FINAL PROJECT REPORT

ON THE

MANAGEMENT OF EMERGING PESTS EUCALYPTUS

CLASSICAL BIOLOGICAL CONTROL OF EUCALYPTUS GALL WASP *LEPTOCYBE INVASA* FISHER & LA SALLE

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ACKNOWLEDGEMENTS

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I am greatly obliged to ***Mr. Narayanaswamy*** *Secretary IPMA for his valuable suggestions, encouragement and guidance and arranging for my sponsor for the training. I also extend my thanks for encouraging me to work on eucalyptus gall wasp in funding the project through IPMA.*

*I wish to express my sincere gratitude to Dr****. H.D.Kulkarni, G.M.(Plantation) ITC ltd.,*** *who has been a guiding force and also co ordinating the work of IPMA, I extend my heartfelt thanks for his help in accompanying me to Israel.*

The help and guidence rendered by Prof. Dr. Zvi mendel and Prof. David brand of Volcani institute along with their collegues during the training is grately acknowledged.

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I am thankful to INDIAN COUNCIL OF AGRICULTURAL RESEARCH (ICAR) *for granting me permission to attend the training at Israel.*

Many more who have helped during workshop at Israel and during the course of the project directly and indirectly are duly acknowledged.

(A.N. Shylesha)
Principal Scientist Entomology

Bangalore

Awareness programme and IPMAs initiative

8th December 2008

A) CONSOLIDATED PROJECT PROPOSAL

1. Project Title:	**MANAGEMENT OF EMERGING PESTS EUCALYPTUS**
2. Executing agency:	**INDIAN PAPER MANUFACTURERS ASSOCIATION NEW DELHI**
3. Broad area:	**Management of pest & Disease of Eucalyptus**
4. Project Duration:	**Two years (extendable by 6months in exigency)**
5. Participating Institution:	**i) Project Directorate of Biological Control P.O. box No. 2491, H.A. Farm post, Bellary Road, Bengaluru -560 024.**
6. **Project Coordinator & Facilitator** :	1. Dr. H.D. Kulkarni, ITC, PSPD, Sarapaka. 2. Dr. Sanjay Tripathi, WCPM, Dandeli. 3. Mr. Manjunath, MPM, Bhadravathi. 4. Dr. R. Seenivasan, TNPL, Kagathipuram. 5. Dr. S.Chinnaraj, TNPL, Kagathipuram.
7. **Scientific Coordinators**:	1. Dr. R.J. Rabindra, Director, P.D.B.C., H.A.Farm Post Bellary road, Bengaluru-560 024. rjrabindra@rediffmail.com
8. **Principal Investigators**:	**1. Dr. A.N. Shylesha, Senior scientist Project Directorate of Biological Control, P.O. box No. 2491, H.A. Farm post, Bellary road, Bengaluru-560024 anshylesha@gmail.com**

B) Introduction:

The growing plantation forestry industry faces several challenges and threats. These threats include those posed by pests and diseases. Damage caused by insect pests and diseases caused by fungi, bacteria and phytoplasma will not only result in tree death but also reduce growth and yield affecting the timber quality. Eucalyptus is an important pulpwood species, which is widely used in the paper and pulp industries. Recently some unknown pests like Blue Gum Chalcid (*Leptocybe invasa*) described by Mendel.(2004), and disease like little leaf have been reported from different parts of India. No clear management strategies were developed to reduce the impact of these pests and diseases. This is of great concern to private tree growers in this country. In the light of recent developments, it is proposed to conduct an organised survey networking three major southern states in Eucalyptus growing areas and identify the key insect pest i.e., wasp (*Leptocybe invasa*) and disease like little leaf to develop suitable management practices. The new gall insect (*Leptocybe invasa*), which has migrated from Australia, has started damaging large areas of Eucalyptus plantation in India, and is considered to be a threat to large scale plantation of Eucalyptus. First reported in Malakampadin area in Tamil Nadu, the damage has now spread to the neighboring States of Kerala, Karnataka and Andhra Pradesh. According to latest reports, it has also invaded northern, western & eastern India. The tiny *Leptocybe invasa* produces galls on leaf mid-ribs petioles and shoot tip which slowly damage and the shoots droop down. Due to this the

normal growth is affected. If it continues uncontrolled then there would be heavy loss of wood production of Eucalyptus and leads to an economic loss.

Management of invasive pests can be ideally attempted through Classical Biological Control involving introducing effective natural enemies from the native home range. This method helps to re establish in the new area the lost balance between the pest and the natural enemies seen in the native home. The introduction is best during the early part of the invasion as it gives best results. Two parasitoids *Aprostocetus sp* and *Megastigmus sp* are found to parasitize *Leptocybe invasa.* These are found to be successful in management of the gall wasps in Israel. The same will be imported to India for the management of the pest after adhering to the quarantine formalities. The impact of the introduction will be assessed by the fellow partners in different states of our country.

Surveys conducted in Eucalyptus growing areas for disease incidence showed that little leaf is wide spread on eucalyptus in India causing more than 1% damage in nurseries (Radhanarayan and Anantha Padmanath, 1977). Incidence of little leaf on different Eucalyptus species in India was reported by Sharma (1983) from Karnataka. The pathogen was identified as phytoplasma which is transmitted by grafting. It can be can be identified by florescence straining of phloem by Dienes staining method. Suppression of symptoms done by application of tetracycline. Macrocone *et al.*, (1997) in Italy have differentiated the phytoplasma of Elm trees from that of phytoplasma of eucalyptus by using RFLP and PCR techniques.

There is no effective control measures available for Gall insect as well little leaf causing organisms and the project envisages to evolve effective management practices.

C) Objectives of the Project:

1. **Management of Eucalyptus gall wasp (*Leptocybe invasa)* through classical biological control**

D) Technical Program for Component-1 (Biological):

Importation of exotic natural enemies, quarantine screening, host specificity test, mass multiplication and release of natural enemies of Eucalyptus gall wasp*, Leptocybe invasa*.:

The Government of India's Plant Protection Advisors for methods & regulations prescribed will be followed for import of potential natural enemies for the classical biological control of the gall formats. Screening the imported natural enemies to rule out hyper parasitisation as well as pathogenic organisms. Host specificity tests will be undertaken following standard protocols.

Multiplication of the parasitoids of the Gall wasp:

The mass production techniques will be developed in two phases

1. Mass production of the host insect *Leptocybe invasa* in the green houses
2. Mass production of the parasites in the laboratory on the host insects
3. Mass multiplication of the parasites in nursery under shade nets

Field release of natural enemies.

Field release of the natural enemies will be attempted by following methods.

a) Gall infested plants :Gall infested plants will be covered by the gall cages and parasitoids will be released
b) Open release method under shade net for containment

Under this method 5 x 5 m shade net cages containing nursery stocks of eucalyptus will be used. For parasitisation the shade nets will be removed to enable parasitoids to disperse to near by areas.

Field release and establishments

Three nurseries under the control of the paper mills (ITC in AP, West Coast Paper Mills in Karnataka and TNPL in Tamil Nadu) will be selected and about 200 parasitoids will be released at monthly interval. The following observations will be recorded. Frequency to be followed at 15 days interval for 6 times.

Percentage parasitization:

50 randomly selected plants 50 leaves per plant will be collected, brought to the laboratory. Gall forming insect as well as the natural enemies will be allowed to emerge under caged conditions. The per cent parasitism will be worked out. Intensity of galls in new flush will be monitored by sampling 50 plants at random and 5 shoots will be selected to work out percentage gall leaves. Such galls released will be brought to the laboratory to assess the extent of parasitism. A close watch will be made to look for hyper parasitism if any.

Monitoring the spread and impact assessment:

Once the field assessment of the natural enemies is established, quarterly survey will be under taken by observing the nearby areas covering perimeter at 1 kms intervals and the same sampling
methods indicated in survey will be followed. Once the spread of the parasite is seen the area of survey can be expanded centrifugally.

F) Facilities Available: Quarantine facility, green house, and laboratory space and other basic requirements for conducting biocontrol experiments are available

G) Benefits of the Project: The principle benefit of the project will be classical biological control of the gall insect in Eucalyptus and reduction in damage to plants.

Budget:

Proposal: MANAGEMENT OF EMERGING PESTS & DISEASE OF EUCALYPTUS

Total Project Cost : Rs. 54,74,200/-
Contribution from IPMA : Rs. 9,74,200/-
Cess Funding : Rs. 45,00,000/-

Components	I year	II year	Total
Component-1	**1851300**	**876700**	**2728000**
Component-2	1545600	1200600	2746200
Grant Total	**3396900**	**2077300**	**5474200**

The proposed project study will be carried out in a most cohesive manner drawing intellectual resources available with IPMA and also from institutes and universities of repute viz. Project Directoreate of Biological Control, Bangalore {ICAR, Ministry of Agriculture} Department of Plant Pathology & Entomology, College of Agriculture, Hyderabad {UGC} Department of Agriculture Entomology, College of Agriculture, Dharwad {UGC} Forest College & Research Institute, Mettupalayam {UGC}

Centre	I year (Rs)	II year (Rs)	Total (Rs)
NBAII (PDBC)	**1521300**	**546700**	**2068000**

Details of PDBC components.

Equipments required (PDBC)

Equipments	Quantity	Cost (in Rs.lakhs)
BOD incubators	2	3.00
Oven	1	0.75
Cages (small)	50	0.50
Cages (big)	25	1.25
Microscope	1	3.50
Total (a)		**9.00**

Staff requirement (PDBC):

Name of the post	Description of post	No. of posts proposed	Scale of Pay	PDBC	Other centers	Total
Scientific	Senior Research Fellows	one	14000-15000	1	3	4
Supporting	Janitor	one	5000	1	---	1

Recurring Expenses (PDBC): (Amount in Rs.)

Head	**N0s**	**I year**	**II Year**	**Total**
Pay and allowances				
1.Senior Res. Fellow	1	193000	207000	400000
2. Janitors	1	60000	60000	120000
TA and POL and Hiring charges of vehicles within country		30000	30000	60000
TA outside the country for Technical Consultation		150000	150000	300000
Contingencies		50000	50000	100000
Total		**483000**	**497000**	**980000**

Budget Abstract for PDBC (Amount in Rupees)

SL.NO	**BROAD HEADS OF ACCOUNT**	**I Year**	**II Year**	**Total**
a.	Pay and allowances	253000	267000	520000
b.	TA	30000	30000	60000
c.	Contingency	50000	50000	100000
d.	TA outside the country for Technical Training	150000	150000	300000
a.	Equipment	900000	0	900000
	Sub-Total	**1383000**	**497000**	**1880000**
f	Institutional Charges (10%)	138300	49700	188000
	Total	**1521300**	**546700**	**2068000**

Funding for PDBC

Institution	**Year 1 (Rs)**	**Year 2 (Rs)**	**Total (Rs)**
PDBC	**1521300**	**546700**	**2068000**

ACHIEVEMENTS

- **INTRODUCTION,QUARANTINING, MASS PRODUCTION AND ESTABLISHMENT OF *QUADRASTICHUS MENDELI* A PARASITOID OF EUCALYTPUS GALL WASP *LEPTOCYBE INVASA***
- **SUCESSFUL BIOLOGICAL CONTROL OF EUCALYPTUS GALLS WASP LEPTOCYBE INVASA IN INDIA.**
- **REDUCTION OF INVASIVE GALL WASP POPULATIONS BELOW PEST STATUS IN INDIA.**

IMPORT PERMIT FOR PROCUREMENT OF

QUADRASTICHUS MENDELI

PQ Form 13

Government of India
Ministry of Agriculture
(Department of Agriculture & Cooperation)
Directorate of Plant Protection, Quarantine & Storage,
NH-IV, Faridabad (Haryana) -121001.

Permit for import of live insects/mites/nematodes/microbial cultures including algae/biocontrol agents.

Permit No. **29/2008**

Date of issue: 10.10.2008
Valid up to: 09**.04.2009**

In accordance with the provisions of clause 7 (3) of the Plant Quarantine (Regulation of Import into India) Order, 2003 issued under Sub-section (1) of Section 3 of the Destructive Insects & Pests Act, 1914 (2 of 1914), I hereby grant permission for import of following insects/mites/nematodes/microbial cultures/biocontrol agents as detailed below:

1.Name and address of importer	2. Name and address of exporter
M/s. Dr. R.J.Rabindra, Project Director, Project Directorate of Biological Control, P.O.Box 2491, H.A. Farm Post, Bellary Road, Hebbal, Bangalore-560024.	**M/s. Prof. Zvi Mendel, Ph.D. Department of Entomology Agricultural Research Organization The Volcani Center, P. O. Box 6, Bet Dagan 50250. Israel.**
3.Country of origin: **Israel**	4. Point of entry – **By Air –Chennai**

Description of organism (Common/Scientific Name	Taxon (Class/Family order etc.)	Stage of organism, host species, if any	No. of specimens/units	Mode of packing
Selitrichodes kryceri Kim & La Salle (Eulophidae)	Hymenoptera	Adults/Pupae.	500 specimens each	Aerated containers

The above permission is granted subject to the following conditions:

(1) No substitute is permitted for the kind of organism permitted for import under this permit.

(2) The consignment shall be accompanied by an official certificate issued by an appropriate authority in the country of origin for freedom from:

(a) xxxxxxxxxxxxxxxxxxxxxxxxxxx ----------

(b) xxxxxxxxxxxxxxxxxxxxxxxxxxx ----------

(3) The consignment of bio-control agents shall be held under post entry quarantine at _____ (Name of Institute / Organisation) for a period of _____ before release for field trials.

(4) The permittee shall intimate the Plant Protection Adviser of any change of address and comply with his instructions.

Date: 10 .10.20008
Place: Faridabad, Haryana India

Name &
Signature:
Designation
Stamp of Issuing Authority

Dr. P. S. CHANDURKAR
Plant Protection Adviser
to the Govt. of India
M/o Agriculture D/o Agri. & Co-opn.
Dte. of P. P. Q. & S.
N. H. IV, FARIDABAD

Report on the visit of Dr. A.N. Shylesha, Principal Scientist Entomology P.D.B.C. Bangalore to Israel sponsored by IPMA For training on Classical Biological Control of Eucalyptus gall wasps.

Wood pulp is a basic raw material for paper industry and Eucalyptus is an important pulpwood species, which is widely used in the paper and Rayon industries in India. The new gall insect, which has migrated from Australia, and spread throughout the world has started damaging large areas of Eucalyptus plantation in South India, and is feared to be posing threat to an estimated 80 lakh hectares of plantation. First reported in Marakkanam and Malakampadin in Villuppuram district area, in 2002 in Tamil Nadu, the damage has now spread to the neighboring States of Kerala, Karnataka and Andhra Pradesh and presently spread to all eucalyptus growing areas of the country.

This eucalyptus gall is caused by a tiny insect *Leptocybe invasa* (blue gum chalcid) produces galls on leaf mid-ribs petioles and shoot tip which slowly damage and the shoots droop down and the growth is stunted. If it continues uncontrolled then there would be heavy loss of wood production of Eucalyptus and leads to an economic loss to the farming community.

Management of invasive pests can be ideally attempted through Classical Biological Control involving introducing effective natural enemies from the native home range. This method helps to re establish in the new area the lost balance between the pest and the natural enemies seen in the native home. The introduction is best during the early part of the invasion as it gives best results.

Many nurseries produce Eucalyptus seedlings on large scale and supply to growers for raising plantations. The nursery stock is severely affected by galls as a result planting rate is coming down gradually. The farmer who is growing clonal Ecalyptus under agroforestry since past two decades is at a loss as his entire field is affected by these galls. There is no remedy available today to the growers and good lots of pesticides are being used with heavy cost leading to environmental hazards and pollution. This is another burden to the growers/farmers.

The Blue Gum Chalcid, *Leptocybe invasa* Fisher & LaSalle, (Hymenoptera: Eulophidae) is a new genus and species that was first recorded in the Middle East in 2000 and has spread to most Mediterranean countries and to many of the *Eucalyptus* areas in northern and eastern Africa, Asia including India (Mendel *et al.*, 2006). The insect causes typical bump-shaped galls on the leaf midribs, petioles and stems of new growth of several *Eucalyptus* species. This wasp occurs in about 22 countries in large areas in the Middle East, the Mediterranean, Africa, Asia and other continents and is a serious pest in young plantations. Heavy galling prevents further development of the infested growth, leaf fall and stunted shoot growth. Though serious damage to young plantations and nursey seedlings have been observed but no mortality of trees have been recorded. *Leptocybe invasa* is only known from females and males are not known except for one record from Turkey (Doganlar, 2005). Eggs are inserted in the epidermis of young leaves, on both sides of the midrib, in the petioles and in the parenchyma of the twigs and the larvae develop inside round galls and adults emerge from round exit holes. Mean length of a gall containing a single wasp is 2.1 mm, leaves of intensively growing trees may carry over 50 galls per leaf. Mean development time from

oviposition to emergence is 132.6 d in room temperature. In Israel the wasp produces two or three overlapping generations annually. It has a thelytokous reproduction (Mendel *et al*., 2006).

The possible pathways of introduction include movement of nursery stock and cut foliage. The adult wasps are very tiny (1.1 –1.4 mm) and thus incapable of long distance flight. The pest has invaded India and was first reported from Marakkanam in Villuppuram district, Tamil Nadu in 2002 and has now spread to several parts of Andhra Pradesh, Karnataka, Kerala, Pondicherry and Tamil Nadu. Very severe infestations have been observed in Chittoor district (AP) and nursery stocks are severely affected.

The pest is native to Australia and the wasps in the introduced countries are free from their principal natural enemies that occur in their native home, where it has been hardly recorded as a pest (Protasov *et al*., 2005). It is suggested that in Australia natural enemies play a significant role in reducing this wasp to below observation threshold (Mendel *et al*., 2006). These may serve as appropriate candidates for classical biological control of the pest in the new ares where the pest has inavded. Recent studies in Australia by scientists from Israel have yieled two natural enemies, *Aprostocetus* sp. (Eulophidae) and *Megastigmus* sp. (Torymidae), and these are being bred in quarantine for further evaluation and field release. These natural enemies could be introduced to India from Israel.

Dr Zvi Mendel has reviewed the taxonomic position of the available parasitoids and finaly confirmed two parasitoids *Quadratichus mendeli* and *Selitrichodes kryseri* to parasitize *Leptocybe invasa* from the native host range from Australia and were brought to Israel and were tested for their efficiency in the Classical Biological control of the same and were found effective and were released sucessfully for the management of eucalyptus gall wasps in Israel. Based on his sucessful experince in Israel and request from different countries for the solution to the above mentioned problem a short duration workshop was proposed during September 2008 and all the concerned people across the globe were invited for the workshop to be conducted from 10th to 17th November 2008.

Although many organisations like ICFRE and other Universities tried to focus the problem of Eucalyptus gall wasps in India, it was Indian Paper Manufacturers Association (IPMA) which took an initiative for the Classical Biological Control of the gall wasps. PDBC being a nodal agency for importaion and classical biological control IPMA felt to sponsor a PDBC Scientist along with faculty from member mills so that the parasitoids could be imported and released for CBC.

From PDBC, Dr. A.N. Shylesha Principal Scientist Entomology, was nominated for participation in the workshop with the funding of IPMA and Dr. H.D. Kulkarni Co ordinator, IPMA R&D and G.M. ITC limited. Import permits for *Quadrastichus mendeli* and *Selitrichodes kryseri* were obtained from Plant Protection and Quarantine GOI New Delhi.

The workshop: Classical Biological control of Eucalytpus gall wasps

The course was jointly organized by Forest Health Unit, KKL-JNF Forestry and Development Department and the Department of Entomology of the Agricultural Research Organization.

Organizers:

1. KKL-JNF: Mr. David Brand, Ms. Nitza Saphir and Dr. Zion Madar
2. ARO : Prof. Zvi Mendel and Dr. Alex Protasov

Prof. Zvi Mendel was the scientific leader of the course.

Mr. David Brand coordinated registration and administration.

Ms. Nitza Saphir was responsible for the course logistics.

The emphasis of this week-long training course in Israel was on two eucalyptus gall wasps and their parasitic wasps, with laboratory and field-based activity that were focused on studying the infestation and biological control by the respective wasps. The emphasis was on sampling, rearing methods, release and recovery, host susceptibility, and other essential aspects of planning biological control projects against gall wasps.

The training course also dealt with the most up-to-date uses of eucalyptus trees in Israel: propagation and use of eucalyptus in nurseries for arid land afforestation and management to familiarize the participants with management strategies concerning eucalyptus cultivation in agro-forestry schemes and arid land afforestation.The other aspects dealt with were disposal of wastewater through irrigation of eucalyptus plantations; and eucalyptus biotechnology (yield and process ability trait introduction by CBD Technologies Ltd.)

The course included a half-day workshop during which the participants also delivered short lectures about plant protection aspects of eucalyptus afforestation and the current status of the gall wasp problem in their own countries, this was done with the purpose of exchanging general and technological information regarding this issue.

A series of lectures from various scientists working in Israel and Turkey covered various aspects of the gall pests and its management. The course was handled by well qualified lecturers from research centers and the Research and Development Unit of the Forest Department of KKL.

This course was attended by participants from 13 countries in Jerusalem at Hotel Mount Zion, from November 10 to 18, 2008.

Training Course Programme: Monday 10th Arrival

Tuesday 11th

08:00 - 12:00 Registration
12:30 – 14:00 Lunch
14:00 – 15:30 General lectures
Lecture A Outline of the training course (Prof. Zvi Mendel, ARO)
Lecture B KKL Forestry Activity in Israel (Dr. Omri Boneh, KKL)
Lecture C Eucalyptus afforestation and cultivation in Israel (David Brand, KKL).

Coffee break
16:00- 17:30
Lecture D Eucalyptus gall wasps and their natural enemies (Prof. Zvi Mendel, ARO); Biology, seasonal history and sampling procedures.

Lecture E Injury and population assessment tools for *Ophelimus maskelli* (Dr. David Nestel, ARO).
19:30 Get together Dinner

Wednesday 12th

Field trip to the North – Main topics: gall wasps and biological control

07:00 - Embarking, Traveling along Judean foot hills
08:30 - Tel Megiddo (Armageddon) and outlook on Izrael Valley.
10:30 - Bet Shean Valley, visit of Nir David Eucalyptus plantations and the natural enemy release site, collection of infested material.
12:00 - Visit to Biological control industries at Sde Eliyahu (Biobee + Biofly)
Lunch at Sede Eliyhu
14:00 - Kefar Harub (or Queshet), Outlook on the Sea of Galilee
15:00 - Qeshet experimental plots, eucalyptus cultivation using affluent water; survey on the damage caused by the gall wasps.
17:00 - Visit in the city of Nazareth
18:30 - Typical Arabic Dinner at Abu Jzenainai

Thursday 13th

Gall wasp and their natural enemies

08:30 - Lecture F Taxonomy of the parasitoids of the gall wasps – Eulophida (Dr. John La Salle, CSIRO, Australia).
09:30 - Lecture G Taxonomy of the parasitoids of the gall wasps -Torymidae (Prof. Miktat Doğanlar, MKU, Turkey).
10:30 - Coffee Break
11:00 - Rearing of the eucalyptus gall wasps and their natural enemies (Prof. Zvi Mendel, Dr. Alex Protasov, ARO).
Laboratory work: identification, propagation and release and recovery of the parasitoids
13:00 - Lunch
14:00 – 19:00 Workshop Situation of the eucalyptus gall wasp issue in the area of the participating countries
Presentation of paper on Gall wasps problems in India- H.D. Kulkarni.
Presentation of paper on Possibility of CBC of eucalyptus gall wasps and Facilities at PDBC Bangalore
16: 00 - Coffee break
19:30 - Dinner

Friday 14th

08:00 - Embarking
09:00 - Greenhouse industry in a desert area
11:00 - Kalia, Dead Sea area
12:00 - lunch
14:00 - Return to Jerusalem
17:00 - Optional tourist activity (night tour in Jewish holy sites)
19.30- Dinner

Saturday 15th

Optional tourist activity (Moslem and Christian holy sites)

Sunday 16th

Field trip to the South –Forestry in arid zones

07:00 - Embarking
08:00 - Small Agroforestry Enterprise in Timurim. Biotic and abiotic diseases of

eucalyptus in Agroforestry and ornamental trees (Dr. Zion Madar) .

09:30 - Commercial nurseries ("Hish-Stil") in Ashkelon

11:00 - Eucalyptus for Bee Forage and visiting the Yad Mordechai Apiary (meeting with Dr. Arnon Dag).

13:00 - Lunch in Gilat forestry center.

14:30 - Watershed management and eucalyptus planting in the Negev (Mr. Yitzhak Moshe).

16:00 - Yatir forest

18:00 - Dinner

19:30 - Return to Jerusalem

Monday 17th

Eucalyptus R&D issues, and meeting on lunch with KKL leaders in Jerusalem

08:00 - Lecture H Production of Eucalyptus saplings from seeds and cuttings. (Prof. Josef Riov HUJ)

Lecture I Cell wall modification, a technological approach for fiber improvement and growth enhancement of commercial eucalyptus**.** Dr. Ziv Shani, CBD Technologies Ltd.

Lecture J Eucalyptus and bee grazing (Prof. Dan Eizikovitz)

Lecture K Eucalyptus cultivation using effluent water (Dr. Pinchas Fine, ARO).

Tuesday 18TH

Departure

Learning from the workshop:

The week long training programme was exhaustive and covered various aspects of eucalyptus from nursery making, varieties, management of nursery and main field, irrigation, fertility management, use of eucalyptus, and also eucalyptus as a bee flora. The main emphasis was given on the two major species of gall wasps damaging the plantations world wide Viz., *Leptocybe invasa* and *Ophilinus muskeli.*

Beside two gall wasps only two other Australian insect species are known from eucalyptus trees in Israel, two borers *Phoracantha semipuctata and Phoracantha recurva*. causing minor damage to eucalyptus plants.

We were also briefed about the other gall insect*:* *Ophelimus maskelli* (Ashmead) (Eulophidae) which is a major leaf gall maker in eucalyptus. The insect is not reported from India till today. The following parasites rearing techniques were also briefed during the training programme. *Stethynium ophelimi* Huber (Mymaridae) (origin NSW, Australia), *Closterocerus chamaeleon* (Girault) (Eulophidae) (origin NSW, Australia)

The host range of *Leptocybe invasa* was restricted to only *Eucalyptus sp.* and was found distributed in Europe (France, Corsica, Greece, Italy, Portugal, Spain), Asia (India, Andhra Pradesh, Karnataka, Kerala, Tamil Nadu), Iran, Israel, Jordan, Syria, Thailand, Turkey, Vietnam), Africa (Algeria, Ethiopia, Kenya, Morocco, Tanzania, Uganda).

The taxonomic details of the eucalyptus gall wasps are *Leptocybe invasa* Fisher and La salle (Hymenoptera: Eulophidae) Leptos=meaning Fine,weak,thin Cybe= meaning head-Signifying weak area on the head around the ocellar triangle,brown in colour with a slight to distinct blue

to green metallic shine. Thelytokus forms-Only females are known so far 1.1-1.4mm. Mean length of a gall =2.1mm. The wasps preffered to oviposit on 0.5 to 5cm length of newly emerged shoots, usually 1-2 weeks of bud opening and eggs are laid at a distance of 0.3-5mm from each other in a lined group. The juvenile shoots are often killed or stunted due to overcrowding of eggs. Over crowding may lead to little leaf kind of symptom. Ooze coming out of plant covers eggs laid. The gall developmental stages can be distinguished in four different stages. One to two weeks after oviposition appearance of cork tissue at oviposition site. Development of typical bump and pink coloration of galls. Fading of green colour, and glossyness. Change in colour to dark red. Emergence holes or emerging wasps. The details of the wasp rearing were discussed and following information was obtained. Collection of infested material. Placing infested material in emergence cages. Collection of *Leptocybe invasa* releasing in cages containing fresh growth or newly emerged shoots of *E. camaldulensis.* The above was demonstrated during the training workshop and we were asked to bring samples from the field and a practical exposure was given on the various aspects of wasp rearing in laboratory.

The following species of Eucalyptus were reported to be succeptible : *E. botryoides, E. bridgesiana E. camaldulensis,E. globulus E. gunii,E. grandis E.saligna E.viminalis*

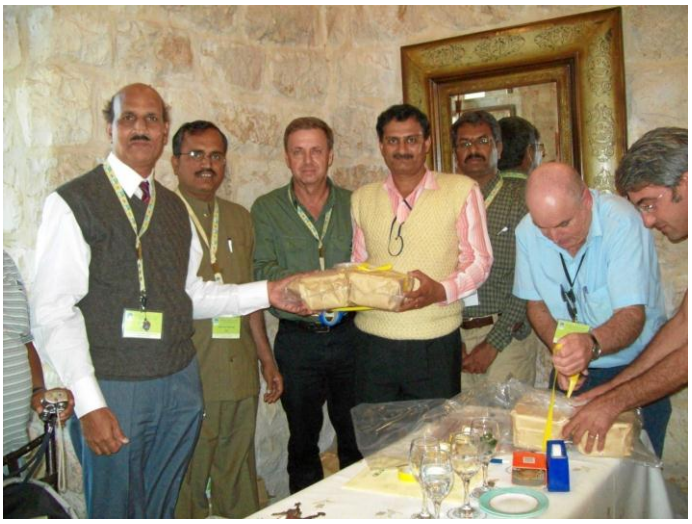
The following species of Eucalyptus were reported to be resistant:*Corymbia- Citriodora, Maculata, Eudesmia - Erythrocorys Symphyomyrtus- Sargentii,Gomphocepahala , torquata, woodwardii, and cladocalyx*

At the end of the course with the import permit the participant were handed over parasitoids required by them depending on the species of gall wasps present in their respective country. Out of 13 countries, only three countries brought the permits and were given the parasitoids. The package with eucalyptus galling material harbored with the parasitoids viz. ***Quadrastichus mendeli*** **Kim & La Salle** (Eulophidae) **,** ***Selitrichodes kryceri*** **Kim & La Salle** (Eulophidae) (origin Queensland, Australia) were brought to India.

We were instructed to set up a rearing of the relevant gall wasp species. Appropriate rearing material is *Eucalyptus camaldulensis* saplings that will fit to the parasitoids rearing cages. The optimal saplings are those harbor galls of different ages were suggested. No other herbivore species infest the galling material

Practical Exposures: We were taken to different areas in Israel where agriculture is being practiced in commercial way. Mechanised green houses, nursery, and the popular system of co-operatives the Kibootz and Mashevas. where people live together and cultivate and share the profits. We were also taken to Bio agents production units producing bumble bees for the use in polination in green houses. We also visited a beekeeping station in the far south of Israel where eucalyptus based honey production is being practiced. Excellent examples near cities using waste water for development of agroforestry were also shown.

Practical sessions



Receiving packed parasitoids
From Dr. Zvi Mendel and David brand

The team of trainers and trainees participated in the training programme at Israel

MANAGEMENT OF EMERGING PESTS EUCALYPTUS

CLASSICAL BIOLOGICAL CONTROL OF EUCALYPTUS GALL WASP *LEPTOCYBE INVASA* FISHER & LA SALLE

INTRODUCTION

Eucalyptus is one of the important plantation crops which play a dominant role in soil conservation and watershed management. There are more than 800 species of eucalyptus (Myrtaceae) belonging to two genera, *Eucalyptus* and *Corymbia.* All the species are endemic to Australia with the exception of *E. deglupta* Blume *and E. urophylla* S. T. Blake, which are native to New Guinea and Timor respectively (Simon, 2007). It produces quality pulpwood for paper, newsprint and rayon industry. It is economically important for timber, fiber, shelter and livestock. Commercial eucalyptus plantations are important global assets providing wood and wood fiber products to modern societies and offer a wide range of social, environmental and economic benefits to millions of people. Eucalyptus meets the requirements of people, industries and has helped to reduce pressure on natural forests. More than 16 million hectares of eucalyptus is planted around the world for various purposes including pulp for paper manufacture, solid wood and structural timbers and as woodlots for fuel. With an area of 8.0 million ha, India ranks first in area with a productivity of 146 t/ha (Vijay and Negi, 2007). Eucalyptus flowers are a source of nectar and help in honey production *viz. E. camaldulensis* Dehnh, *E. melliodora* Maiden, *E. robusta* Johnson and *E. sideroxylon* Chippendale. There is a high demand for the eucalyptus wood in India for various purposes like timber, pulp wood, fire wood and poles (Sexena, 1991).

Eucalyptus plantations and nurseries throughout the tropical and subtropical countries of the world are currently under threat by the gall forming invasive wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae), commonly referred to as the blue gum chalcid (Mendel, *et al*, 2004). In 2000, this gall wasp was recorded from the Mediterranean region where it caused severe injury to young foliage of red river gum, *Eucalyptus camaldulensis*, by inducing galls mainly on rapidly growing shoots. Seedlings in nurseries, saplings in plantations and coppiced shoots in plantations are known to be more susceptible to *L. invasa* attack (Mendel, *et al.*, 2004). The wasp lays eggs in

the petiole and midrib of leaves and stems of young shoots that leads to gall formation. Gall formation by *L. invasa* damages growing shoot tips and leaves of *Eucalypts*, resulting in quicker abscission of leaves and drying up of shoots. Heavy galling prevents further growth of the infested shoots. Its rapid population growth and spread in countries into which *Eucalyptus* has been introduced is attributed to the thelotokous parthenogenetic reproduction and multivoltine development of *L invasa* and the absence of natural enemies (Mendel *et al.,* 2004). *L invasa* is not considered a pest in Australia suggesting that the natural enemies in its native country.

Several plant and animal species of foreign origin have enriched the Indian agriculture. Establishment of these exotics has immensely contributed to the diversity of Indian agriculture. However, periodically some alien species have become 'exotic pests' which pose enormous threat to agriculture. These have invaded new areas due to either accidental or deliberate transport by humans. Increasing trends towards travel and import of plant products suggest that exotic species will continue to invade Indian agriculture in future with unprecedented consequences. Absence of natural enemies and native competitors greatly facilitate the invaders. Though species naturally disperse and colonize new areas, human transport and habitat disturbances have greatly increased the rate and scale of such invasions. Today, more species have become invasive than at any other time in the past. While most introduced species fail to become established, those that do, have become serious pests in agriculture, urban areas and natural landscapes. Annually, alien invasive species cost crores of rupees in control measures and crop damage. They also threaten human health, displace native species or degrade environmental aesthetics.

First noticed in India during 2001 (Anonymous, 2007b), the insect attack has assumed greater significance since its spread in many parts of the country. The gall infestation is very severe in many parts of Karnataka which has destroyed entire nursery and vast areas of eucalyptus coppice. Gall wasp has become a major constraint in eucalyptus production threatening the productivity of paper and rayon Industry. Initially the occurrence was restricted to a small area and attack was not observed to be serious. Currently, the insect attack has assumed greater significance since it has spread to other parts of the country. If the problem is unattended it may become severe in all the eucalyptus growing areas. A preliminary survey showed that *Eucalyptus camaldulensis* plantations raised in Rangareddy, Nizamabad, Warangal, Khammam East and West Godavari, Nellore and Chittor districts of Andhra Pradesh and *E. tereticornis* plantations in Karur, Trichy,

Pudukottai, Sivaganga, Villupuram, Thiruvannamalai, Cuddalore and Coimbatore districts of Tamil Nadu are affected by the gall problem (www.ifgtb.res.in).

The recent outbreak of the invasive gall wasp, *L. invasa* Fisher & La Salle is threatening the productivity of the existing eucalyptus plantations and has become a constraint in the expansion of the plantations throughout India. Even after a decade of its existence no effective control measures exist to manage *L. invasa* menace.

Studies on the biology of the Eucalyptus gall wasp *Leptocybe invasa* Fisher and La Salle:

Common Name: **Eucalyptus gall wasp, Blue gum Chalcid**

Taxonomic position: ***Leptocybe invasa* Fisher & La Salle**

Hymenoptera: Chalcidoidea: Eulophidae

Common names: Eucalyptus gall wasp; Blue gum gall wasp

Description:

Female: Color dark brown with metallic greenish / bluish tinge. Wings hyaline or lightly and uniformly infuscate. Antennal formula 11433 (anelli four-segmented). Mesoscutum without a median line. Scutellum with prominent submedian carinae. Dorsellum prominent, as long as propodeum. Fore wing with two setae on submarginal vein; postmarginal vein very short, only about a quarter as long as stigmal vein.

Male: Similar to female in general appearance and coloration; antennal formula 11343 with scape having a narrow and elongate sensory organ on lateral margin, annelli three-segmented, funicle four-segmented with long latero-terminal bristles and less stout than in female, club three-segmented; abdomen somewhat tubular. Doganlar (2005) described the males as follows: head and mesosoma brown with distinct blue to green metallic shine, metasoma brown with a slightly metallic tinge, legs pale yellow, except mid and hind coxae which have metallic shine, and antennae yellow. Males are fewer and not commonly observed as the wasp is mainly thelytokous. Doganlar (2005) provided a description of the male

Adult longevity: The adults of *L. invasa* lived for 3 to five days. Adults usually fed on the secretions of the plant and when sugar solution was provided the longevity could be increased by one to one and half days. As the frequency of occurrence of males was less longevity for males was not recorded.

Collection of adults: The adult wasps were collected by placing aged infested galls of eucalyptus twigs collected from infested fields. The cages were observed daily for the emergence of adults and the adults were collected and sexes were observed. The populations were used for further biological studies.

Collection and maintenance of adult *Leptocybe invasa*

The wasp reproduces by thelytoky (predominantly producing females). In India males were also collected from the field. So far males are being recorded in turkey and India in all other locations the pest exhibits thelytoky. Eggs are inserted in the epidermis of the upper side of newly developed leaves on both sides of the midrib, petiole and parenchyma tissue of twigs, always in a lined group. The sections of the leaf midrib carrying the eggs turn from green to pink 1-2 weeks after oviposition. The galls are spherical and glossy green initially and later turn from green to light or dark red depending on whether the galls are on leaves or the stem. The emergence holes are light brown on the leaves and reddish brown on the stem.

Observations in the lab and green house on various aspects of the biology and behavior like period of activity, oviposition and gall development of the pest were recorded. To study its biology, infestation free seedlings kept in glass cage were subjected to oviposition for 48 hr. ten adults were released into the cage for oviposition. Later ten plants kept in green house for further observation. Observations on the gall development as per the description given by Mendel *et al*. (2004) were recorded daily.

L.invasa females dominate the population although males are found occasionally in India. Just after emergence from the host tissue, the adult wasps spend some time hovering on the new shoot. They were often found feeding on the sugar solution sprinkled on the twigs. Oviposition started on the same day and eggs are deposited on the new twigs, basal midrib, leaf petiole etc. The plant reaction in the form of ooze is observed and this ooze will cover the egg. Eggs are laid

in a sequence each having a gap ranging from 0.25mm to 0.67mm in a line. Under heavy competition for egg laying the sequence is not maintained and due to heavy overcrowding of the eggs little leaf symptom occurs in the new twigs emerging after Oviposition and development of gall.

Gall development

Five stages of gall development on *E. camaldulensis* trees were identified. Stage 1 begins 1–2 weeks after oviposition, with the first symptoms of cork tissue appearing at the egg insertion spot. This stage is characterized by a small change in the morphology of the attacked tissue, the cork scar becomes bigger and the section of the midrib that carries the eggs often changes its colour from green to pink (**Fig. 1**). Towards the end of the stage the galls are easily recognized by their spherical appearance, their colour is glossy green, and each of the galls can easily be separated from the others. Stage 2 is characterised by development of the typical bump shape and the galls reach 48 days. Stage 3 is characterised by the fading of the green colour on the surface that tends to change to pink while retaining its typical gloss complete 16.5 days. Stage 4 complete 14. 5 days characterised by the loss of the glossiness of the gall surface, with colour changes to light or dark red according to whether the galls are present on the leaves or on the stem. Stage 5 was recognized as soon as emergence holes of the wasps were noticed completes 37.56 days, their colour was light brown when displayed on the leaf and red/brown on the stem (**Figs 1**). Similarly entire life cycle completes 133.56 days.

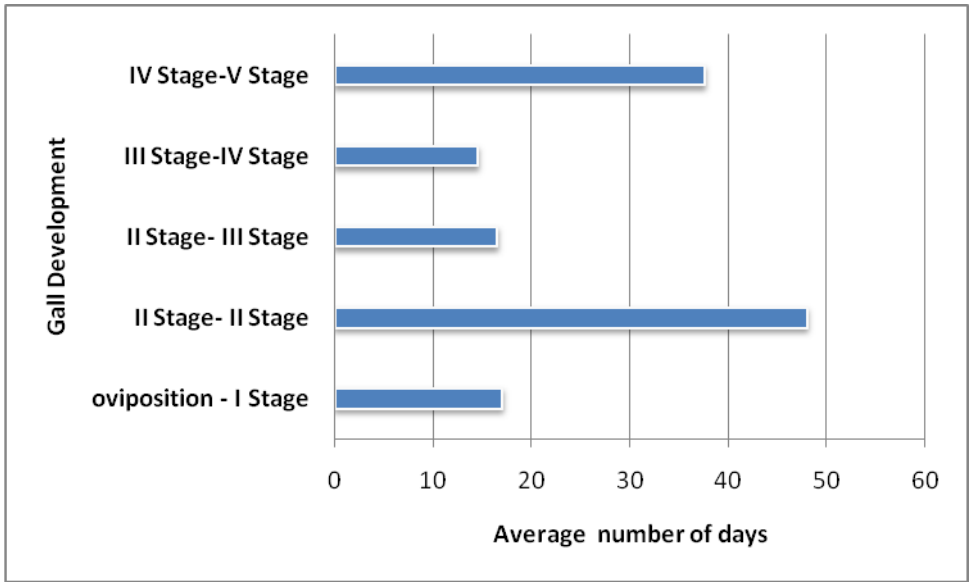


Figure. 1 Stages of eucalyptus gall wasp, *Leptocybe invasa*

Healthy plants of eucalyptus and galled plants

Green galls, leaf midrib galls and pink stage of gall

Dark pink galls with emergence hole, Little leaf symptoms and green galls, Little leaf symptom due to excessive oviposition by *Leptocybe invasa*

Importation and establishment of Parasitoids:

The insect galled twigs of eucalyptus were brought from Israel on the 19th of November 2008. The samples were opened in quarantine laboratory at NBAII for the presence of other unwanted insects and organisms. The samples were sprinkled with water and kept in emergence cages. Before that cleanup of the samples for other insects were done. Out of the two consignments of the parasites 62 *Quadrastichus*, 12 Megastigmus and only one Selitrichodes (male) emerged. The same were released on the plants containing fresh galls of *Leptocybe invasa*. The second generation populations were used for the study.

Biology of *Quadrastichus mendeli*
Adult description:
***Quadrastichus mendeli* Kim and La Salle**

Female Length 1.15–1.35 mm. Antenna light brown (or testaceous). Body mainly yellow with dark brown markings; on ocellar triangle, median area of pronotum, latero-posterior corner of pronotum just in front of prothoracic spiracle, apex of axilla, lateral panel of metanotum, median area of propodeum, transverse stripes on gastral tergites 2–4. Legs pale. Head Post Ocellar Length about 2.8 times as long as Ocellar Ocular Lentht on shriveled head. Frons with a median area dorsally, bordered laterally by sutures which extend from frontal suture halfway to level of toruli. Vertexal suture very weak and extending from lateral ocellus to eye. Frons with median area but without median carina. The ventral margin of torulus slightly lower than level of ventral margin of eyes. Subantennal groove present as a fine line beneath torulus, curved outwardly and extending over half of the distance from torulus to clypeal margin. Gena swollen and malar sulcus distinctly curved. Anterior clypeal margin subtruncate, only with a small lobe slightly protruding. Antenna with three funicular segments and one large anellus. All funicular segments longer than wide and almost equal in length and width; Scape reaching to vertex, but not extending over vertex. Mesosoma Pronotum about 0.3 the length of the mid lobe of mesoscutum in dorsal view. Mid lobe of mesoscutum with distinct median line and with 3–4 adnotaular setae on each side. Scutellum wider than long (L/W = 0.7); submedian lines and sublateral lines present. 2 pairs of setae on scutellum, anterior seta situated slightly behind midlength of scutellum. Mesosternum nearly flat in front of trochantinal lobe; precoxal suture weak and extending about 0.3–0.4 length of mesopleuron. Dorsellum semicircular in shape and about 0.3 the length of scutellum. Propodeum about 0.5 the length of dorsellum and nearly smooth without distinct median carina or paraspiracular carina. Propodeal spiracle partially covered by a raised lobe of callus.

Propodeal callus with 2 setae. Fore wing Submarginal vein with 1 seta, situated slightly basal to the middle. Costal cell without setae. Parastigma and stigmal vein without a hyaline break. Postmarginal vein rudimentary. Gaster, Slightly longer than the head plus mesosoma. Hypopygium extending about 0.3–0.4 the length of gaster, reaching up to the posterior margin of the third gastral tergite. Cercus with 3 setae, the longest one slightly curved and about 1.3 as long as the others, which are subequal in length. Ovipositor sheath slightly protruding, very short in dorsal view. ***Males of the species are unknown so far.***

Biological studies on *Quadrastichus mendeli* Kim and La Salle (Hymenoptera: Eulophidae: Tetrastichinae)

Rearing procedure:

Parasitoids were released onto saplings of *E. camaldulensis* on which about 25% of the branches and their leaves were galled by *L. invasa*, in 30 × 30 × 90- cm cages. The age of the galls ranged from 35 to 90 days (mature larvae and prepupae). In the quarantine facility the cages were kept in a closed room. Later, in the mass-rearing stage, they were kept under semi-controlled temperature in poly house.

Parasitoid development was studied by dissecting several dozen randomly chosen galls of *L. invasa*, 45– 65 days after gall inducer oviposition, Three sets of galls were exposed to the parasitoids in the cages, i.e. galls of stage 45-50 days old, 51-60 days old and 60-65 days old maintaining five replications in each. 5 plants with galls formed single replications and 10 adults were released per cage and observations were carried out by teasing the galls under the microscope after 15, 25, 35 and 40 days after Oviposition by the parasitoid and for parasitization. The numbers of parasitoid adults emerging from the plants were also recorded. Percent parasitization was calluculated based on the total galls in the plant and the number of parasitoids emerged. The tests were conducted in a ventilated polyhouse at an average temperature of 28°C and relative humidity ranging from 50 to 75%.

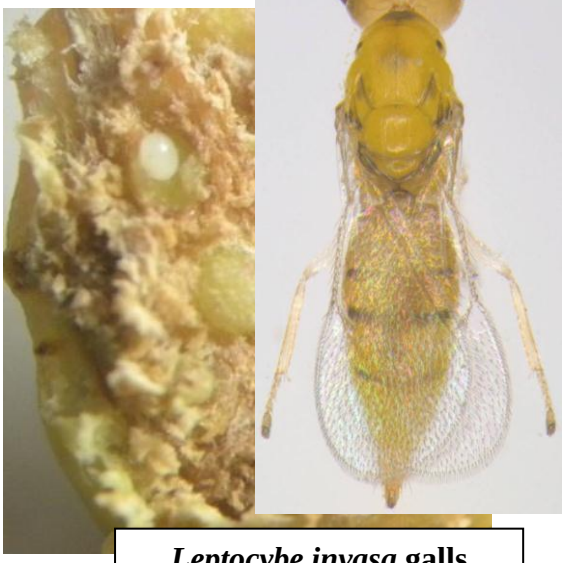
Adult longevity

The longevity of the studied parasitoids was determined by keeping them in cages containing the galled leaf and supplementing with 0.1% honey solution on the leafs and also providing cotton wads soaked in honey solution.

Suitable stage galls for Oviposition by Quadrastichus mendeli

Life cycle of *Quadrastichus mendeli*

Early stage larva

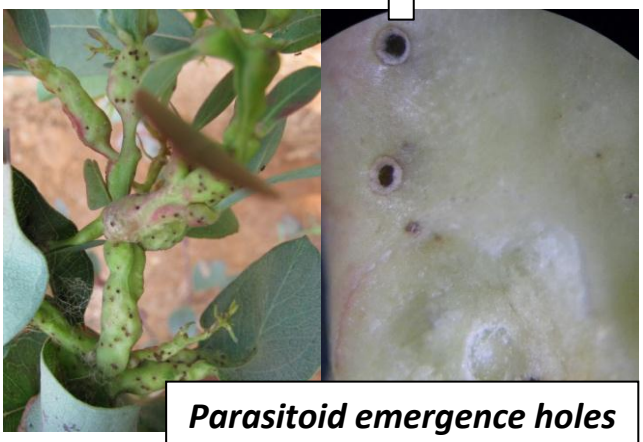


Grownup larva

Pupa within the host tissue

***Leptocybe invasa* galls within host tissue and exposed parasitoid larva**

Quadrastichus mendali



Parasitoid emergence holes

ELIMINATION OF THREATS IN QUARANTINE

Ophelimus maskelli (Ashmead)
Closterocerus chamaeleon (Girault)

Ophelimus maskelli (Ashmead) (Hymenoptera: Eulophidae), an invasive species was found as the major contaminant in the sample received from Israel. This is one of the most destructive pests on eucalyptus found in other countries and not in India. The pests along with its parasitoids are completely incinerated in the quarantine. A report to the effect was conveyed to the supplying agency.

Quarantine screening and multiplication of parasitoids:

A meeting was held on 25th February 2009 under the chairmanship of Dr. N. Krishnakumar, Director, IFGTB, Coimbatore, for finalization of the plant species and other organisms to be included for quarantine screening and safety to non target species from *Quadrastichus mendeli* introduced parasitoid of *Leptocybe invasa* from Israel. The meeting was attended by 19 experts from various organizations in addition to 30 scientists from IFGTB celebrated on the different plants and other organisms to be included in the quarantine screening and finally came out with the following report which was strictly followed and included in the screening schedule for the imported parasitoid screening for safety.

Quadrastichus mendeli, are light yellow wasps belonging to eulophidae, and only females are known. As they were uniparental the same was provided with honey and fresh green galls of the age between 35- 40 days were provided in cages and released for oviposition. The longevity of the adults was recorded and the time taken for the wasps to emerge was also recorded. Adults of the second generation emerged after 39-45 days and were used for studying various safety tests in the quarantine.

Host specificity studies:

The parasites were evaluated for host specificity using a wide range of hosts which are listed below. The parasitoids were released in cages containing different plants and observations were recorded on the behavior of the parasitoids. Any injury caused by the parasitoid was also recorded during the entire longevity of the adults. The plants were kept in cages and observed for a period of 75 days so that any damage on the plants were done by the parasitoid were clearly seen and also for emergence of the same species if they use it as host either by galling or by any other means.

Species selected for safety testing for *Quadarstichus mendeli*

Since a close relative of *Q.mendeli* viz., *Q. erythrinae* is phytophagous attacking species of *Erythrinae* plant species were also included for safety testing of the insect. The list included at least 2 species of Erythrina, some economically important species of plants belonging to Myrtaceae, representatives crop plants from cereals, pulses, oil seeds, fiber, plantation species (particularly export oriented crop) vegetables, fruits, ornamental plants, economically important forest trees, medicinal plants and at least 2 plants from red listed species of sandal and mulberry

Erythrina sp.

1. *Erythrina variegata*
2. *Erythrina strictus.*

Myrtaceae

1. *Syzygium aromaticum* (Clove)
2. *Psidium gujava*
3. *Syzygium cumini*

Forest trees

1. *Melia dubia*
2. *Gmelina arborea*
3. *Butea monosperma*
4. *Mangifera indica*
5. *Anacardium occidentale*
6. *Tamarindus indica*
7. *Phyllanthus emblica*
8. *Terminalica chebula*
9. *Accacia* hybrid
10. *Casuarina equisettifolia*
11. *Morus alba*

Vegetables

1. *Solanum tuberosum*
2. *Lycopersicum esculentum*
3. *Capsicum annum*
4. *Glycine max*

5. *Lagenaria siceraria*
6. *Cicer aerientinum*
7. *Cajanus cajan*
8. *Cucumis sativus*
9. *Solanum melongena*
10. *Citrus aurentifolia*

Oil seeds

1. *Arachis hypogea*
2. *Helianthus annus*
3. *Brassica juncea*

Plantation crops

1. *Camellia sinensis*
2. *Coffea arabica*
3. *Havea braziliensis*

Fibre

4. *Gossipium hirsutus*

Ornamental

Rosa sinensis

Weeds

1. *Chromolaena odorata*

Red listed trees

1. *Santalum album*
2. *Pterocarpus santalinus*

In addition, the following economically important insects will also be included

Insects

1. Honey bees- *Apis ceran indica*
2. Lac insect- *Lassifera lacca*
3. Silk worm- *Bombyx mori.*

Parasitoids

a) Chalcids- *Brachymeria sp*
b) Bethylid- *Goniozus nephantidis*
c) Eulophid- *Tetrastichus shoenobi, Trichospilus pupivorous*
d) Selionid- *Telenomus remus*
e) Trichogramatid- *Trichograma chilonis*

Predator

a) *Chrysoperla zastrowi silimi*
b) Coccinella septumpunctata
c) Crypholaemus montrouzieri

Erythrina gall wasp: *Quadrastichus erithrinae*

The results of the safety to host plants indicated that the parasitoids did not take any of the above mentioned plants or insects or insect parasitoid and predator as hosts. There was absolutely

no parasite induced injury in above said plant and insect species. Thus the eucalyptus gall wasp parasitoid was completely safe to plants and insects.

Safety to selected non target organisms:

Safety of the parasitoids to the following non target beneficial organisms was tested., *Micromus igorotus, Chrysoperla* carnea, *Brumoides* sp *Cryptolaemus* montrouzieri, *Goniozus nephantidis, Trichogramma chilonis, T. japanicum, Bombyx mori, Apis cerana indica, Scymnus coccivora,* and *Spalgis epius.* Five pairs of hosts were exposed to 5 pairs of parasitoids and observed for oviposion and adult emergence by the parasitoids. Observations on the host mortality and other parasite related injury were also recorded.

The results indicated that the parasitoid tested was not harmful to these beneficial insects. There was no probing for oviposition on the different hosts provided and no mortality or injury was observed on all the non target organisms tested proving that the parasite (*Quadrastichus mendeli*) is safe to these non target organisms studied.

Conclusion:

1. The parasitoid imported from Israel (Quadrastichus mendeli) was found to be pure cultures with no hyper parasitoids or other contaminant arthropods.
2. The parasitoid was host specific and did not parasitize or take as host any of the plant and insect species tested.
3. The parasitoid did not parasitize larvae of *Micromus igorotus, Chrysoperla* carnea, *Brumoides* sp *Cryptolaemus* montrouzieri, *Goniozus nephantidis, Trichogramma chilonis, T. japanicum, Bombyx mori, Apis cerana indica, Scymnus coccivora,* and *Spalgis epius.* Thus proving its host specificity. No parasite induced injury was recorded on these natural enemies.

In view of the above the parasitoid Quadrastichus mendeli is considered safe for release in eucalyptus orchards infested with *Leptocybe invasa* and the proposed release sites are as follows.

Limited area release permit for *Quadristichus mendeli*

Locations:

1**. Andhra Pradesha**	Andhra Pradesh Paper Mills.
	ITC- Bhadrachalam
	BiLT
2. **Karnataka**	–WCPM Dhandeli
	-MPM--- Shimoga
3. **Tamil Nadu**	-TNPL-Karur
	-Sheshashayee Paper mills
	-IFGTB- Coimbatore
4. **Orissa**	-JKPM
	-BILT
5. **North**	-Haryana and Punjab
6. **Gujarat**	-J K Paper mills
	-State forest Department
7. **UP**	- Century Paper Mills
8. **Uttaranchal**….	Forest Department

Protocol for release of parasitoids *Quadrastichus mendeli* in the field for biological control of *Leptocybe invasa* causing Eucalyptus galls

1. NBAII, Bangalore has obtained the parasitoides from Israel in 2008 November and 2010 February and multiplied /quarantined including some major species for host specificity.
2. The parasitoids are now ready for release under limited area release; hence we are giving some culture of the parasitoids for further multiplication in the nurseries of respective IPMA member mills/Institutions.
3. I request you to kindly follow the protocol for multiplication of the parasitoids before it is released to fields.
4. Indigenous parasitoids *Megastigmus* species are also multiplied and will be supplied to the requesting persons.

Protocol:

a. **Maintenance of Plants: Eucalyptus plants with galls of *Leptocybe invasa* preferably of 30 - 45days old (Green galls only and avoid pink and reddish galls with emergence holes) which are showing just eye spots should be separated and maintained in a separate block of the nursery. Please do not spray any insecticide or pesticide even botanicals as they hinder the production of parasitoids or may kill the host and parasites.**
b. **We are not recommending plastic mesh or any cover as the emerged insects are found to perch on the screen and oviposition rate was reduced. Normal watering of plants to be undertaken and avoid too much sprinkler water and liquid fertilizer spray. How ever you can provide fertilizer through soil media.**
c. **The parasitoids will be given in a tray of live plants having galls with parasitoids. Keep this tray amidst the plants maintained as in item no. a. For this purpose.**
d. **The seedling trays can be taken in a closed box carefully till they are kept in the nursery.**
e. **Maintain the plants for minimum 45 days so that we can observe for the emergence of the parasitoids from the new plants. Once we observe the adult activity of the parasitoids, we can move the plants for further infestation and spread in the field or desired locations. NBAII will be responsible for starter culture of the parasitoids. Till establishment we will be supplying the needed culture.**
f. **As the parasitoids given are only females and no males are found in them there is no need for collection and mating and re release. Just after emergence of the parasitoids they go in-search of the host and lay eggs.**
g. **Please do not release the given parasitoids directly in field as they may not be sufficient for management in field conditions kindly multiply further and use. If it escapes on its own to field do not bother.**
h. **For further clarification and guidance kindly contact Dr. A.N. Shylesha, Principal scientist NBAII, H.A. Farm Post, Bellary road, Hebbal, Bangalore-560 024. Phone: 080- 2351198. Mobile: 9480424706.**

Mass production of Eucalyptus gall wasp parasitoids (*Q. mendeli* and *Megastigmus* sp)

Classical biological control involves the use of co-evolved natural enemies of the alien pest. Therefore, for the management of the papaya mealybug National Bureau of Agriculturally Important Insects (NBAII) imported three Eulophidae parasitoids *viz*., *Q. mendeli* and *Megastigmus* sp. In order to carry out the mandatory tests of specificity and safety, these parasitoids were multiplied in the qurantine facility of NBAII at Bangalore on the Eucalyptus gall wasp.

Seasonal incidence of eucalyptus gall wasp, *Leptocybe invasa* Fisher and Lasalle

To study the seasonal incidence and relative abundance of insect pests, Eucalyptus (Variety: *E. camaldulensis*) nursery was established at NBAII, Bangalore during September, 2010 to January, 2011. The Eucalyptus crop was raised by following all the recommended package of practices except plant protection measures. Randomly selected ten plants from the nursery incidence of galls recorded separately on top, middle and bottom portion of the sample with a different gall stages recorded during different months is presented in **Table 1.**

The number of galls recorded on top portion of the shoot ranged from 11.50 (November 2010) to 30.75 (January 2011). Middle portion of the sample recorded maximum and minimum gall incidence in June (42.00) and November 2010 (22.75) respectively. Gall incidence at bottom portion of the sample ranged from 21.00 in January, 2011 to 54.75 in June 2010 respectively.

Table 1. Seasonal incidence of eucalyptus gall wasp, *Leptocybe invasa* at Mallur during 2010-2011

Month	Mean no of different stage galls per 10 cm shoot length					
	Top potion					
	I	II	III	IV	V	Total
March -2010	5.00	8.25	4.50	5.00	0.00	22.75
April	6.50	6.50	5.75	3.25	2.75	24.75
May	7.00	7.50	5.00	0.00	0.00	19.50
June	8.00	8.75	4.50	0.00	0.00	21.25
July	9.75	5.50	3.25	0.00	0.00	18.50
August	7.00	6.00	6.75	0.00	0.00	19.75
September	3.88	7.00	8.50	0.00	0.00	19.38
October	4.05	8.25	5.75	0.00	0.00	18.05
November	4.50	3.75	3.25	0.00	0.00	11.50
December	3.25	5.75	5.00	4.00	0.00	18.00
January -2011	4.00	6.00	7.00	6.25	7.50	30.75

February	4.25	5.00	6.50	5.50	0.00	21.25
Mean and STD	5.60±2.01	6.52±1.49	5.48±1.55	2.00±2.57	0.85±2.24	-
Middle potion						
March - 2010	0.00	10.00	4.50	5.75	6.75	27.00
April	5.25	9.00	7.50	7.50	6.25	35.50
May	3.50	9.00	7.25	5.50	6.00	31.25
June	4.25	14.00	8.75	11.25	3.75	42.00
July	2.00	16.25	7.75	8.50	6.00	40.50
August	2.75	9.25	8.00	5.00	4.25	29.25
September	0.00	11.00	11.00	8.50	3.25	33.75
October	0.00	9.00	8.25	10.00	5.75	33.00
November	2.75	6.50	5.75	4.25	3.50	22.75
December	4.75	5.25	15.00	6.50	13.00	44.50
January 2011	1.50	9.50	7.00	8.50	10.50	37.00
February	4.25	7.75	6.00	10.50	8.50	37.00
Mean and STD	2.58±1.90	9.00±2.99	8.06±2.72	7.64±2.27	6.45±2.94	-
Bottom potion						
March 2010	0.25	16.50	8.25	9.50	9.75	44.25
April	0.25	8.00	9.75	7.00	6.25	31.25
May	0.25	8.00	9.50	6.75	7.25	31.75
June	0.25	12.00	17.75	11.75	13.00	54.75
July	0.50	9.50	13.50	12.75	8.75	45.00
August	0.25	12.25	13.00	14.00	11.75	51.25
September	0.00	12.00	8.75	11.75	11.00	43.50
October	0.75	9.00	11.00	14.25	10.25	45.25
November	0.00	6.75	4.75	5.50	6.00	23.00
December	0.25	7.50	6.00	13.25	14.25	41.25
January 2011	0.25	6.75	3.50	4.00	6.50	21.00
February	0.50	9.50	11.00	5.75	7.00	33.75
Mean and STD	0.29±0.21	9.81±2.89	9.73±3.97	9.69±3.71	9.31±2.80	-

The parasitoid was brought in pupal stage with in the galls of eucalyptus and emergence was monitored. Pure culture of the parasitoids emerged were collected and second generation parasitoids were raised using Eucalyptus plant galls of the age of 35-40 days. The plant debris was incinerated using the incinerator facility at the Quarantine laboratory of NBAII. A total of 67 parasitoids were obtained and was used for further studies.

Telegram: PROTECTION

Tel: 0129 2418506
Fax: 0129-2412125

F.No. 99-3/2010-PQD
Government of India
Ministry of Agriculture
(Department of Agriculture & Cooperation)
DIRECTORATE OF PLANT PROTECTION, QUARANTINE & STORAGE
NH IV, FARIDABAD – 121 001 (Haryana)

Dated : 29.8.2011

To

The Director,
National Bureau of Agriculturally Important Insects,
P.O. Box 2491, H.A. Farm Post, Bellary Road,
Hebbal, Bangalore-560024 (Karnataka)

SUB: Permission for limited area release of *Quadraistichus mendeli*

***

Sir,

Please refer to your letter no. NBAII/PME-63/2010-11/2581 dated 28th June, 2011 on the above cited subject. On the recommendations of members of the Expert Committee, Director, National Bureau of Agriculturally Important Insects, Bangalore is hereby granted permission for field release of ***Quadraistichus mendeli*** at following selected locations in the states of Andhra Pradesh ,Karnataka, Tamilnadu,, Orissa , North India , Gujrat, UP and Uttaranchal :

1. Andhra Pradesh : Andhra Pradesh Paper Mills, ITC- Bhadracahalam
2. Karnataka : WCPM Dhandeli, MPM –Shimoga
3. Tamilnadu : TNPL- Karur, Shashashayee Paper Mills, IFGTB- Coimbatore
4. Orissa : JKPM, BILT
5. North : Haryana and Punjab
6. Gujrat JK Paper Mills, State Forest Department
7. UP Century Paper Mills
8. Uttaranchal Forest Department

It is advised to provide post release observations/results of such releases to this Directorate.

Yours faithfully,

(K.C. Gupta)
Joint Director (PP)
for Plant Protection Adviser

Important document
PMG / Dr. Shylesha / PS
8/9

Emergence of *Leptocybe invasa* and local parasitoid (*Megastigmus viggiani*) in sample collected from different locations

A roving survey was conducted in different parts of Karnataka *viz.* Tumkur, Kanakapura, Nelamangala, Kolar, Devanhalli, Chikkaballapur, Mandya, Mysore, Doddaballapur, Hoskote and Mallur were surveyed twice in a month. Infested eucalyptus plant material was collected at random interval and the observations were recorded intensity of gall development on number and stage of the galls on 30 cm length of plant sample separately from top, middle and bottom portion (10 cm each). The gall stages recorded were as per the description given by Mendel *et al*. (2004). Mean and standard deviation of stage galls was worked out. The plant sample was kept for adult emergence in a pin holed polythene bags. The adult emergence of the pest and parasitoids was recorded at three days interval till the cessation emergence. Apart from the general emergence recorded above, samples from few places were separately maintained to generate additional data on parasitoid emergence. Parasitization per cent was worked out using the following formula (Kim *et al*., 2008).

$$\text{Per cent parasitization} = \frac{\text{No. of parasitoid adults emerged}}{\text{Total no. of adults (gall wasps + parasitoids)}} \times 100$$

The samples collected from different locations emergence of pest and parasitoid in the month of June 2010 and December, 2010. Differential distribution of the pest *L. invasa* adults that from different samples ranged from 126 (Mallur) to 60 (Mysore) on june 2010 and 145 (Hoskote) to 71 (Mysore) from December, 2010 respectively. The parasitoid, *Megastigmus viggainii* recorded from sixteen locations ranged from 41 (Doddaballapur) to 23 (Tumkur) on June 2010 and 47 (Nelmangla) to 22 (Dovaspete) from December, 2010 respectively. The per cent parasitization among different locations was recorded Mysore (31.82%) followed by Mandya (30.97 %) in on June 2010 and Tumkur (29.55) followed by Kankapura (29.17 %) from December, 2010 respectively (Table 2).

Table 2. Emergence of *Leptocybe invasa* and local parasitoid (*Megastigmus viggiani*) in sample collected from different locations

Sl No	Locations	Emergence- June, 2010			Emergence- December , 2010		
		L I	M V	% Parasitization	L I	M V	% Parasitization
1	Tumkur	104	23	18.11	93	39	29.55
2	Kanakapura	89	34	27.64	102	42	29.17
3	Nelmangala	98	31	24.03	120	47	28.14
4	Kolar	120	29	19.46	135	29	17.68
5	Devenhalli	113	37	24.67	141	31	18.02
6	Chikkaballapur	123	40	24.54	131	27	17.09
7	Mandya	78	35	30.97	84	34	28.81
8	Mysore	60	28	31.82	71	29	29.00
9	Doddaballapur	109	41	27.33	123	37	23.13
10	Hoskote	121	37	23.42	145	41	22.04
11	Mallur	126	29	18.71	140	33	19.08
12	GKVK	97	26	21.14	102	29	22.14
13	Rajankuntte	103	37	26.43	121	23	15.97
14	Kanakapura	91	30	24.79	98	31	24.03
15	Ramanagar	78	25	24.27	85	27	24.11
16	Dovaspete	107	31	22.46	132	22	14.29
Mean		101.06	32.06	24.36	113.94	32.56	22.64

Plant height (cm) after release of *Quadrastichus mendeli* on eucalyptus gall was under laboratory condition

Infested seedlings kept in glass cage were subjected to parasitization for 48 hr. ten adults were released into the cage for oviposition. Later ten plants kept in green house for further observation. Ten plants was selected and counted the number of gall on the plants and plant height was before and after release of parasitoid, *Q. mendeli* and also plant height recorded.

The results obtained from study before release of parasitoid plant height and number of galls ranges from 20-28 cm and 91-151 galls respectively. Similarly after release of parasitoid plant height and number of galls range from 120-178 cm and 06 -30 galls respectively **(Table 3).**

Table 3. Plant height (cm) after release of *Quadrastichus mendeli* on eucalyptus gall wasp under laboratory condition

No. of plants	Plant Height (cm)	No. of gall/plant	After release of parasitoid plant height	After release of parasitoid No. of gall/ plant
1	22	151	178	30
2	26	145	170	14
3	20	91	155	27

4	28	102	120	06
5	21	96	135	19
6	23	130	141	09
7	23	121	139	22
8	25	109	143	11
9	28	98	127	09
10	28	113	130	20

Per cent parasitization of *Quadristicus mendali* on Eucalyptus gall wasp, *Leptocybe invasa*

Five groups of five to ten plants an 18-month-old saplings were exposed to one-day-old adult *L. invasa* in ventilated glass cages for 24 h. Ten gall wasps per sapling were introduced into each cage. After gall develop counting the number of gall on the leaf midrib, petiole and stem of the plant. Five pairs of *Q. menadli* were then introduced for 24 h into each cage. The test was conducted in a ventilated greenhouse with temperature ranging from 25^0C and relative humidity from 75%. After 35 days the gall growths were removed and placed in transparent glass cages in order to monitor the emergence of the wasps. Percentage mortality was calculated from the number of emerging parasitoids divided by the total number of emerging wasps (gall inducers + parasitoids) for each cage.

$$\text{Per cent parasitization} = \frac{\text{No. of parasitoid adults emerged}}{\text{Total no. of adults (gall wasps + parasitoids)}} \times 100$$

Plants	No of galls	No of parasitoids released	No. of parasitoid adults emerged	% Parasitism
1	68	10	47	81.74
2	105	10	86	90.05
3	71	10	63	94.03
4	65	10	57	93.44
5	79	10	61	87.14
6	76	10	57	85.71
7	63	10	51	89.47
8	68	10	47	81.74
9	70	10	58	90.63

10	71	10	57	89.06

Table 7. Green house study of per cent parasitization of *Q. mendeli* against eucalyptus gall wasp

Per cent parasitization of *Q. mendeli* ranges from 94.03 to 81.74 per cent (Table 7)

Host specificity studies on *Q. mendali*

The parasites were evaluated for host specificity using nine different types of crops *viz*., *Rosa simensis, Tamarindus indica, Butea monosperma, Syzygium cumini, Havea braziliensis, Anocardium accidenalis, Acacia hybrid, Citrus aurantifolia* and *Coffea Arabica* common in India. Second generation pure cultures of the parasitoids were used for screening. Five pairs of parasitoids were released and three replications were maintained. The parasitoids were observed for symptoms of gall formation if any from 6-7th day onwards of release of parasitoids till 45-50th day.

The result reviewed that all the plants showing zero gall development.

Table 4. Host specificity/preference study on eucalyptus gall wasp parasitoids (*Quadrastichus mendeli* and *Megastigmus viggianii)*

Host	*Quadrastichus mendeli*	*Megastigmus viggianii*	No of galls on plants
Rosa simensis	**10**	**10**	**0.00**
Tamarindus indica	**10**	**10**	**0.00**
Butea monosperma	**10**	**10**	**0.00**
Syzygium cumini	**10**	**10**	**0.00**
Havea braziliensis	**10**	**10**	**0.00**

Sl.	Month	*Quadrastichus*	*Megastigmus viggiani* (Mean of ten sweep
Anocardium accidenalis	**10**	**10**	**0.00**
Acacia hybrid	**10**	**10**	**0.00**
Citrus aurantifolia	**10**	**10**	**0.00**
Coffea arabica	**10**	**10**	**0.00**
Eucalyptus sp.(control)	**10**	**10**	**78.0**

Distribution of Eucalyptus gall wasp parasitoid and plant materials to Different paper mills

Imported parsitoid, *Q. mendeli* were distributed below different paper mill association in India.

Sl.No.	Name of the Member mill	Adults supplied	Plants no. Of trays
1	ITC Bhadrachalam	10	4 trays
2	JK paper mills Gujarath	10	4 trays
3	TNPL Karoor.	10	6 trays
4	ICFRE(IFGTB)	10	4 trays
5	West coast paper mills	10	6 trays
6	Andra Pradesh Paper mills	10	2 trays
7	Mysore paper mills	10	……..
8	Sheshashayee paper mills	15	4 trays
9	BILT tech Jeypore	10	4 trays
10	Star Paper mills	10	4 trays
11	UAS Dharwad	10	……
12	Harihar poly fibers	10	…….
13	JK paper mills Gujarath	40	5plants
14	Paralal, punjab	120	60 plants
15	Pujab	25	-

Table 8. Distribution of Eucalyptus gall wasp parasitoid and plant materials to Different paper mills

Emergence of eucalyptus gall wasp parasitoid in nursery

Eucalyptus plants were raised in nursery at NBAII, Hebbal, Bangalore the imported (*Q. mendeli*) and local parasitoid (*Megastigmus* sp.) were released on infested plants in open condition. Ten plants were randomly pruned during May and June 2010 and kept for adult emergence in a bulk. Per cent parasitization was calculated as mentioned above.

No		*mendeli* (Mean of ten sweep net)	net)	
			F	M
1	May-2010	11.08±2.13	7.96±1.42	3.76±1.28
2	June	14.60±3.60	7.64±2.97	3.16±1.86

Table 6.

Emergence of eucalyptus gall wasp parasitoids in nursery after release of parasitoids

The results showed that number of *Q. mendeli* and *M. viggainii* male and female adults were sweeped in the insect collection net in the months of May and June2010 the Q. mendeli 11.08±2.13and14.60±3.60. Similarly both male (3.76±1.28) and female (7.96±1.42) activity of M. viggianii May 2010 **(Table 6).**

Release of imported parasitoid, *Qudristichus mendali* against eucalyptus gall wasp at different locations

Location	No of Parasitoids
Tumkur	**120**
Kanakapura	**80**
Nelmangala	**90**
Kolar	**130**
Devenhalli	**100**
Chikkaballapur	**100**
Mandya	**70**
Mysore	**60**
Doddaballapur	**80**
Hoskote	**120**
Mallur	**90**
GKVK	**100**
Rajankuntte	**110**
Kanakapura	**80**
Ramanagar	**85**
Dovaspete	**90**

Molecular characterization and Identification of eucalyptus gall wasp, *Leptocybe invasa* and their parasitoids (*Quadristichus mendeli and Megastimus viggianii*)

The live adult pest and parasitoids of *Leptocybe invasa* (Plate 1 & 2) *Megastimus viggianii* (Plate 3) *and Quadristichus mendeli* (Plate 4) was freezed killed at –80°C and transferred to an Eppendoff

tube and homogenization was done by crushing the adult in 20µl of 5% Chelex 100 MB (BIO–RAD) (Walsh *et al*., 1991). This was followed by incubation for 3 h at 56°C and thenat 100ºC for 10 min. PCR was performed with a total reaction mixture of 50 µl consisting of 10x Taq buffer (complete), 10mM dNTP mix, universal primer HCO1–2198 (20pm/µl), LCO1–1490(20pm/ µl) (Folmer *et al*., 1994), template DNA (50ng), Taq polymerase (1U/ µl) and sterile water. The DNA extracted was amplified under the following conditions: Initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation (94° C for 1 min), annealing (54°C for 1 min), extension (72°C for 1 min) and a final extension step at 72°C for 10 min. The PCR amplification was performed in a thermal cycler (Bio-Rad).

CO1 (556, 518 and 530bp) region of *L. invasa. M. viggianii and Q. mendeli* from Mallur and nursery were visualized on 1.8% gel (Fig. 2) with a low range ladder (Fermentas Mass Ruler 1000bp). The PCR products were purified with MinElute PCR purification kit (Qiagen). The PCR product was sequenced using an ABI prism 310 DNA sequencer by Big Dye Terminator reaction. The sequence was edited by BioEdit software and aligned using BLAST2 and verified. The DNA sequence of *L. invasa. M. viggianii and Q. mendeli* (Mallur and) was submitted to GenBank. The BLAST2 (www.ncbi.nlm.nih.gov) and BioEdit tools were used to find the similarity between the two populations for the conserved CO1 region. The study revealed that the L1: female *L. invasa* (556 bp), L2: male *L. invasa* (556 bp); M3: female *M. viggianii* (518 bp), M4: male *M. viggianii* (518 bp) and Q1; *Q.mendeli* (530 bp) Nursery sample Q2; *Q.mendeli* (530 bp) populations from Mallur are one and the same. The barcode developed for *L. invasa. M. viggianii and Q. mendeli* is a diagnostic tool for the identification of the parasitoid. The CO1 gene has proved to be suitable for species identification in a large range of animal taxa, including butterflies and moths (Hebert *et al*., 2004; Burns *et al*., 2008), spiders (Greenstone *et al*., 2005), mosquitoes (Kumar *et al*., 2007) and wasps (Smith *et al*., 2008).

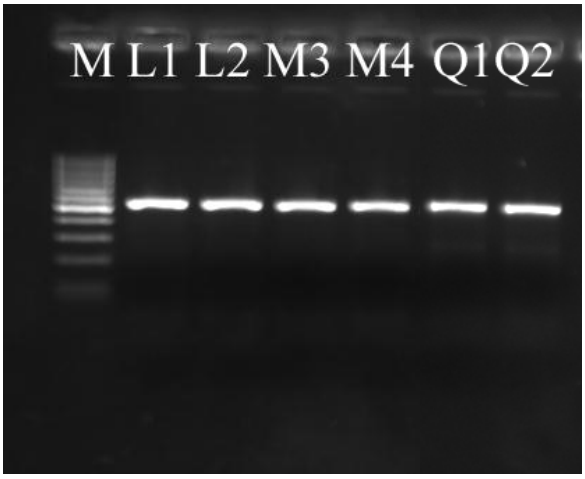


Fig.2 PCR amplification of CO1 region of *Leptocybe invasa*, *Megastigmus viggianii* and *Quadristichus mendeli* (M: 50bp Ladder, L1: female *L. invasa* (556 bp) Mallur, L2: male *L. invasa* (556 bp) Mallur, M3: female *M. viggianii* (518 bp) Mallur, M4: male *M. viggianii* (518 bp) Mallur and Q1; *Q.mendeli* (530 bp) Nursery sample Q2; *Q.mendeli* (530 bp) field sample from Mallur.

Plate 1. Adult male

Plate 2. Adult female

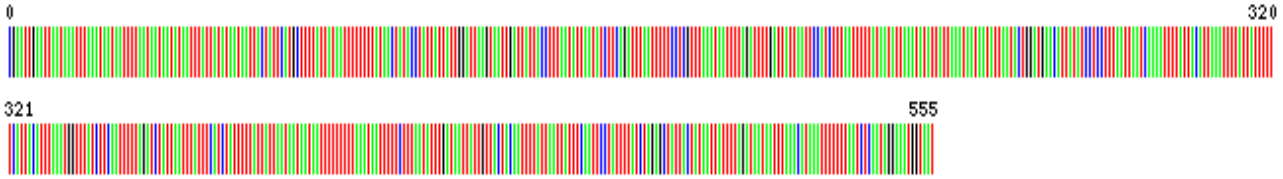
Plate 3. *Megastigmus viggiani*

Plate 4. *Quadrastichus mendeli*

***Leptocybe invasa* (556 bp) Hymenoptera: Eulophidae: Tetrastichinae**

CGAATTGAATTAATAAATTTAAATAATAATTTTAATAATAATATAATTTATTATATAAT
AATTACTATTCATGCTTTATTATAATTTTTTTTATAACTATACCTATTATTATTGGATTA
AGTAATTGATTATTACCTTTAATATTAATATCTTCAGATTTAATTTTTCCTCGTTTAAAT
AATTTTAGATTTTGATTATTAATTCCATCTTTAATTTTTATAATATTAAATATATTATTA
AATAATAATATTAATACTGGATGAACATTATATCCTCCTTTAATTAATCAAAATTTTAT
TACATTAAATTTTATTATTTTTTCATTACATTTAAATGGTTTATCTTCAATTTTTAGATC
TATTAATTTATTTCATCTATTTTTATTATTAATAATAATAATTTTTTTTTTAAATAATTTTT
CTTTAATATTTGATAATTATTGTTACTACAATTTTATTAATTATTTCAATTCCTATTTTA
TCTAGAGCTATTACTATAATTATTTTAGATAATAATTTAAACATAAATTTTTTTAATCT
CAATAGGAAATGGTAAT

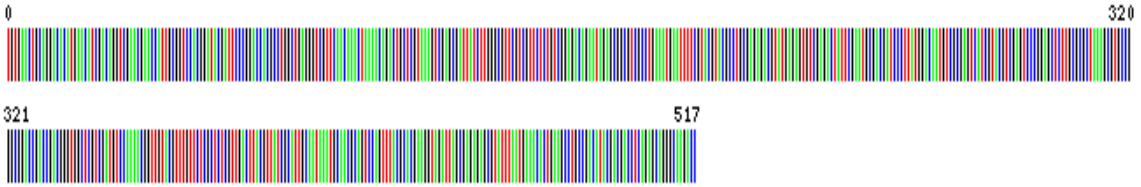
DNA Barcode



***Megastigmus viggiani* (518 bp) Hymenoptera: Eulophidae: Tetrastichinae**

TGTGAACTGCAGGACACATGAACATCGACAGGTCGAACGAACCATTGCGGTCCACGG
ATACGATTCCCGGACCACGCCTGGCTGAGGGTCGTTCAACAAACTAAAACAGACTGCT
CGTAAAGTCGAGCGATTATCTTGGGCGTTCGTCGTTCTCTGTCGGAGACAGAATAGAG
CGGCGTCGCCTGAAATGAATTTTCGTACGTACGCCTGCGAGAGACGTATGAGAGTGTC
GAGACTATTCGAATGCGACGATCCCTTATGGACAATGTCGCGAGTCATCGTACGTCGA
GTCCCGGAGCTCTTGCGCGCTAAAGCGTGCCGGGCGGACCGACCGACGGGTGGCTCGT
CGATGTCCAAAACGGTTGTACGTTTGTTCTCGCTCTCGTTGACTTACGTTATCGCATTG
CAATAAATGCAACGCAGTCCAGTTTACGCACGAAGGTAGATTAGCGAGAGAGCGTAT
TTAATGAAACACTGAAGCCTGCCGACATACGACGACCTCAGAGCAGGGCAAGACC

DNA Barcode



***Quadrastichus mendeli* Kim & La Salle (530 bp) Hymenoptera: Eulophidae: Tetrastichinae**

TGTGAACTGCAGGACACATGTGAACATCGACAGTGCGAACGAACCATTGCGGTCCTCG
GATACGATTCCCGGACCACGCCTGGCTGAGGGTCGTTCATAAGATAAAACCAGACTGC
TCGTCTCTTTGACGAGCGTACGCTTGAGCGCTCGTCGAGTTGTTGTTGTTGTTGTC
GTCCGTGATGATTTTTTTTTTTATCCGACGACGCACTCCTCGGTGTCGCTCTAAATGAA
TCAAACGACTTGTCGAACGTTGCGAAAACGAACGAAAGTCAAAGTCGTACGCACACG
CAATTTCAAAAGCGAAGCGTCTCTCACGTGCGTCGTACGTCAAGTCCCGGAGCTCTTG
TGGTCGCGCTTTCGGCGCGCCCCGAGCGGACCGACGTCTTCCTCGTGAGGTATGACGC
GTTCGCGCGTGCCTCTGCCCTTGACAACTGATTGTTCGGATTTCATTTGAAAGGCTCGT
TCGTGCAAATGGTATTATGTATAATTCTATTATATAACGACAACCTCAAAGCAGGGCA
AAACCA

DNA Barcode