma and Moreno 1988).

The alternation of host plant also has consequences on the ecology and biology of individuals; for example, a variation in life cycle duration and changes in signal traits interfering with mate recognition have been associated to host alternation in membracids (Lin and Wood 2002; Cocroft et al. 2008, 2010). In the case of *A. grossa*, differences in life cycle duration could be related to phenological changes in the host plants (i.e., chemistry) or seasonal changes related to weather (i.e., humidity, temperature) of these zones (Navarro and Maldonado 2002, Hori et al. 2011). It has been suggested that host alternation is related to the onset of diversification via ecological speciation, a hypothesis which states that host-specialized species occur via disruptive selection on host preference with the feedback of the preference of organisms to mate on their favorite host (Futuyma and Moreno 1988, Wood et al. 1999). However, although egg-guarding females of *A. grossa* showed the capability to recognize and defend their clutches, their presence is vital for nymphal survival, and such recognition capacity was not related to host-plant alternation. Reproductive patterns of preference in *A. grossa* in the context of host alternation should be pursued.

Among membracids, it is assumed that species showing maternal care during nymphal development are semelparous (Wood 1984, 1993a; McKamey and Deitz 1996; Lin 2006), while those genera that are iteroparous or at least moderately iteroparous show ant mutualism, i.e., nymphal tending by ants whereby ants obtain honeydew excreted by nymphs and nymphs obtain protection against natural enemies (Nikhat and Imtiaz 1979, Stegmann and Linsenmair 2002, Zink 2003a, Linares et al. 2010). Moreover, Wood (1984) proposes that landscape constancy promotes iteropary in membracids. *A. grossa* differs from most of these patterns by being a species without ant mutualism, which shows a high level of maternal care, but exhibits a moderate level of iteropary. Additionally, *A. grossa* is exceptional because the tribe Hoplophorionini has been characterized as being strictly semelparous (Wood 1993a).

Behavioral traits in *A. grossa,* such as aggregation of two nymphal groups to be tended by one female as well as an important number of clutches with the occasional presence of a mature adult female accompanying the egg-guarding female, could be explained by two distinct behavioral scenarios: one suggesting the occurrence of cooperative care (Lin 2006) and another brood parasitism (Zink 2003b). Under the first scenario, cooperative care could evolve if it is maintained for a considerable time during nymphal development. Nevertheless, the short time that companion females remained in the clutch suggests a brood-parasitism scenario, i.e., a strategy whereby female adults "parasitize" other families and distribute their eggs among various clutches during the short accompanying periods on each (Zink 2003b).

Life and social traits described for *A. grossa* were more complex than expected from descriptions of other members of the tribe Hoplophorionini. The mechanisms and ecological effects associated with host-plant alternation and the biological role of companion females related with social evolution seem to have an ideal model study system in *A. grossa*.

Acknowledgments

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