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# Biological control of Eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) by its native parasitiods

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**ABSTRACT:** Utilization of native parasitoids *viz. Megastigmus dharwadicus* Narendran and Vastrad (Hymenoptera: Torymidae) and *Aprostocetus gala* Walker (Hymenoptera: Eulophidae) for biological control of eucalyptus gall wasp, *Leptocybe invasa* Fisher and LaSalle (Hymenoptera: Eulophidae) is reported. Two native parasitoids multiplied in the greenhouse were released in a severely affected eucalyptus plantation spread over an area of 1000 ha. A total of 14,000 heavily parasitized galled seedlings, 1400 *M. dharwadicus* and 300 *A. gala* were distributed over a period of six months. The per cent parasitization by these native parasitoids was ascertained before distribution of galled seedlings. Though there was a gradual increase in per cent parasitization, the reduction in gall incidence was not evident up to three months. However, drastic reduction in gall incidence and pest emergence accompanied by very high per cent parasitization was evident within eight months. Post release evaluation conducted during June 2011 and May 2012 indicated the successful control of the pest. 

**Key Words:** Biological control, Native parasitoids, Eucalyptus gall wasp, *Leptocybe invasa, Megastigmus dharwadicus, Aprostocetus gala*

## INTRODUCTION

Since its first report from the Middle East during 2000, the Eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle has rapidly spread to other areas throughout the world (Aquino *et al.,* 2011; Aytar, 2006; Branco *et al.,* 2006; Dhahri *et al.,* 2010; Gaskill *et al.,* 2009; Karunaratne *et al.,* 2010; Kim *et al.,* 2008; Mendel *et al.,* 2004; Mutitu, 2003; Nyeko, 2005; **Wiley, 2008).** In India it was first reported during 2001 from Karnataka (Anon, 2007) which subsequently

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spread to the neighboring states (ICFRE News letter, 2007; Jacob *et al.,* 2007; Kumar *et al.,* 2007). Recently the pest has expanded its range to northern India (Anon, 2011). The pest causes galls on midribs, petioles and stems of new shoots of eucalyptus. Heavy infestation leads to deformed leaves, shoots and reduction in growth. Invasive gall wasp has become a major constraint in eucalyptus production threatening an estimated eight million hectares of plantation affecting the productivity of paper and rayon industry due to raw material shortage. In Karnataka, the gall wasp was reported to be on an attacking spree and damaged 25 lakh eucalyptus saplings in the nurseries of two major wood based industries (West Coast Paper Mills and Harihara Polyfibres) (Anon, 2007). Three lakh grown up trees were severely affected by *L. invasa* in Punjab (Anon, 2011).

Even after a decade of its existence no effective control measures exist to manage *L. invasa*. Classical biological control has long been considered an environmentally benign approach to controlling invasive pests. In recent years, however, several studies have shown that some biological control agents have deleterious effects on native non-target species (Louda *et al.,* 2003; Samways, 1997). Michaud (2002) opined that classical introductions are increasingly used as a first line of defence against invasive pests regardless of the need. He concluded that the application of the classical approach as an automatic response to every new pest underestimate the potential resilience of native ecosystems to pest invasions. The indiscriminate use of classical approach is environmentally irresponsible as it exposes the ecosystems to an undue risk of non-target effects, including the potential disruption of biological control systems already in place. In the light of increasing evidence of non-target host use and resultant threat to native biodiversity associated with it, the classical biological control needs to be weighed carefully since many exotic species have been released without considering the use of native species (van Lantern *et al.,* 2006). Further, successful fortuitous biological control of introduced pests (Gautam *et al.,* 2009; Michaud, 2002; Nechols, 2002; Rose and Bebach, 1991-92; Shrestha *et al.,* 2010) may render an ill conceived classical approach redundant resulting in waste of time and money. Australian species of *Megastigmus* associated with eucalyptus has been recently reviewed with description of new species (Doganlar and Hassan, 2010). *Megastigmus* sp. and *Aprostocetus gala* Walker were found parasitizing *L. invasa* in India (Vastrad *et al.,* 2010). This native *Megastigmus* sp. parasitizing the invasive eucalyptus gall wasp was later described as *M. dharwadicus* Narendran and Vastrad (Narendran *et al.,* 2010). These native parasitoids were successfully mass multiplied and used to manage gall wasp under green house conditions (Kulkarni *et al.,* 2010). This paper highlights the utilization of native parasitoids to manage the invasive eucalyptus gall wasp in a large monoculture eucalyptus plantation spread over 1000 ha severely affected by the invasive eucalyptus gall wasp since 2007 (Fig 1).

## MATERIALS AND METHODS

### Mass multiplication

Heavily galled seedlings supplied by The West Coast Paper Mills nursery were used for the mass multiplication. Parasitoids that emerged from eucalyptus samples collected during the routine survey were released on six month old galled seedlings kept in the green house for mass multiplication following the methodology of Kulkarni *et al.* (2010). A total of 2305 *M. dharwadicus* and 82 *A. gala* were released in the green house between July 2010 and February 2011 (Table 1). Seedlings were kept in the green house for a minimum of 45 days before they were distributed in the plantation. Parasitized galled seedlings and the adult parasitoids collected from the green house were used for field release. Before the field release the extent of parasitization was ascertained from 25 randomly selected seedlings as described by Kim *et al.* (2008).

**Table 1: Parasitoids released in the greenhouse for mass multiplication**

| Months | *Megastigmus dharwadicus* | *Aprostocetus gala* | Total |
|---|---|---|---|
| July 2010 | 845 | 00 | 845 |
| August | 201 | 00 | 201 |
| September | 206 | 00 | 206 |
| October | 624 | 00 | 624 |
| November | 153 | 39 | 192 |
| December | 71 | 26 | 97 |
| January 2011 | 07 | 17 | 24 |
| February | 198 | 00 | 198 |
| **Total** | **2305** | **82** | **2387** |

**Field release**

The release site belonging to The West Coast Paper Mills, Dandeli consisted of 2-6 year old clones mostly derived from *Eucalyptus tereticornis* spread over an area of 1000 ha in Kulwalli village (located between 15$^{o}$ 32' 07.57" and 15$^{o}$ 34' 06.52" N, 74$^{o}$ 47' 34.04" and 74$^{o}$ 47' 50.51 E) (Fig 1). A total of 14,000 parasitized galled seedlings were distributed at 20 randomly selected spots between September 2010 and March 2011. In addition, 1400 *M. dharwadicus* and 300 *A. gala* collected from the greenhouse were released in the centre of the plantation during October and November 2010 (Table 2). The impact of field release on gall incidence and per cent parasitization was recorded over a period of nine months. Galled samples were randomly collected from four locations on the day on which field release were made. In each location 30

**Table 2: Number of parasitized galled seedling distributed and adult parasitoids released at Kulwalli during 2010-11**

| Month | No. of parasitized alled seedlings | Adult emergence from 25 randomly selected galled seedlings | | | Parasitization (%) recorded on galled seedlings before distribution | | | No. of parasitoids released | |
|---|---|---|---|---|---|---|---|---|---|
| | | L | M | A | M | A | Total | M | A |
| 22 September 2010 | 500 | 75 | 108 | 38 | 48.86 | 17.19 | 66.05 | - | - |
| 7 October 2010 | 12,500 | 17 | 100 | 04 | 82.64 | 3.30 | 85.94 | 860 | 250 |
| 6 November 2010 | 500 | 19 | 34 | 31 | 40.47 | 36.90 | 77.37 | 540 | 50 |
| 7 March 2011 | 500 | 41 | 35 | 65 | 24.82 | 46.09 | 70.91 | - | - |
| **Total** | **14,000** | **152** | **277** | **138** | **48.85** | **24.33** | **73.19** | **1400** | **300** |

L-*Leptocybe invasa,* M-*Megastigmus dharwadicus,* A-*Aprostocetus gala*

**Table 3: Gall incidence and per cent parasitization recorded at Kulwalli following the field release of parasitoids**

| On the day of first release (September 2010) | | | | Second release (October 2010) | | | | Third release (November 2010) | | | | Fourth release (March 2011) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of galls/ 30 cm shoot | Per cent parasitization | | | Number of galls/ 30 cm shoot | Per cent parasitization | | | Number of galls/ 30 cm shoot | Per cent parasitization | | | Number of galls/ 30 cm shoot | Per cent parasitization | | |
| | M | A | Total | | M | A | Total | | M | A | Total | | M | A | Total |
| Top portion of the sample | | | | | | | | | | | | | | | |
| 9.80 ± 1.66 | 0.00 | 0.00 | 0.00 | 13.80 ± 1.32 | 0.00 | 0.00 | 0.00 | 9.80 ± 1.66 | 0.00 | 0.00 | 0.00 | 2.30± 1.30 | 0.00 | 0.00 | 0.00 |
| Middle portion of the sample | | | | | | | | | | | | | | | |
| 7.80 ± 1.40 | 47.61 | 0.00 | 47.61 | 7.80 ± 1.60 | 31.81 | 0.00 | 31.81 | 7.80 ± 1.40 | 47.61 | 0.00 | 47.61 | 3.10± 2.15 | 90.09 | 4.95 | 95.04 |
| 4.60± 1.35 | 35.00 | 0.00 | 35.00 | 5.20 ± 1.16 | 58.33 | 0.00 | 58.33 | 4.60 ± 1.35 | 65.00 | 0.00 | 65.00 | 1.40± 1.01 | 0.00 | 0.00 | 0.00 |
| Cumulative | | | | | | | | | | | | | | | |
| 16.50± 1.66* | 42.86 | 0.00 | 42.86 | 15.40 ± 2.85 | 48.27 | 0.00 | 48.27 | 16.30± 2.51 | 53.12 | 0.00 | 53.12 | 4.55± 3.35 | 90.09 | 4.95 | 95.04 |

M-*Megastigmus dharwadicus,* A-*Aprostocetus gala* centimetre apical shoots from ten eucalyptus plants were randomly collected. The samples were equally divided into top, middle and bottom portion (10 cm each). Different gall stages were recorded on each sample as described by Mendel *et al.* (2004). Based on the number of gall stages recorded, mean gall incidence was worked out for each spot from thirty samples. Later the samples were kept in pin holed polythene bags for pest and parasitoid emergence. Adult emergence of the pest and parasitoids was recorded daily till the cessation of adult emergence. The per cent parasitization was worked out as mentioned in the section 2.1.

## RESULTS

### Gall incidence and per cent parasitization

### On the day of first release

Before the release of parasitoids, mean gall incidence was 16.50 galls/30 cm shoot. Top portion harboured more number of galls (9.80 galls/10 cm) than middle and bottom portions (7.80 and 4.60 galls/30 cm shoot, respectively). Similarly, parasitization by *M. dharwadicus* was 35.00 and 47.61 per cent on bottom and middle portion respectively. During all the observations no parasitization was recorded on top portion of the sample. Total parasitization recorded on the entire sample was 42.86 per cent. Parasitization by *A. gala* was not noticed (Table 3).

### Second and third release

No appreciable reduction in the total number of galls was evident during second and third release (15.40 and 16.30 galls/30 cm shoot, respectively). While the number of galls on the middle and bottom portion of the sample remained unchanged, slight increase in gall incidence was noticed on top portion of the sample on the day of second release. Parasitization by *A. gala* was not recorded during the period. Gradual increase in overall per cent parasitization from 42.86 (on the day of first release) to 53.12 was recorded on the day of third release. Similar trend was noticed on the bottom sample. However, about 16.00 per cent decline in parasitization over previous release was noticed on the middle portion of the sample on the day of second release (Table 3).

### Fourth release

Impact of field release of parasitoids was clearly evident as indicated by the drastic reduction in the number of galls (4.55 galls/30 cm shoot) and substantial increase in the per cent parasitization. Parasitization due to *M. dharwadicus* and *A. gala* was 90.09 and 4.95 per cent respectively, accounting for 95.04 per cent total parasitization. Effect of such high levels of parasitization resulted total elimination of the gall incidence and as a result no parasitization was recorded three months after fourth release. Since June 2011, no gall wasp incidence and pest and parasitoid emergence has been noticed in the study area.

While overall gall incidence did not change during first three months, gradual decline in pest emergence following field release was clearly evident. Large scale field release on 7 October 2010 resulted in highest recovery of adult *M. dharwadicus* during March 2011. *Aprostocetus gala* not encountered during the previous observations was also recovered in March 2011 (Fig 2). The true impact of field release within six months was indicated by the drastic reduction in the number of galls and adult emergence coupled with significant increase in per cent parasitization. Among the native parasitoids used for the biological control of the gall wasp *M. dharwadicus* was the most dominant.

**Post release evaluation**

Very few galled plants containing late stage galls were observed during the survey conducted in May 2012. Fresh oviposition damage and early stage galls were conspicuously absent. A single female *M. dharwadicus* emerged from these samples. Emergence of pest was not noticed indicating the effectiveness of native parasitoids one year after the last field release (Plate 1).

## DISCUSSION

Classical biological control has been a preferred approach for management of alien insects. However, concerns have been raised about the risks of classical biological control (Howarth, 1991; Samways, 1997; Louda *et al.,* 2003; van Lantern *et al.,* 2006). In the light of concern raised about the risks of classical biological control, present investigations reinforce the utility of native natural enemies to manage invasive pests. The literature is replete with many examples of native parasitoids exploiting the exotic hosts (Aebi *et al.,* 2006; Cooper & Rieske, 2007; La Salle & Pena, 1997). The recently described *M. dharwadicus* has emerged as an efficient parasitoid of *L. invasa* to combat its spread. Contrary to the reported inefficiency of *Megastigmus* spp against *L. invasa* (Protasov *et. al*., 2008) the present findings have conclusively established the effectiveness of native *M. dharwadicus* in combating the menace of the invasive eucalyptus gall wasp. However, classical biological control of the pest has been successfully attempted elsewhere (Kim *et al.,* 2008).

Among the two parasitoids released, *M. dharwadicus* was the most dominant and mainly responsible for reduction in pest incidence. The impact of field release was evident within a couple of months. During the initial period though the decline in gall incidence was negligible, the par cent parasitization increased from 42.86 to 53.12 per cent. The true impact of parasitoid release was clearly evident three months after third release as indicated by substantial reduction in the number of galls (4.55 galls/30 cm shoot) coupled with significant increase in per cent parasitization (95.04%). Faster turn over of parasitoid generation (~ 45 days) compared to the pest (~120 days) has contributed to the overwhelming of the pest by the parasitoids. By the end of June 2011 no fresh oviposition damage and gall incidence was noticed resulting in spectacular control of the pest. While the present study highlights a massive host shift by newly described *M. dharwadicus*, the question of its local host still remains unresolved. However, the other parasitoid used in the study *viz. A. gala* has been reported on *Stenodiplosis*

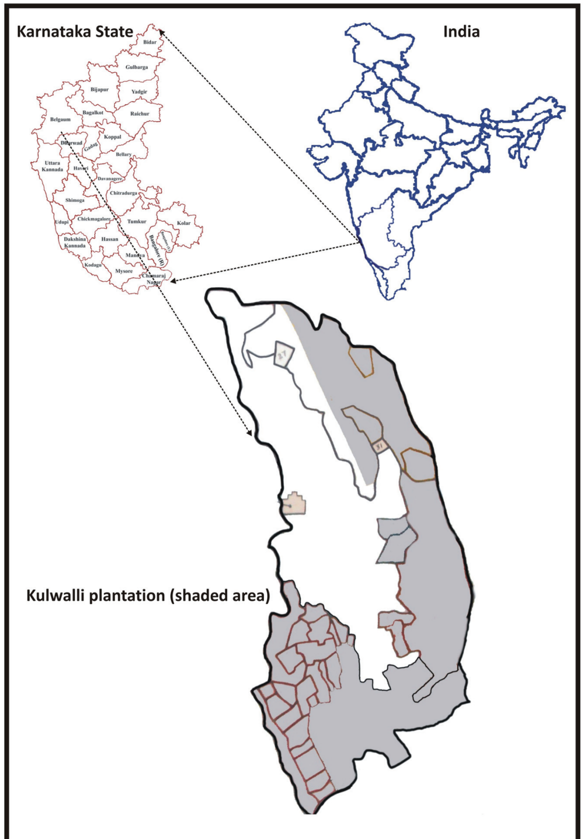


**Fig. 1: Site map of study area**

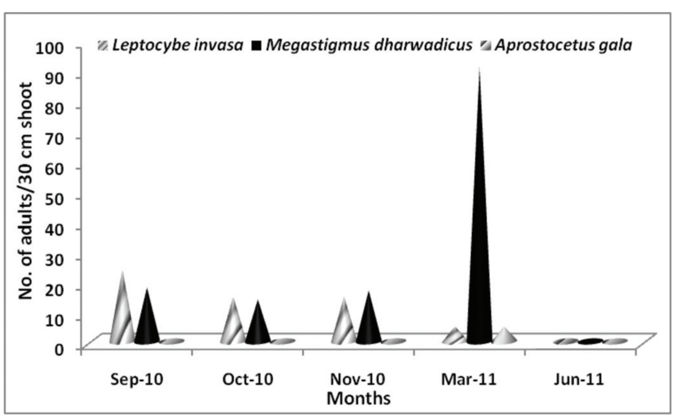


**Fig 2: Emergence of the pest and parasitoid adults during 2010-2011**

Severely infested eucalyptus plantation at Kulwalli during 2007

Recovery of plantation at Kulwalli during 2012

Plate 1: Impact of native parasitoids on invasive eucalyptus gall wasp

*sorghicola* and *Diaprepes abbreviatus* (Hall *et al.,* 2001; Nwanze *et al.,* 1998) and we have recovered it from the galls on *Xylia xylocarpa* Roxb. and *Pongamia glabra* Vent.

Though the native parasitoids were recorded as early as 2008, their impact on gall incidence was not discernible. Despite 42.86 per cent parasitization recorded in the beginning of the study and gall incidence still remained high (16.50 galls/30 cm shoot) three months after the first release. The augmentative biological control through repeated field releases of parasitoids resulted in successful control of the pest. Post release evaluation conducted during May 2012 revealed that there has been no resurgence of the pest one year after the last field release. The present study is a rare example of the native parasitoids halting the ravages of an invasion resulting in substantial financial savings on control measures and avoided large scale negative environmental impact due to use of insecticides. It also highlights the importance of considering the use of native parasitoids before embarking on classical biological control.

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