



Studies on the life cycle patterns of treehopper and its obligatory mutualistic association with Godzilla ant, *Camponotus compressus*

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Abstract

The beneficial interaction between individuals of two species, commonly referred as mutualism, is recognized as a common and important ecological interaction. Ant-hemipteran mutualisms are wide spread in nature in which homopterans produce honeydew collected by ants, and ants provide protection or other services. Hence the present investigation has been conducted to assess the life cycle of thorn mimic treehopper and to determine the effects of mutualistic strength between Thorn Mimic Treehopper, *Leptocentrus taurus* (Hemiptera: Membracidae) and the Godzilla ant (*Camponotus compressus*). It was observed that the secretion of honey dew occurred by tree hopper during evening hours of the day when compared to morning hours. The more the numbers of tickling by the ant, the more honey dew was secreted. There was a significant reduction in the nitrogen and phosphorous level in the host plant after the attack of the host plant by the tree hopper.

Keywords: Mutualism, Thorn Mimic Treehopper, *Leptocentrus taurus*, Godzilla ant, *Camponotus compressus*,.

Introduction

Mutualism is thought to have driven the evolution of much of the biological diversity and co-evolution between groups of species. However mutualism has previously received less attention than other interactions such as predation and parasitism (Addicott, 1978). The outcomes of conditional mutualisms often depend on varying ecological factors. Such conditionality is thought to be widespread in ant-homopteran mutualisms, in which homopterans produce excretions (honeydew) collected by ants, and ants provide protection or other services (Baker & Baker, 1973.). Ant attendance can definitely affect

the size of a homopteran aggregation individuals on a host plant depending on several factors including ant abundance and natural enemy levels. (Baker *et al*; 1978) Ant attendance can also influence characteristics of individual homopterans such as growth rate by inducing changes in feeding behaviour and the quantity or quality of honeydew that homopterans produce. (Bach, 1991). The mechanisms underlying the distribution and strength of ant-homopteran associations can be discovered by locating these individual-level effects, and determining their causes and consequences of these associations.

Beneficial interaction between individuals of two species, commonly referred as mutualism, is recognized as a common and important ecological interaction. (Beamer, 1930) Food-for -protection mutualism between ants (Hymenoptera: Formicidae) and honeydew-producing insects in the hemipteran is one of the most familiar examples. These ant-hemipteran mutualisms are very common interactions in terrestrial communities from temperate to tropical latitudes. Honeydew is a sugary excretion of carbohydrates, amino acids and water derived from plant phloem upon which many hemipterans feed (Way, 1963). Ants are attracted to honeydew as a predictable, renewable food resource and, consequently, 'tend' honeydew-producing hemipterans, protecting them from predators and parasitoids. (Cocroft *et al*, 2000). Ants tend honeydew-producing hemipterans on an extremely wide range of plants. The interactions between ants and honeydew-producing hemipterans are also very common in managed habitats like agricultural fields in addition to being widespread in natural habitats from grasslands to forests (Eberhard, 1986).

The Thorn Mimic Treehopper, *Leptocentrus Taurus* (Hemiptera: Membracidae) is frequently tended by ants. The honeydew droplets that fall on lower leaves and ground from tree hopper aggregations could serve as cues to potential tending ants. (Herre *et al*;1999). This behaviour pattern would be particularly important for the survival of developing brood by minimizing mortality due to predation and parasitism at early stages. Ants are dominant eusocial organisms in tropical lowland ecosystems. In addition to extra-floral nectar, honeydew produced by Hemipterans and other insects provides an important nutrient resource for tropical ant communities (Cushman, 1991). Although other Hymenopterans such as bees and wasps are associated with treehoppers, interactions with ants are the most pervasive. In addition to protection, treehoppers gain a benefit from ant attendance by increased feeding rates resulting in a greater developmental rate, adult size, and survivorship (Loye, 1982).

A colony of Common Godzilla ants (*Camponotus compressus*) were meticulously tending to a colony of Tree hoppers (Family: Membracidae), as would farmhands do to cows and goats (Anderson and McShee, 2000). The tending nature of the ants is primarily for food alone that the hoppers are looked after so carefully. The hoppers by their very proximity to these fierce fighter ants, get all the protection they would ever need from predators. (Del- Claro and

Oliveira, 1996.). The mandibles of the Godzilla ant are strong enough to slice our skin, and the sight of a major worker ant running in to attack is indeed awe inspiring. The ants were coaxing the hopper to do something.... rubbing them gently with their feelers... running over them softly....on a one to one basis and in groups(Wood,1984). One of the hopper nymphs seemed to get the idea, and raised its posterior end. The ant, immediately sensing it, attached its mouth to the raised end, and partook of an amber coloured drop of secreted sweetness, which it then proceeded to share with another of its kind(Dennis,1961). The ants were getting 'manna' in exchange for the unknowing protection they offered by their mere presence. In Greek, 'Trophikos' means nourishment, and 'biosis' is nothing but 'life'..... and the word 'Trophobiosis' was coined to explain this magical phenomenon.

The ant-hemipteran 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. Hence an investigation has been conducted to determine the effects of trophobiosis strength between Thorn Mimic Treehopper, *Leptocentrus taurus* (Hemiptera: Membracidae) and Common Godzilla ant (*Camponotus compressus*).

Materials and Methods

Culture of thorn mimic treehopper (*Leptocentrus taurus*)

Cassia fistula, an abundant shrub in the study area that frequently hosts aggregations of *Leptocentrus taurus* near the apical meristem of the principal stem was selected for the present investigation. The thorn mimic treehoppers were collected from *Cassia fistula* tree and reared in the laboratory to avoid potentially confounding effect of dietary history on host plant preference.

Introduction of Common Godzilla ant(*Camponotus compressus*) into the host plant

The Common Godzilla ant (*Camponotus compressus*) were collected and were introduced into the rearing cage of the thorn mimic treehopper.

Laboratory Bioassays Biology of Tree hopper

The adult pairs were provided with their host twigs as substrata for egg laying. For assessing the duration of

larval development, the eggs were separated and observations were made on their incubation period and duration of each larval stage when fed on their different ages of host leaves. The biology of the tree hopper was keenly observed and studied to analyse the life cycle patterns of the insect.

Fecundity Studies

Freshly emerged adult male and female pairs were reared on the host plant parts and host plant was provided for oviposition. Fecundity was assessed on the basis of the oviposition period and the number of eggs laid during the lifetime was noted. The mean longevity of male and female, which were reared on different host plants, were also noted. Reproduction per day was calculated by dividing the fecundity by the reproductive period.

$$\text{Reproduction per day} = \frac{\text{Fecundity}}{\text{Reproductive period}}$$

Life history parameters

Life tables were constructed for tree hopper on the host plant and on suspected alternative hosts. Host plant was systematically searched every day. Duration of each moulting was recorded and changes occurring at the time of larvae, pupal and adult period was noted frequently.

Plant Sap and Honey dew secreting behaviour of the tree hopper

The plant Sap and honey dew secreting behaviour of the tree hopper were observed. The time taken for plant juice sucking of each tree hopper was noted separately. These observations were noted in the morning, afternoon and evening hours. The behaviour of the tree hopper during the study period was recorded separately. The honey dew secreting preferences of the tree hopper was noted in the morning, afternoon and evening separately.

Behavioural patterns of the tree hopper and the black ant

Behavioural data on membracids and black ant newcomers were simultaneously gathered from 0900 to 1400 hours. For 1 min, at 1-h intervals, the number of ants inside a 0.6-m diameter circle on the plant and the number of ants climbing onto the plant are counted. The mutualistic relation between the tree hopper and the black ants were recorded.

Estimation of biochemical parameters in the host plant and in tree hopper

The shrub with treehoppers and ants was used for the present study. The treehoppers on each plant after initiating the experiment were counted every 3–4 days thereafter for 24 days. After 24 days, the leaves and the tree hoppers were collected from each plant. The tissue analyses were made to n samples dried at 60°C for 48 h. The subsamples of ground leaves and whole treehoppers for carbon and nitrogen concentrations using a Perkin–Elmer 2400 CHN analyser (Perkin–Elmer, Wellesley, MA, USA), and phosphorus concentration using persulfate digestion and ascorbate–molybdate colorimetry were performed. The soluble protein concentration in treehoppers were also estimated using the direct Lowry procedure. In the phosphorus and soluble protein assays, treehoppers were gently crushed to expose tissues to reagents.

In an another assay, the ants were removed from the experimental plant by applying sticky trap to the base of stems to prevent ants from climbing the shrub. The biochemical parameters of the tree hopper and the plant were estimated as the same procedure adopted before.

Results and Discussion

An investigation has been conducted to determine the effects of trophobiosis strength between Thorn Mimic Treehopper, *Leptocentrus Taurus* (Hemiptera: Membracidae) and Common Godzilla ant (*Camponotus compressus*). The aggregations of adults, nymphs, and females on eggs or with nymphal aggregations were tagged individually with tapes on the branches and observed every 3 h from 8 am to 5 pm for 8 consecutive days. All life stages of *Leptocentrus taurus* were found on *Cassia fistula*. A single clutch of eggs is deposited on the under side of stems toward the apical portion of the branches. The females appear to lay eggs on branches that lack ovipositions from other females. Nevertheless, two egg masses were found on a single stem, one deposited about 3 cm below the other.

Table 1 shows the life cycle duration of tree hopper. Individuals required an average of 75 days to go from egg to adult, with each instars requiring 7–10 days on average (Table 1). The longevity of the adults varied to a maximum of 60 days. The mature ovarian eggs were 0.03 mm long and 0.35 mm wide. When newly deposited they were white and were sub cylindrical with the basal end sharp and the distal end rounded.

Table-1 Life history parameters of tree hopper, *Leptocentrus taurus*

Biological parameters	Duration of each stage (in days)
Fecundity (No of eggs)	35
Total larval duration	70
Nymphal duration (days)	18
Adult tree hopper	18

The average number of eggs laid was 45, with a range of 24 to 72. The first instar nymph is elongate, sub ovoid colour when newly emerged from egg (or recently molted later instars) yellow with transparent legs, and ventral part of abdomen reddish orange. The colour of the second instar nymph is well maintained with ventral portion of abdomen reddish orange on sides and middle part yellow. The fourth instar nymph has waxen white coloured wing pad apices and the rest of the body colour was similar to that of third instar and the ocelli was also visible. The fifth instar nymph was horn brownish. The posterior pronotal process was reddish and was extended to first abdominal segment. The horn length was sexually dimorphic, shorter in males and not exceeding the anterior edge of the head.

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. Daily turnover of ant species at a given treehopper aggregation was observed on *Cassia fistula*. The typical diurnal ants such as *Camponotus compresus* and *C. aff.blandus* were most active at treehopper aggregations from 0800-1800 h.

Table 2 and 4 shows the honey dew sucking behaviour of Godzilla ant *Camponotus compressus*. In *Cassia fistula*, thousands of ants were observed on the spikes at any time during the day. Without exception, ants were slowly running up and down the spikes, frequently stopping and inserting their heads into the spikes. Often several ant species visited a single spike simultaneously or in succession. The examination of the interior of ant-visited spikes revealed nymphal aggregations of the tree hopper on *Cassia* branches. The nymphs of the treehopper feed on phloem sap. The aggregations comprised 10-12 nymphs per branch, but only occasionally adults. The secretion of the honey dew was more frequent in the evening when compared to morning hours.

Table 3 shows the amount of honeydew secretion per tickling of Godzilla ant *Camponotus compressus* on tree hopper *Leptocentrus taurus*. Both in the field and in the laboratory, tree hopper nymphs were observed to slowly excrete droplets of honey dew followed by the tickling of the Godzilla ant. When a nymph remained in one place for some time, several droplets fused into a bigger drop. In the field, Godzilla ant species were observed imbibing droplets, by tickling the abdomen of tree hopper. It was observed that 1ml of honey dew was secreted following 110 ticklings by the tree hopper.

Table-2 Honey Dew Sucking Behaviour of Godzilla Ant *Camponotus compressus*

Name of the tree hopper	Name of the Host plant	Morning	Afternoon	Evening
<i>Leptocentrus taurus</i>	<i>Cassia fistula</i>	++	+	+++

Table -3 Amount of honey Dew secretion per tickling of Godzilla ant *Camponotus compressus* on tree hopper *Leptocentrus Taurus*

Number of tickling by ant	Amount (ml) of honeydew secreted
110	1
220	2
330	3
440	4
550	5

Table- 4 Time frequency table of tickling performed by Godzilla ant *Camponotus compressus*

Tickling Organism	Morning	Afternoon	Evening
<i>Camponotus compressus</i>	++	+	+++

A recent study shows that ant attendance also affected treehopper stoichiometry (Kay *et al.* 2004). Treehoppers tended by ants contained lower nitrogen concentrations than those without ants. Unlike insects such as some species of Lycaenid butterfly larvae that form obligate relationships with ants, most treehopper–ant relationships appear to be facultative relative to ant species (Wood 1977). Nevertheless, further research on natural history, particularly of the tropical fauna, will be necessary to understand the degree (facultative or obligate) and relationships

(range from mutualism to parasitism) of ant association in treehoppers.

Table 5, 6 and Fig 1 demonstrates the biochemical components of tree hopper and Cassia leaves. There was a remarkable reduction in nitrogen and phosphorous level in Cassia leaves after the attack of the host plant by the tree hopper. It was observed that protein and carbohydrate level increases in the haemolymph of treehopper followed by the consumption of the plant sap by the insect.

Table -5 Biochemical components of tree hopper *Leptocentrus taurus* before and after sap sucking

Biochemical parameter	Amount present in the tree hopper before sap sucking	Amount present in the tree hopper after sap sucking
Protein(g)	1.52	2.12
Lipids (% g)	0.52	0.72
Total carbohydrate(g)	3.62	4.21
Nitrogen (mg)	1.25	1.45
Carbon(mg)	0.35	0.36
Phosphorous (mg)	0.79	0.81

Table- 6 Biochemical components of host plant *Cassia fistula* before and after plant sap sucking by the tree hopper

Biochemical parameter	Amount present in the plant before sap sucking	Amount present in the plant after sap sucking
Protein(mg)	1.94	1.05
Lipids (mg)	0.78	0.54
Total carbohydrate(mg)	6.68	3.64
Fibre	3.02	3.02
Ash(mg)	0.78	0.78
Nitrogen (mg)	1.52	0.09
Moisture Content (%)	0	0
Phosphorous (mg/100mg)	0.96	0.75

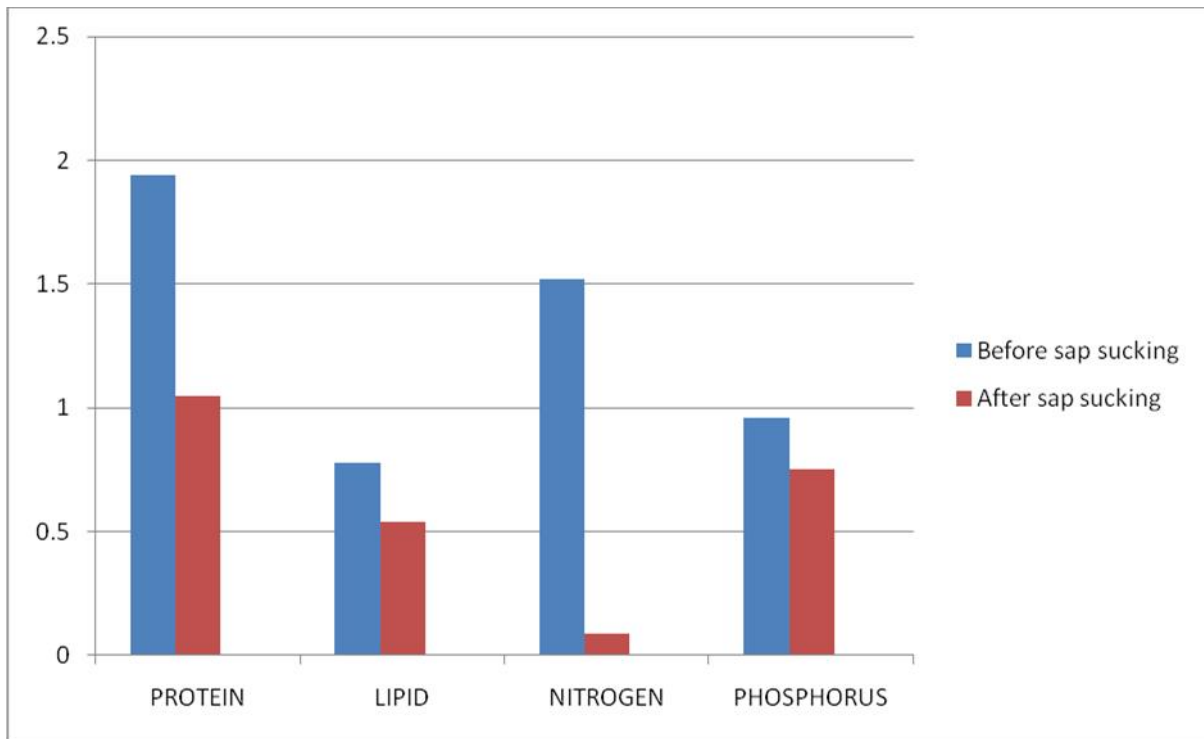


Figure 1 Comparative biocomponents of host plant *Cassia fistula* before and after plant sap sucking by the tree hopper

Ants and treehoppers had an interactive effect on leaf nutrient concentrations that may have resulted from tree hopper feeding. Amino acid concentrations in leaves often decrease after attack by phloem feeders (Oliveira, 1997), which could explain the uniformly low concentrations in leaves on which treehoppers were feeding at the time of collection. Damage on these leaves from ant excluded and control plants may have been similar because of the similar number of treehoppers per leaf in each treatment. On plants with ants, leaf nitrogen levels were also lowing leaves without treehoppers. However, such leaves were rare and were close to leaves being fed upon – they thus may also have been recently attacked. In contrast, many leaves on ant-excluded plants may have escaped recent attack because treehoppers have limited mobility and were rare on these plants (McEvoy,1979). Regardless of the mechanism, ant presence was associated with lower leaf nitrogen concentrations in plants because (1) leaves with treehoppers had low nitrogen concentrations,(2) plants with ants had proportionally more leaves with treehoppers on them, and (3) leaves without treehoppers had much lower nitrogen concentrations on plants with ants than on plants without ants. Plant quality is thought to mediate ant-hemipteran

interactions through its bottom–up effect on honeydew quality (Wood, 1974). Our results suggest that ant presence may have a top–down effect on plant quality that could increase the cost of producing attractive honeydew, thereby contributing to the dynamic nature of these interactions.

Conclusion

The Thorn Mimic Treehopper, *Leptocentrus Taurus* (Hemiptera:Membracidae) is frequently tended by ants .The honeydew droplets that fall on lower leaves and ground from tree hopper aggregations could serve as cues to potential tending ants. This behaviour pattern would be particularly important for the survival of developing brood by minimizing mortality due to predation and parasitism at early stages. Ants are dominant eu-social organisms in tropical lowland ecosystems. In addition to extra-floral nectar, honeydew produced by Hemipterans and other insects provides an important nutrient resource for tropical ant communities. Although other Hymenopterans such as bees and wasps are associated with treehoppers, interactions with ants are the most pervasive.

The behavioural changes observed during the trophobiotic interaction between thorn mimic treehopper (*Leptocentrus taurus*) and Common Godzilla ant (*Camponotus compressus*) and their influence on the host plant, *Cassia fistula* reveals that the secretion of honey dew occurred during evening hours of the day when compared to morning hours. The more the numbers of ticklings, the more honey dew was secreted. The secretion of honey dew is dependent on the tickling of the Godzilla ant. The presence of ant in the host plant may have a negative effect on plant quality but it increased the production of honeydew, thereby causing the dynamic nature of these plant-animal interactions. There was a remarkable reduction in the nitrogen and phosphorous level in *Cassia* leaves after the attack of the host plant by the tree hopper. It was observed that protein and carbohydrate level increases in the haemolymph of treehopper followed by the consumption of the plant sap by the insect. The membracids alone decrease plant growth and seed set in *Cassia* leaves. In the absence of membracids, the ants defend the plant against external herbivores, and their presence increases plant growth and it can be concluded that the interaction between ants and membracids exerts an overall negative effect on plant growth.

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