INSECTA MATSUMURANA

NEW SERIES 53 : 117-134 MARCH 1997

BIOLOGY AND KARYOLOGY OF A CECIDOGENOUS PSYLLOID, TRIOZA FLETCHERI MINOR (HOMOPTERA : PSYLLOIDEA) AND MORPHOGENESIS OF GALLS ON THE LEAVES OF TERMINALIA TOMENTOSA AND T. ARJUNA (COMBRETACEAE)

By ANANTANARAYANAN RAMAN, R.N. SINGH,
and ANNA MARYANSKA-NADACHOWSKA

Research Trips for Agricultural and Forest Insects in the Subcontinent of India, Scientific Report No. 52.

Abstract

RAMAN, A., SINGH, R.N., and MARYANSKA-NADACHOWSKA, A. 1997. Biology and karyology of a cecidogenous psylloid, *Trioza fletcheri minor* (Homoptera : Psylloidea) and morphogenesis of galls on the leaves of *Terminalia tomentosa* and *T. arjuna* (Combretaceae). *Ins. matsum. n. s.* 53 : 117-134, 5 tabs., 26 figs.

A subtropical plant louse, *Trioza fletcheri minor*, induces leaf galls on at least five species of *Terminalia* in the Indian subcontinent. Taking *Terminalia tomentosa* and *T. arjuna*, this paper describes cecidogenetic interactions between them and the psylloid. *T. f. minor* completes its life cycle in *c.* 34 days, and the cecidogenetic process lasts 20-22 days, correlating with the developmental phases of the nymphal instars. Nymphal I instars initiate galls by settling on stomatal apertures of the host leaf, and the host plant responds by producing a covering growth enclosing the nymphal instars. Karyological study of the triozids raised independently from galls shows that the same species induces galls on both *tomentosa* and *arjuna*. In spite of that, probably due to genetic and physiological variation in host plants, subtle differences in the expression of mature gall form and production of trichomatous covering growth during early stages of cecidogenesis are evident. Largely, morphogenetic and metabolic responses within growing gall systems are identical. Shorter egg pedicels of *T. f. minor* appear to be an evolutionary adaptation to the host plants of mesic distribution.

Authors' addresses. RAMAN, A.: Loyola College, Madras 600 034, India. SINGH, R.N.: Central Tasar Research and Training Institute, Ranchi, India. MARYANSKA-NADACHOWSKA, A.: Polish Academy of Sciences, 31-016 Krakow, Poland.

Address for Correspondence: Professor A. Raman, 3/40 Cunningham Street, Dubbo, NSW 2830, Australia

Contents

INTRODUCTION

Galls induced by *Trioza fletcheri minor* Crawford on the leaves of *Terminalia tomentosa* (Roxb.) W. & A. and *T. arjuna* W. & A. are common along the foothills of the Himalaya and Indo-Gangetic plains (Crawford 1912, Mathur 1935, Hodkinson 1983). These galls occur with equal frequency in the subtropical and deciduous forests of lower montane regions and plains of peninsular India (Mani 1974).

Unlike a majority of gall-inducing psylloids that are generally host and site specific (Hodkinson 1984), *T. f. minor* is known not only from the leaf galls of *T. tomentosa* and *T. arjuna*, but also from those of *T. catappa* Linn., *T. paniculata* Roth, and *T. tomentosa*×*T. arjuna* hybrids (Mathur 1975). Keeping the ability of this psylloid to induce galls on more than one species of *Terminalia* in view and using its cecidogenetic interactions particularly with *T. tomentosa* and *T. arjuna*, this paper attempts to seek answers to the following: (a) Are there any critical differences in the nymphal behaviour and developmental durations while inducing galls on the leaves of two host species? (b) How do the leaves of *T. tomentosa* and *T. arjuna* morphogenetically and metabolically respond to the stimulus of *T. f. minor* during galling? (c) Because gall-inducing psylloids are generally known to be host-specific, are the triozids inducing galls on *T. tomentosa* and *T. arjuna* really conspecific?

MATERIAL AND METHODS

Galls from *T. tomentosa* and *T. arjuna* in different developmental stages were obtained periodically from the gardens of Central Tasar Research and Training Institute (Ranchi; 23°23′N; 85°23′E) during April 1991–July 1992. To raise adult triozids and establish the developmental durations of nymphal instars, cut branches from both host plants were individually maintained in Knop's nutrient solution. Galls were periodically slit vertically under a stereo-binocular microscope to extract the nymphal instars and this was used to determine gall age. To verify laboratory results, branches bearing galled leaves of both hosts were enclosed in muslin bags (30×12 cm) in their natural habitats to obtain adults.

Morphometric evaluations. Length, breadth, and height of nymphs were measured with a caliberated ocular micrometer after their extraction from galls. Fifty nymphal instars of each developmental stage from the galls of *T. tomentosa* and 50 from those of *T. arjuna* were examined. Since differences between those from *T. tomentosa* and *T. arjuna* were statistically insignificant, the values were pooled and means were scored. Nymphal mass was obtained by collectively weighing subsample pools of 10 nymphal instars of the same developmental stage in a monopan balance (0.001 g sensitivity) and subsequently dividing the gross value by 10. Weighing was repeated for the remaining nine subsample pools of 10 nymphs each and mean readings were obtained. Galls of every developmental stage, after the removal of insect material were individually weighed (*T. tomentosa*: n=50; *T. arjuna*: n=50) and the mean mass was calculated.

Microscopy. Gall and comparable normal-leaf tissues of established age were fixed in the field in formal-acetic-alcohol (FAA). Fixed materials were processed through customary methods of dehydration and wax embedding. Sections were cut

at 5-6 μm in a rotary microtome. Most of the sections were contrasted with 1.0% toluidine blue in 1.0% aqueous borax for bright-field microscopy, while a few were prepared unstained for phase-contrast microscopy. Histochemical tests done for the evaluation of some of the metabolites are indicated at appropriate points in the Observations section.

Karyology. To establish the taxonomic status of the psylloids from *T. tometosa* and *T. arjuna*, nymphal instars and adults raised in individual rearing chambers were fixed in 1 : 3 acetic acid : ethanol. The fixed materials were stored in 90% ethanol at 10-12°C. Gonads dissected from the insects were squashed in 50% acetic acid for observations. Cover slips were removed after freezing in dry ice. Slides were air-dried and chromatin materials were contrasted with 2.0% acetic-orcein.

Biochemical estimations. A hundred galls of known age and a hundred comparable portions of normal leaves for use as control were collected randomly. Since the tissues came from natural habitats, they were rinsed in distilled water for 10 min to remove dust and surface debris; water wash was done quickly to minimize any possible leaching from cut surfaces of tissues. They were air-dried by spreading on Whatman (# 1) filter paper for 25-30 min. Each gall sample was trimmed carefully with a sharp razor to avoid any normal leaf tissue; inhabiting nymphal instars were extracted by slitting the galls partially. From this, a subsample pool of 20 gall and 20 normal (control) leaf tissues were used for each biochemical assay; evaluations were repeated four more times using the remaining subsample pools of 20 each. Mean data were generated examining the five values of every biochemical assay of both control and gall tissues, considering the standard error. Values obtained as mg/g of control and gall tissues were compared subjecting to t-test of significance.

The materials were boiled in 80% ethanol. An extract obtained by grinding the ethanol-boiled materials in an agate mortar and filtering first through cheese cloth and later through Whatman (# 1) filter paper was used for assaying total proteins, free amino acids, reducing sugars, phenols, and ortho-dihydroxy phenols, following the methods of Lowry *et al.* (1951), Moore and Stein (1948), Nelson (1944), Bray and Thorpe (1958), and Johnson and Schaal (1952), respectively.

Observations

Natural history of the psylloid and morphology of galls

Adult males and females at emergence are pale brown with black terminal antennal segments, but a progressive colour change to deep brown follows with maturation. Under laboratory and field rearings from both host plants, the nymphal instar duration is 20-22 days and adult longevity is 10-12 days (Tables 1 and 2). Nymphal V instar escapes from the gall for moulting into adult outside. In 3-4 days, mating takes place and the gravid females lay eggs on tender shoots of host plants.

Galls of *T. f. minor* on the leaves of *T. tomentosa* generally occur isolated as epiphyllous pustules (Fig. 1) with usually one and rarely two nymphal chambers. On the adaxial side of the leaf, they are expressed as hemispherical swellings, while along the abaxial side a younger gall appears like a cone with a narrow slit-like ostiole (Fig. 2). In mature galls this ostiole widens, enabling the escape of the final

Table 1. Mean longevity of developmental stages of *Trioza fletcheri minor* (from *Terminalia tomentosa*: n=50*; *T. arjuna*: n=50*).

Developmental state	Days
Egg	2.7-3.2
Nymphal I instar	3.1-3.7
Nymphal II instar	5.8-6.4
Nymphal III instar	2.9-3.4
Nymphal IV instar	2.8-3.4
Nymphal V instar	1.2-2.3
Adult	10.0-12.0

Table 2. Morphometric data of the nymphal instars of *Trioza fletcheri minor* (from *Terminalia tomentosa*: n=50*; *T. arjuna*: n=50*).

Nymphal instar stage	Length (mm)	Breadth (mm)	Height (mm)	Mass** (mg)
I	0.20	0.16	0.12	0.004
II	0.35	0.28	0.20	0.007
III	0.56	0.46	0.38	0.014
IV	0.83	0.79	0.56	0.018
V	1.70	1.4	0.90	0.031

* Since differences in values between the psylloids from *T. tomentosa* and *T. arjuna* were statistically insignificant, data were pooled as n=100.

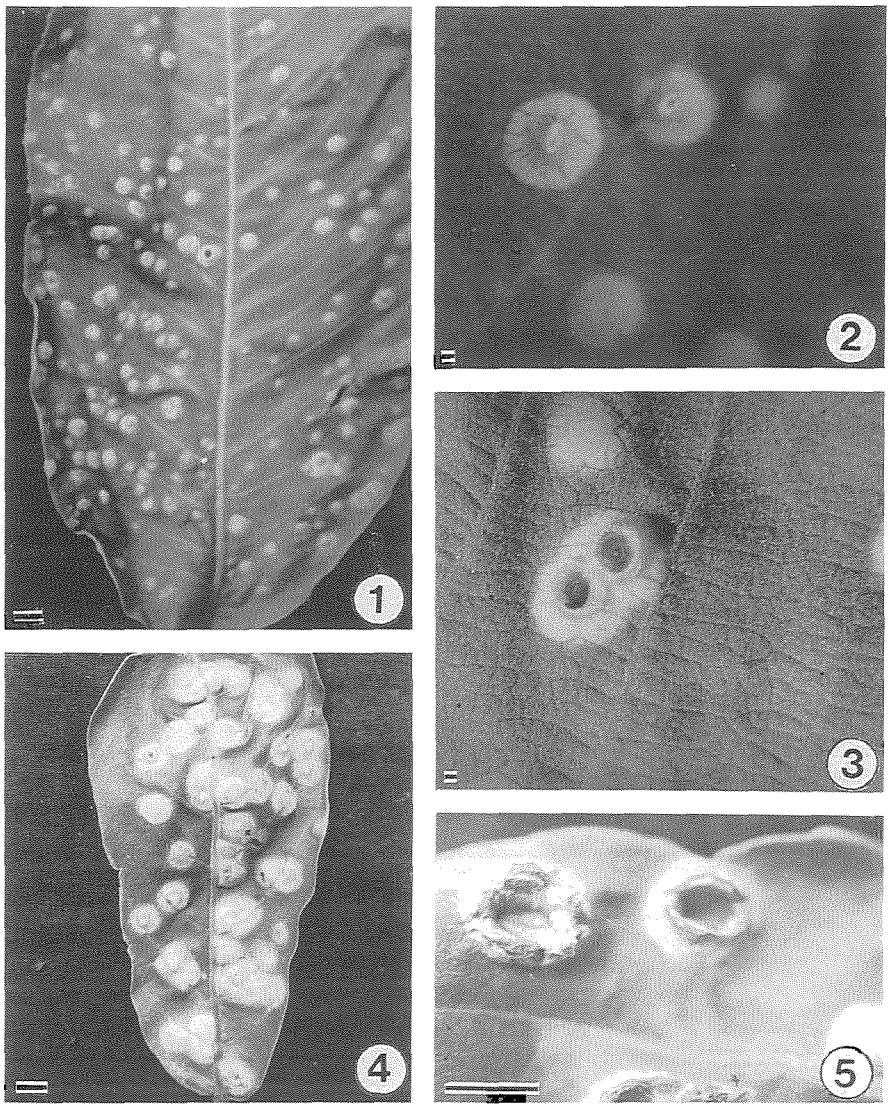
** Means from weighing of 10 subsample pools of 10 nymphs each.

Table 3. Mean fresh mass (mg) of developing galls, each housing one nymph.

Gall age in days	Nymphal instar stage	*T. tomentosa* (n=50)	*T. arjuna* (n=50)
3-4	I	0.010	0.012
5-10	II	0.020	0.036
11-13	III	0.030	0.062
14-16	IV	0.095	0.108
15-18	V	0.104	0.218
19-22	Dehisced gall	0.123	0.229

nymphal instars (Fig. 3; Table 3).

Mature galls on *T. arjuna* are morphologically similar to those on *T. tomentosa* except the former are at least three times larger than the latter. Galls occur rather closely and sometimes the outlines of two neighbouring galls coalesce with each other (Fig. 4). The conical growth on the abaxial side of the galls such as that on *T. tomentosa* is conspicuously absent. Gall dehiscence similar to that on *T. tomentosa*, but the widened ostiolar margin is rough (Fig. 5; Table 3).



Figs. 1-5. Morphology of galls (bar=1 cm). 1, Leaf of *Terminalia tomentosa* (upper side view). 2, Conical covering growth with slit-like ostioles of *T. tomentosa* galls (lower side view). 3, Dehisced gall of *T. tomentosa* (lower side view). 4, Leaf of *T. arjuna* (upper side view). 5, Dehisced gall of *T. arjuna* (lower side view).

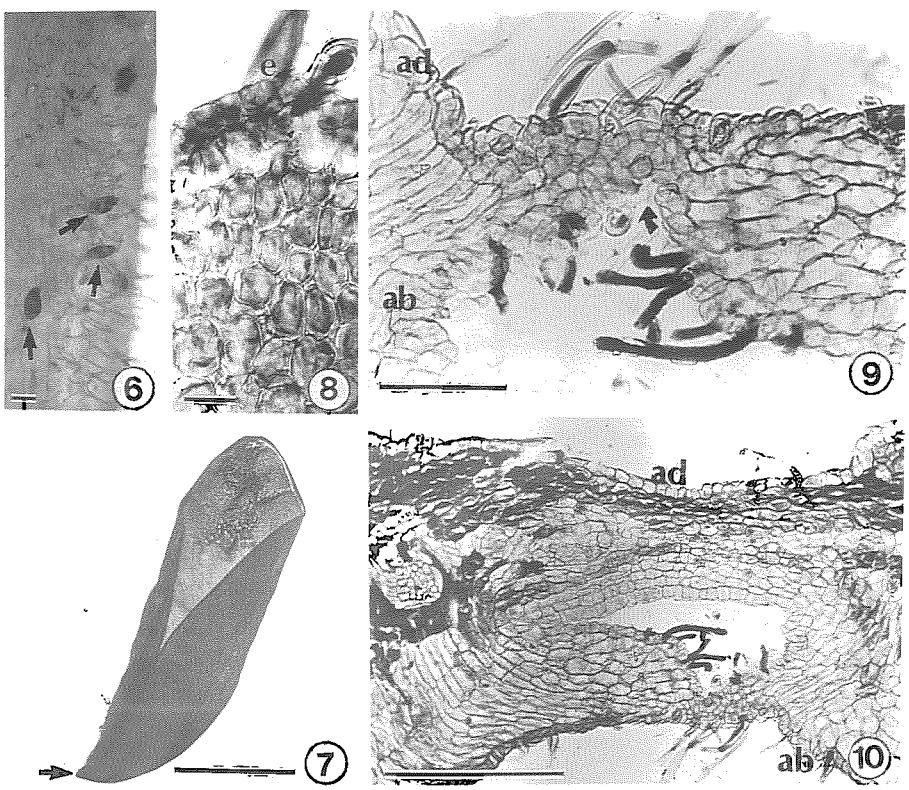
Cecidogenesis

Terminalia tomentosa

Gravid females of *T. f. minor* deposit eggs (*c.* 0.6×0.2 mm ; n=100) on the stem axis and petioles of young vegetative branches (Fig. 6). When laid freshly, each egg stands perpendicular to the horizontal axis of the substratum with its pedicel buried in the host tissue (Fig. 7). The short cylindrical pedicel (200-220 μm ; n=100) penetrates the host tissue only up to the second or third layer of cortical paren-

chyma. Subcellular alterations, such as split vacuoles and intensified cytoplasm, normally seen at the oviposition sites of triozids, are not evident here; but the cortical cells, in and around the pedicel, have thicker walls than those away (Fig. 8). In about 48 hours after oviposition, as a result of embryo growth, the egg inclines itself in such a way that the operculum touches the plant surface, facilitating easy migration of nymphs after the dehiscence of the egg.

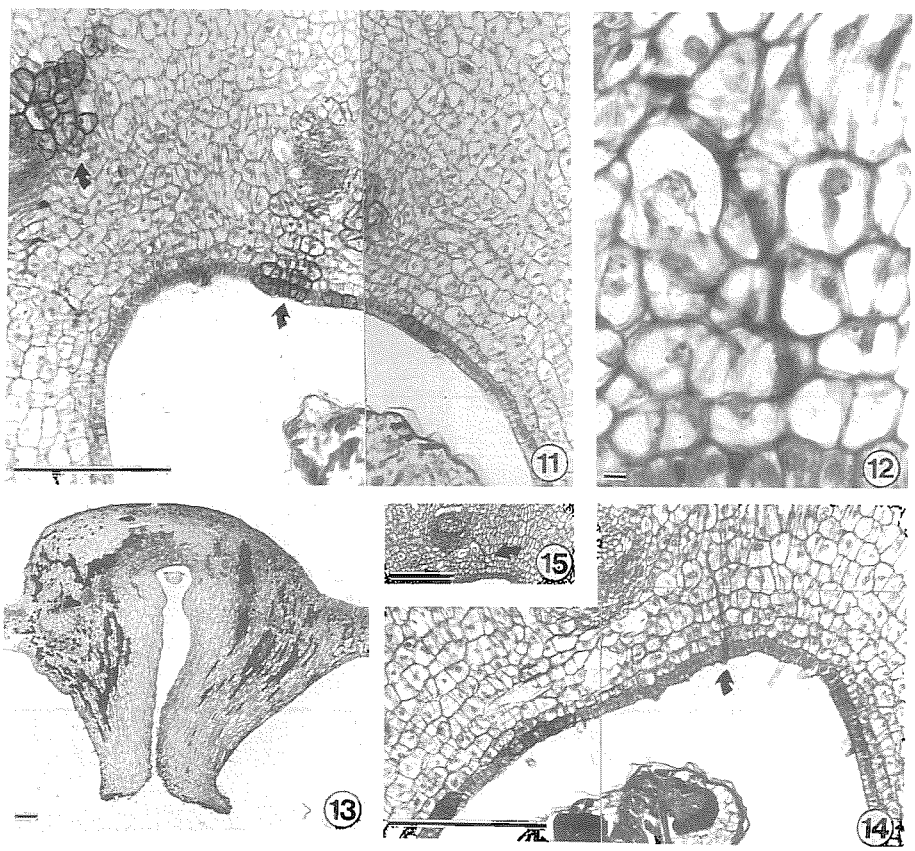
Nymphal I instars crawl from shoot/petiolar axes towards venal angles along the abaxial surfaces of differentiating leaves and settle with their mouth parts lying on stomata. By inserting their stylets through stomatal apertures, these stationary nymphs feed on the primordial mesophyll parenchyma cells which become metabolically active, as evidenced by hypertrophied cells and nuclei besides enriched cytoplasm, indicating the activation of cellular metaplasia. Feeding activity triggers growth in the host leaf, especially around the nymphal bed, thus making the gall



Figs. 6-10. Eggs of *Trioza fletcheri minor* and early stages of cecidogenesis in *T. tomentosa* (bar=100 μm, except Fig. 8 where bar=50 μm). 5, Randomly distributed eggs (arrows) on tender shoots. 7, Egg with its pedicel partially broken (arrow). 8, Transverse sectional view of shoot axis showing the egg with embedded pedicel (e). Cortical cells thick walled. 9, First recognizable cecidogenetic event. Arrow - nymphal bed surrounded by trichomens with abundant polyphenolic materials. Transverse sectional view of a primordial leaf; ad - upper side and ab - lower side. 10, Young gall. Covering growth completed. Transverse sectional view of host leaf; ad - upper side.

recognizable (Fig. 9). The adaxial epidermis of the leaf part accommodating the nymph responds actively by producing cylindrical trichomes that are filled completely by polyphenolic materials (confirmed by 2.5% alcoholic ferric chloride solution test - Schneider 1964). Mesophyll cells adjoining these trichomes show intense hypertrophy and hyperplasia and thus contribute cells to the labial growth around the nymph. In 4-6 days, labial growth in the form of a cone encloses the nymph, meeting along the abaxial side of the host leaf, though there is no fusion at the tip. By this time, the nymphal chamber gets organized with well-defined parenchyma cells bordering its inner perimeter. Mesophyll cells lying around the developing gall, particularly along the adaxial side of the host leaf include dense polyphenolic materials (2.5% alcoholic ferric chloride solution test) (Fig. 10).

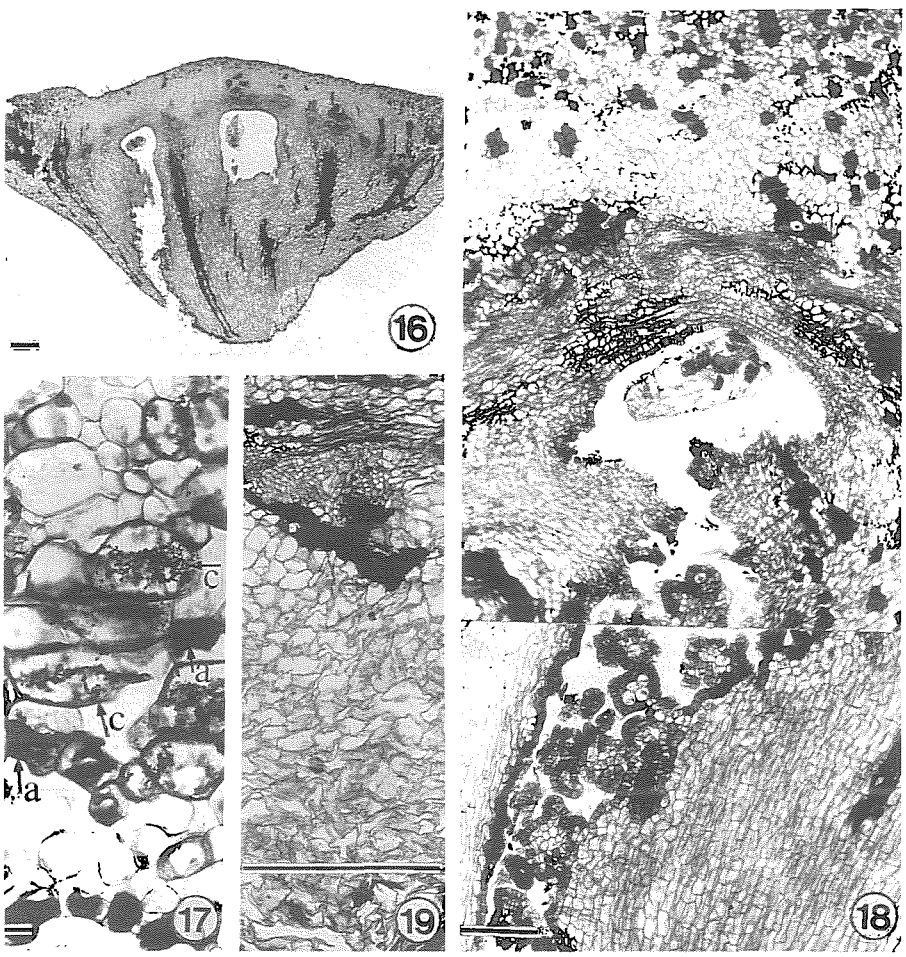
With the moulting of the nymph into second instar, growth is intense in the mesophyll region of the gall and the gall volume rapidly increases. The number of



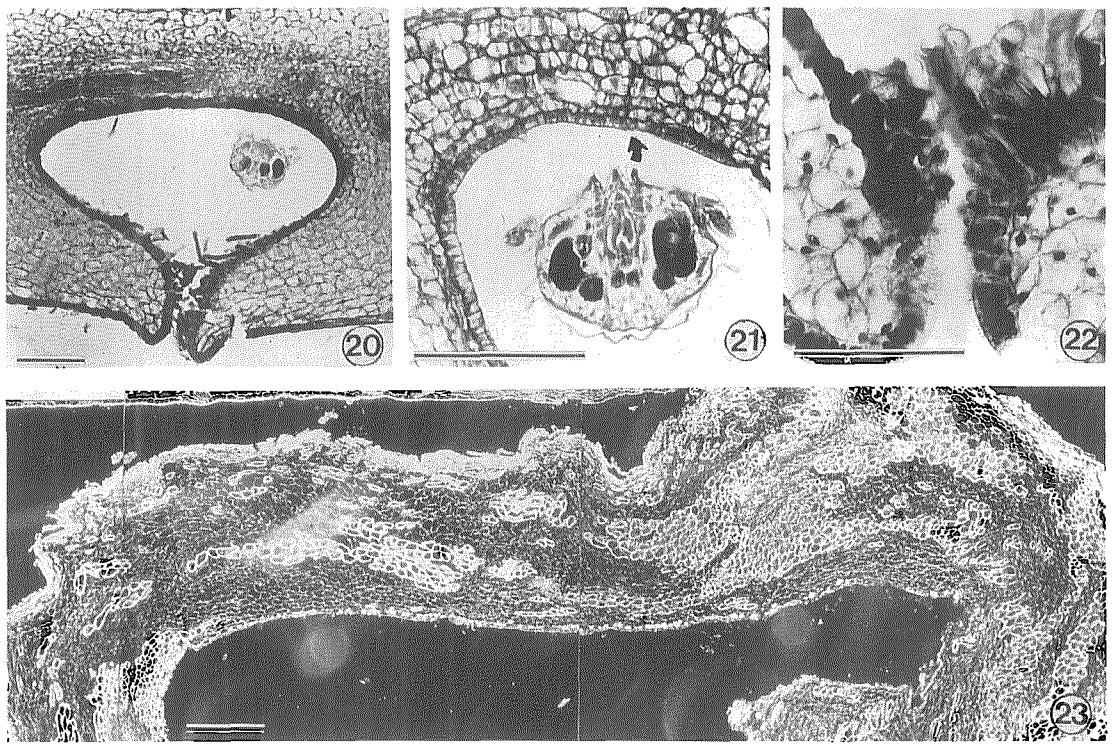
Figs. 11-15. Galls of *T. tomentosa* and nutritive tissues of nymphal II and III instars (transverse sectional views of host leaves; bar=100 μm, except Fig. 12, where bar= 10 μm). 11, Parenchymatous nutritive tissue (arrows) close to and away from nymphal II instar. 12, Intercellular passage of stylet in the parenchymatous region of the gall. 13, Gall inhabited by nymphal II instar. 14, Stylet track of late nymphal III instar (arrow). 15, Same as Fig. 14. Stylet track (arrow) reaching phloem.

layers between the adaxial epidermis and nymphal chamber increases to nearly 25, composed of compactly arranged isodiametric parenchyma cells (30-40 μm dia) (Fig. 11). Concurrently with the increase in gall volume, evident as a hemispherical swelling along the adaxial side of the leaf, the nymphal chamber too increases in volume offering adequate space to the rapidly-growing nymph. The nymph feeds on the parenchyma cells of the gall wall that lie either close to itself or at a depth of 8-10 cells (Fig. 12). The nutritive parenchyma cells are rich in cytoplasm. With toluidine blue staining, colour display is more intense in the walls of these cells than in those of nearby cells.

Following the moult to third instar, the gall attains its final shape including the



Figs. 16-19. Mature galls of *T. tomentosa* (nymphal IV and V instars) (transverse sectional views of the host leaf; bar=100 μm, except Fig. 17, where bar=10 μm). 16, Section showing two chambers housing mature nymphal instars. 17, Erstwhile nutritive parenchyma with crystaline (c) and amorphous (a) polyphenolic materials. 18, Widenning ostiole with localized, parenchymatous ingrowths. 19, Degenerating parenchyma cells of an old gall.



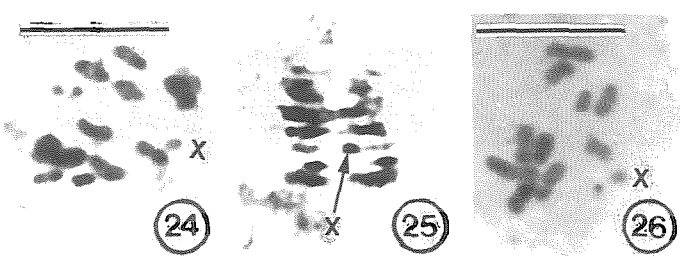
Figs. 20-23. Galls of *T. fletcheri minor* on the leaves of *T. arjuna* (transverse sectional views of host leaves; bar=100 μm). 20, Gall of nymphal II instar showing the flat covering growth along the lower side of the leaf. 21, Stylet track (arrow) of early nymphal IV instar shifting from parenchyma to phloem. 22, Covering growth with shorter trichomes. 23, Dehiscing gall with the roof expanding laterally. Bright cells - sclerenchyma (phase-contrast microscopy).

prominent conical covering growth along the abaxial side and distinct bulge along the adaxial side of the host leaf (Fig. 13). Because of rapid increase in the mass and linear dimensional values, the nymph has just enough space in the nymphal chamber. Characteristically, at this stage of development, the nymph shifts its feeding site from parenchyma cells (Fig. 14) to vascular phloem (Fig. 15).

Moulting to the fourth nymphal instar stage marks a number of vital morphogenetic changes in the gall. The erstwhile nutritive parenchyma and cells in their neighbourhood begin to accumulate starch (I-KI reaction - Johansen 1940) and polyphenolic materials (2.5% alcoholic ferric chloride solution test) either as crystalline or viscous inclusions (Fig. 16 & 17). Such cells are distributed rather irregularly in the gall mesophyll. Cells bordering the narrow space in the conical ledge proliferate at random points thereby the widening pathway is narrowed (Fig. 18). This can be seen as an adaptation in the gall system to prevent the possible entry of a predator. Synchronizing with the moulting of the fifth nymphal instar, the adaxial region of the gall (gall roof) expands laterally (as in vertical longitudinal sections of the gall) either by producing new growth centres that divide or expand. Galls ready for dehiscence include 10-12 rows of horizontally distributed sclerified elements. The parenchyma cells lying close to the nymphal chamber (the erstwhile nutritive cells) shrink and degenerate (Fig. 19). This results in the widening of the ostiole in the abaxial cone and enables the escape of the final nymphal instar for moulting into adult outside.

Terminalia arjuna

The cecidogenetic response is broadly similar to that of *T. tomentosa* and the more-important differences alone are highlighted here: (a) The abaxial covering growth expresses itself as a flattened structure (Fig. 20). (b) Only the fourth nymphal instar shifts its feeding site from parenchyma to vascular phloem (Fig. 21). (c) The ledges of abaxial covering growth include trichomes developed by the modification of epidermal cells and include abundant polyphenolic materials. These are shorter than those of *tomentosa* (Fig. 22). (d) At maturity, the gall roof (adaxial portion of the mature gall) widens laterally, more by expansion of the constituent sclerenchyma elements than by the division of parenchyma cells (Fig. 23).



Figs. 24-26. Chromosomal profiles of *T. f. minor* (bar=10 μm). 24, From galls of *T. arjuna* (I metaphase). 25, From galls of *T. arjuna* (I anaphase). 26, From galls of *T. tomentosa* (I metaphase).

Table 4. Mean values (±SE) of metabolic components of the galled (G ; n=5) and control (C ; n=5) leaf tissues of *T. tomentosa*.

Stage of the inhabiting nymphal instar (gall age in days)	Total proteins (mg/gm dry tissue mass)		Amino acid (mg/gm dry tissue mass)		Reducing sugars (mg/gm fresh tissue mass)		Total phenols (mg/gm dry tissue mass)		Ortho-dihydroxy phenols (mg/gm of fresh tissue mass)	
	C	G	C	G	C	G	C	G	C	G
I (3-4)	137.3 ±5.3	360.0* ±5.7	93.3 ±0.0	244.0* ±2.4	375.3 ±11.0	920.0* ±3.0	18.3 ±0.7	50.8* ±0.9	0.26 ±0.02	0.17* ±0.01
II (4-10)	68.5 ±4.6	86.6** ±4.1	95.0 ±6.1	145.8* ±0.0	247.0 ± 0.0	376.7* ±1.9	15.5 ±0.6	24.0* ±1.1	0.25 ±0.01	0.24^{NS} ±0.02
III (10-13)	143.0 ±3.9	199.5* ±7.3	68.3 ±8.2	97.5* ±5.0	195.3 ± 2.7	286.0* ±3.0	12.3 ±1.1	16.6* ±0.7	0.30 ±0.0	0.29 ±0.0
IV (13-16)	34.6 ±0.7	38.5** ±0.6	25.2 ±2.5	28.0^{NS} ±2.0	47.3 ± 0.4	51.4* ±0.3	2.7 ±0.1	3.0** ±0.1	0.39 ±0.0	0.32* ±0.01
V (16-18)	26.9 ±2.3	29.9^{NS} ±0.0	43.0 ±0.0	47.9* ±0.0	32.4 ± 1.8	33.8^{NS} ±4.1	3.9 ±0.1	4.3^{NS} ±0.4	0.45 ±0.01	0.42^{NS} ±0.01
Dehisced gall (18-22)	18.6 ±0.0	19.1* ±0.31	22.0 ±3.2	25.0^{NS} ±0.3	22.0 ± 0.0	23.3^{NS} ±0.6	2.3 ±0.0	4.2* ±0.2	0.55 ±0.01	0.51^{NS} ±0.01

*P<0.01 ; **P<0.05 ; NS - Not significant.

Table 5. Mean values (±SE) of metabolic components of the galled (G ; n=5) and control (C ; n=5) leaf tissues of *T. arjuna* (expressed in mg/gm of dry tissue mass).

Stage of the inhabiting nymphal instar (gall age in days)	Total proteins (mg/gm dry tissue mass)		Amino acid (mg/gm dry tissue mass)		Reducing sugars (mg/gm fresh tissue mass)		Total phenols (mg/gm dry tissue mass)		Ortho-dihydroxy phenols (mg/gm of fresh tissue mass)	
	C	G	C	G	C	G	C	G	C	G
I (3-4)	277.5 ±7.0	392.0** ±7.1	182.5 ±7.3	277.0* ±4.9	757.51 ±10.3	924.0* ±8.0	44.26 ±0.99	50.0^{NS} ±6.81	0.28 ±0.02	0.18* ±0.01
II (4-10)	178.6 ±4.1	320.0* ±2.6	134.8 ±3.3	228.9* ±4.1	320.0 ±49.5	495.5* ±5.4	20.3 ±4.1	34.0* ±0.9	0.28 ±0.01	0.25^{NS} ±0.1
III (10-13)	276.2 ±9.8	592.5* ±1.0	165.2 ±2.7	167.2^{NS} ±10.5	268.3 ±9.7	410.0* ±0.6	13.6 ±1.2	18.4* ±0.0	0.35 ±0.01	0.31* ±0.0
IV (13-16)	146.1 ±3.2	236.9* ±10.4	100.4 ±6.6	150.0* ±1.0	157.3 ±2.5	234.4* ±0.4	18.3 ±0.7	23.1* ±0.9	0.48 ±0.01	0.38* ±0.01
V (16-18)	25.2 ±1.7	47.3* ±0.6	33.1 ±0.9	45.8* ±2.4	26.7 ±0.4	47.2* ±0.7	3.9 ±0.4	6.8* ±0.3	0.57 ±0.01	0.44* ±0.02
Dehisced gall (18-22)	10.9 ±0.1	17.4* ±0.4	15.9 ±1.2	17.7^{NS} ±1.2	13.3 ±0.4	19.4* ±0.3	3.1 ±0.1	4.6* ±0.1	0.56 ±0.03	0.43* ±0.0

*P<0.01 ; **P<0.05 ; NS - Not significant.

Karyology of the Psylloid

Triozid from the galls of *Terminalia arjuna* (2n ♂ = 24 + XO)

At first metaphase (MI) stage, 13 elements exist including 12 autosomal bivalents and X-univalent. All chromosomes are rather small and gradually decrease in size. The X-chromosome is more or less similar in size to the smallest chromosome (Fig. 24). At first anaphase (AI), the X-chromosome moves to one pole behind the autosomes (Fig. 25).

Triozid from the galls of *T. tomentosa* (2n ♂ = 24 + XO)

At MI, 12 bivalents and the unpaired X-chromosomes are present. Dimensionally, all the bivalents and the X-chromosome are similar to those obtained from the triozid in galls on *T. arjuna* (Fig. 26).

Assays of Metabolic Components

Terminalia tomentosa (Table 4)

Total protein content is very high initially (nymphal I instar ; 3-4 days). It drops down abruptly in the next 4-10 days (nymphal II instar) and peaks again (nymphal III instar ; 10-13 days) ; subsequently, the values decline to the minimum. At dehiscence stage, the values are more or less similar to those of the control. Free amino acids in gall tissues show a decreasing trend from early to late gall stages. Only during the late gall stage (nymphal V instar) does a modest rise occur, a process shown by the control tissues as well. Values of reducing sugars in young gall tissues are nearly 2.5 times more than their normal counterparts. With further development, reducing sugars decline steeply almost equalling that of control. Reaching the equilibrium at dehiscence stage. Total phenols are significantly higher than their counterparts especially during early cecidogenesis. Values drop abruptly particularly during the inhabitation periods of second and fourth nymphal instars. On the contrary, ortho-dihydroxy phenols are low at initial stages and increase gradually with gall growth, similar to the trend evident in the control.

Terminalia arjuna (Table 5)

The pattern of changes is similar to that of *T. tomentosa*. Random comparisons of both control and gall tissue values of *T. arjuna* and *T. tomentosa* did not reveal any significant differences.

Discussion

A remarkable trait of gall-inducing insects is their host specificity. A large number of them are so specific to their host plant genera as to be considered efficient indicators of plant taxa (Shorthouse and Rohfritsch 1992, Raman 1994). Cecidogenous psylloids are no exception to this. Some species such as *Diaphorina truncata* Crawf. and *Psyllopsis fraxini* (Linn.) belonging to Psyllidae - a group that includes relatively few gall inducers - display a high level of fidelity to their respective host plants (Balakrishna and Raman 1992, Nguyen 1970). Among the known species of Triozidae - a group that predominently includes gall inducers -, *T. f. minor* seems to be the only Indian psylloid that can induce galls not only on leaves,

but also on flowers (Kapil and Sokhi 1990) of more than one species of *Terminalia*.

Mani (1974) refers to galls of *T. tomentosa* and *T. arjuna* as induced by *T. fletcheri* subsp. *minor* and *T. fletcheri*, respectively. However, Mathur (1975) implicates *T. f. minor* as the gall inducer on species of *Terminalia* and considers *T. fletcheri* as the galler on *Trewia nudiflora* Linn. (Euphorbiaceae) from Southern India. Results of our karyological analyses do not allow the recognition of triozids from the galls of *tomentosa* and *arjuna* as taxonomically-distinct entities. [This observation needs to be seen in the light of the following comment of D. Burckhardt (personal communication): "For detecting and separating two 'hidden' biological species, I find karyological data are not very appropriate. .....Probably a biochemical approach could tell more...".] Individuals raised independently from both *tomentosa* and *arjuna* galls show the same number of chromosomes (2n ♂ = 24 + XO), which seems to be common for Psylloidea, and probably is the modal number for the group (Maryanska-Nadachowska and Hodkinson 1993), notwithstanding the fact that only about 50 species belonging to 24 genera and six families have been karyotyped up to now (Maryanska-Nadachowska *et al.* 1992, 1993).

Ovipositional behaviour of *T. f. minor* does not appear to be as specialized as that of its other triozidine relatives. For example, *T. jambolanae* Crawf. and *T. alacris* Flor lay eggs in precise serial rows along the marginal sutures of leaves of *Syzygium cumini* (Linn.) Skeels and *Laurus nobilis* Linn., respectively (Bouyjou and Nguyen 1974, Raman 1991). On the other hand, *T. f. minor* lays eggs on the shoot and petiolar axes rather randomly. Taylor (1992) considers the long stalks (*c.* 600 μm) of *Schedotrioza distorta* Taylor to be an adaptation to absorb maximum moisture from the leaf tissue of *Eucalyptus leucoxylon,* a species that invariably occupies dry and humid parts of Australia. Against this assumption, the shorter egg pedicel of *T. f. minor* (*c.* 200 μm) appear normal, because species of *Terminalia* generally occur in regions of modest to high ground-water table and reasonably good rainfall (1,200–2,200 mm per year). It is but appropriate to recall here Taylor's (1992) comment that shorter egg pedicels of Psylloidea are a secondarily and recently-evolved character.

Cortical tissues of shoots and petioles of *T. tomentosa* and *T. arjuna* do not show any marked metaplastic changes as do the young leaves of *Syzygium* with the oviposition of *T. jambolanae* (Raman 1991). However, the subsequent developmental behaviour of the eggs of *T. f. minor* and *T. jambolanae* are identical in that in both species with the growth of the embryo, the eggs incline themselves along the plant surface enabling easier migration of instar I nymphs towards stomatal apertures of leaves. Like other cecidogenous triozidine (Hodkinson 1984, Raman 1991), diaphorinine (Balakrishna and Raman 1992), and pachypsylline (Walton 1960, Lewis and Walton 1964) psylloids, the first nymphal instars of *T. f. minor* insert their stylets through stomata of young host leaves after migrating from the petiolar and shoot axes and initiate galls by feeding on undifferentiated mesophyll parenchyma. *T. f. minor* shares this trait with other gall-inducing psylloids in terms of preferring tender leaves and generating complex, prosoplasmatic galls (Lewis and Walton 1964, Taylor 1987, Raman 1987, 1991). This behaviour contrasts sharply with that of non-cecidogenous spondyliaspidine (Woodburn and Lewis 1973) and weakly cecidogenous diaphorinine psylloids (Pande 1971, Balakrishna and Raman 1992), which prefer mature host leaves.

Developmental periods of nymphs as well as morphometric changes during development in *T. f. minor* from both *T. tomentosa* and *T. arjuna* galls are nearly identical. The only obvious biologically-significant difference is the nymphal behaviour during the shift of feeding sites from gall parenchyma to vascular phloem: triozids in galls of *T. tomentosa* shift, when they are in the late third nymphal instar stage, whereas those in galls of *T. arjuna* do so only as fourth instars. It is rather difficult to decide whether *T. f. minor* in *T. tomentosa* galls is precocious in development or that inhabiting *T. arjuna* galls is slower, for no worthwhile change either in further developmental pattern or biomass is evident when raised from both host plants under natural and laboratory conditions. However, what is important is the shift in feeding sites from parenchyma to phloem which initiates metabolic changes in the gall tissue: parenchyma cells lining the nymphal chamber shrink due to snapping of water transport mechanism (Raman and Singh, unpublished data, see also Raman 1991), while those away from the nymph turn into sclerenchymatous elements with the accumulation of starch and polyphenolic materials. Such a morphogenetic response of the host tissue mediated by physiological alterations (Raman 1987, 1991, Balakrishna and Raman 1992) facilitates gall dehiscence.

Following the settling of first nymphal instars, the foremost response of the host leaf, be it that of *T. tomentosa* or *T. arjuna*, is to generate uniseriate trichomes by the modification of abaxial epidermal cells. These cells containing abundant polyphenolic materials grow over the nymphs and offer protection. Compared with the other known triozidine galls, this response appears unique to *Terminalia* galls. Considering that instar I nymphs do not move once they have commenced feeding, growth of such a trichomatous cover is biologically significant. Most other gall-inducing psylloids like their free-living relatives run the risk of being exposed to climatic vagaries or natural enemies during the earliest stage of cecidogenesis (Taylor 1992). The gross shape of abaxial covering growths enclosing early nymphal instars varies subtly in that galls of *T. tomentosa* have a pointed, conical structure, whereas those of *T. arjuna* have a flat contour. Such variation in the response pattern of *T. tomentosa* and *T. arjuna* galls suggests host-plant characteristics as critical factors in cecidogenesis, given that the same psylloids induce galls on both host-plant species.

Assay of some of the primary and secondary metabolic components in both *T. tomentosa* and *T. arjuna* galls, from the inhabitation time of the first nymphal instar (3-4 days) to adult emergence (18-22 days) display similar patterns. By and large, total proteins, free amino acids, reducing sugars, and total phenols show high values at the start of gall growth, and decline progressively with ageing. On the other hand, ortho-dihydroxy phenols increase gradually from the beginning. Secondly, when compared with the values obtained from their respective controls, gall tissues provide statistically-significant readings.

Total proteins alone shoot up rather abruptly during the inhabitation time of the third nymphal instar and this is one of the potential reasons for the near doubling of both nymphal and gall mass at this stage of cecidogenesis. In many psylloid galls, the third nymphal instar is the most critical developmental stage capable of redirecting gall growth substantially, from a phase of quantitative increment to that of qualitative differentiation of plant tissues, thus preparing the gall for dehiscence (Mani and Raman 1994). Accordingly, values of many metabolites decrease abrupt-

ly at the inhabitation time of either the late third nymphal instar (*T. tomentosa*) or early fourth nymphal instar (*T. arjuna*). The steep drop from 199.5 mg (*T. tomentosa*) and 592.5 mg (*T. arjuna*) (10-13 days of gall growth) to 38.5 mg (*T. tomentosa*) and 236.9 mg (*T. arjuna*) explains the lower protein requirments of subsequent nymphal instars. The free amino acid pattern complements that of proteins and very high values during initial stages of gall development, especially with the settling of first nymphal instar. This metabolic behaviour of the host plant can be playing a critical role in the insect's preference for *Terminlia* (Beck 1965).

The level of reducing sugars, particularly at the initial stages of gall development, is nearly 2.5 times more in *T. tomentosa* galls and 1.2 times in *T. arjuna* galls than their corresponding normal tissues. During final stages of gall growth (18-22 days), values of the gall reach values similar to those in control tissues. Higher values at the initial stages emphasize the greater need for primary sugars to combat the feeding stress of nymphs.

Elevated levels of phenolics during development of psylloid galls are not uncommon (Balakrishna and Raman 1992, Mani and Raman 1994). Ortho-dihydroxy phenols oxidize into quinones which very possibly react with tryptophan released by the hydrolysis of host-plant proteins during cecidogenesis and ultimately facilitate gall growth by the synthesis of IAA. Ortho-dihydroxy phenols known for their role as auxin promotor (Tandon and Arya 1980), protect IAA from being oxidized by polyphenol oxidase (Ingram 1958).

Acknowledgements

We thank Dr Daniel Burckhardt (Museum d'Histoire Naturelle, Geneva, Switzerland), Professor Carl W. Schaefer (University of Connecticut, Storrs, U.S.A.), Dr Hans C. Roskam (University of Leiden, Leiden, Holland), and Dr Odette Rohfritsch (Institut de Biologie Moleculaire des Plantes - CNRS, Strasbourg, France) for their critical reviews of this paper. AR and RNS thank Professor T.N. Ananthakrishnan (Loyola College, Madras, India) and Dr K. Thangavelu (Central Tasar Research & Training Institute, Ranchi, India), respectively, for facilities.

Literature

Balakrishna, P. and Raman, A. 1992. Cecidogenesis of leaf galls of *Strychnos nux-vomica* (Loganiaceae) induced by the jumping plant louse species, *Diaphorina truncata* (Homoptera : Psylloidea : Psyllidae). Entomologia Generalis 17 : 285-292.

Balakrishna, P., Raman, A., and Raman, K.J. 1991. On the morphometrics and dynamics of growth of the leaf galls of *Syzygium cumini* (L.) Skeels (Myrtaceae) induced by *Trioza jambolanae* Crawford (Insecta : Homoptera : Psylloidea). Phytomorphology 41 : 109-113.

Beck, S.D. 1965. Resistance of plants to insects. Annual Review of Entomology 10 : 207-232.

Bouyjou, B. and Nguyen, T.X. 1974. Observations sur la morphogênèse et la structure de la galle de *Trioza alacris* Flor (Homoptera - Psylloidea) sur *Laurus nobilis* L. Marcellia 33 : 237-254.

Bray, H.C. and Thorpe, W.V. 1958. Analysis of phenolic compounds of interest in metabolism. Methods in Biochemical Analysis 1 : 27-52.

Crawford, D.L. 1912. Indian Psyllidae. Records of the Indian Museum 7 : 419-437.

Hodkinson, I.D. 1983. The psyllids (Homoptera : Psylloidea) of the Austro-Oriental, Pacific and Hawaiian zoogeographical realms : An annotated checklist. Journal of Natural History 17 : 341-377.

Hodkinson, I.D. 1984. The biology and ecology of gall-forming Psylloidea. In : Ananthakrishnan, T.N. (ed.), Biology of Gall Insects. Oxford & IBH Publishing Company Private Limited, New Delhi, India. pp. 59-77.

Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill Publishing Company, New York, U.S.A. 523 pp.

Johnson, G. and Schaal, L.A. 1952. Relation of chlorogenic acid to scab resistance in potatoes. Science 115 : 627-629.

Ingram, L.L. 1958. Reaction inactivation of polyphenol oxidases : catechol and oxygen dependence. Journal of the American Chemical Society 77 : 2875-2876.

Kapil, R.N. and Sokhi, J. 1990. Ontogeny of the male and female gametophytes of *Terminalia arjuna* as affected by *Trioza fletcheri minor*. In : Maheswari Devi, H., Rao, P.N., and Rao, K.V.M. (eds.), Recent Advances in Developmental Morphology of Crop Plants. Bishen Singh Mahendra Pal Singh, Dehra Dun, India. pp. 177-184.

Lowry, O.H., Rosenbrough, N.J., Farr., A.L., and Randall R.J. 1951. Protein measurement with Folin-phenol reagent. Journal of Biological Chemistry 193 : 265-275.

Lewis, I.F. and Walton, L. 1964. Gall-formation on leaves of *Celtis occidentalis* L. resulting from material injected by *Pachypsylla* sp. Transactions of the American Microscopical Society 33 : 62-78.

Mani, M.S. 1974. Plant Galls of India. Macmillan India, New Delhi, India. 353 pp.

Mani, T. and Raman, A. 1994. Biochemical changes in relation to growth in two leaf gall systems induced by *Trioza jambolanae* and *Microceropsylla longispiculata* (Homoptera : Psylloidea). Phytophaga 6 : 59-64.

Maryanska-Nadachowska, A., Kuznetsova, U.G., and Warchalowska-Sliwa, E. 1992. Karyotypes of Psyllina (Homoptera), I. New data and checklist. Folia Biologica 40 : 15-25.

Maryanska-Nadachowska, A., Glowacka, E., and Warchalowska-Sliwa, E. 1993. Karyotypes of Psylloidea (Homoptera). III. Chromosomal number of eight species belonging to the families Aphalaridae, Psyllidae, Homotomidae and Triozidae. Folia Biologica 41 : 7-10.

Maryanska-Nadachowska, A. and Hodkinson, I.D. 1993. Karyotypes of Psylloidea (Homoptera). II. Chromosome numbers of nine mediterranean species from Mallorca (Spain). Folia Biologica 41 : 1-5.

Mathur, R.N. 1935. On the biology of Psyllidae. Indian Forest Records 1 : 35-70.

Mathur, R.N. 1975. Psyllidae of the Indian subcontinent. The Indian Council of Agricultural Research, New Delhi, India. 429 pp.

Moore, S. and Stein, W.H. 1948. Photometric ninhydrin method for use in chromatography of amino acids. Journal of Biological Chemistry 176 : 367-386.

Nelson, N. 1944. A photometric adoption of the Somogyi method for the determination of glucose. Journal of Biological Chemistry 153 : 375-380.

Nguyen, T.X. 1970. Recherches sur la morphologie et la biologie de *Psyllopsis fraxini* (Hom., Psyllidae). Annales de Societé d'Entomologie de France 6 : 757-773.

Pande, Y.D. 1971. Biology of *Citrus* psylla, *Diaphorina citri* Kuwayama (Hemiptera : Psyllidae). Israel Journal of Emtomology 6 : 307-311.

Raman, A. 1987. On the cecidogenesis and nutritive tissues of leaf galls of *Garuga pinnata* Roxburgh (Burseraceae) induced by *Phacopteron lentiginosum* Buckton (Pauropsyllinae : Psyllidae : Homoptera). Phytophaga 2 : 141-160.

Raman, A. 1991. Cecidogenesis of the leaf galls of *Syzygium cumini* (L.). Skeels (Myrtaceae) induced by *Trioza jambolanae* Crawford (Homoptera : Psylloidea). Journal of Natural History 25 : 653-663.

Raman, A. 1994. Adaptational integration between gall-inducing insects and their host plants. In : Ananthakrishnan, T.N. (ed.), Functional Dynamics of Phytophagous Insects. Oxford & IBH Publishing Company Private Limited, New Delhi, India. pp. 249-275.

Schneider, F.L. 1964. Qualitative Organic Microanalysis. Academic Press Incorporated,

New York, U.S.A. 543 pp.
Shorthouse, J.D. and Rohfritsch, O. (eds.). 1992. Biology of Insect-Induced Galls. Oxford University Press, New York, U.S.A. 285 pp.
Tandon, P. and Arya, H.C. 1980. Presence of auxin protectors in *Eriophyes* induced Zizyphus stem galls. Experientia 36 : 958-959.
Taylor, G.S. 1987. The gall forming Psylloidea of *Eucalyptus obliqua* in the Mount Lofty Ranges of South Australia. Journal of the Australian Entomolgical Society 26 : 223-228.
Taylor, G.S. 1992. The structure of the eggs of some Australian Psylloidea (Hemiptera). Journal of the Australian Entomolgical Society 33 : 109-117.
Walton, B.C.J. 1960. The lifecycle of hackberry gall-former *Pachypsylla celtidis-gemma* (Homoptera : Psyllidae). Annals of the Entomological Society of America 52 : 265-277.
Woodburn, T.L. and Lewis, E.E, 1973. A comparative histological study of the effects of feeding by the nymphs of four psyllid species on the leaves of eucalypts. Journal of the Australian Entomological Society 12 : 134-138.