Biology of leaf gall-inducing *Thlibothrips manipurensis* 
*MURALEEDHARAN, 1982 on Ardisia sp. (Myrsinaceae) in north-eastern India* 

*(Thysanoptera: Tubulifera: Phlaeothripidae)*

With 11 figures

**HEISHNAM TAPTAMANI, RAMAIYER VARATHARAJAN and ANANTANARAYANAN RAMAN**

**Summary**

Biology of the epiphyllous roll-gall-inducing *Thlibothrips manipurensis* was studied on *Ardisia* sp. under laboratory conditions. *T. manipurensis* laid eggs linearly along the margins of tender leaves. Eggs hatched in 6.8 d and the larval duration was 3.4 and 8.2 d for larvae I and II, respectively. After 20.2 h as prepupa, *T. manipurensis* grew into pupa; adult emergence occurred in 4 d. Each female laid 34±7 eggs and the mean longevity of the adult was 10.2 d. Increase in thrips numbers correlated with the age of the gall: 15 individuals/gall occurred in young (4–10 d) galls, whereas 28 occurred in mature (20 d) galls, and 36 in old (25 d) galls. Male–female ratio in old galls was 1:5. Mature galls included a homogeneous tissue structure, made of 12–15 layers of parenchyma cells with no distinction into spongy and palisade cells. The gall-mesophyll cells adjacent to the upper and lower epidermises developed essentially through anticlinal divisions of the primordial cells, whereas those in mid regions developed through periclinal divisions; the latter facilitates the blade to roll inwards. Cells in the mid region of the galled lamina were larger than those occurring adjacent to the epidermises. Bulk of the cells in the mid-region of the galled lamina included darkly staining inclusions, which, in high likelihood, were phenolic materials. *Thlibothrips* is an ally of *Gynaikothrips*. *Thlibothrips* and *Gynaikothrips* are the better known gall-inducing taxa. The roll galls induced by *Thlibothrips manipurensis* on *Ardisia* sp. (Myrsinaceae, Asterids, Eudicots) are highly similar to those induced by *Gynaikothrips flavian ten natus* on the leaves of *Casearia elliptica* (Salicaceae: Sabuaceae, Rosids, Eudicots) in southern India. Because the Rosids and Asterids are considered related phylogenetically, the possible pathways taken by species of *Gynaikothrips* and *Thlibothrips* to radiate exploring different, but related plant taxa appear striking.

**Zusammenfassung**


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Key words

*Ardisia* sp., galls, marginal-leaf rolls, Myrsinaceae, Phlaeothripidae, population patterns through development, *Thlibothrips manipurensis*, Thysanoptera, Tubulifera

Introduction

*Thlibothrips* *Priesner*, 1951 belongs to *Gynaikothrips— Liophlaeothrips— Liothrips* complex (*sensu* *Ananthakrishnan & Muraleedharan* 1974). It is distinguished by elongated fusiform eighth antennal segment and enlarged posterior ommatidia. Seven species of *Thlibothrips* are known today (*Mound* 2007). *Thlibothrips isunoki* *Okajima*, 1981 and *T. nigricauda* (*Karny*, 1915) are the confirmed gall-inducing taxa on the leaves of *Symlocos myrtaea* *Siebold* et *Zuccarini*, 1870 (Symlocaceae) (*Haga* et al. 1989) and *Drypetes sepiaria* (*Wight* et *Arnold*) *Pax* et *K. Hoffmann*, 1922 (= *Hemicyclia sepiaria* *Wight* et *Arnold*, 1833) (Euphorbiaceae) (*Ananthakrishnan* 1978), respectively. *Thlibothrips atavus* (*Karny*, 1915), *T. antennalis* *Okajima*, 1981, *T. primitivus* (*Karny*, 1922), *T. malloti* *Ananthakrishnan*, 1961, and *T. manipurensis* Muraleedharan, 1982 have been recorded as associates in undetermined galls; their role — whether inducers or inquilines — in galls was not established (*Ananthakrishnan* 1978). The present paper confirms the role of *T. manipurensis* as the inducer of the marginal-roll galls on the leaves of a possible new species of *Ardisia* *Swartz*, 1910 (suspected to be near *Ardisia paniculata* *Roxburgh*, 1824 — a common taxon on north-eastern India, *Devi* & *Yadava* 2006), with notes on their populations during gall development.

Materials and Methods

Leaves of *Ardisia* sp. bearing young (4–10 d), mature (20 d), and old (>25 d) galls, and stages showing gall initiation (0–3 d) were collected randomly from Moreh (Chandel district, Manipur, India, 300 amsl, 24°40′N; 93°50′E) in the Indo—Myanmar biodiversity hotspot of north-eastern India in August 2009, based on previous field observations that the life cycle of *T. manipurensis* is completed in 25–28 d (*Taptamani & Varatharajan*, unpublished observations). The collected materials were transported to the Life-Sciences laboratory of Manipur University at Canchipur (c. 90 km from Moreh) in polythene bags stored in a transportable ice chest. In the laboratory, galls in different developmental stages were unrolled carefully under a stereobinocular microscope (Olympus Magnus MS 24/13, No. 5D 0347, Olympus India, New Delhi, India) for obtaining population data of different development stages of *T. manipurensis*. Eggs laid along the partly in-rolled margins of tender leaves were counted. Cut ends of gall-bearing branches were stood in glass jars containing tap water. Galled leaves were enclosed individually in polythene bags with pinholes to permit aeration. The bags were tightened at pedicel points to prevent thrips escape. The polythene bags were carefully opened every day to dry accumulated moisture, to count numbers of moulted larvae, pupae,
and adults, and to assess brood sizes. Five such cultures were maintained in the laboratory (18±2°C min, 26±1.5°C max, 72±5 % RH, 12:12 L:D). Numbers (mean±SE) of different life stages of *T. manipurensis* from the maintained cultures were obtained.

To establish the relationship between gall size and brood size, 15 tender leaves of *Ardisia* sp. with their pedicels, after ensuring that each leaf included at least one gravid female of *T. manipurensis*, were individually stood in tap-water cultures in the laboratory, enabling galls to grow. In 25 d, every gall maintained in water culture, was unrolled to assess the brood size. Fifteen mature and old galls were measured from leaf tip to pedicel using a scientific centimetre ruler. The data pertaining to gall size versus brood size were recorded from each culture and then processed for Pearson-correlation analysis using the Statistical Package for Social Sciences (2001, 11.5 software; http://www.spss.com; Chicago).

Microtome sections were prepared from similar-aged leaves (i) those bearing galls and (ii) those did not (hereafter referred as 'normal') from determined sheets of *Ardisia* sp. near *paniculata* stored in the herbarium of the School of Life Sciences, Manipur University. Leaf materials were revived by boiling them for 60 min in water added with a few drops of commercial detergent and glycerol. Revived materials were sliced (3 mm²) and fixed FAA (70 % alcohol 90 ml, formalin 5 ml, acetic acid 5 ml). Fixed specimens were processed for microtomy, following protocols described in Bradbury (1973). The 10 μm sections obtained were mounted on glass slides and stained with safranin—fast green combination, mounted in DPX, and photographed (Olympus Trinocular Research Microscope, CX 31, Camera E-520, Manila, The Philippines).

**Results**

The earliest infestation by *T. manipurensis* on the leaves of *Ardisia* sp. in Moreh, under field conditions occurred in August 2009 and earliest stages of 'galls' included 2–3 adults/leaf, with at least one adult female on each infested leaf. On an average, 15 individuals (11±1 larvae and 4±1 pupae)/gall occurred in young galls, 28 (13.5±1 larvae, 8.5±1 pupae, and 6±1 adults)/gall in mature galls, and 36 (3.5±0.5 larvae, 3.5±0.5 pupae, and 29±2 adults)/gall in old galls. The sex ratio in 25 d old galls was 1:5 (♂:♀).

*Thlibothrips manipurensis* laid eggs linearly along the adaxial edges of tender leaves, with their edges showing early signs of rolling commencing from the leaf tip (Fig. 1). Eggs hatched in 6.8 d; larval duration was 3.4 and 8.2 d for larvae I and II, respectively. After 20.2 h as prepupa, *T. manipurensis* grew into pupa and adult emergence occurred in 4 d. Each female laid 34±7 eggs and the mean longevity of the adult was 10.2 d (Figs 1-6). The gravid female laid the first batch of eggs (4.8±0.8) on the third day of adult eclosion on the adaxial side of tender leaves and guarded her eggs until larval emergence. On the fifth day, the second batch of eggs (8.2±0.7) was laid. Meanwhile the already started rolling extended towards the pedicel of the infested leaf, with several individuals of *T. manipurensis* feeding at those points. Subsequently, each female laid 4.4±1 eggs/day from d 6 to 10. However, brood size depended on the length of the leaf roll. Length measurements of old galls (added value of galls along both edges of the same leaf blade) revealed that 18.3±2 cm long galls included 34±2 eggs, whereas 15.3±1 cm long galls included 27±2 eggs, and 12.2±0.4 cm long galls 23±2 eggs. Fig. 7 illustrates the relationship between the two tested variables and also that the brood size is directly dependent.
on gall length \((r=0.877, \ p<0.01, \ n=15)\). Mating period was 10–20 min and they displayed a wing-combing and grooming behaviour (Ellington 1980, Varatharajan 1984, Mound & Morris 2001) using their mid and hind legs.

All gall-bearing leaves, maintained under laboratory conditions, from initiation stage included only populations of *T. manipurensis*. Neither in the samples obtained from the field nor in the laboratory cultures we could observe any other taxon that was either a predator or a parasitoid or an inquiline. Each mature gall \((n=15)\) included 13.5±1 larvae, 8.5±1 pupae, and 6±1 adults of *T. manipurensis*, whereas each old gall included 3.5±0.5 larvae, 3.5±0.5 pupae, and 29±2 adults. More individuals of *T. manipurensis* occurred in the inner roll, until the 25 d of gall growth. Thereafter adults started moving out of the gall and occupied either the corners of polythene bag or the partly dry leaf areas.

*Thlibothrips manipurensis* induce galls (Fig. 8) on the leaves of *Ardisia* sp., wherein the blades roll towards the midrib on the upper side. Mature normal leaves showed a dorsiventrally differentiated structure with one layer of short palisade cells and 5–6 layers of spongy mesophyll (Fig. 9). Mature galls included a homogeneous tissue structure, made of 12–15 layers of parenchyma cells with no distinction into spongy and palisade cells (Fig. 10). The mesophyll cells lying close to the upper and lower epidermises in mature galls developed essentially through anticlinal divisions of the primordial cells, whereas those in the mid regions developed through periclinal divisions. Cells in the mid region of the galled leaf blade showed a greater level of cell enlargement than those occurring close to the epidermises. The periclinal divisions and hypertrophied cells in the mid regions of the galled leaf blade enabled rolling. Bulk of the cells in the mid-region of the gall leaf blade included darkly staining cell inclusions, which, in high likelihood are phenolic substances (Fig. 11).

**Discussion**

*Thlibothrips* is an ally of *Gynaikothrips* (Zimmermann, 1900) and *Litotetothrips* Priesner, 1929 (Okajima 1981). *Gynaikothrips* and *Thlibothrips* are the better known gall-inducing taxa, whereas *Litotetothrips* is an established non-gall-inducing taxon. *Gynaikothrips australis* Bagnall, 1929, *G. ficorum* (Marchal, 1908), and *G. uzeli* Zimmermann, 1900 induce leaf-fold galls on *Ficus macrophylla* Desfontaines ex. Persoon, 1807, *F. microcarpa* Linnaeus, 1781 (= *F. retusa* Linnaeus, 1767), and *F. benjamina* Linnaeus, 1767 (Moraceae), respectively, especially in warmer parts of the world (Ananthakrishnan & Raman 1989), whereas *G. flaviantennatus* Moulton, 1929 induces roll galls on the leaves of *Casentria elliptica* Willdenow, 1799 (= *C. tomentosa* Roxburgh, 1832) (formerly under Samydaceae, now under Salicaceae [Samydeae] sensu Breteleur 2008) – a widely occurring taxon in the Indian subcontinent (Raman et al. 1978). Mature galls induced by *Thlibothrips manipurensis* on the leaves of *Ardisia* sp. are closely similar to those induced by *G. flaviantennatus* on *C. tomentosa*, in terms of their gross morphology and tissue organization.

A majority of known gall-inducing insects demonstrate a high degree of orientation to specific trophic niches and display a high level of fidelity to particular species of plants (Raman 1996, 2010). Salicaceae and Moraceae are presently treated under Rosids (Eudicots), whereas Myrсинaceae under Asterids (Eudicots); Rosids and Asterids are considered to be related phylogenetically (Angiosperm Phylogeny Group 2003). This indicates how species of *Gynaikothrips* and *Thlibothrips* have possibly adapted to radiate among taxa of the Rosids and Asterids, exploring varied ecological niches.

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That *T. manipurensis* completes its life cycle in 20–25 d matches with the noted biologies of many other Indian Phlaeothripidae: *Crotonothrips dantahasta* Ramakrishna, 1928, *G. flaviantennatus*, *Schedothrips orientalis* (Ananthakrishnan, 1968), and *Teuchothrips longus* (Schmutz, 1913), which induce leaf-roll galls on *Memecylon edule* Roxburgh, 1798 (Melastomataceae), *C. tomentosa*, *Ventilago madraspatana* Gärtner, 1788 (Rhamnaceae), and *Pitvetta zeylanica* (Hook. F.) Gamble, 1921 (= *P. hispidula* Wight & Arnold, 1880) (Rubiacaeae), respectively (Ananthakrishnan & Raman 1989). Besides being closely similar to the galls induced by *G. flaviantennatus*, the external and internal morphologies of galls induced by *T. manipurensis* are also similar to those induced by *S. orientalis* and *T. longus* on the leaves of *V. madraspatana* and *P. hispidula*, respectively. Mature galls induced by *G. flaviantennatus*, *S. orientalis*, and *T. longus* also include densely staining secondary compounds (Raman & Ananthakrishnan 1983), similar to those that occur in galls induced by *T. manipurensis*. Another striking example is the leaf-roll gall induced by *Alocothrips hadrocerus* (Karny, 1926) (Phlaeothripidae: Phlaeothripinae) on *Maytenus senegalensis* (Lamarck) Exell., 1952 (Celastraceae), but structures of the galls induced by *A. hadrocerus* differ from those induced by *T. manipurensis*, *S. orientalis*, and *T. longus*. Galls induced by *A. hadrocerus* include multiple layers of sclereids (Raman & Ananthakrishnan 1979, Raman 2011a), which are absent in the galls induced by *T. manipurensis*, *S. orientalis*, and *T. longus*. Given that sclereid differentiation is regulated by sugar–hormone modulation (Uggla et al. 2001) and also that the physical feeding action among Phlaeothripidae is considerably similar (Raman 2011b), the preceding comparison points to that the salivary composition of *T. manipurensis*, *S. orientalis*, and *T. longus* is different from that of *A. hadrocerus*. The saliva of *T. manipurensis*, *S. orientalis*, and *T. longus* includes no molecular-trigger factor capable of altering the sugar–hormone modulation in their respective leaf environments, and thus modify the mesophyll parenchyma to differentiate into sclereids.

Margins of tender leaves of *Ardisia* sp. (0–3 d) already showed signs of rolling, when examined for eggs, similar to the behaviour of *Sacothrips corycids* Mound, 1971 (Phlaeothripidae: Phlaeothripinae) inducing leaf-margin roll galls on *Geijera parviflora* Lindley, 1848 in eastern parts of subtropical Australia (Mound 1971). Such a response in the leaves of *Ardisia* indicates that the gravid females of *T. manipurensis* trigger gall induction by their feeding action before oviposition. In the present study we have shown that gall lengths correlate with brood sizes of *T. manipurensis*. Although our findings align with the present understanding that the morphology of thrips-induced galls is regulated by the impact of feeding thrips populations documented data (Crespi et al. 1997, Raman 2003, 2010, 2011a), we refer to Laurence Mound's remark (email 21 December 2010) that a majority of thrips-induced galls on *Acacia* in Australia reach their maximum size before oviposition. This reinforces the need for a reconsideration of the effects of brood size of thrips on gall morphology; the gravid female in high probability initiates the gall, similar to the behaviour in the gall-inducing Sternorrhyncha (Rohfritsch 1992).

Leaf galls induced by *Gynaikothrips ficorum* and *G. australis* on subtropical-Australian *Ficus microcarpa* and *F. macrophylla*, respectively, include either the kleptoparasitic *Mesothrips jordani* Zimmermann, 1900 or the inquilinous *Gynaikothrips additamentus* (Karny) Mound, 2008 (Tree & Walter 2009). Leaf galls induced by *Gynaikothrips uzeli* on *Ficus benjamina* are commonly invaded by predatory *Androthrips ramachandranii* Karny, 1926 (Phlaeothripidae: Phlaeothripinae) in tropical Asia (Ananthakrishnan 1978) and the Americas (Boyd & Held 2006, Cambero-Campos et al. 2010). Why any such predatory, parasitic, and/or inquilinous thrips does not occur in *T. manipurensis*–induced galls on *Ardisia* sp. in Manipur, which are similar open systems with the gall chamber communicating with external environment, is unclear. Probably *Thlibothrips manipurensis* also discharges volatile compounds (e.g., β–myrcene) similar to *T. isunoki* that induces galls on the leaves of *Symplocos myrtacea* in Japan that repel invading arthropods and restrict contaminating fungi (Suzuki et al. 2004).
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Literature


Figs 1–6: Life stages of T. manipuresis. Fig. 1. Eggs along the margin of a young leaf; arrow – inward rolling of the blade (Bar: – 500 µm). Fig. 2. Emerging larva; e – egg) (Bar: 500 µm). Fig. 3. Larva II. (Bar: – 500 µm). Fig. 4. Prepupa (Bar: – 200 µm). Fig. 5. Pupa (Bar: – 220 µm). Fig. 6. Adult (Female). (Bar: – 200 µm). Figs 8–11: Normal and T. manipuresis-infested leaves of Ardisia sp. Fig. 8. infested mature galled leaf (Bar: 1 cm); arrow – thrips colony. Fig. 9. Normal leaf (C.S.): DI – dark inclusions (likely phenolics); LE – lower epidermis; UE – upper epidermis (Bar: 100 µm). Fig. 10. Galled leaf (C.S.): LE – lower epidermis; UE – upper epidermis (Bar: 800 µm). Fig. 11. A portion of the leaf roll enlarged; * – larval chamber (Bar: 100 µm).

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Authors' addresses:

Dr. Heishnam Taptamani
Department of Life Sciences
Manipur University
Imphal 795 003, India
e-mail: tapta18jan@gmail.com

Prof. Ramaıyer Varartharajan
Department of Life Sciences
Manipur University
Imphal 795 003, India
e-mail: rvrajanramya@gmail.com

Dr. Ananthanarayanan Raman
Charles Sturt University & E H Graham Centre for Agricultural Innovation
PO Box 883
Orange, NSW 2800, Australia
e-mail: araman@csu.edu.au

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