RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2009.35.113

ScienceAsia 35 (2009): **113–117**

Susceptibility of 18 eucalypt species to the gall wasp *Leptocybe invasa* in the nursery and young plantations in Vietnam

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Received 9 Dec 2008
Accepted 18 May 2009

ABSTRACT: The gall wasp, *Leptocybe invasa*, is a devastating pest of eucalypt plantations, woodlots, and urban trees in the Middle East, the Mediterranean basin, Africa, India, South-East Asia, and China. It was first observed in Israel in 2000 and has since spread rapidly south and east. It reached southern Vietnam in 2002 and has since moved northward devastating nurseries and young plantations. Eighteen species of eucalypts were selected for a sentinel trial in northern Vietnam to determine their susceptibility to pathogens in the region. They were also rated for the impact of *Leptocybe invasa*. *Leptocybe invasa* was capable of feeding and forming galls on 13 species from six sections within *Eucalyptus* and one species of *Corymbia*. The impact was greater in the nursery. Five of the *Eucalyptus* spp. which were susceptible in the nursery were not damaged in the field. The most susceptible hosts in Vietnam were *E. camaldulenis*, *E. grandis*, and *E. tereticornis*, both in the nursery and field trial. Variation in the susceptibility of provenances of *E. camaldulensis*, *E. urophylla*, and *E. grandis* were observed, which is promising for breeding for resistance and long-term control.

KEYWORDS: *Eucalyptus* section, provenance variability, tolerance, sentinel plants

INTRODUCTION

In Vietnam, *Eucalyptus* spp. plantations occupy about 500,000 ha, with another 50,000 ha scattered in urban and home gardens[1]. *Eucalyptus* spp. have been planted on a large scale in many provinces in mountainous and costal areas of Vietnam and have played an important role in economic and social development of Vietnam, especially in providing income for local people living in remote areas[2]. Eucalypts have been selected for planting on such a large scale in Vietnam because they can tolerate and grow well on degraded, unfertile soils where it is very difficult to establish other tree species. Moreover, eucalypt plantations have a short rotation in Vietnam of 5–7 years[3] and thus return on investment is relatively rapid. The wood from forest plantations can be used for industrial materials and for the production of goods for the large international and domestic market[4]. *Eucalyptus* species have been chosen for wood production in nine ecological zones with plans for over 5 million ha of new plantations[3].

Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae), a gall-inducing wasp on several *Eucalyptus* spp. (Fig. 1a), was first reported from the Middle East in 2000 and has subsequently spread throughout many countries in the Mediterranean basin, Africa, and Asia[5]. Occurrence of the wasp has been reported from Algeria, France, Israel, Italy, Jordan, Morocco, Spain, Turkey, Kenya, South Africa, Thailand, Syria, Tanzania, Uganda, Iran, India, and Vietnam[5–8]. Eucalypts occupy 8 million ha in India and spread of the gall wasp is of huge economic concern to that country[6]. The young shoots of seedlings and coppice crops form an ideal breeding site for the wasp and heavy infestations can damage an entire plantation if timely control measures are not adopted[6].

The biology of *L. invasa* has been studied in detail[5]. Adult females of *L. invasa* oviposit on young shoot tips and midribs of juvenile leaves (1–2 weeks old) leaving scars on both sides of the midrib. The eggs hatch and the larvae feed and then pupate. The adults emerge from the gall within a period of 4–5 months. Galls reach their full size while larvae are feeding and gall size is correlated to the number of wasps developing in the gall. Mature galls are usually deep pink in colour. Gall formation by *L. invasa* on leaves of eucalypts causes deformation of terminal shoots and leaves and results in quicker abscission of leaves and drying up of shoots (Figs. 1b and c). Seedlings in the nursery and young plantations are

Fig. 1 (a) Adult *Leptocybe invasa* (length = 1.2 mm); (b) and (c) damage in field to seedlings of *E. grandis*.

particularly susceptible. The impact is less on trees over two years of age. A heavy infestation of the wasp results in loss of vigour and growth retardation in clones and seedlings. In Iran, Israel, and Turkey, two to three overlapping generations of the wasp per year have been observed. Ten *Eucalyptus* spp. were found to be suitable hosts in Israel: *E. botryoides*, *E. bridgesiana*, *E. camaldulensis*, *E. globulus*, *E. gunii*, *E. grandis*, *E. robusta*, *E. saligna*, *E. tereticornis*, and *E. viminalis*.

In Vietnam, *L. invasa* was first noticed in 2002, causing damage to eucalypt nurseries and young plantations in southern Vietnam[8]. At present, the eucalypt plantation industry in Vietnam is reliant on only a few clones; clones U6, PN2, PN14 of *E. urophylla* and less than six clones of *E. camaldulensis*[8]. These clones are all seriously damaged by *L. invasa* in many nurseries and young plantation in northern, central and south-eastern Vietnam and it is becoming increasingly difficult to find seedlings to establish new plantations. The wasp has been problematic in *E. camaldulensis* plantations and nurseries in Thailand[9] and recently has been recorded in Guangxi Province, P.R. China (2007: Xu Daping, pers. comm.) and central Lao PDR (2008: Viengxay Vue, pers. comm.). There is an urgent need to screen a wide range of *Eucalyptus* species, clones and hybrids for resistance or tolerance to *L. invasa*. Without new material the eucalypt plantation industry in Vietnam is under serious threat. This article reports on the susceptibility of 18 eucalypt species, to *L. invasa* in the nursery and young plantations.

MATERIALS AND METHODS

Trial design

Seeds of 23 provenances belonging to 18 eucalypt species, were obtained from the Australian Tree Seed Centre (CSIRO, PO Box E4008, Kingston ACT 2604, Australia) (Table 1). Species selected represent two species, *Corymbia* and *Eucalyptus*, three sections and series within *Corymbia* and four sub-genera within *Eucalyptus* (Table 1). The majority of species of *Eucalyptus* are from the sub-genus *Symphyomyrtus* and 4 sections and 9 series within this group are covered (Table 1). Seeds were germinated in river sand and after one week, each sapling was planted into a pot (5×12 cm) containing soil and granular NPK (2% by weight). Pots were watered 2–3 times per week for 3 months in the nursery.

After three months (in July 2007) seedlings were planted at the Dai Lai, Vinh Phuc province in a randomized complete block design with 3 reps and 10 seedlings per rep in row plots. The tree and row spacing were 2 m and 3 m, respectively. Site was prepared by clear cutting vegetation from previous eucalypt trial, the dimension of the planting holes were $40 \times 40 \times 40$ cm. 200 g of NPK fertilizer was placed in the bottom of the hole when planting.

Assessment of damage in the nursery

After three months in the nursery, and prior to transplanting to the field, the severity of damage caused by *L. invasa* was determined. For each provenance 100 seedlings were evaluated. Damage index (D.I.) and their criteria are showed as follows:

D.I.	Severity scale and percentage leaves infected
0	healthy tree, no damage
1	$<$ 25% leaves and twigs of crown infected
2	25-50% leaves and twigs of crown infected
3	51-75% leaves and twigs of crown infected
4	$>$75% leaves and twigs of crown infected

Based on the severity level of every seedling, damage incidence (percentage of trees affected) and average damage index were calculated. The average damage index was calculated according to the following formula:

$$R = \frac{\sum_i n_i v_i}{NV},$$

where R is the average damage index, n_i the number of trees infected at damage index i, v_i the damage index at level i, N number of trees assessed, and V the highest damage level (4).

Based on the average damage index, a damage severity level was identified as follows; nil (average

Table 1 Classification of the eucalypts species and provenances selected for the trial. Classification based on Ref. 10.

Seedlot	Genus	Sub-genus	Section	Series	Species	Collection location
20539	Corymbia		Ochraria	Maculatae	*Corymbia henryi*	SSO Barclays Deniliquin, NSW
20012	Corymbia		Ochraria	Maculatae	*C. citriodora citriodora*	Dawson Range, QLD
12967	Corymbia		Blakella	Tesselares	*C. tessellaris*	NW of Mareeba, QLD
16616	Corymbia		Rufaria	Polycarpae	*C. polycarpa*	Derideri to Arufi, WP, PNG
13888	Eucalyptus	Monocalyptus	Renantheria	Pilulares	*Eucalyptus pilularis*	Gallangowan SF Gympie, QLD
15607	Eucalyptus	Northocalyptus			*E. microcorys*	11 km W of Beerburrum, QLD
13565	Eucalyptus	Idiogenes			*E. cloeziana*	Cardwell, QLD
19284	Eucalyptus	Symphyomyrtus	Maidenaria	Globulares	*E. globulus maidenii*	Yambulla SF, NSW
20702	Eucalyptus	Symphyomyrtus	Maidenaria	Viminales	*E. smithii*	Tallaganda, NSW
20408	Eucalyptus	Symphyomyrtus	Adnataria	Moluccanae	*E. moluccana*	Long Mile Range Creek, NSW
12469	Eucalyptus	Symphyomyrtus	Adnataria	Oliganthae	*E. coolabah*	5 km N Barringun, QLD
12384	Eucalyptus	Symphyomyrtus	Transversaria	Resiniferae	*E. pellita*	S Helenvale, QLD
10144	Eucalyptus	Symphyomyrtus	Transversaria	Resiniferae	*E. urophylla*	N of Aileu, Indonesia
17841	Eucalyptus	Symphyomyrtus	Transversaria	Resiniferae	*E. urophylla*	Piritomas W of Alor, Indonesia
08989	Eucalyptus	Symphyomyrtus	Transversaria	Resiniferae	*E. urophylla*	36 km S of Dili, Timor
14129	Eucalyptus	Symphyomyrtus	Transversaria	Salignae	*E. robusta*	ESE of Nambour, QLD
16942	Eucalyptus	Symphyomyrtus	Transversaria	Salignae	*E. saligna*	20 km N Helido, QLD
13023	Eucalyptus	Symphyomyrtus	Transversaria	Salignae	*E. grandis*	20 km E of Gympie, QLD
20758	Eucalyptus	Symphyomyrtus	Transversaria	Salignae	*E. grandis*	Eungella, QLD
14212	Eucalyptus	Symphyomyrtus	Exsertaria	Tereticornes	*E. tereticornis tereticornis*	5–12 km S Helenvale, QLD
12499	Eucalyptus	Symphyomyrtus	Exsertaria	Rostratae	*E. camaldulensis*[a]	Ward R NW Char'Ville, QLD
13701	Eucalyptus	Symphyomyrtus	Exsertaria	Rostratae	*E. camaldulensis* var. *obtusa*	Waverley CK, MT ISA, QLD
16546	Eucalyptus	Symphyomyrtus	Exsertaria	Rostratae	*E. camaldulensis simulata*	Palmer River, QLD

[a] var. *camaldulensis* QLD = Queensland

damage index = 0), low damage (average damage index < 1.0), medium damage (average damage index 1.1–2.0), severe damage (average damage index 2.1–3.0) and very severe damage (average damage index 3.1–4.0).

Assessment of field trial

The field trial was rated for survival three months after planting, and damage caused by *L. invasa* assessed as follows; nil (no seedlings with galls), low damage (<25% of seedlings with galls), medium damage (25–75% of seedlings with galls) and high damage (>75% of seedlings with galls).

RESULTS

Damage in the nursery

The average damage incidence, damage index, and damage severity of the eucalypt species caused by *L. invasa* are presented in Table 2. The resistance of eucalypt species differed greatly. Of the 15 species represented by a single provenance, four species, *C. henryi*, *C. citriodora*, *C. tessellaris*, and *E. cloeziana* were resistant to *L. invasa* and no galls were produced. Another 9 species showed a low damage severity: *E. pellita*, *E. microcorys*, *E. pilularis*, *E. robusta*, *E. coolabah*, *E. globulus*, *E. smithii*, *E. moluccana*, and *C. polycarpa*. Moderate damage was observed for one species, *E. saligna* and severe damage was observed for *E. tereticornis*.

There were 2 provenances of *E. grandis* and 3 provenances of *E. camaldulensis* and all were severely damaged. There were 3 provenances of *E. urophylla*, no damage was observed for one provenance and low damage for the other two.

Seedling survival and *Leptocybe* damage in the field trial

Seedling survival varied greatly between species. This is due to several factors, including (a) the suitability of the climate for that tree species, (b) susceptibility to various leaf pathogens, such as *Quambalaria* spp. and *Cytospora* sp. and (c) susceptibility to attack by *L. invasa*. Less than 50% survival was observed for seven species: *C. henryi*, *E. cloeziana*, *E. pilularis*, *E. smithii*, *E. saligna*, *E. grandis* (seedlot 13023), and *E. camaldulensis* (seedlot 12499).

Survival aside, incidence of attack by *L. invasa* was mostly similar in the field trial to that observed in the nursery. Six species with low damage in the nursery showed no damage in the field; *E. pellita*, *E. microcorys*, *E. pilularis*, *E. globulus*, *E. moluccana*, and *E. urophylla* (seedlot 17841). *Eucalyptus robusta*, which showed low damage in the nursery, appeared to be more susceptible in the field and showed medium damage. Some minor gall development was observed on *E. urophylla* seedlot 10144, which showed no damage in the nursery. Damage in the field for *E. grandis*, *E. tereticornis*, and *E. camaldulensis* were high with all individual seedlings affected (Fig. 1). The two seedlots with poor field survival, *E. grandis* (seedlot 13023) and *E. camaldulensis* (seedlot 12499), showed only medium damage in the field for surviving seedlings. However, the dead seedlings were still in

Table 2 The impact of *Leptocybe invasa* after 3 months in the nursery, as determined by the damage incidence, damage index and damage severity, and seedling survival and severity of *L. invasa* damage in the field

Seedlots #	Species	Damage incidence (%)	Average damage index	Damage severity	Survival in field (%)	Field Assessment
20539	*Corymbia henryi*	0	0	nil	44.4	nil
20012	*C. citriodora ssp. citriodora*	0	0	nil	70.4	nil
12967	*C. tessellaris*	0	0	nil	55.6	nil
16616	*C. polycarpa*	15	0.38	low	88.9	low
13888	*Eucalyptus pilularis*	9	0.21	low	48.2	nil
15607	*E. microcorys*	10	0.33	low	59.2	nil
13565	*E. cloeziana*	0	0	nil	44.4	nil
19284	*E. globulus ssp. maidenii*	25	0.88	low	96.3	nil
20702	*E. smithii*	14	0.75	low	7.4	low
20408	*E. moluccana*	36	0.56	low	55.6	nil
12469	*E. coolabah*	31	0.54	low	74.1	low
12384	*E. pellita*	12	0.19	low	85.2	nil
10144	*E. urophylla*	0	0	nil	85.2	low
17841	*E. urophylla*	24	0.80	low	88.9	nil
08989	*E. urophylla*	19	0.37	low	70.4	low
14129	*E. robusta*	26	0.41	low	74.1	medium
16942	*E. saligna*	41	1.34	medium	37.0	medium
13023	*E. grandis*	92	2.55	severe	44.4	medium
20758	*E. grandis*	65	2.69	severe	59.3	high
14212	*E. tereticornis ssp. tereticornis*	81	2.52	severe	77.8	high
12499	*E. camaldulensis var. camaldulensis*	80	2.02	severe	44.4	medium
13701	*E. camaldulensis var. obtusa*	76	2.43	severe	96.3	high
16546	*E. camaldulensis spp. simulata*	88	2.27	severe	85.2	high

the ground and it could be seen that they had suffered from heavy infestation by *L. invasa*.

DISCUSSION

Leptocybe invasa was capable of feeding and forming galls on 13 species from 6 sections of *Eucalyptus* and one species of *Corymbia*. The impact was greater in the nursery and 5 of the susceptible *Eucalyptus* spp. in the nursery were not affected in the field. Mendel et al (2004) observed fewer host species susceptible to *L. invasa* in Israel, with species from only 3 sections of *Eucalyptus* being suitable for the development of *L. invasa*. Combining our results with those of Mendel et al (2004), *E. camaldulensis*, *E. tereticornis*, *E. grandis*, *E. robusta*, *E. saligna*, and *E. globulus* were suitable hosts, whilst *C. citriodora* was not. In addition, we found *C. polycarpa*, *E. microcorys*, *E. smithii*, *E. moluccana*, *E. coolabah*, *E. pellita*, and *E. urophylla* were suitable hosts in the nursery. Of these, *C. polycarpa*, *E. smithii*, *E. coolabah*, and *E. urophylla* also supported *L. invasa* after 3 months under field conditions.

The most susceptible hosts in Vietnam were *E. camaldulenis*, *E. grandis* and *E. tereticornis*. This is most unfortunate, as along with *E. urophylla*, clones and hybrids of these species are the most commonly planted *Eucalyptus* spp. in the region[3]. *Eucalyptus urophylla* was tolerant of *L. invasa*, with only a low level of damage; however *E. urophylla* is greatly impacted by leaf (*Kirramyces destructans*), and bark (*Kirramyces zuluense*) diseases[11–15].

Variation in the susceptibility of provenances of *E. camaldulensis*, *E. urophylla* and *E. grandis* were observed. Variation with provenances is promising for breeding for resistance and long term control. Another strategy will be the development of biological control. This work is underway in Israel and South Africa and several potential control agents have been identified. However, there is an urgent need to save the mother plants that are being used commercially in Vietnam and the only option is chemical control.

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