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Incidence of Galls Induced by *Leptocybe invasa* on Seedlings of *Eucalyptus camaldulensis* and *E. tereticornis* from Different Seed Sources in Southern India

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ABSTRACT

Levels of susceptibility and resistance measured in terms of intensity of gall incidence induced by the invasive wasp *Leptocybe invasa* Fisher & La Salle on seedlings of *Eucalyptus camaldulensis* Dehnh. and *E. tereticornis* Sm. raised from nine different seed sources in southern India have been compared. Seedlings raised from different seed sources showed variation in percentage of gall incidence, number of galls, and growth of seedlings, besides tissue-specific variation in the incidence of galls on seedlings. Under identical environmental conditions, seedlings from the seed sources Ongole red, Kennedy River, Pudukkottai, Rudrapur were severely affected, bearing several galls on petiole, midrib, and stems of new branches, whereas seedlings of the seed sources Sathyavedu 1 showed resistance to gall induction with a few isolated galls occurring on either petioles or midribs and rarely on stems. Severely affected seedlings were growth retarded. Anatomy of galls showed that in the seedlings that were 'resistant', the eggs were deposited in the cortical region outside the vascular ring, whereas in susceptible seedlings the eggs occurred in the parenchymatous tissue within the vascular ring.

Key Words: Eucalyptus, Gall Chamber, Nutritive Tissue, Seed Source, Susceptibility, Resistance

INTRODUCTION

Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae) induces galls on eucalypts (Mendel et al. 2004). *L. invasa*, originally from Australia where it is not designated as a 'pest', is spreading rapidly in the Mediterranean region, Africa, and Asia inflicting considerable damage to at least 10 species of *Eucalyptus* in both nurseries and plantations. Recently, the outbreak of *L. invasa* on species of *Eucalyptus* in nurseries and plantations was noticed in the states of Tamil Nadu, Karnataka, Kerala, and Andhra Pradesh in India. *L. invasa*-induced galls, in southern India, were first found in Marakkanam (12° 11' N; 79° 57' E) within the state of Tamil Nadu in 2004 (Jacob et al. 2007), which was subsequently found in Aligarh (27°30' N; 79°40' E) in the state of Uttar Pradesh in 2007 (V. V. Ramamurthy, pers. comm., email, 29 June 2007).

Eucalyptus plantations occur extensively (*c.* 1.3 M ha) in India. Several species of *Eucalyptus* are planted for production of pulp to meet the increasing demand from paper and related industries. Because of their excellent coppicing ability, after the pure-crop harvest, 2–3 rotations are usually managed from the coppiced shoots by interested parties (e.g., state-owned forest departments, individual planters). The coppiced shoots are ideal breeding sites for *L. invasa*, which by building into massive populations on young shoots destroy them and thus force planters to abandon extensive areas of the crop, if appropriate and timely management measures are not implemented.

Leptocybe invasa induces galls on shoot tips, petioles, and midribs in seedlings maintained in nurseries, and on coppiced shoots and in juvenile plants in plantations. Its developmental time from oviposition to adult emergence is about 132 days with

2–3 overlapping generations in a year (Mendel et al. 2004). Although information regarding the biology and ecology of many Australian Eulophidae associated with galls are available (Bouèek 1988, Raman and Withers 2003) that pertaining to structural changes during development of galls induced by *L. invasa* on *Eucalyptus* is lacking. Moreover, field observations showed considerable variation in the patterns of incidence of galls in eucalypt clones and plants raised from different seed sources. Because characters such as resistance to insects are critical in tree-improvement programmes, an understanding of the mechanisms involved in imparting resistance to insects in *Eucalyptus* is necessary to develop resistant–candidate germplasm through tree breeding. Therefore, in this paper we report variations in terms of gall incidence in the seedlings of *E. camaldulensis* and *E. tereticornis* raised from seed sources from different nurseries in southern India and relate those variations to resistance and susceptibility traits of those seed sources. Efforts have also been made to document the structural changes during development of galls from different seed sources and variation in biochemical parameters of the seed sources and relate to susceptibility and resistance to *L. invasa*.

MATERIALS AND METHODS

Study Sites, Measurements of Infestation, and Gall Intensity

Field observations were made on 4-month old seedlings of *E. camaldulensis* and *E. tereticornis* raised from different seed sources in the nursery of the Institute of Forest Genetics and Tree Breeding (IFGTB, Coimbatore, India; 11°0'N, 76°58'E). Seeds from different seed orchards in the states of Tamilnadu, Andhra Pradesh, and Kerala included those from pedigreed trees raised from native Australian provenances of *E. camaldulensis* and *E. tereticornis*. Data from these seedlings have been compared with those obtained from unpedigreed local sources (Kaikini, 1961) from the states of Uttar Pradesh and Andhra Pradesh (Table 1). Seed orchards form a part of the breeding programme for improving the productivity of *E. camaldulensis* and *E. tereticornis* in India including the superior provenances identified in provenance trials (Varghese et al. 2002).

Incidence of galls was observed in 4-month old seedlings in IFGTB nursery in March 2007. Nursery beds with 200 seedlings in each bed were assessed for percentage of infestation and categorized into the following 'intensity classes' based on the percentage of gall incidence per plant, following Mathew (1995): up to 30% — low, between 30% and 70% — medium, and >70% — high. An assessment of percentage infestation was repeated on 6-month old seedlings in June 2007 to quantify fresh gall incidence using the following parameters: shoot lengths (= height of the aerial part of seedling), and distribution of galls on stems, petioles and midribs in infested beds [random sampling method, Mathew 1995]. Since variation in gall incidence on shoot tips of seedlings among different seed sources was evident, a comparison of gall infestation intensity was also made by counting exit holes on tagged galls in the stem region in the first week of July 2007 when adults were emerging from the galls. Counting was repeated every 15 days until the first week of August 2007, when all the insects from tagged galls had emerged. The counted galls were spotted red, using commercial nail varnish, to avoid erroneous re-counting.

Table 1. Seed sources of *E. camaldulensis* and *E. tereticornis* screened for incidence of galls induced by *L. invasa*

Species	Source
E. camaldulensis	Pudukkottai, Tamilnadu (10.28°N ; 78.5°E;)
E. tereticornis	Pudukkottai, Tamilnadu (9.2°N; 78.5°E)
E. camaldulensis	Panampalli, Kerala (11° 17 N; 77° 07'E)
E. camaldulensis	Sathyavedu-1, Andhra Pradesh (13.26°N ; 79.57°E)
E. camaldulensis	Sathyavedu-2, Andhra Pradesh (13.26°N ; 79.57°E)
E. camaldulensis	Kennedy River, Pudukkottai, Tamilnadu (9.2°N ; 78.5°E)
E. camaldulensis	Ongole commander (white), Andhra Pradesh (15.33° N; 80.02°E)
E. camaldulensis	Ongole local (red), Andhra Pradesh (15.33° N; 80.02°E)
E. tereticornis	Rudrapur, Uttar Pradesh (29°30' N; 79°28' E)

One-way ANOVA was used to assess the significance level of the percentage of seedling damage

in materials from the nine seed sources, followed by Tukey–Kramer test for pair-wise comparisons of datasets. In all analyses, significance was determined at $p<0.05$. All analyses were done with KyPlot (version 2.0 –13–32 bit, Kochi Yoshioka® 1997–2000). Seedlings from the nine identified sources were graded on the intensity of gall incidence. Samples from those seed sources that showed low, medium, and high intensity classes were only used in studies examining growth comparison, anatomy, and biochemistry, reported in the following sections.

Gall Development

Three sets of fresh leaf and stem samples from the seedling beds of low, medium, and high intensity classes were collected for anatomical investigations. To maintain consistency in the samples collected in terms of age and growth of galls, the fifth leaf from the shoot tips and stem between the fifth and sixth leaves from the shoot tip were used. Developmental stages of galls were determined following Mendel et al. (2004). During the earliest stage (1–2 weeks after oviposition) the leaves show corky tissues at oviposited sites (considered first stage in this study). Well expressed, round and greenish galls become evident in the following 45 days (considered second stage in this study). Gradually, gall colour changes from green to pale pink and the galls remain so for 15 days (considered third stage in this study). Subsequently gall colour changes to deep red colour (considered fourth stage in this study). Galls with exit holes were considered final stage in this study. Sections were cut at 7 m thickness using a kryostat 1720 Digital (Leica Microsystems GmbH, Wetzlar, Germany). Sections were stained with saffranin–fast green, observed in a compound microscope Nikon™ Optishot 2 (Tokyo, Japan), and photomicrographs were obtained.

Biochemical Analysis

Leaf and stem samples were collected from the nursery beds with low, medium, and high-infestations for biochemical assays. To maintain consistency, samples were collected as described earlier (Section – Gall Development). 0.5 g of freshly collected leaves and stems were dried in an incubator at 37ºC until a constant mass was achieved. The difference in mass was calculated and the moisture content was represented as percentage. Method described in Sadasivam and Manickam (1996) was followed to assess total phenols, tannins, and cellulose. Total lipids were estimated following Folch et al. (1957). Six replicates were assessed in each analysis and the means obtained were used.

RESULTS

Assessment of Infestation and Intensity of Galls

Percentage of infestation by *L. invasa* on seedlings raised from different seed sources is provided in Table 2. A comparison among the different seed sources showed significant difference among seed sources ($df=8, F=14.5, p<0.001$). Four-month old seedlings of Sathyavedu 1 source (*E. camaldulensis*) showed the lowest percentage ($df=8, F=14.5, p<0.01$), whereas the Ongole–red source (*E. camaldulensis*) showed the highest percentage ($df=8, F=14.5, p<001$) (Table 3). Seedlings of Pudukottai source (*E. camaldulensis*) showed an intermediate trend. Intensity of gall incidence on individual seedlings showed a trend similar to the percentage of infestation. A replicate of the assessment of gall incidence after two months (i.e., on 6-month old seedlings) yielded similar results, although a 5–8% of increase in gall numbers in two months in all seed sources was evident, except the Ongole–red source (*E. camaldulensis*), which showed a 20% increase. The Sathyavedu 1, Pudukottai, and Ongole–red (all *E. camaldulensis*) sources, therefore, could be classed as low, medium and high, respectively, in terms of percentage gall incidence and intensity of galls on individual seedlings. All other seed sources remained distributed among these categories. Seed sources Panampalli (*E. camaldulensis*), Pudukkottai (*E. tereticornis*) and Sathyavedu 2 (*E. camaldulensis*) were more resistant than the Pudukkottai seed source (*E. camaldulensis*), whereas seed sources Ongole commander (white) (*E. camaldulensis),* Rudrapur (*E. tereticornis),* Kennedy River Pudukkottai (*E. camaldulensis)* showed increasing levels of susceptibility towards Ongole–red (*E. camaldulensis)*

Average seedling growth in six month old seedlings, measured as height, showed that the Sathyavedu 1 (*E. camaldulensis*) was 74.1±2.49 cm, whereas that of Ongole–red (*E. camaldulensis*) was 36.8±2.74 cm. The Pudukottai source showed 50.1 ± 2.34 cm height.

Variation in the Distribution of Galls on Seedlings

An assessment of the distribution of galls on stems, petioles and midribs showed that in the seed source Satyaveedu 1, 70% of galls occurred on petioles and 20% occurred on stems. In the Ongole–red seed source, 90–100% of galls occurred on midribs, petioles, and stems, which indicated that this seed source was highly susceptible to *L. invasa* infestation, irrespective of the plant organ. In the Pudukottai seed source, both midribs and petioles showed greater numbers of galls compared than those on stems (Figure 1).

Anatomy of Gall Development

A comparison of the galled region in the stems of seed sources Sathavedu 1 and Ongole–red that were bearing low and high infestations, respectively, showed that *L. invasa* preferred tender shoot terminals, petioles, and midribs for oviposition. External morphologies of galls on the three seed sources, viz., Sathyavedu 1, Pudukkottai and Ongole–red, varied. On the seedlings of high and medium-level susceptible sources, many cylindrical galls occurred closely on the stems, petioles and midribs. On the seedlings of low susceptible Sathyavedu 1 seed source, isolated 1–2 galls on stems (evident from the single emergence holes) occurred mostly on petioles and midribs. The anatomy of galls at different developmental stages is described below:

Stage 1: After either successful oviposition or attempts to oviposit by *L. invasa* randomly on the sides of midribs or petioles or shoot tips, a patch of dead epidermal and sub-epidermal cells became apparent at the point of egg insertion in seedlings. A variation in the cuticle of stems among different seed sources was evident. The Sathavedu 1 seedlings had 4.5 µm thick cuticle and seedlings of Ongole red had 2 µm thick cuticle. During the earliest stage of gall initiation, the width of the gall chamber was 40 µm in the widest context.

Stage 2: As the egg hatched, the surrounding parenchyma cells divided, the gall became evident as a swelling on the host organ. In susceptible plants (Ongole–red), several galls developed, coalesced (Figure 2), whereas in resistant plants (Sathyavedu 1), galls developed singly, mostly on either midribs or petioles and rarely on stems. At this stage, a nutritive tissue developed in the larval chamber enveloping the larva (Figure 3). Galls on Ongole–red seedlings had a 15 cell wide (160 µm) nutritive tissue, whereas the galls on Sathavedu 1 seedlings included a 8–10 cell wide (60 µm) thick nutritive tissue. Elongated and hypertrophied nutritive cells with prominent nuclei were occurring along the inner perimeter of the gall chamber (Figure 4). A 2–3 layered sclerenchyma tissue surrounded the nutritive tissue in the larval chambers of galls on Sathavedu 1 seedlings, whereas in the Ongole–red seedlings, the sclerenchyma tissue was seldom apparent, and if at all present, was often single layered (Figure 5 & 6). At this stage the width of the gall chamber increased to 140 µm.

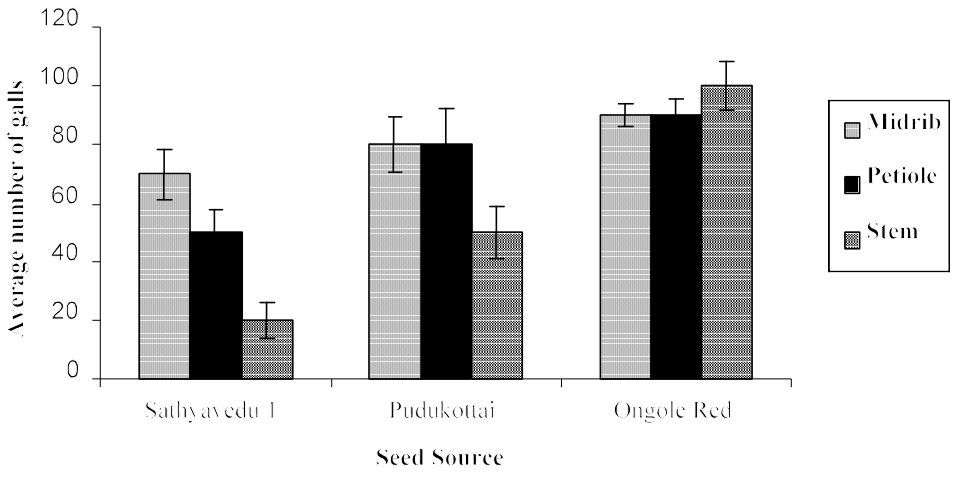
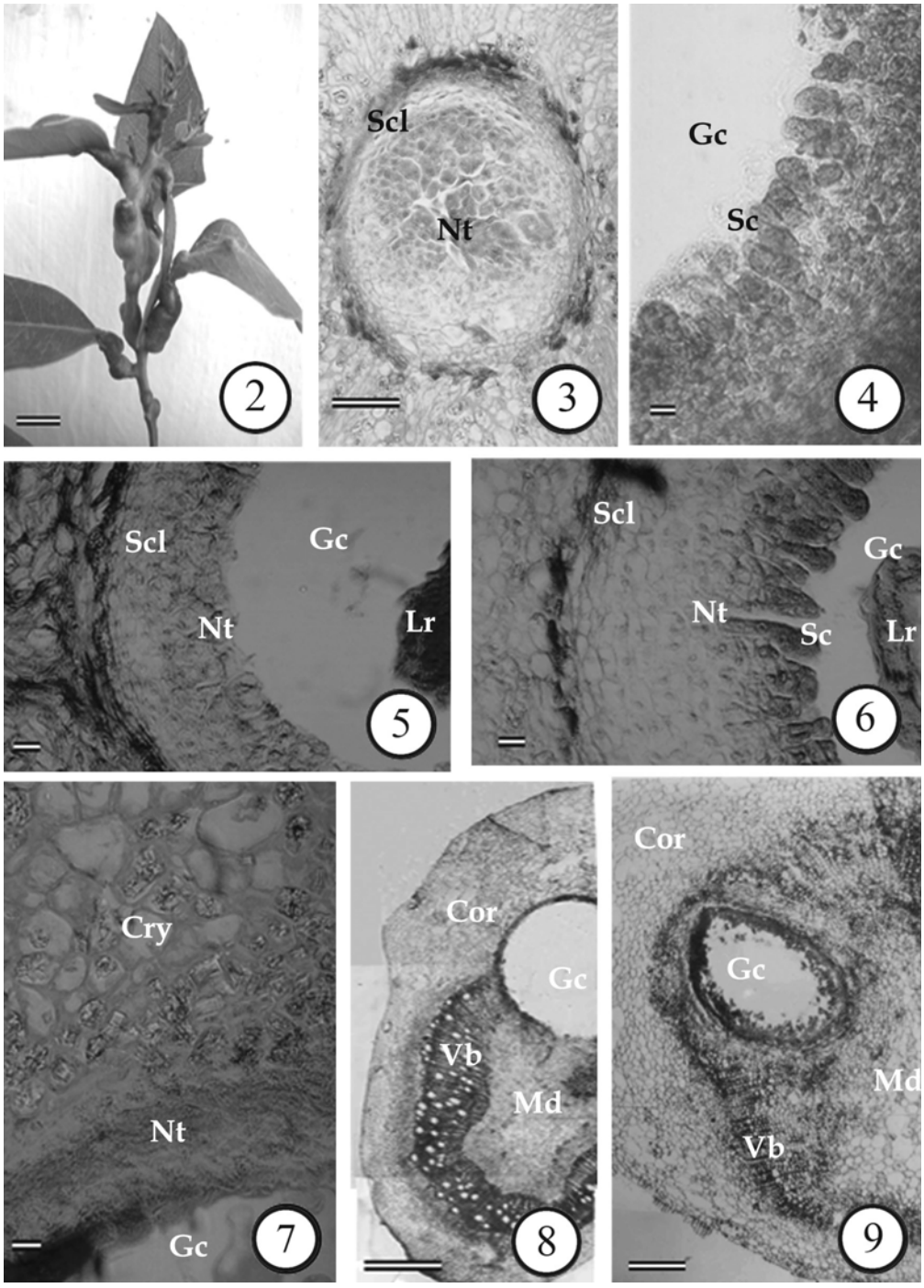


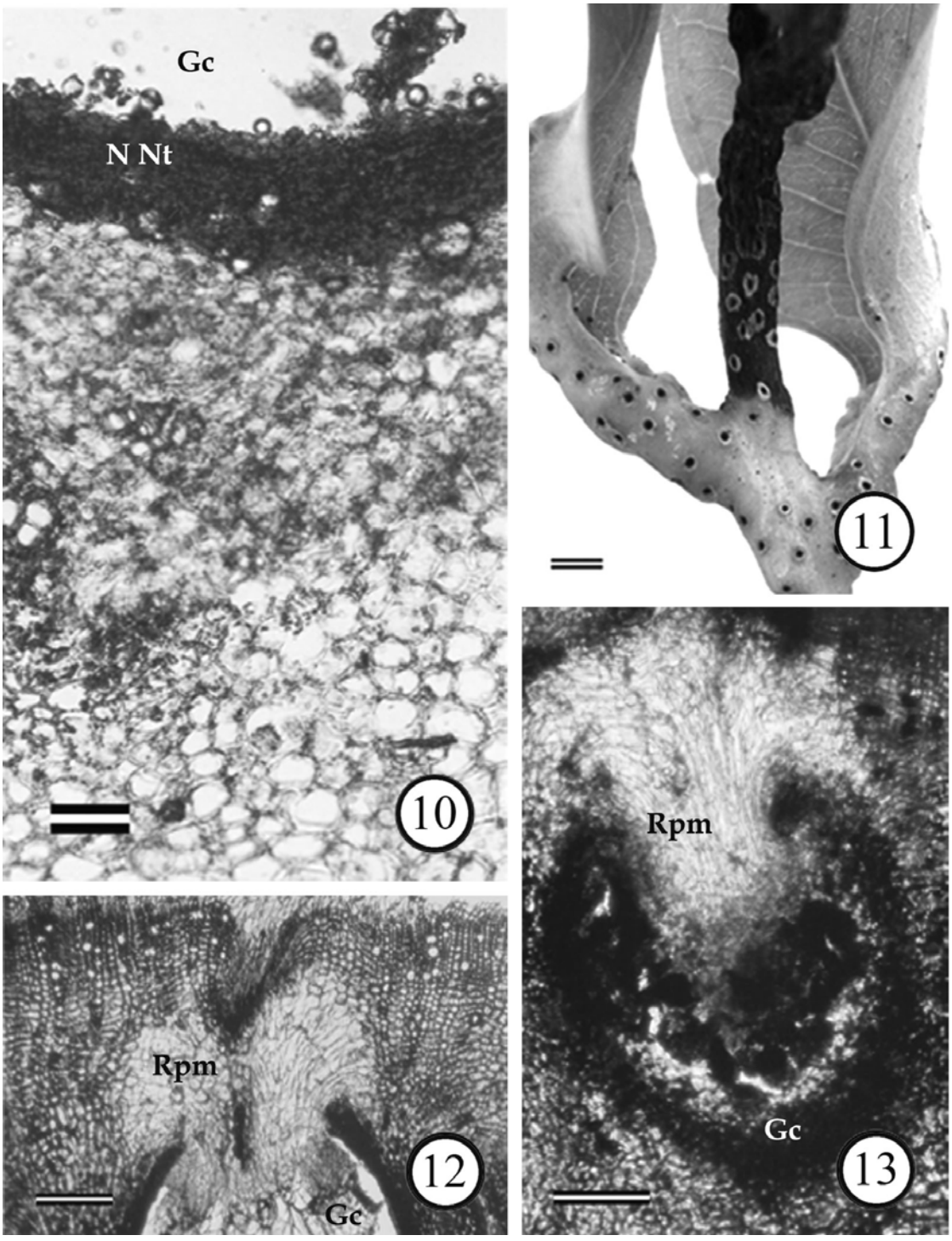
Figure 1. Distribution of *L.invasa*-induced galls on plant parts of *Eucalyptus* seedlings



Figures 2-9.

2. Several galls coalesce in Ongole local (red) seedling (Scale bar = 12cm).
3. CS of stem showing well developed nutritive tissue layer enveloping the larval chamber (Scale bar = 40□m).
4. Enlarged view of secretary cells in the nutritive layer (Scale bar = 4□m).
5. CS of gall chamber in Sathyavedu-1 seed source (Scale bar = 4□m).
6. CS of gall chamber in Ongole red seedlings (Scale bar = 4□m).
7. CS of stem in Ongole red seedlings showing crystals around the gall chamber (Scale bar = 4□m).
8. Gall chamber in the medulla region in Sathyaveedu-1 seedlings (Scale bar = 200□m).
9. Gall chamber in the cortex region in Sathyaveedu-1 seedlings (Scale bar = 200□m).

Scl- Sclerenchyma cells, Nt – nutritive tissue, Sc- secretary cells, Lr- larva, Cor – Cortex, Md – medulla, Vb -Vascular bundle, Gc- gall chamber.



Figures 10-13.
10 Necrotised nutritive layers inside the gall chamber (Scale bar = 4□m). 11 Drying of gall-bearing stem after *L. invasa* adult emergence from seedlings (Scale bar = 12cm). 12 & 13 CS of stem showing larval chamber getting filled with regenerative parenchyma after the exit of the adult (Scale bar = 8□m). N Nt- necrotized nutritive tissue, Rpm- regenerative parenchyma, Gc – gall chamber.

Table 2. Percentage of gall incidence on *Eucalyptus* seedlings from different seed sources.

Percentage of gall infestation (Average number. of galled seedlings /30 seedlings)

SPECIES Seed Source Level	*E camaldulensis* Sathyavedu_1	*E camaldulensis* Panampalli	*E camaldulensis* Sathyavedu-2	*E. tereticornis* Pudukkottai	*E camaldulensis* Pudukkottai	*E camaldulensis* Ongole Commander (white)	*E camaldulensis* Kennedy River Pudukkottai	*E. tereticornis* Rudrapur	*E camaldulensis* Ongole local (Red)
				Four-month old seedlings					
Low	25	22	22	20	17	18	19	15	4
Medium	5	7	5	10	10	9	6	13	10
High	0	1	3	0	3	3	5	2	16
				Six-month old seedlings					
Low	21	19	18	15	14	13	13	10	2
Medium	8	9	8	12	7	13	10	16	2
High	1	2	4	3	9	4	7	4	26
				Average number of exit holes/30 seedlings					
Early July 2007	11	15	14	17	11	10	16	10	26
Mid-July 2007	8	7	8	10	9	15	12	16	12
Early August 2007	1	2	4	3	9	4	7	8	2

Table.3. Tukey–Kramer results for percentage of gall infestation on different seed sources of *Eucalyptus**

Seed sources	Pudukkottai 1	Pudukkottai	Panampalli	Sathyavedu-1	Seed Sources Sathyavedu-2	Kennedy River Pudukkottai	Ongole commander (white)	Ongole local (red)	Rudrapur
Pudukkottai (*E. camaldulensis*)	N.S.	N.S.	N.S.	P<0.05	N.S.	N.S.	N.S.	P<0.001	N.S.
Pudukkottai (*E. tereticornis*)			N.S.	N.S.	N.S.	N.S.	N.S.	P<0.001	N.S.
Panampalli				N.S.	N.S.	N.S.	N.S.	P<0.001	N.S.
Sathyavedu 1					N.S.	P<0.05	P<0.01	P<0.001	P<0.05
Sathyavedu 2						N.S.	N.S.	P<0.001	N.S.
Kennedy River Pudukkottai							N.S.	P<0.001	N.S.
Ongole commander (white)								P<0.001	N.S.
Ongole–red									P<0.001

P values included, 'N.S.': not significant.

Stage 3: As the larva grew, the larval chamber increased to 300 μm width. The parenchymatous outer cortical cells grew larger and the external colour of the gall changed to pale red. Actively feeding larvae moved around the chamber. In Ongole–red seedlings, calcium oxalate crystals around the gall chamber occurred, whereas such crystals were absent in the gall chambers in Sathavedu 1 seedlings (Figure 7).

Stage 4: Outer colour of the gall changed to deep red. In susceptible seedlings the gall chamber developed within the vascular cylinder, whereas in resistant seedlings the gall generally remained outside the vascular cylinder (Figures 8 and 9). The width of the gall chamber increased to 520 μm.

Stage 5: The width of the gall chamber increased to 700 μm. Soon after pupation, the nutritive tissue degenerated (Figure 10). Due to the disruption of the vascular tissue during adult emergence, drying of the gall-bearing organ (stem, petiole, midrib) occurred (Figure 11). In a few of the investigated materials, the larval chamber — after the exit of the adult — got filled with regenerative parenchyma (Figures 12 and 13). No difference in the size of the gall chamber was evident among the examined galls from different seed sources.

Biochemical Studies on Leaves and Stems

Moisture.
No variation in the moisture content of the leaves and stems in the Sathyavedu 1, Pudukottai, and Ongole-red seed source existed (Table 4). The moisture content of leaves ranged from 65 to 67% and that of the stem from 63 to 65%.

Lipids.
Variations occurred in the total lipid content in leaves and stems. Total lipid content in the stems was 80 mg g^{-1} dry mass, whereas on leaves was 300 mg g^{-1} dry mass. When the lipid content among the Sathyavedu 1 Pudukottai, and Ongole–red seed sources were compared, the seed source Sathyavedu 1 with lesser infestation than the other two had the maximal total lipid content per g dry mass (300 mg g^{-1}). A decreasing trend in the lipid content in leaves was evident as the susceptibility to gall infestation increased in seed sources. The susceptible seed source Ongole red showed 220 mg g^{-1} dry mass. A reverse trend occurred in lipid contents in the stems. The highly susceptible seed source showed a high lipid content of 80 mg g^{-1} dry

Table.4. Biochemical parameters of leaf and stem of *E. camaldulensis**

Seed Source	Moisture (%)		Lipid (mg g^{-1})		Phenol (mg g^{-1})		Tannin (mg g^{-1})		Cellulose (mg g^{-1})	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Sathyavedu-1	65±1.3	65± 2.3	300± 5.2	20± 1.4	9.17± 0.38	7.19± 0.54	0.57± 0.10	2.30± 0.3	9.29± 0.02	5.36± 1.0
Pudukottai	67±2.1	65± 1.8	260± 3.9	60± 3.1	9.36± 0.81	6.71± 0.28	0.95± 0.05	2.06± 0.25	13.48± 0.52	4.98± 0.53
Ongole– red	65±1.9	63± 1.2	220± 3.5	80± 5.1	10.29±0.52	6.51± 0.62	1.09± 0.61	1.78± 0.62	17.59± 1.6	4.84± 0.81

*Values represent mean of 12 replicates.

mass, whereas the least susceptible source Sathyavedu 1 showed 20 mg g^{-1} dry mass (Table 4).

Phenols and Tannins.
The total phenol content, in general, was higher in leaves than in stems. Leaves of highly infested seed source showed the highest value (10.25 mg g^{-1} dry mass). Although not significant, the total phenol contents, the seed sources Sathyavedu 1 and Pudukottai showed 9.17 and 9.36 mg g^{-1} dry mass, respectively. No significant variation in the phenol contents was evident in the stems of Sathyavedu 1 Pudukottai, and Ongole-red sources (Table 4). Significant difference occurred in the level of total tannins between leaves and stems. A gradual increase in the tannin content was observed in the leaves as the susceptibility to gall infestation increased: from 0.57 mg g^{-1} dry mass in the less-infested Sathyavedu 1, tannin contents increased to 1.09 mg g^{-1} dry mass in the highly susceptible Ongole–red. However, the tannin content did not show any significant variation in the stem region among the three seed sources (Table 4).

Cellulose.
The cellulose content varied significantly between stems and leaves. Leaves of highly susceptible Ongole-red showed high cellulose content of 17.5 mg g^{-1} dry mass. The less-susceptible Sathyavedu 1 recorded 9.29 mg g^{-1} dry mass of cellulose, which increased to 13.48 mg g^{-1} dry mass in the moderately susceptible Pudukottai source. Cellulose content in the stems of all the three seed sources remained at 4.8–5.3 mg g^{-1} dry mass levels.

DISCUSSION

Gall-inducing insects are generally not easily amenable for any conventional control tactic, such as application of insecticides because of their embedded/concealed nature. In such a context, one long-term strategy in the management of *L. invasa* that induces stem and leaf galls on *Eucalyptus camadulensis* and *E. tereticornis* would be to determine genetically stable characters that code for resistance in eucalypts and develop trees that would bear the resistant genes.

Gall induction by *L. invasa* has a major impact on the seedlings of *Eucalyptus* as well, although *L. invasa* outbreak usually occurs in mature plants in plantations (Mendel et al. 2004, Jacob et al. 2007). In the plantation industry that caters to pulpwood demands, several thousands of propagules of *Eucalyptus* are raised annually either through clonal method or through seeds collected from genetically superior trees. The present study illustrates that among the several seed sources of *Eucalyptus*, the Ongole–red, Kennedy River, Pudukkottai, Rudrapur are highly susceptible to infestations of *L. invasa*. Trees raised from these seed sources act as reservoirs for *L. invasa*. Seed sources such as Sathyavedu 1 are less susceptible to *L. invasa.* Seedlings of *Eucalyptus* susceptible to *L. invasa* bear galls on shoot tips, petioles, and midribs. Adult females of *L. invasa* oviposit on terminals of young shoots and midribs of juvenile leaves; larvae develop within cylindrical galls on plant parts where oviposition occurred. The life cycle of the wasp is *c*. 132 days. Gall development damages growing shoot terminals and leaves of *E. camaldulensis* and *E. tereticornis* inducing rapid abscission of leaves and drying of shoot tips. An intense infestation by *L. invasa* results in loss of vigour and growth retardation in seedlings. The morphology and development of galls induced by *L. invasa* on *E. camaldulensis* and *E. tereticornis* in southern India is similar to those on leaves of *E. saligna* by *Ophelimus eucalypti* (Hymenoptera: Eulophidae) (Raman and Withers 2003).

Capability of gall-inducing insects in distinguishing between plant hybrids and genotypes is well known (Raman 1994, Floate and Whitham 1995, Ollerstam et al., 2002, Raman et al., 2005). Variation in distribution of galls induced by *L. invasa* in different plant parts such as midrib, petiole and stem was evident in different seed sources of *Eucalyptus* (*camaldulensis* and *tereticornis*). Whereas maximal preference for gall induction was on stems, petioles and midribs of both high and moderately susceptible seed sources of *E. camaldulensis* and *tereticornis*, viz., Ongole–red, Kennedy River, Rudrapur and Pudukkottai, occurrence of galls was comparatively less in the stems of the less-susceptible seed source Sathyavedu 1 (*E. camaldulensis)*. Morphological features and frequency of incidence of galls induced by *L. invasa* varied among the different seed sources. Gall-inducing behaviour of *L. invasa* on seedlings of different seed sources revealed that the adults of *L. invasa* exhibit preference for oviposition among the different seed sources as well as specific locations on a plant. *Ophelimus maskelli* (Hymenoptera: Eulophidae) showed a tendency to oviposit on developed, immature leaves of *E. camaldulensis*, preferring to oviposit on the leaf blade close to the petiole (Protasov et al. 2007). Although *L. invasa*-induced galls on the less-susceptible seed sources

such as Sathyavedu 1, these galls remained isolated, sporadic, and occurred in limited numbers on the stem, petiole and midribs demonstrating traits of resistance to *L. invasa*.

Insects respond to diverse stimuli when selecting their host plants for either feeding or oviposition (Ananthakrishnan et al. 1985). Gall-inducing insects have the ability to choose the right host plant and the right host tissue for oviposition initially and feeding subsequently (Raman et al. 2005). Adults of *L. invasa* prefer to oviposit on young shoot tips, midribs and petioles of juvenile leaves of seedlings raised from different seed sources. In *E. camaldulensis* seedlings raised from Sathyavedu 1 seed sources, oviposition preference of *L. invasa* was more to midribs than either to the petiole or to the stem. By directing their movements not only towards preferred seed sources, but also towards specific sites and tissues within the seed sources, *L. invasa* demonstrate adaptive tactics in the selection and oviposition behaviour. Meristematic tissues in the seedlings of *E. camaldulensis* and *E. tereticornis* attract *L. invasa* and provide suitable substrate for oviposition — a common behaviour in many gall-inducing Hymenoptera (Shorthouse et al. 2005).

The present study illustrates that seed sources with severe infestation show poor growth rate and form. Severe infestation by *O. eucalypti* retarded the growth of *E. saligma* and *E. botryoides* (Walsh 1996, Raman and Withers 2003). Retardation of growth in highly susceptible seedlings could be due to siphoning of nutrients to the sink site, viz., the gall, from normal plant-growth activity (Rohfritsch 1992).

Exomorphic features of host plants (e.g., trichomes, organ, and tissue toughness) are responsible for either attraction to or repulsion from feeding or ovipositing insects; the plant cuticle is one of such key phenotypic manifestations that imparts a critical level of resistance by acting as an effective barrier to plant-feeding insects (Schoonhoven et al. 2005). Thickness of the cuticle in the stems of seed sources varied in *E. camaldulensis* from 2 □m in Ongole red seed source (*E. camaldulensis*) to 4.5 □m in Sathyavedu 1 seed source (*E. camaldulensis*). Failure of development of multiple galls in Sathyavedu 1 seed source (*E. camaldulensis*) suggests that the thicker cuticule restricts oviposition efforts of *L. invasa*.

Petioles and stems include patches of necrosed cells at the point of oviposition in both susceptible and resistant seed sources. Moreover, locations of gall chambers in both susceptible and resistant seed sources of *E. camaldulensis* were different, with the Sathyavedu 1 source (with low level infestation) bearing the gall chambers mostly in the cortical region whereas in the Ongole–red source, the gall chambers were in the inner cortex. In the resistant clones, the adult female could not insert its ovipositor, deeper into the plant tissue, and such a behaviour regulates gall induction in resistant seedlings. In the present study, we found that the nutritive cells were active in *L. invasa* induced galls. In *O. eucalypti*-induced galls on *E. saligna* in New Zealand, those along the abaxial surface of the gall have been found to be more active than those along the adaxial surface (Raman and Withers 2003).

The tendency of different species of eucalypts to hybridize easily (Morrow et al. 1993) results in some level of uniformity in the biochemistry of eucalypts, which enable the feeding insects to either survive or oviposit on more than one species of eucalypts. However variations in the quality of nutrients (e.g., sugars, proteins, lipids) and non-nutrients (e.g., phenolic materials) between plant species or even within a plant influences food preference, growth rate, developmental time and hence the biotic potential of the insect (Slansky 1982). In the present study quantitative variation in individual phenols, tannins, lipids and cellulose in different seed sources of *E. camaldulensis* coupled with physical characters like cuticle thickness plays an active role in regulating either resistance or susceptibility of seedlings to *L. invasa.* Higher levels of phenols and tannins in the stems of Sathyavedu 1 seed source inhibit gall induction by *L. invasa*. A strong inverse correlation has been indicated between the concentration of total phenols and suitability of a tree for gall development by aphids (Zucker 1982). Differences in condensed and hydrolyzable tannins, total phenolics, lignin, cellulose, hemicellulose and nitrogen influenced the occurrence of gall-inducing cynipids on species of *Quercus* (Abrahamson et al. 2004). No significant variation in the cellulose content in the stem of Sathyavedu 1, Pudukottai and Ongole–red sources were evident in the present study. Lipid content in the leaves of all the three seed sources was higher than those in the stem. Levels of leaf oil content in *Eucalyptus* were shown to influence insect feeding and growth (Morrow and Fox 1980). Levels of nutrients and secondary compounds in gall tissue are usually markedly different to those of surrounding plant tissue, and that gall-inducing insects may produce species-specific and temporally variable changes in the chemical composition of gall tissue (Hartley 1998).

Variation in relative susceptibility of seedlings of different seed sources of eucalypts has been observed in the nursery beds, which suggests existence of clones with natural resistance. In the present study, the incidence of *L. invasa* was total and all the seedlings were exposed equally to the wasp in the nursery. The percentage of gall infestation by *L. invasa* as well as the tissue specific distribution of the gall within a plant indicates that *E. camaldulensis* seedlings raised from Sathyavedu 1 seed source could be treated as seed source that bear the least attack from *L. invasa*.

In conclusion, considerable evidence has been mounted through this study to demonstrate the variation in the physical characters in several seed sources of *Eucalyptus camadulensis* and *E. tereticornis* in southern India to varying levels of susceptibility to the gall inducing *L. invasa*. Molecular verification of these traits should provide useful data in building resistance against *Leptocybe invasa*, a gall-inducing taxon, the populations of which are generally hard to manage because of its concealed and embedded nature within plant tissue.

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REFERENCES

Abrahamson W. G, Hunter M. D, Melika, G., and Price P.W. 2003. Cynipid gall-wasp communities correlate with oak chemistry. Journal of Chemical Ecology 29: 209–223.

Ananthakrishnan, T. N., Dhileepan, K. and Padmanaban, B. 1985. Behavioural responses in terms of feeding and reproduction in some grasshoppers (Orthoptera: Insecta). Proceedings of Indian Academy of Science (Animal Sciences) 94: 443–461.

Bouèek, Z. 1988. Australasian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species. Commonwealth Agricultural Bureaux International, Oxford. 832 pages.

Floate, K. D. and Whitham, T. G. 1995. Insects as traits in plant systematics: their use in discriminating hybrid cottonwoods. Canadian Journal of Botany 73: 1–13.

Folch, J., Less, M. and Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497–506.

Hartley S. E. 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? Oecologia 113: 492–501.

Jacob J. P., Devaraj, R. and Natarajan, R. (2007). Outbreak of the invasive gall inducing wasp *Leptocybe invasa* on eucalypts in India. Newsletter of the Asia–Pacific Forest Invasive Species Network 8: 4–5.

Kaikini, N. S. 1961. *Eucalyptus* in Mysore state. Proceedings of the 10th All-India Silvicultural Conference, Dehra Dun, India. 546–553.

Mathew, G. 1995. Field performance of some indigenous tree species with respect to insect pest incidence in Kerala (India), Indian Journal of Forestry 18: 133–140.

Mendel, Z., Protasov, A., Fisher, N. and La Salle, J. 2004. Taxonomy and biology of *Leptocybe invasa* gen. & sp. n. (Hymenoptera: Eulophidae), and invasive gall inducer on *Eucalyptus*. Australian Journal of Entomology 43: 101–113.

Morrow P.A. and Fox L. R. 1980. Effects of variation in *Eucalyptus* essential oil yield on insect growth and grazing damage. Oecologia 45: 209–219

Morrow, P.A., Whitham, T.G., Potts, B.M., Ladiges, P., Ashton, D.H. and Williams J.B 1993. Gall forming insects concentrate on hybrid phenotypes of *Eucalyptus*. Pages 121-134. In: Price P.W, Mattson, W.J and Baranchikov T.N. (Editors) The ecology and evolution of gall forming insects. General Technical Report no. NC-174, USDA, Forest Service, St Paul, Minnesota, USA.

Ollerstam O., Rohfritsch O., Hoglund S., and Larsson S. 2002. A rapid hypersensitive response associated with resistance in the willow *Salix viminalis* against the gall midge *Dasineura marginemtorquens*. Entomologia Experimentalis et Applicata 102: 153–162.

Protasov, A. La Salle J., Blumberg D., Brand D., Saphir N., Assael F., Fisher N. and Mendel Z. 2007. Biology, Revised taxonomy and impact on host plants of *Ophelimus maskelli,* an invasive gall inducer on *Eucalyptus* spp. in the Mediterranean area. Phytoparasitica 35: 50–76.

Raman, A. 1994. Genetic, developmental, and physiological variation in individual plants: strategies to counter insect herbivores. Phytophaga 6: 97–100.

Raman, A. and Withers, T.M. 2003. Oviposition by the introduced *Ophelimus eucalypti* (Hymenoptera: Eulophidae) and morphogenesis of female-induced galls on *Eucalyptus saligna* (Myrtaceae) in New Zealand. Bulletin of Entomological Research 93: 55–63.

Raman A., Schaefer C.W., and Withers T.M. 2005. Biology, Ecology, and Evolution of Gall-inducing Arthropods. 2

Volumes; Science Publishers, New Hampshire, USA. 817 pages.

Rohfritsch O., 1992. Patterns in gall development. Pages 60–86. In: Shorthouse, J.D and Rohfritsch, O. (Editors) Biology of Insect-induced Galls. Oxford University Press, Oxford, UK.

Sadasivam, S., and Manickam, A. 1996. Biochemical Methods (Second edition). New Age International (P) Limited, Publishers. New Delhi, India. 256 pages.

Schoonhoven, L.M., van Loon, J.J.A., and Dicke, M. 2005 Insect—plant Biology. Oxford University Press, New York, USA. 440 pages.

Shorthouse, J.D., Leggo, J.J., Sliva, M.D., and Lalonde, R. G. 2005. Has egg location influenced the radiation of *Diplolepis* (Hymenoptera: Cynipidae) gall wasps on wild roses? Basic and Applied Ecology 6: 423–434.

Slansky, F. Jr. 1982. Insect nutrition: an adaptationist's perspective. Florida Entomologist 65: 45–71.

Varghese M., Nicodemus A., Nagarajan B., Ravi N., and Hegde R. 2002. A breeding programme for improving productivity of *Eucalyptus camaldulensis* and *E. tereticornis* in India. Pages 19–28. In: Bagchi S.K., Varghese M., and Siddappa (Editors) Recent Eucalypt Research in India. Institute of Forest Genetics and Tree Breeding, Coimbatore, India.

Walsh P. J. 1996. Gall wasp on *Eucalyptus botryoides* and *E. saligna* and possibilities for biological control. New Zealand Forestry 10: 40–41

Zucker W. V. 1982. How aphids choose leaves: The roles of phenolics in host selection by a galling aphid. Ecology 63: 972–981