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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. © 2015 Association for Advancement of Entomology

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° 32' 07.57" and 15° 34' 06.52" N, 74° 47' 34.04" and 74° 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*

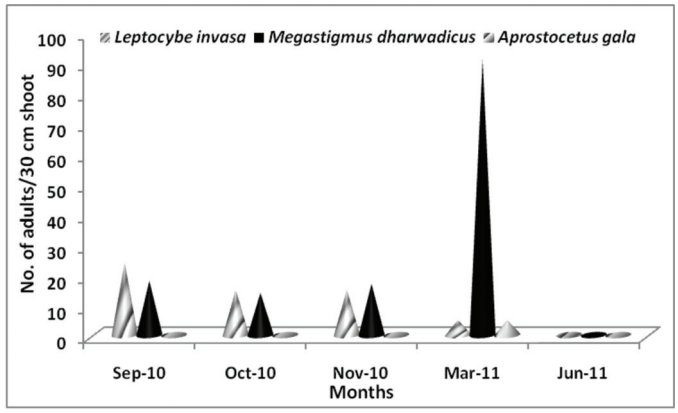

Fig 2: Emergence of the pest and parasitoid adults during 2010-2011

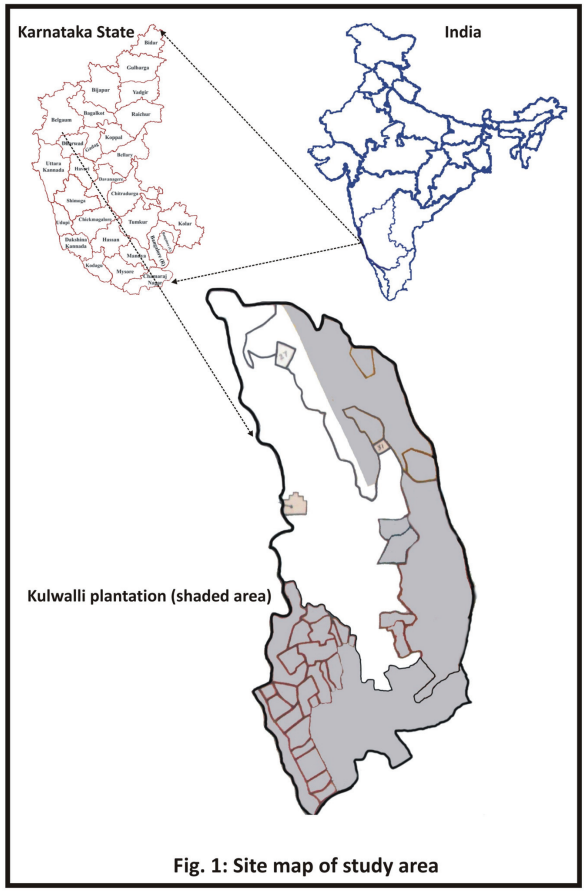

Fig. 1: Site map of study area

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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REFERENCES

Aebi A., Schnrogge K., Melika G., Alma A., Bosio G. and Quacchia A. (2006) Parasitoid requirement to globally invasive chestnut gall wasp, *Dryocosmus kuriphilus*. *In: Galling Arthropods and their associates: Ecology and evolution* (eds: Osaki K, Yukava J., Ohghushi T and Price P. W.) Springer, Japan, pp. 103-121.

Anonymous (2007) Eucalyptus facing severe attack from 'gall insect' Sirsi, UNI: Deccan Herald Tuesday, June 19, 2007.

Anonymous (2011) Eucalyptus gall disease spreads in Punjab, elsewhere. The Indian Express Friday, August 12, 2011.

Aquino D.A., Botto E.N., Loiacono M.S. and Pathauer P. (2011) Avispa de la agalla del eucalipto, *Leptocybe invasa* Fischer & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). en Argentina *RIA* 37 (2): 159-164.

Aytar F. (2006) Natural History, Distribution and hosts of Eucalyptus gall wasps in Turkey. 1Poster Presentation in VIII European Congress of Entomology, on September 17-22.

Branco M.C., Valente J., Franco C. and Mendel Z. (2006) Localization, *Boletin de Sanad vegetal.* De Plagas 32: 199-202.

Cooper W.R. and Rieske L.K. (2007) Community associates of an exotic gall maker *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in eastern North America. Annals of Entomological Society 100: 236-244.

Dhahri S., Ben Jamaa M.L. and Lo Verde G. (2010) First record of *Leptocybe invasa* and *Ophelimus maskelli* eucalyptus gall wasps in Tunisia. Tunisian Journal of Plant Protection 5: 229- 234.

Doganlar M. and Hassan E. (2010) Review of Australian species of *Megastigmus* (Hymenoptera: Torymidae) associated with *Ecalyptus,* with descriptions of new species. Australian Journal of Basic and Applied Science 4 (10): 5059-5120.

Gaskill D.A., Hung S.E. and Smith T.R. (2009) Florida CAPS Blue Gum Chalcid Survey Report. Florida Cooperative Agricultural Pest Survey. Program Report No. 2009-03-BGCW-01. 7 pp.

Gautam R.D., Suroshe S.S., Gautam S., Saxena U.,Fand B.B. and Gupta T. (2009) Fortuitous Biological control of exotic mealy bug, *Phenacoccus solenopsis*- A boon for Indian growers. Annals of Plant Protection Sciences 17: 473-474.

Hall D.G., Pena J., Franqui R., Nguyen R., Stansly P., McCoy C., Lapointe S.L., Adair R.C. and Bullock B. (2001) Status of biological control by egg parasitoids of Diaprepes abbreviatus (Coleoptera: Curculionidae) in citrus in Florida and Puerto Rico. BioControl 46: 61-70.

Howarth F.G. (1991) Environmental impacts of classical biological control. Annual Review of Entomology 36: 485-509.

ICFRE Newsletter (2007) Outbreak of alien forest invasive species, *Leptocybe invasa* on Eucalyptus in Madhya Pradesh. ICFRE Newsletter 7: 1-8.

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

Karunaratne W.A.I.P., Edirisinghe J.P. and Ranawana K.B. (2010) Rapid survey of damage due to Gall Wasp infestation in a coppiced *Eucalyptus camaldulensis* plantation in Maragamuwa, Naula in the Matale District of Sri Lanka. Ceylon Journal of Science 39 (2): 157-161.

Kim I.K., Mendel Z., Protasov A., Blumberg D. and La Salle J. (2008) Taxonomy, biology and efficacy of two Australian parasitoids of the eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). Zootaxa 1910: 1-20.

Kulkarni H., Kavitha Kumari N., Vastrad A.S. and Basavana Goud K. (2010) Release and recovery of parasitoids Eucalyptus against gall wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae) under green house. Karnataka Journal of Agricultural Science 23 (1): 91-92.

Kumar S.S., Kant S.K. and Emmanuel T. (2007) Emergence of gall inducing insect *Leptocybe invasa* (Hymenoptera: Eulophidae) in *Eucalyptus* plantations in Gujarat, India. Indian Forester 133 (11): 1566-1568.

La Salle J. and Pena J.E. (1997) A new species of *Galeopsomyia* (Hymenoptera: Eulophidae: Tetrastichinae): A fortuitous parasitoid of the citrus leafminer, P*hyllocnistis citrella* (Lepidoptera: Gracillariidae). Florida Entomologist 80 (4): 461-470.

Louda S.M., Pemborton R.W., Johnson M.T. and Follet P.A. (2003) Non target effects - The Achillis' heel of biological control? Retrospective analyses to reduce risk associate with biocontrol introductions. Annual Review of Entomology 43 (2): 101-113.

Mendel Z., Protasov A., Fisher N. and La Salle J. (2004) Taxonomy and biology of *Leptocybe invasa* gen & sp. (Hymenoptera: Eulophidae) an invasive gall inducer on Eucalyptus. Australian Journal of Entomology 43 (2): 101-113.

Michaud J.P. (2002) Three targets of classical biological control in the Caribbean: Success, contribution, and failure. *In* Proceedings of the 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii (Ed: Van Driesche R. G.), pp. 335-342.

Mutitu K.E. (2003) A pest threat to Eucalyptus species in Kenya, KEFRI., Technical Report, p. 12.

Narendran T.C., Girish Kumar P. and Vastrad A.S. (2010) Two new species of *Megastigmus* dalman (Hymenoptera: Torymidae) from India, with a revised key to Indian species. Record of Zoological Survey of India 110 (2): 1-6.

Nechols J.R. (2002) Biological control of the spherical mealy bug on Guam and in the Northern Marianas islands: A classic example of Fortuitous biological control. *In* Proceedings of the 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii (Ed: Van Driesche, R. G.), pp. 324-329.

Nwanze K.F., Reddy Y.V.R., Nwilene F.E., Kausalya K.G. and Reddy D.D.R. (1998) Studies on

tritrophic interactions involving sorghum genotypes, midge (*Stenodiplosis sorghicola*) and the predominant parasitoids (*Aprostocetus* spp.). Crop Protection 17: 165-169.

Nyeko P. (2005) The cause, incidence and severity of a new gall damage on *Eucalyptus* species at Oruchinga refugee settlement in Mbarara district, Uganda. Uganda Journal of Agricultural Science 11: 47–50.

Protasov A., Doganlar M., La Salle J. and Mendel Z. (2008) Occurrence of Two Local *Megastigmus s*pecies Parasitic on the Eucalyptus gall wasp *Leptocybe invasa* in Israel and Turkey, Phytoparasitica 36 (5): 449-459.

Rose M. and Debach P. (1991-1992) Biological control of *Parabemisia myricae* (Kuwana) (Hymenoptera: Aleyrodidae) in California. Israel Journal of Entomology 25-26: 73-95.

Samways (1997) Classical biological control and biodiversity conservation: what risks are we prepared to accept? Biodiversity conservation 6: 1309-1316.

Shrestha B.B., Poudel A., Kc J., Karki D., Gautam R.D. and Jha P.K. (2010) Fortuitous biological control of *Parthenium hysterophorous* by *Zygogramma bicolorata* in Nepal. Journal of Natural History Museum 25: 333-338.

van Lenteren J.C., Bale J., Bigler F., Hokkenen H.M.T. and Loomans A.J.M. (2006) Assessing risks of releasing exotic biological control agents of arthropod pests. Annual Review of Entomology 51: 609-634.

Vastrad A.S., Basavanagoud K. and Kavitha Kumari N. (2010) Native parasitoids of eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle (Eulophidae: Hymenoptera) and implications on the biological control of the pest. Entomon 34 (3): 197-200.

Wiley J. (2008) *Eucalyptus* pest, *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae), Genus and Species New to Florida and North America http://www.doacs.state.fl.us/pi/enpp/pi-pest-alert.html

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