THE BIO-ECOLOGICAL DYNAMICS AND EFFICACY OF A FIELD INTEGRATED CONTROL STRATEGY OF INVASIVE PEST, *Leptocybe invasa* Fisher & La Salle, IN KENYA

BY

KENNETH OPIYO ODHIAMBO
Bsc. (Hons), M.Phil. (MU)

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DECLARATION

Declaration by the candidate

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Kenneth Opiyo Odhiambo Date

SC/ DPHIL./ 11/ 04

Declaration by supervisors

This thesis has been submitted for examination with our approval as University supervisors.

_____ _____

Prof. B.M. Khaemba, Date

Biological Science Department,

University of Eldoret,

P.O. Box 1125, Eldoret.

_____ _____

Prof. F.M.E. Wanjala, Date

Biological Sciences Department,

University of Eldoret,

P.O. Box 1125, Eldoret.

DEDICATION

This thesis is dedicated to Caroline, Idah, Roseline, Sarah and Lindsey for their unfathomable love and support.

ABSTRACT

Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae) has been recorded in many tropical and sub-tropical regions of the world as a gall wasp attacking *Eucalyptus* species. Chemical control strategy is hampered by the fact that *L. invasa* completes much of its life cycle inside eucalyptus tissue and only expensive systemic insecticides may be palliative. It is not known whether integrated control of *L. invasa* would be feasible due to insufficient information on the insect's biology and ecology. The urgent need for more information on the biology and ecology to pave way for its control generated the current study. The studies reported here investigated the colonization and the rearing procedure for *L. invasa;* its life cycle, oviposition requirements, foraging, potential of selected herbaceous plants as IPM components against the pest and variability of its attack on different Eucalyptus germplasm. Caged infested *E. saligna* seedlings were used as sources of *L. invasa* while caged healthy seedlings were used in *L. invasa* life cycle and susceptibility experiments with gall formation and mean gall numbers per seedling as response variables. *Leptocybe invasa* took nineteen weeks (128-131 days) from oviposition to adult stage at a room temperature of 25.5 ^{o}C with ten infested and caged *Eucalyptus* seedlings giving an output of 30± 6 insects per cage per day. *L. invasa* egg is white to light yellow in colour and round in shape. Egg diameter ranges from 0.09 mm to 0.14 mm (mean of 0.11± 0.01 mm). Eggs are laid singly, normally in a row beneath the epidermis of the host tissue (leaf midrib, petiole and succulent shoots). A white substance occurs on the oviposition spots 1.50 ± 0.29 days after oviposition. The larva of *L. invasa* is a minute, white legless grub. Larval stage takes 33 – 42 days with four larval instars and body length ranges from 0.13- 0.19 mm with a mean (± SD) of 0.16 ± 0.02 mm. The pupa of *L. invasa* has mummy-like appearance and is dark brown to black in colour. Mature pupa measures 1.1 – 1.2 mm in length. Pupal stage takes about 14 days. Adult *L. invasa* are black in colour and small in size. The mean (mean ± SD) body measurements of unsexed insects are as follows: Body length = 1.13 ± 0.07 mm; Head capsule width (eye to eye) = 0.25 ± 0.01 mm; Antennal length = 0.25 ± 0.00 mm; Number of antennal segments = 0.37 ± 0.02 mm; Wing length = 0.77± 0.09 mm; Abdominal length = 0.42 ± 0.07 mm; and Abdominal width 0.29 ± 0.01 mm. Adult stage takes 3-4 days under natural conditions but can be lengthened to 16 days if the adults are fed on 15 % sucrose. More eggs are laid by *Leptocybe invasa* (Hymenoptera: Eulophidae) in response to cues for oviposition in relation to olfaction stimuli than visual stimuli ($p < 0.05$) and patch residence time was greater than time spent in foraging from patch to patch ($p < 0.05$). Low nitrogen fertilization and moderate watering regime lowered the severity of attack by the gall wasp (2.6 ± 0.9 galls per seedlings) ($p < 0.05$) while high levels of nitrogen fertilization and high watering regimes increased the severity of attack by the pest (13.1 ± 0.9 galls per seedling) ($p < 0.05$). *Leonotis nepetifolia*, *Schkuria pinnata* Kuntz ex Thell and *Tagetes erecta* L. were repellent to *L. invasa*. *E. saligana* grown together with *T. erecta* had the least number of galls (4.2± 0.8), followed by those grown together with *S. pinnata* (6.0 ± 1.3), *L. nepetifolia* (7.4 ±1.8) and *E. saligna* grown alone (10.8 ± 1.7) ($p < 0.05$). Unlike *S. pinnata*, the planting of *L. nepetifolia* and *T. erecta* as companion plants significantly reduced height growth of the E. saligna ($p < 0.05$). *Eucalyptus saligna* was the most susceptible species to *L. invasa* attack (15.43± 0.29 galls per seedling) while *E. globulus* and *E. citriodora* were resistant, having significantly fewer galls per seedling (86±0.07 and 0.94±0.07 galls respectively; $p <$

0.05). Whereas *E. camaldulensis* seemed resistant in the presence of *E. saligna*, it was slightly susceptible to *L. invasa* attack when exposed to the insects alone. In the presence of *E. saligna*, gall count per seedling on *E. camaldulensis* was 3.21±0.33 while 7.11±0.24 galls per seedling was recorded when the species was alone ($p < 0.05$). This study has provided a first report on *L. invasa* larval instars. The use of *S. pinnata* in particular and other herbaceous hosts studied as companion plants to *E. saligna* as part of IPM strategy against *L. invasa* has been recommended. Research geared towards elucidating the potential of biological control agents against the pest is recommended.

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GLOSSARY OF ABBREVIATIONS

AAS:	African Academy of Sciences
AFORNET:	African Forestry Research Network
ANOVA:	Analysis of variance
BC:	Biological Control
CABI:	Commonwealth Agricultural Bureau International
CI	Confidence interval
DF (or df)	Degree of freedom
GOK:	Government of Kenya
Ht:	Height
ICIPE:	International Centre for Insect physiology and Ecology
IPM:	Integrated Pest Management
IUPAC:	International Union of Pure and Applied Chemistry
KEFRI:	Kenya Forestry Research Institute
KFS:	Kenya Forest Service
LSD	Least Significanat Difference
m.a.s.l.:	Metres above sea level
MS	Mean Square
min:	Minute(s)
SD:	Standard deviation
SS	Sum of Squares
SE:	Standard error
sec:	Second(s)
SV:	Source of variation

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CHAPTER ONE
INTRODUCTION

1.1. Background information

Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae), commonly known as the blue-gum chalcid, is an invasive, gall-inducing insect pest of *Eucalyptus* trees (Myrtaceae), particularly *E. saligna, E. grandis, E. robusta* and *E. camaldulensis*. It is currently widely recognized in many tropical, sub-tropical and Mediterranean regions of the world as a gall inducer attacking *Eucalyptus* species, particularly seedlings (Ananthakrishnan, 2009, Nyeko *et al.* 2009; Protasov *et al*., 2007; Nyeko, 2005; Hesami *et al.*, 2005; Doganlar, 2005). *Eucalyptus species* are native to Australia but are widely planted in South America, Africa, Europe, and Asia.

The blue-gum chalcid is native to Australia and its occurrence was first recorded in the Middle East in 2000 and it has since spread to most of the Mediterranean countries, India and Africa (Mendel *et al*., 2004). In East Africa, the pest was first reported in western Kenya and eastern Uganda in 2002. By 2007 the pest had spread to southern parts of Africa, including South Africa (Mendel *et al*., 2004; CABI, 2007; Gupta & Poorani, 2009; Nyeko *et al*., 2009). It causes typical galls; appearing as swellings on leaf midribs, petioles and twigs or stems, particularly of new re-growths as shown in Figure 1. Repeated pest attack leads to twisted and knobbed appearance of the leaves. Although mainly concentrated in western parts of Kenya, the pest has been reported along the Kenyan coast on *Eucalyptus* trees but is almost absent in high altitude areas of the country (Mutitu *et al*., 2007a).

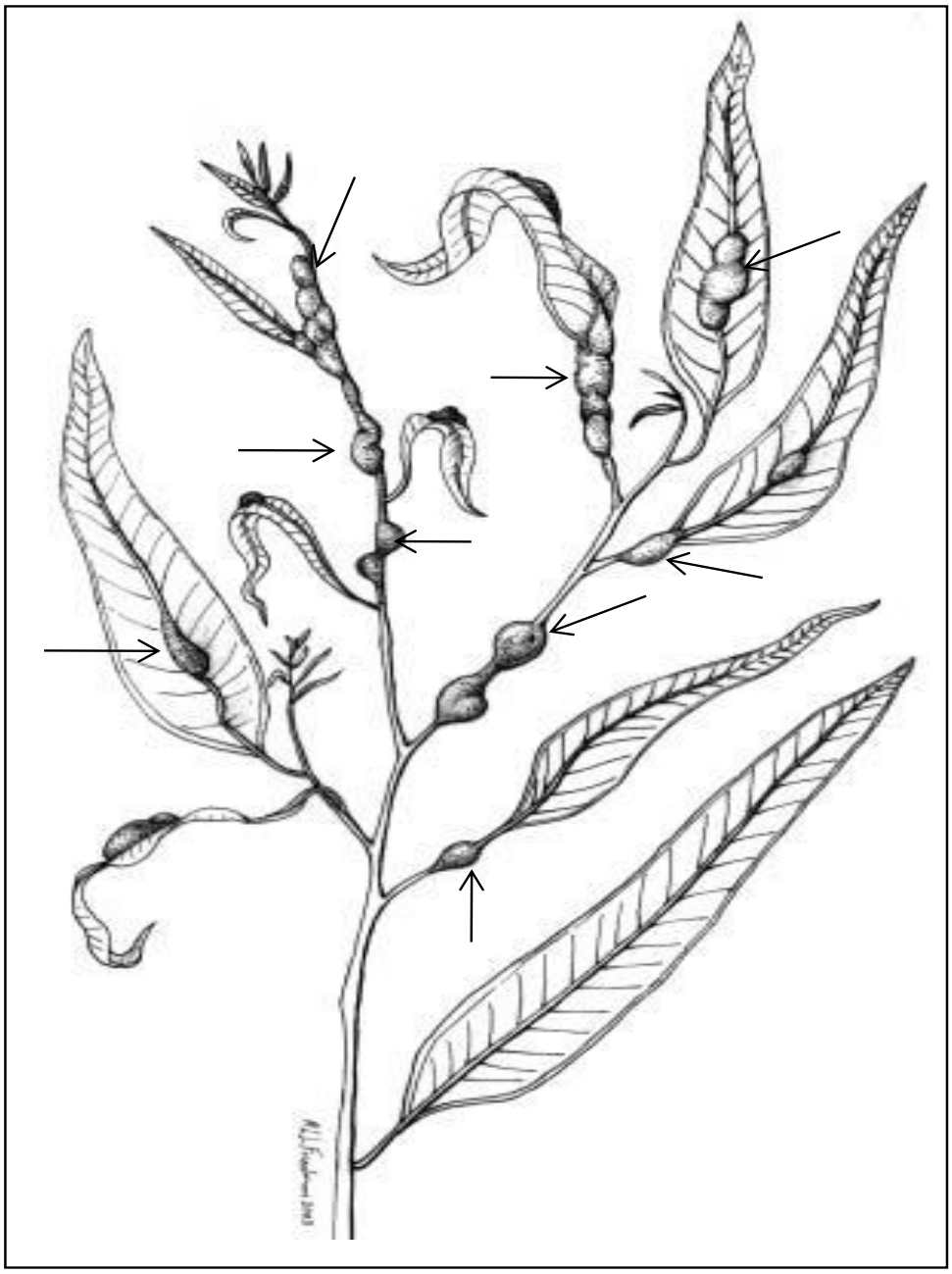


Figure 1: Typical galls, induced on *Eucalyptus species* (Myrtaceae) by *Leptocybe invasa* (Hymenoptera: Eulophidae): Source: Mendel *et al* (2004) modified by arrowing.

Several major pest species from Australia, including gall-making wasps have invaded all major regions in the world where *Eucalyptus* is grown and examples of such pests include *Thaumastocoris peregrines, Quadrastichodella nova* Girault, *Ophelimus eucalypti* (Gahan), *O. maskelli* (ashmead), *Gonoptherous spp., Epichrysocharis burwelli* Schauff, *Phoerecantha spp., Leptocybe invasa* Fisher & La Salle, *Colletogloeopsis zuluense, Nambouria xanthops* Berry &Withers and *Leprosa milga* Kim & La Salle (Withers *et al*., 2000; Berry and Withers, 2002; Branco *et al.,* 2005; Protasov *et al*., 2006; Ramanagouda *et al*., 2010). The insect pests of eucalyptus cause economic losses in terms of increased operational costs, reduced timber yield and reduced provision of non-market ecosystem services (Holmes *et al.,* 2009).

Eucalyptus saligna is commonly known in Kenya as the blue-gum tree where it is a major industrial and farm forestry tree species but unfortunately is one of the most susceptible species to *L. invasa* attack in the country. It is extensively grown in the country (Oballa and Wamalwa, 2007) and is put to a variety of uses: fuel wood, construction material and poles. Being the most extensively grown tree species in Kenya, and particularly in western region, *E. saligna* occupies a critical segment of the hydrological cycle of Lake Victoria basin and influences the socio-economic set-up of the region.

1.2. Statement of the problem

Eucalyptus is the third widely planted tree genus in Kenya, after *Pinus* (Pineaceae) and *Cupressus* (Cupressaceae). The genus is currently attacked by *L. invasa* insect pest, particularly in dry lowland areas such as the western region bordering Lake Victoria and some parts of the Kenyan coast. Young trees and seedlings are the most

affected age classes by the pest, thus threatening the continued propagation of the trees. It is estimated that 15,000 ha of Eucalypts are grown by Kenya Forest Service (KFS) and 35,000 ha by private sector while small-scale farmers, urban and county councils have also put substantial areas of land under *Eucalyptus* trees (Oballa and Wamalwa, 2007). A survey by Mutitu *et al*. (2007b) covering five Districts in western Kenya (Bungoma, Busia, Nyando, Nandi and Vihiga) showed that *L. invasa* pest attack is one of the constraints to *Eucalyptus* growing in the region where 60,000 ha are under threat, other constraints being limited land, effect of drought and other insect pests like termites.

Elsewhere, several control methods have been prescribed for various insect pests, including gall-forming types (Thacker, 2002; Davies, 1985; Coulson and Witter, 1984). With regard to *L. invasa*, classical biological control could be one of the control options although little effort, if any, has been put to explore this control option, mainly due to scanty information that is available about the pest's biology and ecology (Protasov *et al.,* 2007). Cultural control methods such as pruning and destroying plant parts is an effective, but labour-intensive way to minimize gall problems (Buss, 2003) and leads to stress of plants, increasing their susceptibility to attack by other pests and diseases. Chemical control strategy is generally hampered by the fact that *L. invasa* insect pests lives and completes much of its life cycle inside host tissue, well out of reach of contact insecticides. While systemic insecticides could be suited for use in tree nurseries, from economic point of view, increasing concern on the adverse effects of chemical insecticides lowers their feasibility as recommended means of controlling *L. invasa* pest. Integrated control of *L. invasa* seems to be the best option but the success of this control method is hampered by non-

availability of sufficiently generated techniques that are pegged on knowledge of the insect's biology and ecology. There is an urgent need to develop an array of techniques for use in the integrated control strategy for the pest in Kenya. It is deemed that knowledge on its bio-ecology would form part of the prerequisites to the development of its control by blending together compatible techniques if any.

1.3. Objectives of the study

1.3.1. Major objective

The main objective of the study was to provide data and information on the eco-biology of *L. invasa* and outline an integrated control strategy against it.

1.3.2. Specific objectives

The specific objectives of the study were to:

1. Devise appropriate colonization and rearing procedures for *L. invasa*.
2. Investigate the ecology, oviposition and foraging of *Leptocybe invasa.*
3. Elucidate the potential of selected herbaceous plant species as IPM components against *L. invasa.*
4. Assess the variability in *L. invasa* attack in current major *Eucalyptus* germplasm.

1.3.3. Working hypotheses

1. There is an appropriate procedure for colonization and rearing of *Leptocybe invasa* (Hymenoptera: Eulophidae).
2. Aspects of the biology and ecology of *L. invasa* can be investigated and used as tools for prescribing its integrated control.

3. Certain herbaceous plant species containing volatile insecticidal chemicals can be grown together with *Eucalyptus spp.* as an IPM component against blue-gum chalcid pest, *L. invasa* in a pull-push strategy.
4. There is wide variability in eucalyptus germplasm to attack by *L. invasa*.

CHAPTER TWO

LITERATURE REVIEW

2.1. Identity and Taxonomy of *Leptocybe invasa*

L. invasa is a hymenopteran insect belonging to the superfamily Chalcidoidea and believed to have originated from Australia. It is commonly known as blue-gum chalcid. It is a new genus and species of the family Eulophidae and sub-family Tetrastichinae (Mendel *et al*., 2004). It is small in size (about 1 mm in length), black in colour and winged. The name *Leptocybe* is derived from two Greek words: *leptos* meaning fine, weak, and thin and *kybe* meaning head; together signifying the weak area on the head around the ocellar triangle.

The genus *Leptocybe* is similar to *Baryscapus* Förster and *Oncastichus* La Salle with many respects except that: (i) Compared to *Baryscapus* it has propodeum with a raised lobe of the callus that partially overhangs the outer rim of the spiracle; spiracular depression open to anterior margin of propodeum (in *Baryscapus* the propodeal spiracle has entire rim exposed); and the setae on the mesoscutum consist of a single row of 2-3 adnotaular short, weak setae (*Baryscapus* is variable in this character, but often has more than a single row adnotaular setae). (ii) It differs from *Oncastichus* in having the post marginal vein less than 0.25 the length of the stigmal vein (0.6-1.0 in *Oncastichus*) (Mendel *et al*., 2004).

There are approximately 115,000 described species of hymenoptera (Sharkey, 2007). This places them behind the Coleoptera and Lepidoptera although some hymenopterists (Grissell, 1999) argue that if the undescribed species were included, the hymenoptera would be more species-rich than all other orders.

Eulophidae (Hymenoptera: Chalcidoidea) is one of the largest families of parasitic wasps containing over 4472 described species placed in 297 genera (La Salle, 1994; Gumovsky, 2002; Noyes, 2008; Hesami *et al.*, 2010). A diagram of a typical eulophid insect is shown in Figure 2. Although majority of the species are parasitoids, particularly of holometabolous insects, the family also contains phytophagous and predator species. Eulophids attack a variety of insects, and occasionally mite and spider hosts. Their larvae act as koino-or idiobionts, gregarious or solitary, ecto- or endoparasitoids; they attack eggs, larvae or pupae of their hosts. Some species are phytophagous and are mainly gall-formers on Eucalypts (Gumovsky, 2002).

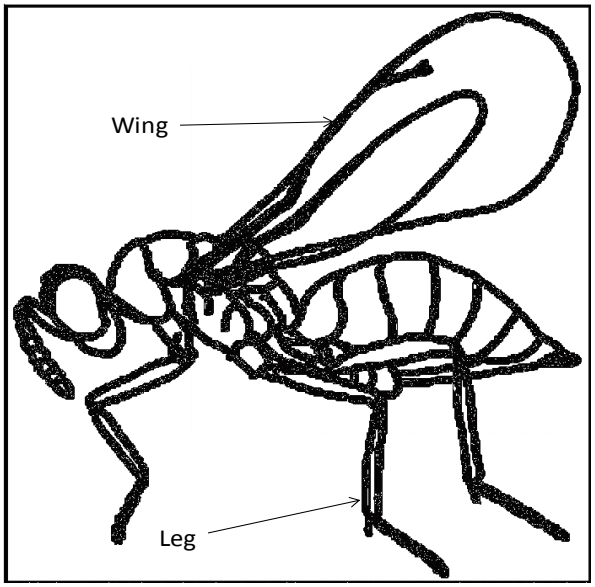


Figure 2: Diagram of a typical Eulophid insect(Body length =1.0 mm), X 110.

2.2. Biology and Ecology of *Leptocybe invasa*

Studies on the biology of *L. invasa* have of late elicited interest in many investigators perhaps primarily because the insect is a new species (Mendel *et al*., 2004; Doganlar, 2005; Ananthakrishnan, 2009; Nyeko *et al.*, 2009). *L. invasa* is a stenophagous feeder developing on eucalyptus plant species (Mendel *et al*., 2004). Female insects of the species lay eggs in young tissues of host plants, especially near apical meristems, leaf petioles and midrib of leaves. A whitish-yellowish substance is formed and block oviposition punctures a day after oviposition (Anonymous, 2005). The eggs develop into minute, white legless larvae within the host tissue. Infected parts of the host plant develop galls with time and eventually adults emerge out of the galls. Other detailed aspects of the insect's biology are generally lacking.

2.3. The pest status of *L. invasa* on *Eucalyptus* and other host plants

L. invasa causes injury to young foliage of *Eucalyptus species* by inducing galls on rapidly growing shoots. Severely attacked trees show twisted and knobbed appearance, stunted growth and dieback which eventually lead to death (Plate 1). In Kenya, *E. saligna*, *E. grandis* and *E. camaldulensis* are the most susceptible hosts while *E. citriodora*, *E. maculate* and *E. paniculata* seem resistant (Mutitu, 2007). Nursery stage Eucalyptus trees (seedlings) are more susceptible to the pest attack than older trees.

Gall induction by *L. invasa* is similar to that of other phytophagous Eulophids like *Ophelimus maskelli* (Ashmead) and *Leprosa milga* Kim & La Salle gen. & sp. nov. (Hymenoptera: Eulophidae: Tetrastichinae). Attempts have been made to control *O. maskelli* in Israel by the introduction of *Closterocerus chamaeleon* (Girault)

(Hymenoptera: Eulophidae) as a biological control agent with considerable success (Protasov *et al.*, 2007). Not much has been done to control *L. milga*, a gall inducer in seed capsules of *Eucalyptus* that has become established in South Africa and Italy (Kim & La Salle, 2008).

Plate 1: Galls on *Eucalyptus saligna* induced by blue-gum chalcid pest, *Leptocybe invasa*, in a farm near Yala Township, Kenya. (Source: Author, 2006)

2.4. Control strategies of *L. invasa*

2.4.1. Cultural control of *L. invasa*

Cultural control methods such as pruning and destroying plant parts are effective, but labour-intensive way to minimize gall problems (Buss, 2003) and lead to stress of plants, increasing their susceptibility to attack by other pests and diseases. Farmers having infested *Eucalyptus* woodlots in Kenya cut off and burn heavily infested branches as a control strategy (Mutitu *et al*., 2007b). The use of healthy seedlings as planting stock and quarantine have been recommended by KEFRI as ways of controlling *L. invasa* attack of *Eucalyptus* plants (Mutitu *et al.,* 2004).

2.4.2. Chemical control of *L. invasa*

Literature on chemical control of *L. invasa* is lacking. However, an evaluation of effectiveness of insecticides against *L. invasa* by Jhala *et al.* (2010) showed that application of Carbofuran 3G or Phorate 10G at 1 g/ plant in the soil 45 days after transferring the seedlings to polyethylene bags followed by spray application of dimethoate at 0.03 % or Phosphamidon at 0.04 % or Methyl-o-demeton at 0.025 % or Acephate at 0.075 % at 15 days interval starting after one month of granular application could be an effective strategy to check the infestation by the pest in eucalyptus nursery. However, these products leave undesirable residues in the soil and have side effects.

2.4.3. Biological control of *L. invasa*

Experimental studies have shown the existence of promising biological control agents. Studies by Kim *et al.* (2008) indicated that *Quadrastichus mendeli* Kim & La Salle sp. nov. and *Selitrichodes kryceri* Kim & La Salle sp. nov. (Introduced from Australia as

part of biological control programme to counter severe levels of damage caused by *L. invasa* to *Eucalyptus* plantations throughout the Mediterranean basin) successfully parasitized the insect pest. Greehouse studies in India of native parasitoids of *Leptocybe invasa*, namely *Megastigmus sp* and *Aprostocetus gala* Walker, during 2008-09 indicated 10-28 % parasitization by *Megastigmus* and 16 % parasitization by *Aprostocetus gala* (Kulkarni *et al*., 2010). No previous studies have been conducted on biological control of the pest in Kenya.

2.4.4. Integrated control of *L. invasa*

Ecological studies of agroecosystems have demonstrated both significant environmental problems associated with the intensive physical and chemical control of highly simplified crop production systems, and the largely untapped opportunities for knowledge-intensive bioecological design and management of more complex systems (Hill *et al*., 1999). In light of this, integrated pest management (IPM) has become the dominant paradigm that guides most apects of current research in and implementation of insect pest management (Zalucki *et al*., 2009).

The key features of IPM are as follows (Thacker, 2002): (i) is based on the principles of ecology and pest status of the insects; (ii) involves use of combinations of various tactics either as alternative or complementary pest control methods; (iii) its functional goal is to reduce or maintain pest population at low levels as judged by ecological, economic and social values; (iv) is a component of the total forest resource management; (v) Its effectiveness is judged by basically on benefit-cost analyses, which in turn are based on evaluation of impacts and available treatment (i.e. control) tactics; (vi) it relies heavily on data for monitoring pest populations and forest stand

conditions; (vii) Its adoption results from an evaluation of treatment (pest control) options available and analyses of impacts; (viii) may but does not usually involve use of chemical insecticides due to the adverse effects the chemicals have on environment.

The detailed major steps in establishing IPM programme for an insect pest are pest-specific but are principally as follows (Dent, 1991; Thacker, 2002): step 1: Define the ecosystem and define the pest complex and predator complex present; step 2: Identify the pest and study its biology and behaviour; its original wild host as well as alternative hosts; step 3: Measure pest numbers and determine economic thresholds (ET); step 4: Establish a monitoring system for pest species; Step 5: Determine cost-benefit ratio of intended control measures so as to select best measure(s); Step 6: Establish systems of monitoring numbers of predatory species available and monitor their population density in the field; Step 7: Augment environmental resistance; Step 8: Use pesticides selectively, i.e. reduced rates, strip spraying, precision spraying, e.t.c.

Integrated control of *L. invasa* seems to be the best option but the success of this control method depends on availability of sufficient knowledge of the insect's biology and ecology. No knowledge on *L. invasa* is readily available for Kenya.

2.5. Plant galls and gall forming insects

Plant galls, elicited by insects to supply the developing food and shelter, have often been referred to as hyperplasiasis, overgrowths, or as abnormal growths (Mani, 1994; Williams, 1994). Gall formation (i.e. Cecidogenesis) was occasionally likened to plant

tumourigenesis. Stimulus for gall formation is usually provided by the feeding stage of the insect, but in some insects the ovipositing female provides the stimulus when she lays eggs on the plant (Coulson & Witter, 1984). The exact cause of gall is poorly understood, but the stimulus for gall formation is believed to be a growth-regulating chemical (Stubbs, 1987; Spooner, 1990; Redfen, 1992). For instance, the distribution and composition of membrane glycerolipids and phosphoglycerides is affected by the interaction of the gall insect with the host plant tissue (Bayer, 1994). Such modifications in the structure of membrane lipids may be responsible, in part, for the regulation of some of the metabolic activities initiated in the host by gallicolous insect.

The most common insects causing galls on trees are found in five orders: Hymenoptera (e.g. cynipid wasps), Diptera (e.g. gall midges), Homoptera (e.g. Adelgids, Aphids and Psyllids), Coleoptera (e.g. some Cerambycids) and Lepidoptera (e.g. some Olethreutids) (Ronquist and Liljeblad, 2001). Among hymenopterous insects the common gall makers are in three families, namely Cynipidae, Tenthredinidae and Eurytomidae. Cynipid wasps, often called gall wasps, are the most common hymenopterous gall makers (Buss, 2003). Gall wasps, or cynipids, form the second largest radiation of galling insects with more than 1300 described species (Ronquist and Liljeblad, 2001). The Gall wasps hijack the physiology of their host plant to produce galls that house wasps throughout their immature stages (Cooper and Rieske, 2009).

2.6. Colonization and rearing of *L. invasa*

L. invasa populations have been reared on artificial diet (honey or sucrose) under different environmental conditions and with varying levels of success (Mendel *et al*., 2004; Doganlar, 2005; Hesami *et al*., 2005) with varying degrees of success.

Insect rearing activity usually aims at multiplying and/ or keeping alive a sufficient number of insects (of a given species, life cycle stage, age, population or progeny) for a predetermined objective (Hill, 1994; Bandah, 1994).

Since *L. invasa* is a new pest in many *Eucalyptus* growing zones of the world (Mendel *et al*., 2004), there is no universal protocol for its colonization and rearing. Being a gall-inducing insect, basic procedures for rearing phytophagous insects are used (Ochieng'- Odero,1994) although the insect has its specific diet requirements, diet application methods and optimum environmental conditions, all of which can vary in time and space.

2.7. Optimum foraging theory

Optimal foraging theory is based on the premise that animals should forage to maximize intake rate (amount/ time) of limiting resources (Belovsky, 1978; Pyke, 1984). The limiting resource is often assumed to be energy and the forager has to make a choice between food types and location (patches). When exploiting a depletable food patch, aforager must decide when to abandon the present patch and seek another (Charnov, 1976; Mc Namara, 1982; Brown, 1988). A forager should stay in a patch as long as its yield in that patch is above the average yield over all patches

(MacArthur and Pianka 1966). Also, time taken by a forager at at a patch may be shortened by pressure from predators (Casas and Aluja, 1997).

Proponents of optimal foraging theory attempt to predict the behaviour of animals while they are foraging; this theory is based on a number of assumptions, some of which are as follows (Krebs and Davies, 1993; Pyke, 1984): (i) An individual's contribution to the next generation (i.e. fitness) depends on its behaviour while foraging; (ii) it is assumed that there should be a heritable component of foraging behaviour, i.e. an animal that forages in a particular manner should be likely to have offspring that tend to forage in the same manner; (iii) the relationship between foraging behaviour and fitness is known. This relationship is usually referred to as "currency" of fitness. In general, any such currency will include a time scale, although in some cases it may be assumed that fitness is a function of some rate.

The evolutionary fitness of an animal depends significantly upon an optimal diet in both quantity and quality. Foraging strategies are therefore rigorously shaped by natural selection (Hassell & Southwood, 1978). Any decision of foraging behaviour is complicated by the forager's perceiving the environment at several hierarchical levels. Such levels can be classified as follows (Hassell & Southwood, 1978): the habitat, the patch, and the food item. According to classical foraging theories, foragers maximize energetic gains by selectively exploiting patches rich in resources, and by minimizing foraging time in poor sites (Stephens & Krebs, 1986). Models such as forager's gain curves (Olsson *et al.*, 2001) have been put forth to predict animal's foraging behaviour and expected benefits and, by extension fitness.

Foraging can be for either suitable food sources or suitable oviposition sites (Almohamad *et al.,* 2009) and current approaches for studying host selection by phytophagous insects are mainly based on optimal oviposition theory, i.e. the preference-performance hypothesis (Scheirs, 2001). For instance, habitat, host physical characteristics and semiochemicals among other factors have been shown to be involved in the selection of oviposition site by aphidophagous hover flies (Almohamad *et al.,* 2009). No previous studies on foraging behaviour of *L. invasa* have been done.

2.8. Herbaceous plants as *E. Saligna* IPM strategy against *L. invasa*

Several herbaceous tropical plants have been recognized to possess chemicals with insecticidal activity, either as secondary metabolites in their tissues or as volatile oils that produce odour plumes that are unpleasant or lethal to insects. For instance, essential oils of Marigold, *Tagetes patula* (Family Asteraceae or Compositae) are larvicidal on larvae of several mosquito species (Dharmagadda *et al*., 2005) and essential oils of *T. minuta* (Family Asteraceae), *Hyptis suaveolens* (Family Labiatae), *Ocimum canum* (Family Labiatae), *O. basilicum* (Family Labiatae) and *Piper guineense* (Family Piperaceae) are insecticidal on some insects, including cow pea weevil, *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). Aromatic plant species, particularly those in the family Labiatae (or Lamiaceae) and Compositae (or Asteracea), are among the most widely used plants in insect pest control (Lambert *et al,* 1985; Shaaya *et al*., 1997; Kéïta *et al*., 2000).

2.9. Plant resistance to insects

The cultivation of plants that are resistant to insects is a plant protection technique that has been used for several hundreds of years (Painter, 1951). Plant resistance to

insects is composed of genetically inherited qualities that result in a plant of one cultivar or species being less damaged than is a susceptible plant, which lacks these qualities (Smith, 1989). The effects of resistant plants on insects can be manifested as antibiosis, in which the biology of the pest insect is adversely affected, or as antixenosis, in which the plant acts as a poor host and the pest insect then selects an alternative host (Guthrie *et al*., 1978; Davies, 1985). The inherent genetic qualities of the plant itself may aid it in expressing tolerance to the pest insect and afford it the ability to withstand or recover from insect damage.

CHAPTER THREE

MATERIALS AND METHODS

3.1. General procedures

3.1.1. Site description

Field materials for this study were collected from Kenya Forest Service Zonal tree nursery at Kisumu County. Kisumu County lies between 1130 – 1835 metres above sea level (m.a.s.l.) with a mean annual rainfall ranging from less than 1000 mm to 1630 mm. The County has mean annual maximum temperature of 25^0 – 30^0 C, and mean annual minimum temperature of 9^0 – 18^0 C (GOK, 1997a). Laboratory experiments were conducted in the facilities of University of Eldoret. The University is situated in Uasin Gishu County, Kenya. Uasin Gishu County lies between 1200 – 2100 m.a.s.l. with a mean annual rainfall of 960 mm. The county has mean annual maximum temperature of 24^0 – 26^0 C, and mean annual minimum temperature of 6^0 – 10^0 C (GOK, 1997b).

3.1.2. General insect rearing procedure

3.1.2.1. Insect colonization

Twenty potted, infested *E. saligna* seedlings measuring 10 – 25 cm in height were randomly collected from K.F.S. zonal tree nursery in Kisumu County and used as sources of insect larvae for colony establishment.

In a well lit laboratory the seedlings were divided into two groups of ten and each group randomly arranged in 1 m^3 ventilated glass emergence cages as shown in Plate 2. The cages were managed till insect emergence occurred, which took four and a half (4.5) months. Detailed procedure has been outlined in appendix 1.

Plate 2: Some of the glass cages used for rearing *Leptocybe invasa* in *Eucalyptus saligna* seedlings. (Source: Author, 2006).

3.1.2.2. Room temperature measurements

Temperature measurements inside cages were taken using thermometers centrally held from the roof of each cage. Mean room temperature was determined from three thermometers held outside the cages by means of retort standsands. Temperature measurements were taken thrice a day at 8:00 a.m., 12:00 noon and 4:00 p.m.

3.1.2.2. Cage management

Cage management included daily cleaning, watering, weeding and fertilization of the seedlings. Cage floor was cleaned thoroughy to remove abscissed leaves, excess water and soil. Watering was done after every two days by adding 20 cm^3 of water to each polythene tube containing the seedlings. Weeds were removed by hand daily, i.e. immediately they germinated. Fertilizer application involved putting five (5) pellets of urea 4 cm from the root collar of each seedling and was done once after four weeks.

3.1.2.3. Insect rearing

Once insects emerged from caged *E. saligna* seedling they were immediatey tranfered to 250-ml beakers where they were reared on artificial diet (15% sucrose solution). The beakers containing the insects were covered with cotton cloth and 15% sucrose was supplied on ball of cotton wool placed on the cloth cover (Plate 3). The insects, thus, fed from beneath the cloth cover. This was done to avoid accidental insect mortalities due to insects getting stuck on sucrose solution. An insecticide in powder formulation was sprinkled round a set of beakers containing *L. invasa* in order to prevent other crawling insects like ants from reaching the wools soaked in sucrose solution.

3.2. Experiment 1: Colonization and rearing of *Leptocybe invasa*

This experiment was done to devise an appropriate colonisation and rearing procedures for *L. invasa*. Peak emergence and optimum temperature for emergence of *L. invasa*, survival of *L. invasa* under different diets and the mating behaviour of the insect were elucidated under this aspect.

3.2.1. Optimum emergence temperature and peak emergence of a generation of *Leptocybe invasa*

Daily records of diurnal temperature inside the cages and the room were kept. Temperature measurements were taken thrice a day (at 8:00 a.m., 12:00 noon and 4:00 p.m.) and means computed to obtain diurnal temperatures. Once *L. invasa* emergence began the following parameters were measured and recorded daily for each cage: date of emergence, number of emergent insects and temperature inside the cage at the time of emergence. Emergent insects were collected daily and used in the following experiments.

3.2.2. Survival of *Leptocybe invasa* under ten different diet application methods

One hundred insects were divided into twenty groups of five and put in 250-ml glass beakers. Using a completely randomised design (CRD) the insects were subjected to at least one of ten treatments (T) with two replicates as follows: T1: Beaker covered by dry cotton wool plug; ball of cotton wool moistened with 10% sucrose and put inside the beaker. T2: Beaker covered by dry cotton wool plug; ball of cotton wool moistened with plain water and put inside the beaker. T3: Beaker covered by cotton wool plug moistened with 10% sucrose solution. T4: Beaker covered by cotton wool plug moistened with plain water. T5: Beaker covered by dry cotton wool plug. T6: Beaker covered by dry cotton cloth; ball of cotton wool moistened with 10% sucrose and put inside the beaker. T7: Beaker covered by dry cotton cloth; ball of cotton wool moistened with plain water and put inside the beaker. T8: Beaker covered by cotton cloth moistened with plain water. T9: Beaker covered by cotton cloth moistened with 10% sucrose. T10: Beaker covered by dry cotton cloth.

Room temperature was recorded from a thermometer held above the beakers. Fresh diet was supplied daily at 8:00 a.m., 12:00 noon and 4:00 p.m. The number of insects alive in each beaker was noted daily at 10: 00 a.m. till all of the insects were dead. The treatment that achieved longer insect survival was chosen as a standard for a further diet experiment. It was however modified by placing a ball of cotton wool soaked in diet on the cotton cloth that covers the beaker instead of moistening the entire cloth. Ants, which were attracted to the beakers by sucrose, were kept away by using super grain dust insecticide (0.1% Bifenthrin and 99.9% Inert) sprinkled on a line surrounding the beakers.

3.2.3. Survival of *Leptocybe invasa* under eight different diet treatments

One hundred and sixty insects were divided into thirty-two groups of five and put in 250-ml glass beakers different from the above preceeding study set up. Using a completely randomised design (CRD) the insects were subjected to at least one of eight treatments (T) with four replicates as follows: T1: 5% Sucrose solution. T2: 10% sucrose solution. T3: 15% sucrose solution. T4: 20% sucrose solution. T5: Mixture of 5% sucrose and host plant extract in the ratio of ratio 1:1 by volume. The plant extract was made by finely crushing four (4) leaves of a third internode from the apex of eucalyptus seedling using pestle and mortar and adding 2 cm^3 of distilled water. T6: Host plant extract alone. T7: Control (No feeding). T8: Plain water (plate 3).

Plate 3: Beakers used to keep and feed *L. invasa* on artificial diet (10 % sucrose). (Source: author, 2006)

3.2.4. Mating behaviour of *Leptocybe invasa*

Two random samples of six unsexed insects were drawn from each cage daily and kept in 250-ml glass beakers covered by cotton cloth. The insects were closely monitored for three days for any of the following forms of mating behaviour: aggregation, swarming, courtship and pairing. This was done for 10 minutes thrice a day from 11:00 a.m., 1:00 p.m. and 5:00 p.m.

Various forms of mating behaviour that were looked for were as follows: Agroup of the insects occurring close to each other in the same place constituted aggregation. When a group of insects remain more or less stationary, flying over one spot (e.g. due to a reaction to a feature of the environment), they are said to be swarming. Visual displays and feeling of each other (at close range) were distinguished as courtship. Males and females coming together and one sex mounting on the back of the other, or in end-to end position was reckoned to constitute pairing (Chapman, 1982).

3.2.5. Data analysis

Descriptive statistics was used to compute mean diurnal temperature (and their standard errors) inside and outside the cages using MS Excel software. The hypothesis that the numbers of emergent adults from the two cages were not different was tested by two-sample *t*-test ($\propto = 0.05$) assuming unequal variances. One –way ANOVA ($\propto = 0.05$) was used to test the hypothesis that the survival (i.e. number of days alive) of *L. invasa* under various diet treatments and diet application methods were the same. This was done using STATISTICA 6.0 software. Means of treatments and application methods were separated by Fisher's LSD test and plotted. No analysis

was done on mating behaviour of *Leptocybe invasa* because no incident of aggregation, swarming, courtship and pairing was noted.

3.3. Experiment 2: Biology of *Leptocybe invasa*

Four sets of experiments were conducted in the laboratory (from November, 2005 to March, 2006; March to August, 2006; November, 2006 to March, 2007 and from March to July, 2008respectively) to determine the life cycle of *L. invasa* under room conditions. For each set of experiment, infested *E. saligna* seedlings with mature galls were collected from Kisumu County and transferred to glass cages as in preceded studies at Chepkoilel University College to yield adult *L. invasa* for use in subsequent life cycle studies (Sections 3.31 and 3.32).

3.3.1. Time taken from oviposition to emergence of adult *Leptocybe invasa*

Infected *E. Saligna* seedlings were used in cages to obtain a new generation of *L. invasa* insects. Newly emerged insects were immediately used to infect a healthy group of three weeks old twenty (20) caged *E. Saligna* seedlings under room conditions. Watering of the seedlings was done after every two days and standard cage management was done as outlined in section 3.1.2.2. The seedlings were observed daily for initiation of gall formation and emergence of the insects.

3.3.2. Life cycle of *Leptocybe invasa*

Two groups of caged twenty (20) healthy *E. saligna* seedlings of 10 cm – 15 cm in height were exposed to thirty (30) *L. invasa* to stimulate attack and then managed and observed to investigate the life cycle of the insect pest. Cage and room temperatures were recorded daily at three regular time intervals (8:00 a.m., 12:00 noon and 4:00

p.m). The following observations were made on one of the groups of the seedlings and recorded from infestation date (date of exposure): (i) time (days) to appearance of white/ yellowish substance on infected surfaces of seedlings, indicating successful oviposition; (ii) time (days) to appearance of galls; (iii) time (days) to insect emergence.

The other group of seedlings were used to monitor life history of the insect: each seedling was sacrificed after every 7 days and its galls dissected and observed for egg, larval and pupal development and measurements. The following observations and measurements made: (i) Egg: shape, colour, size, numbers per oviposition event, location of oviposition within plant tissue. (ii) Larva; shape, body length, head capsule length and head capsule width. Head capsule widths of larvae were measured using a micrometer eye-piece fitted in a binocular microscope and used to calculate growth ratio, which indicated number of larval instars according to Dyar's law (Dyar, 1890; Klingenberg & Zimmermann, 1992). (iii) Pupa: shape and size. (iv) Adult: The following measurements on individuals of each group of adult *L. invasa* emerging from galls were taken: Body length and width, Head capsule (eye to eye) length, Antennal length and number of segments of antenna, Wing length, and abdominal length and width.

3.4. Experiment 3: Ecology of *Leptocybe invasa*

3.4.1. Oviposition requirements of *Leptocybe invasa*

These experiments were conducted to investigate cues for oviposition by *L. invasa* in relation to visual and olfaction stimuli. Twenty trials using artificially fed insects in twelve (24) beakers (one insect per beaker) under room temperature (26^0C) and subjected to eight treatments with three replicates as follows:

Treatment A: An insect put together with a piece of host plant leaf (1 x 1 cm) in a vial.

Treatment B: An insect put together in a vial with a piece of host plant leaf (1 x 1 cm) that had been smeared with clear vanish and left overnight for the vanish odour to diffuse away.

Treatment C: An insect put together in a vial with a piece of host plant leaf (1 x 1 cm) and a piece of filter paper (1 x 1 cm) soaked in leaf extract.

Treatment D: treatment B was repeated with a piece of filter paper soaked in leaf extract added into the vial.

Treatment E: An insect put together in a vial with a piece of host plant leaf (1 x 1 cm) and a piece of filter paper (1 x 1 cm) not soaked in leaf extract.

Treatment F: treatment B was repeated with a piece of filter paper not soaked in leaf extract added into the vial.

Treatment G: An insect put together in a vial with a piece of filter paper (1 x 1 cm) soaked in leaf extract.

Treatment H: An insect put together in a vial with a piece of filter paper (1 x 1 cm) not soaked in leaf extract.

Oviposition behaviours shown by the insects were noted. Pieces of leaves and filter papers were removed from the vials after four days of exposure and inspected for oviposition punctures under microscope (x 100). The leaf tissue was then dissected under a microscope (x100) and any eggs revealed were counted. The resulting set of data (Appendix 5) was summarized and subjected to one-way ANOVA using SPSS version 17 software, and differences determined using multiple comparisons of Tukey test (Zar, 1984; Stolion, 1981).

3.4.2. Foraging and patch use by adult *Leptocybe invasa*

An equal number of singly caged adult *L. invasa* were provided with two different types of habitat structures repeated twelve times (12 trials). The first set of habitat structure presented vertical structure comprising four (4) seedlings each of *Eucalyptus saligna, Cupressus lusitanica* and *Grevillea robusta* ranging from 10 cm to 15 cm. a total of twelve (120 seedlings were therefore kept in each cage and exposed to *L. invasa* insect. The second set of habitat structure presented horizontal structure comprising four sets of petri dishes and each petri dish containing randomly arranged five 1 cm by 1 cm pieces of filter papers. Each of the filter papers had one of the following treatments: dry, moist (soaked in water), soaked in *E. saligna* leaf extract, soaked in *G. robusta* leaf extract, and soaked in *C. lusitanica* leaf extract. Patch use was predicted from marginal value theorem (Brown, 1988). Each was closely monitored one at a time with each set up lasting 20 minutes for the following variables: patch type landed on, time taken at a patch and time taken from one patch to another.

3.4.3. Host condition in relation to successful attack by *Leptocybe invasa*

E. saligna seedlings of two age categories were used. One week old *E. saligna* seedlings constituted one age category while six weeks old *E. saligna* seedlings another age category. For each age category, a randomized complete block design (RCBD) was used to subject groups of nine caged seedlings to all possible combinations of the following treatments for two months: Factors: watering regime, and N-fertilization regime. Each factor had the following levels: none, low and high based on the scale presented in appendix 7a. Thereafter, an equal number of ten *L. invasa* were introduced into the cages and monitored for gall formation. Numbers of galls per seedling were recorded at week 20 from exposure time. The resulting data were subjected to univariate ANOVA and means separated by Tukey HSD test sing SPSS version 17.0 software.

3.5. Experiment 4: The potential of selected herbaceous plant species as IPM components against blue-gum chalcid pest, *Leptocybe invasa*

In these studies the potential of three local herbaceous plant species namely lion's ear *Leonotis nepetifolia* (Family Labiatae or Lamiaceae); dwarf marigold, *Schkuria pinnata* Kuntz ex Thell (Compositae or Asteraceae) and marigold, *Tagetes erecta* L. (Family Asteraceae or Compositae) (Plates 4–6) were evaluated for their use in an IPM strategy for *L. invasa* as companion plants of *E. saligna*. Also, these studies investigated the preferred positions on *E. saligna* for oviposition by *L. invasa* and the effect of the three companion plant species on growth of *E. saligna* seedlings. Fifty muslin cloth cages were set within the greenhouse in a completely randomized design (CRD) with each cage enclosing potted seedlings of healthy *E. saligna* either mixed

with each one of the test plants in alternating rows or enclosed alone as shown in Plate 7.

A total of 50 unsexed adults were introduced into each cage over a successive period of 5 days (10 adults daily). The insects were then confined with the plants for two weeks before the cages were removed to minimize cage effects on plant growth. The insects had all died when the cages were removed. The herbaceous plants were monitored for five months for gall development.

(a) (b)

Plate 4: *Leonotis nepetifolia* (Family Labiatae or Lamiaceae): (a) Whole plant (b) Flowers of the plant. (Source: Wikimedia, 2007)

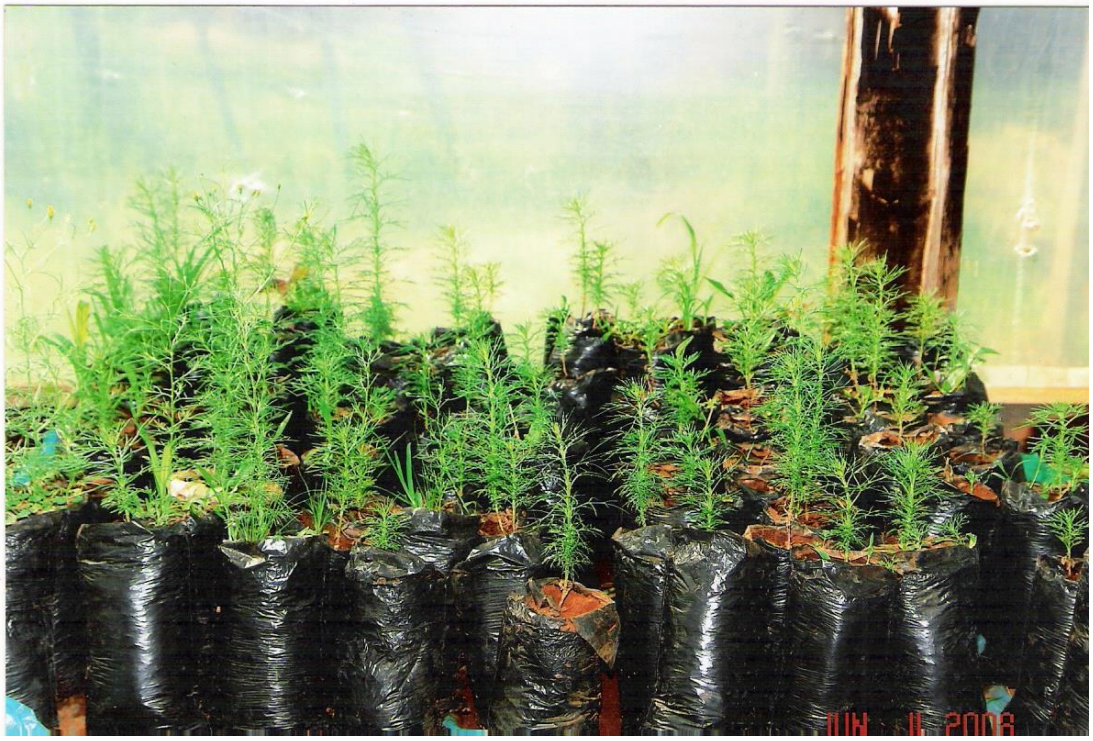


Plate 5: *Schkuria pinnata* Kuntz ex Thell (Compositae or Asteraceae). (Source: Author, 2008).

Plate 6: *Tagetes erecta* L. (Family Asteraceae or Compositae). (Source: Author, 2008).

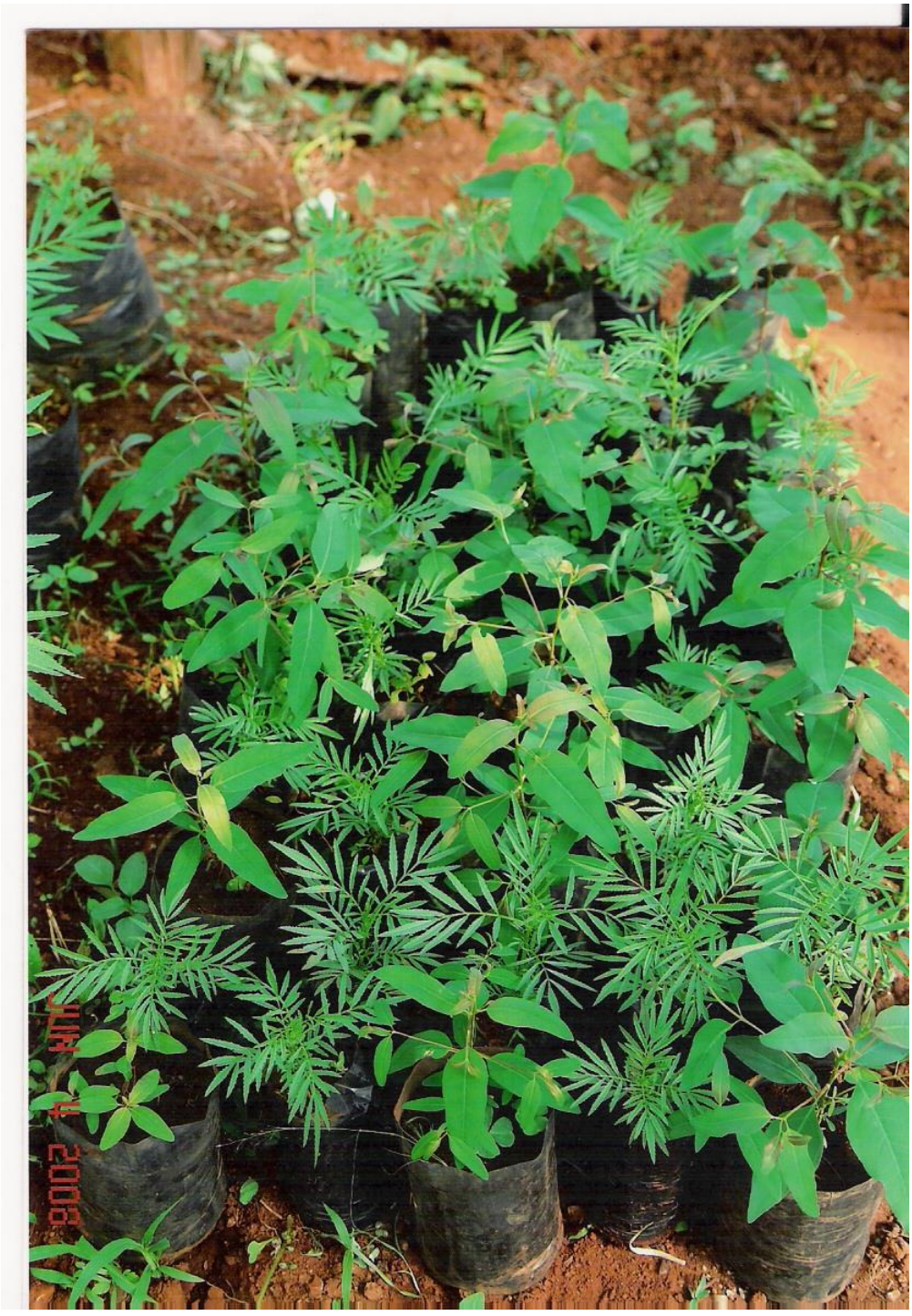


Plate 7: *Eucalyptus saligna* seedlings grown with *Tagetes erecta* as companion plants against *Leptocybe invasa* (Hymenoptera: Eulophidae). (Source: Author, 2008).

There were five groups (four treatments and a control) in total:

1- *E. saligna* and *L. nepetifolia* seedlings with *L. invasa*;

2- *E. saligna* and *S. pinnata* seedlings with *L. invasa*,

3- *E. saligna* and *T. erecta* seedlings with *L. invasa*;

4- *E. saligna* with *L.* and

5- *E. saligna* alone (control).

Height (Ht) and root collar diameter (RCD) growth of *E. saligna* were measured weekly for five months while the number of galls occurring on leaf mid-ribs, petioles and twigs of the seedlings were recorded for three months from the onset of gall induction.

3.5.1. Data analysis

Data on height (cm), root collar diameter (mm) and number of galls that developed on *E. saligna* were subjected to ANOVA at 95 % confidence interval and means separated by Tukey test using SPSS for Windows version 17 software.

3.6. Variability in *L. invasa* attack between major *Eucalyptus* germplasm.

A total of forty (40) caged three weeks old seedlings of four *Eucalyptus species* (*E. saligna, E. camaldulensis, E. citriodora* and *E. globulus*) of 12.5 ± 2.5 cm height were divided into five groups of eight (8) as follows:

(i) Eight (8) *E.saligna* seedlings caged a lone;

(ii) Eight (8) *E. camaldulensis* seedlings caged alone;

(iii) Eight (8) *E. citriodora* seedlings caged alone;

(iv) Eight (8) *E. globules* seedlings caged alone; and

(v) Two (2) of each of the four seedlings caged as a mixture (i.e. eight seedlings of different species caged together).

Seedlings in each cage were then exposed to *L. invasa* by releasing ten (10) of the insects into the cages. The seedlings were managed till gall development occurred. The number of galls per seedling was recorded and the resulting data subjected to one-way ANOVA at 95 % confidence interval using SPSS version 17 software. Means were separated by Tukey HSD test (Zar, 1984).

CHAPTER FOUR

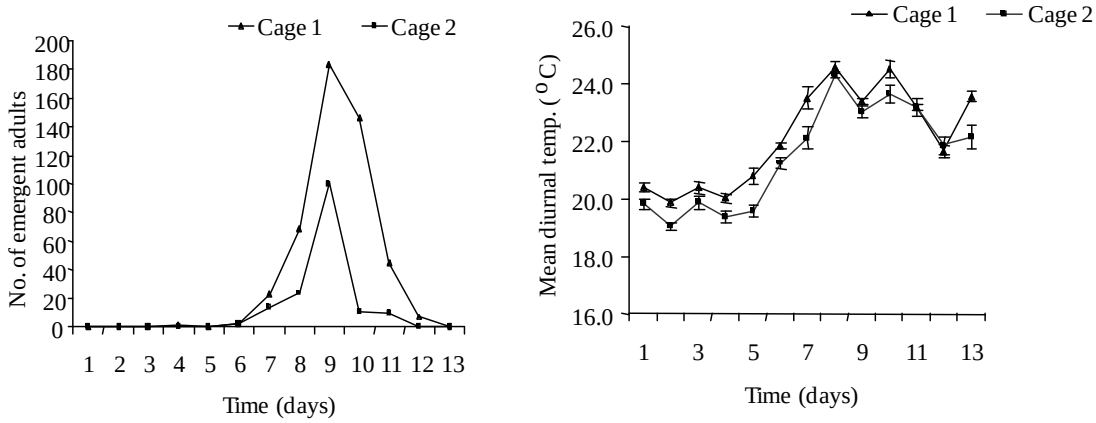
RESULTS

4.1. Colonization and rearing of *Leptocybe invasa*

4.1.1. Peak emergence and optimum temperature for *L. invasa* emergence

Data showing number of emergent adult *L. invasa* under room temperature are presented in figure 3. Data showed that once adult emergence began, less than 20 adults emerged between the first and the sixth day of emergence. However, 20-24 adults emerged per day between the sixth and seventh day, 40-100 between the eighth and ninth day and peaked in the ninth day of emergence when > 100 – 190 adults were recorded.

The largest number of adults emerged (24 insects per cage of ten seedlings) at room temperature of 26 ^{0}C (Figure 4). The optimum temperature for *L. invasa* emergence was 25.5 ± 0.6 ^{o}C and differences between numbers of emergent insects at different diurnal temperatures were significant at 95% CI ($P < 0.05$). These results indicated that environmental temperatures lower than 25 ^{0}C and higher than 26 ^{0}C curtailed reproductive potential of *L. invasa*.



(a) Numbers of emergent *Leptocybe invasa* **(b) Mean (±SE) weekly diurnal temperature**

Figure 3: Numbers of emergent *Leptocybe invasa* adults from infected caged *Eucalyptus saligna* seedlings maintained at different temperatures.

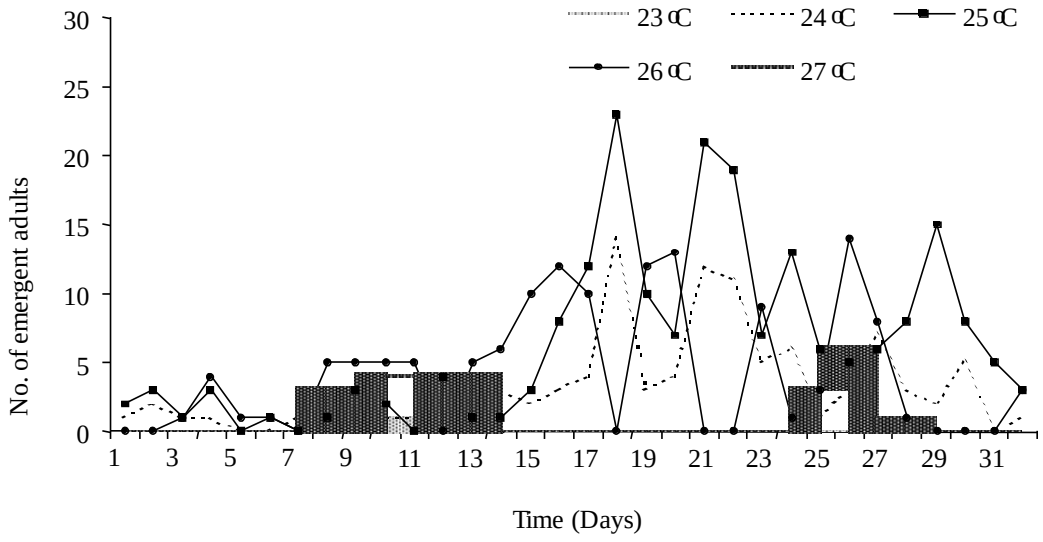


Figure 4: Adult *L. invasa* emergence at different diurnal temperatures (^{o}C).

4.1.2. Survival of *L. invasa* under various diet application methods

Table 1 shows survival of *L. invasa* (Mean days alive) under various diet application methods while their corresponding LSD tests are presented in Table 2. Mean diurnal room temperature was 22.4 ± 0.1 ^{o}C (Figure 5a). Differences between treatment effects were significant ($P < 0.05$). Beakers covered by cotton cloth moistened with 10% sucrose (Treatment 9) achieved the highest survival of the insect (6 days; 2.0 ± 1.0 insects alive), followed by beakers covered by cotton cloth moistened with plain water (Treatment 8) and then beakers covered by dry cotton cloth (Treatment 10) (Table 2 and Figure 5b). These results showed that other than nutritional value of artificial diet, the method of presenting the diet to the insects influenced survival of the latter. They also indicated that the life span of *L. invasa* was 4-6 days.

Table1: Mean survival of *Leptocybe invasa* under various diet treatments and application methods

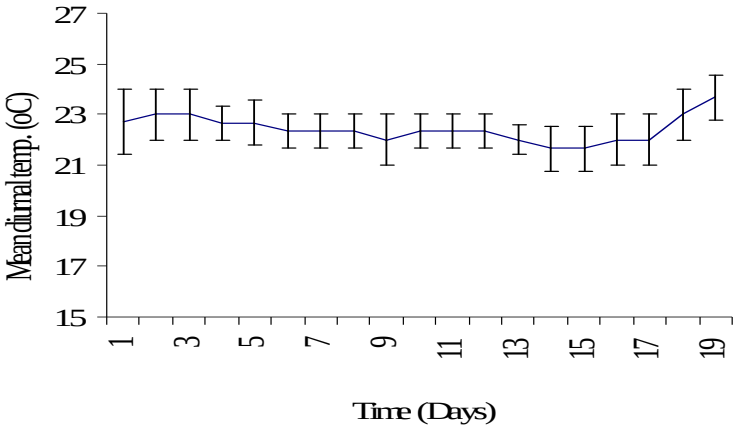
	Number of *L. invasa* adults surviving within one week							
T/D	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
T1	5.0 ± 0.0	2.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T2	5.0 ± 0.0	3.5 ± 0.5	1.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T3	5.0 ± 0.0	1.5 ± 0.5	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T4	5.0 ± 0.0	2.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T5	5.0 ± 0.0	1.5 ± 0.5	1.0 ± 1.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T6	5.0 ± 0.0	3.5 ± 0.5	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T7	5.0 ± 0.0	5.0 ± 0.0	3.5 ± 0.5	2.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T8	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	4.5 ± 0.5	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
T9	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	2.5 ± 0.5	2.0 ± 1.0	0.0 ± 0.0
T10	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0

T – Treatment, **D** – Day. **T1**: Beaker covered by dry cotton wool plug; ball of cotton wool moistened with 10% sucrose and put inside the beaker, **T2**: Beaker covered by dry cotton wool plug; ball of cotton wool moistened with pain water and put inside the beaker, **T3**: Beaker covered by cotton wool plug moistened with 10% sucrose solution, **T4**: Beaker covered by cotton wool plug moistened with plain water, **T5**: Beaker covered by dry cotton wool plug, **T6**: Beaker covered by dry cotton cloth; ball of cotton wool moistened with 10% sucrose and put inside the beaker, **T7**: Beaker covered by dry cotton cloth; ball of cotton wool moistened with plain water and put inside the beaker, **T8**: Beaker covered by cotton cloth moistened with plain water, **T9**: Beaker covered by cotton cloth moistened with 10% sucrose, **T10**: Beaker covered by dry cotton cloth

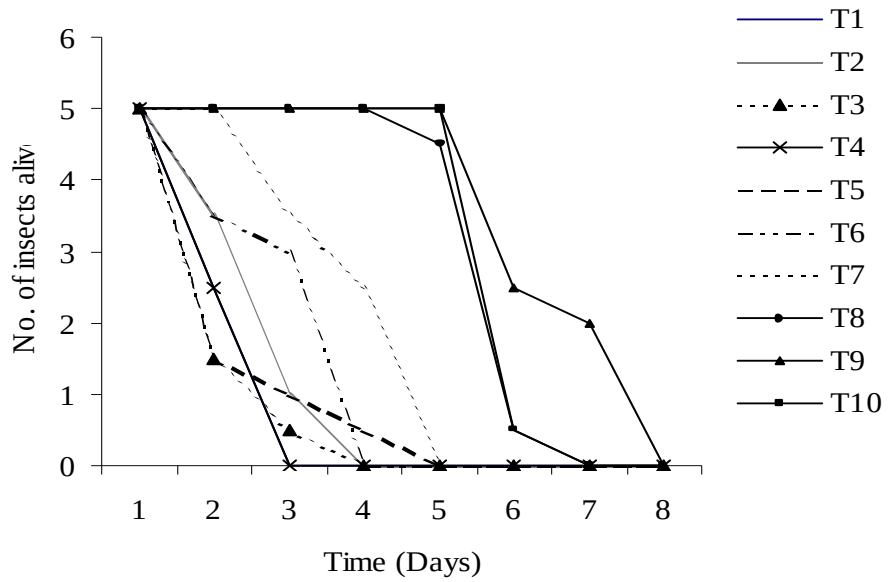
Table 2: Fisher's LSD test on mean survival of *Leptocybe invasa* under various diet treatments and application methods at room temperature (22.4 ± 0.1 ^{o}C).

Treatment	{1} 0.938	{2} 1.188	{3} 0.875	{4} 0.938	{5} 1.000	{6} 1.438	{7} 2.000	{8} 0.313	{9} 3.688
2	0.809								
3	0.952	0.762							
4	1.000	0.809	0.952						
5	0.952	0.856	0.904	0.952					
6	0.628	0.809	0.586	0.628	0.672				
7	0.305	0.432	0.278	0.305	0.335	0.586			
8	0.037*	0.064	0.032*	0.037*	0.043*	0.105	0.278		
9	0.009*	0.018*	0.008*	0.009*	0.011*	0.032*	0.105	0.586	
10	0.032*	0.056	0.028*	0.032*	0.037*	0.093	0.252	0.952	0.628

Error: Between MS = 4.2330, d.f. = 70 *Significant ($\propto$ = 0.05).



(a) Room temperature



(b) Survival of *L. invasa*

Figure 5: Survival of *Leptocybe invasa* (Mean days alive) under different diet treatments and application methods at room temperature (22.4 ± 0.1 ^{o}C).

4.1.3. Survival of *L. invasa* under various diet treatments

Results presented in figure 6 showed that *L. invasa* survived longer (16 ± 1 days; 2.0 ± 0.6 out of initial 5.0 insects alive) on 15% sucrose than the other diets while control insects had the shortest survival (3.5 ± 1 days; 2.0 ± 0.6 out of initial 5.0 insects alive). Pooled data for treatments 1, 2 and 3 indicated that mean number of survaival days for *L. invasa* on 5% - 15% sucrose solution was fifteen (15) days (1.0 ± 0.6 out of initial 5.0 insects alive) (Table 3). Differences between treatment effects on *L. invasa* survival were significant ($P < 0.05$) and Fisher's LSD (Table 4) exposed 15% sucrose solution (Treatment 3) as the most effective in achieving high survival (15 days) in *L. invasa* among the other investigated diets. These results indicate that natural conditions that may avail sugar solution (e.g. nectar) in adult *L. invasa* environments are likely to enhance the insect's survival, and thus increased infestation of host plants.

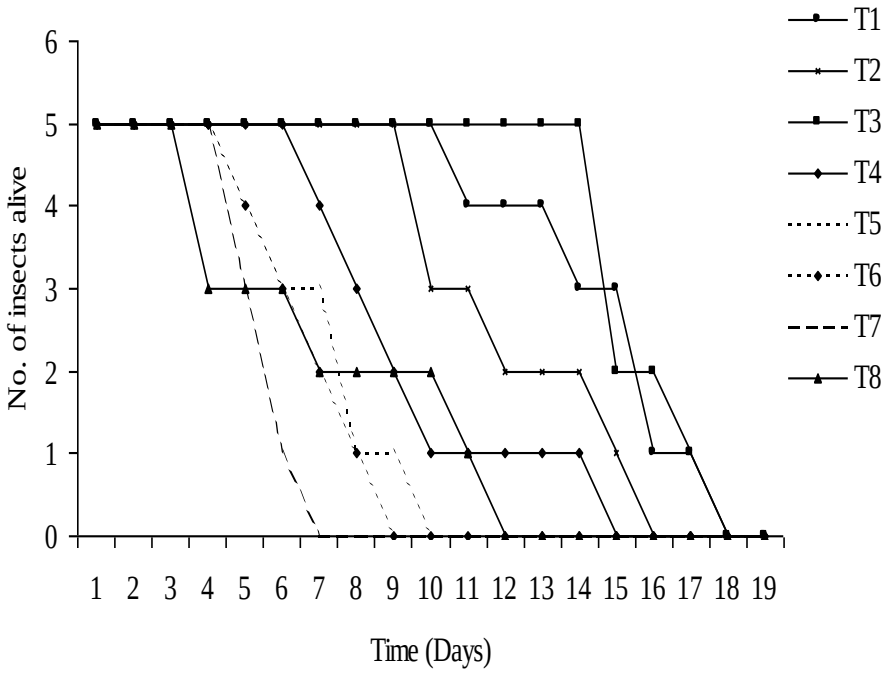


Figure 6: *L. invasa* survival under various artificial diet treatments at room temperature (22.4 ± 0.1 ^{o}C). Treatments were as follows; T1: 5% sucrose solution, T2: 10% sucrose solution, T3: 15% sucrose solution, T4: 20% sucrose solution, T5: Mixture of 5% sucrose solution and host plant leaf extract (ratio of 1:1 vol.), T6: Host plant leaf extract alone, T7: Control (no feeding), T8: Plain water.

Table 3: Pooled data showing mean (±SE) survival of *L.invasa* subjected to three different concentrations of sucrose diet at room temperature (22.4 ± 0.1 ^{o}C).

Day	Treatment (Sucrose solution as diet)			Mean number of *L. invasa* alive
	5%	10%	15%	
0	5	5	5	5.0 ± 0.0
1	5	5	5	5.0 ± 0.0
2	5	5	5	5.0 ± 0.0
3	5	5	5	5.0 ± 0.0
4	5	5	5	5.0 ± 0.0
5	5	5	5	5.0 ± 0.0
6	5	5	5	5.0 ± 0.0
7	5	5	5	5.0 ± 0.0
8	5	5	5	5.0 ± 0.0
9	5	3	5	4.3 ± 0.7
10	4	3	5	4.0 ± 0.6
11	4	2	5	3.7 ± 0.9
12	4	2	5	3.7 ± 0.9
13	3	2	5	3.3 ± 0.9
14	3	1	2	2.0 ± 0.6
15	1	0	2	1.0 ± 0.6
16	1	0	1	0.7 ± 0.3
17	0	0	0	0.0 ± 0.0
18	0	0	0	0.0 ± 0.0

Table 4: Fisher's LSD test for differences in sucrose diet treatment effects on survival of *L. invasa*

Treatment	{1}	{2}	{3}	{4}	{5}	{6}	{7}
	3.6842	**3.0526**	**3.9474**	**2.3158**	**1.6842**	**1.5789**	**1.2632**
2	0.980						
3	1.000	0.874					
4	0.426	0.952	0.202				
5	0.048*	0.426	0.013*	0.980			
6	0.029*	0.326	0.008*	0.952	1.000		
7	0.006*	0.115	0.001*	0.749	0.998	1.000	
8	0.061	0.480	0.017*	0.988	1.000	1.000	0.996

Error between MS = 4.0987, d.f. = 144. *Significant ($\propto$ = 0.05). Treatments were as follows; **T1**: 5% sucrose solution, **T2**: 10% sucrose solution, **T3**: 15% sucrose solution, **T4**: 20% sucrose solution, **T5**: Mixture of 5% sucrose solution and host plant leaf extract (ratio of 1:1 vol.), **T6**: Host plant leaf extract alone, **T7**: Control (no feeding), **T8**: Plain water.

4.1.4. Mating behaviour of *Leptocybe invasa*

The results on mating behaviour of *L. invasa* obtained during the investigations on aggregation, swarming, courtship and pairing are presented in Table 5. According to these data (Table 5) it was apparent that the pest did not engage in mating behaviour based on the parameters tested. These results indicated that all the well documented phenomena of sexual reproduction did not apply in *L. invasa*. The pest's mode of reproduction, therefore, could be parthenogenetic although this need to be confirmed by means other rhan mating behaviour.

Table 5: Observation of mating behaviour on groups of six *L. invasa* monitored for ten minutes three times a day for three days

Group	Mating behaviour			
	Aggregation	**Swarming**	**Courtship**	**Pairing**
1	*	*	*	*
2	*	*	*	*
3	*	*	*	*
4	*	*	*	*
5	*	*	*	*
6	*	*	*	*
7	*	*	*	*
8	*	*	*	*
9	*	*	*	*
10	*	*	*	*

Key: + Mating behaviour observed

*No mating behaviour observed

4.2. Biology of *Leptocybe invasa*

4.2.1. Time taken from oviposition to emergence of adult *Leptocybe invasa*

The results of studies on oviposition, gall development and emergence of *Leptocybe invasa* (Hymenoptera: Eulophidae) from caged *E. Saligna* seedlings (Table 6) indicated that the developmental time taken by the pest from oviposition to emergence is 129 ± 1.41 days (4.2 – 4.3 months). During these studies insect emergence per cage per day ranged from 20-30 individuals. It was concluded from the data that obtained during these investigations that L. invasa had a life cycle of approximately four months pointing to its immense potential for rapid population build-up over three generations in a year.

Table 6: Observations on oviposition, gall development and emergence of *Leptocybe invasa* (Hymenoptera: Eulophidae) from caged *E. Saligna* seedlings

S/No.	Observation	Cumulative no. of days observed in each set of experiment						
		I	II	III	IV	n	**Mean no. of days**	**SE of mean**
1.	Exposure date	0	0	0	0	4	**0.00**	**0.00**
2.	Appearance of white/ yellowish substance on different parts of midrib, leaf petiole and stems of seedlings	1	2	2	1	4	**1.50**	**0.29**
3.	Galls appear.	12	13	12	13	4	**12.50**	**0.29**
4.	Bump-shaped galls	24	25	27	25	4	**25.25**	**0.63**
5.	Green, shinny galls begin to turn pinkish	29	31	35	38	4	**33.25**	**2.02**
6.	Glossy galls lose their shininess	124	120	128	126	4	**124.50**	**1.71**
7.	Adult *L. invasa* insects begin to emerge	125	131	131	129	4	**129.0**	**1.41**
8.	Emergence of adult *L. invasa* continues	130	132	134	130	4	**131.5**	**0.96**
9.	No adult *L. invasa* emergence occurs	132	133	135	138	4	**134.5**	**1.32**

4.2.2. Egg and oviposition of *Leptocybe invasa*

Data on egg characteristics of *L. invasa* obtained during these studies are presented in Table 7 and figure 7. The diameter of eggs averaged 0.11± 0.01 mm (range 0.09 mm to 0.14 mm).

It was further observed that eggs were whitish to yellowish in colour when laid and were normally laid in singles in rows beneath the epidermis of the host tissue. A whitish substance appeared at oviposition spots 1.50 ± 0.29 days later (Appendix 2b). A single *L. invasa* laid an average (±SE) of 6.33 ± 0.29 eggs in four days (n=60) on *E. Saligna* leaf tissue (Appendix 5b). Egg incubation period was 8.2 ± 5.1 days.

Table 7: Egg measurements (diameter in mm) of *Leptocybe invasa*

S/NO.	Replicates			
	Set I	Set II	Set III	Set IV
1	0.10	0.11	0.11	0.11
2	0.09	0.10	0.12	0.09
3	0.12	0.12	0.10	0.12
4	0.11	0.11	0.09	0.11
5	0.09	0.10	0.10	0.10
6	0.11	0.09	0.09	0.12
7	0.09	0.14	0.11	0.11
8	0.12	0.09	0.09	0.12
9	0.09	0.11	0.09	0.09
10	0.10	0.12	0.09	0.10
11	0.09	0.09	0.12	0.11
12	0.10	0.12	0.11	0.09
13	0.11	0.09	0.10	0.09
14	0.10	0.14	0.11	0.12
15	0.10	0.08	0.09	0.10
16	0.12	0.09	0.12	0.11
17	0.11	0.12	0.12	0.12
18	0.12	0.09	0.10	0.12
19	0.11	0.12	0.09	0.11
20	0.09	0.11	0.11	0.09
n = 20	**mean=0.10** **SD =0.01** **SE = 0.00**	**mean =0.11** **SD =0.02** **SE = 0.00**	**mean =0.10** **SD =0.01** **SE = 0.00**	**mean =0.11** **SD =0.01** **SE = 0.00**

Mean of means ± SD = 0.11 ± 0.01

Mean of means ± SE = 0.11 ± 0.00

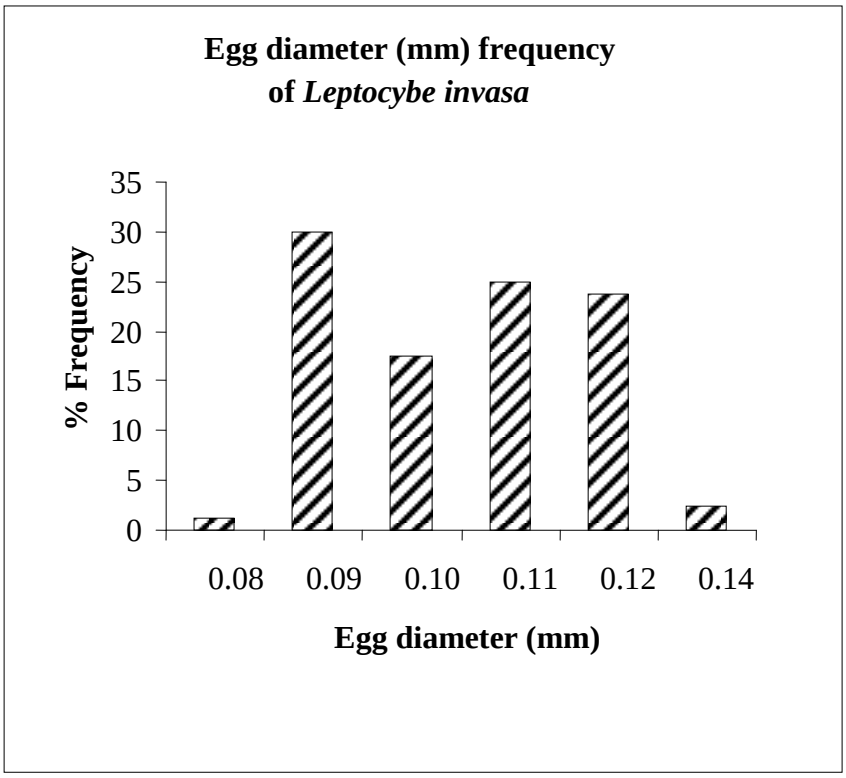


Figure 7: Percent (%) frequency of egg diameter *of Leptocybe invasa*

4.2.3. Larva and Pupa of *Leptocybe invasa* (Hymenoptera: Eulophidae)

Data on larval and pupal characteristics of *L. invasa* obtained during these investigations are presented in appendix 4a-c and figure 8.

The larva of *L. invasa* was minute, whitish and legless. The larval stage took 89.1 ± 5.1 days; its body length ranged from 0.13- 0.19 mm with a mean (± SD) of 0.16 ± 0.02 mm (Appendix 4c). Application of Dyar's law (Dyar, 1890; Klingenberg & Zimmermann, 1992) to larval measurements indicated the existence of four larval instars (Figure 8; Appendix 4c). The pupa of *L. invasa* had mummy-like appearance and was dark brown to black in colour. Mature pupa measured 1.1 – 1.2 mm in length. Pupal stage took 28.4 ± 1.2 days.

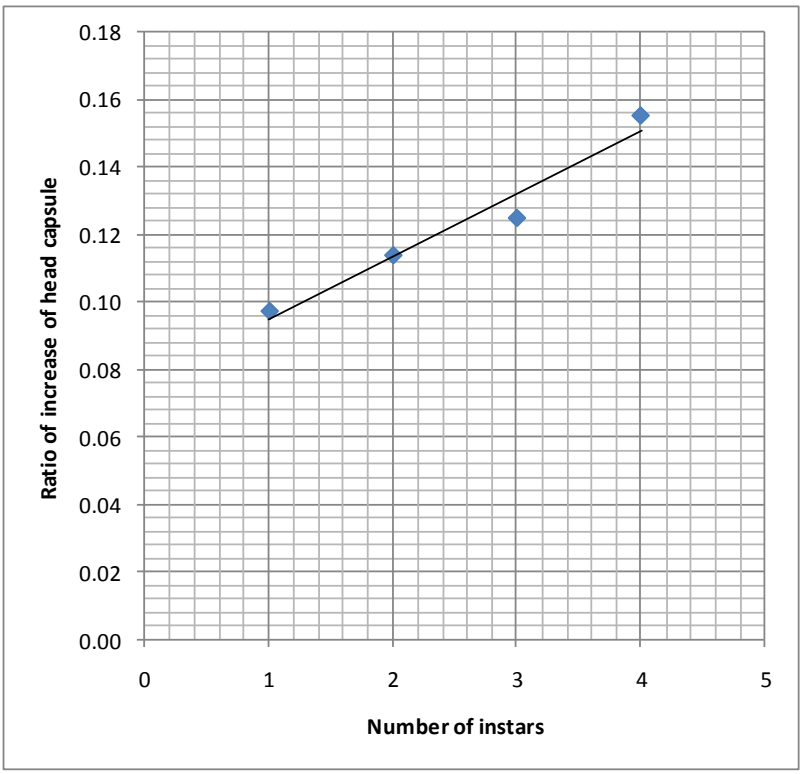


Figure 8: Ratio of increase of head capsule of *Leptocybe invasa* larvae as indicator of numbers of larval instars

4.2.4. Body characteristics of adult *Leptocybe invasa*

Data on body characteristics of adults of *L. invasa* are summarized in appendix 4c. The mean (mean ± SD) body measurements of unsexed insects (n =159) were as follows: Body length = 1.13 ± 0.07 mm; Head capsule width (eye to eye) = 0.25 ± 0.01 mm; Antennal length = 0.25 ± 0.00 mm; Number of antennal segments = 0.37 ± 0.02 mm; Wing length = 0.77± 0.09 mm; Abdominal length = 0.42 ± 0.07 mm; and Abdominal width 0.29 ± 0.01 mm.

It was observed that the adults, blackish in colour, emerged by eating their way out of the galls in which they matured thereby creating emergence holes. Emergent adults cleaned themselves, flapped their wings and flew to new succulent host plant (*Eucalyptus saligna*) shoots on which they oviposited a few minutes after emergence. Adult stage took 3-4 days under natural conditions and was lengthened to 15 days when the adults were fed on 15 % sucrose (Figure 6).

4.3. Ecology of *Leptocybe invasa*

4.3.1. Oviposition requirements of *Leptocybe invasa*

Number of eggs laid by *Leptocybe invasa* (Hymenoptera: Eulophidae) in response to cues for oviposition in relation to visual and olfaction stimuli is tabulated in Appendix 5a. The highest mean (± SE) *L. invasa* egg count (6.33 ± 0.29) was recorded on unvarnished piece of *Eucalyptus saligna* leaf presented together with a piece of filter paper not soaked in *E. saligna* leaf extract (Figure 9; Appendix 5b). The lowest egg count (0.47 ± 0.06) was recorded on piece of filter paper not soaked in *E. saligna* leaf extract and presented together with vanished piece of *E. saligna* leaf. Vanished pieces of *E. saligna* leaf presented

together with unsoaked pieces of filter paper had higher *L. invasa* egg counts (2.92 ± 0.15) as compared to unsoaked pieces of filter paper presented alone (1.12±0.10) (Appendix 5b). There were significant differences between effects of the twelve treatments on mean *L. invasa* egg count ($p < 0.05$; Appendix 5c). *L. invasa* had a preference for vanished *E. saligna* leaf over unsoaked filter paper as surface for oviposition (Table 8; Appendices 5d and 5e). These results indicate that host finding and oviposition by *L. invasa* is greatly influenced by olfaction, and thus volatile chemical compounds emanating from host plants (*E. saligna*).

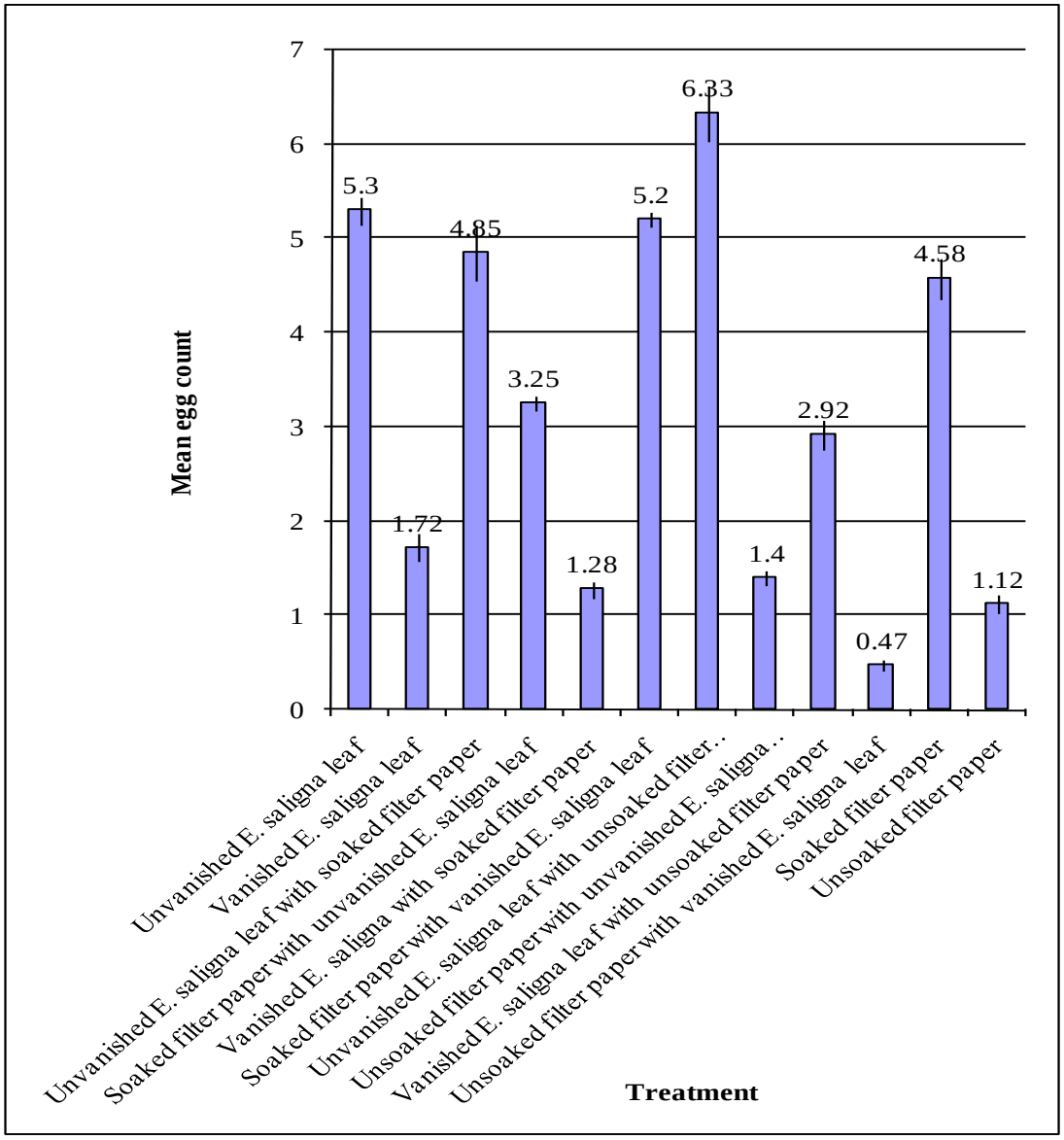


Figure 9: Mean *L. invasa* egg count following four days different treatments

Table 8: Tukey HSD* homogenous subsets of mean *Leptocybe invasa* egg count following four days of exposure to different treatments

S/ NO.	Treatment	n	Subset for alpha = 0.05				
			1	2	3	4	5
1	Unsoaked filter paper with vanished *E. saligna* leaf	60	0.47a				
2	Unsoaked filter paper	60	1.12a	1.12b			
3	Vanished *E. saligna* with soaked filter paper	60		1.28b			
4	Unsoaked filter paper with unvanished *E. saligna* leaf	60		1.40b			
5	Vanished E. saligna leaf	60		1.72b			
6	Vanished *E. saligna* leaf with unsoaked filter paper	60			2.92c		
7	Soaked filter paper with unvanished *E. saligna* leaf	60			3.25c		
8	Soaked filter paper	60				4.58c	
9	Unvanished *E. saligna* leaf with soaked filter paper	60				4.85c	
10	Soaked filter paper with vanished *E. saligna* leaf	60				5.20c	
11	Unvanished *E. saligna* leaf	60				5.30c	
12	Unvanished *E. saligna* leaf with unsoaked filter paper	60					6.33d
	P		0.195	0.307	0.960	0.095	1.000

Means for groups in homogeneous subsets are displayed. Those with same letters are not significantly different.

* Uses Harmonic Mean Sample Size = 60.000.

4.3.2. Foraging and patch use by adult *Leptocybe invasa*

Data on foraging and patch use by adults of *L. invasa* are presented Appendix 6 and figure 10. Travel time from patch to patch was longer (4 - 6 min) when *L. invasa* landed on plants other than *E. saligna* than when the insect landed on *E. saligna* (1-2 min) (Appendix 6). For both vertical and horizontal habitat structures, time taken by *L. invasa* on the patch of *E. saligna* (i.e. residence time) was longer and and marked by shorter travel time from patch to patch compared with corresponding duration when the insect was on other patch types (i.e. *G. robusta* and *C. lusitanica*) (Figures 10a and 10b). These results suggest that polycultures of *E. saligna* and other non-host plant species can increase travel time from plant to plant for *L. invasa* and lower residence time taken on a given plant. In effect this would lower chances for host finding and ovipositing by the pest.

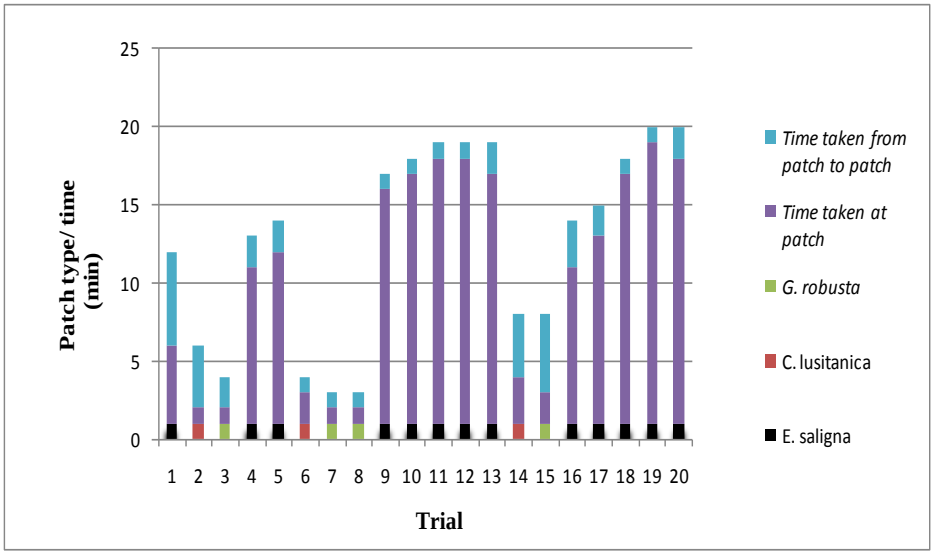


Figure 10a: Travel and residence time taken by *L. invasa* in relation to different vertical patch types

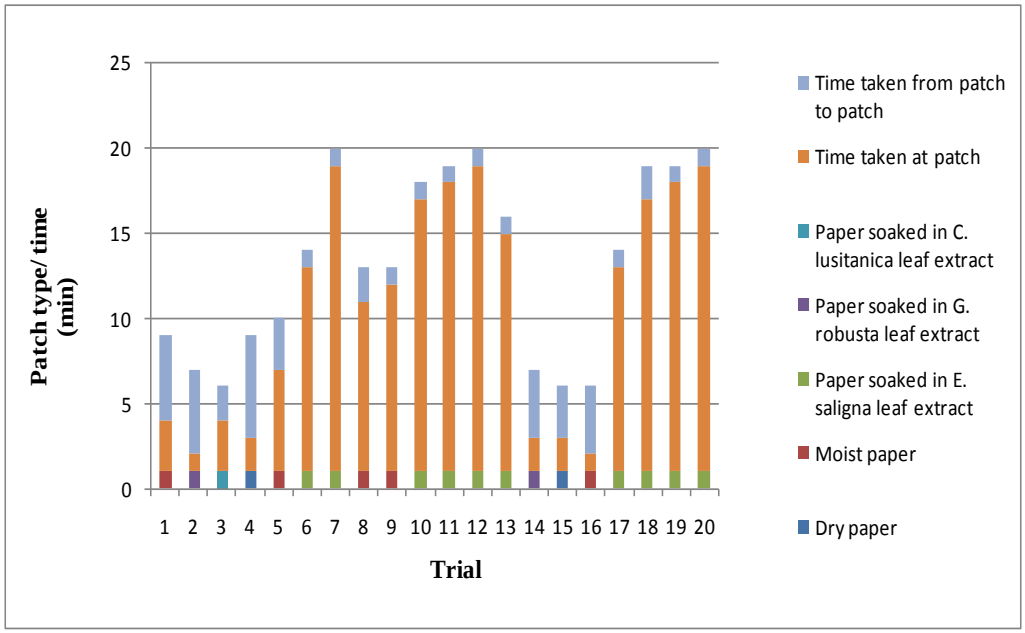


Figure 10b: Travel and residence time taken by *L. invasa* in relation to different horizontal patch types

4.3.3. Host condition in relation to successful attack by *Leptocybe invasa*

Mean gall numbers per seedling as a response to host condition in relation to attack by *L. invasa* are presented in Table 9. Also, factors of host condition (age, water and nitrogen fertilization) and treatments that were given to host plants (*E. saligna*) prior to exposure to *L. invasa* are presented in Appendices 7a and 7b.

High nitrogen fertilization and high watering regime for newly pricked out (transplanted) *E. saligna* seedlings rendered the plants more susceptible to attack by *L. invasa* as indicated by higher counts of galls per seedling (13.1 ± 0.9 galls per seedling). Older seedlings (six weeks old) subjected to low regimes of nitrogen fertilization and watering were less susceptible to attack by the pest (2.6 ± 0.9 galls per seedlings) than younger seedlinds subjected to higher regimes of nitrogen fertilization and watering (Figure 11; Appendix 7d). The differences in mean gall numbers per seedling as a response to host condition in relation to attack by *L. invasa* were significant ($p < 0.05$; Appendix 7c).

These results indicate that high levels of nitrogen fertilization and watering of *E. saligna* seedlings increased the host's susceptibility to attack by the pest, probably due to enhanced plant tissue succulence and nutritional value.

Table 9: Mean gall numbers per seedling in relation to *E. saligna* host condition for successful attack by *L. invasa*

Treatment	Mean ± SE
A1W1N1	6.3 ± 0.9abcde
A1W1N2	7.8 ± 0.9bcdef
A1W1N3	7.3 ± 0.9bcdef
A1W2N1	9.5 ± 0.9cdefg
A1W2N2	11.0 ± 0.9fg
A1W2N3	11.2 ± 0.9fg
A1W3N1	10.1 ± 0.9defg
A1W3N2	10.6 ± 0.9efg
A1W3N3	13.1 ± 0.9g
A2W1N1	7.1 ± 0.9bcdef
A2W1N2	6.8 ± 0.9abcdef
A2W1N3	4.9 ± 0.9ab
A2W2N1	2.6 ± 0.9a
A2W2N2	3.4 ± 0.9ab
A2W2N3	4.1 ± 0.9abc
A2W3N1	5.1 ± 0.9abc
A2W3N2	5.9 ± 0.9abcd
A2W3N3	5.3 ± 0.9b

Means with same letters are not significantly different at 95% CI

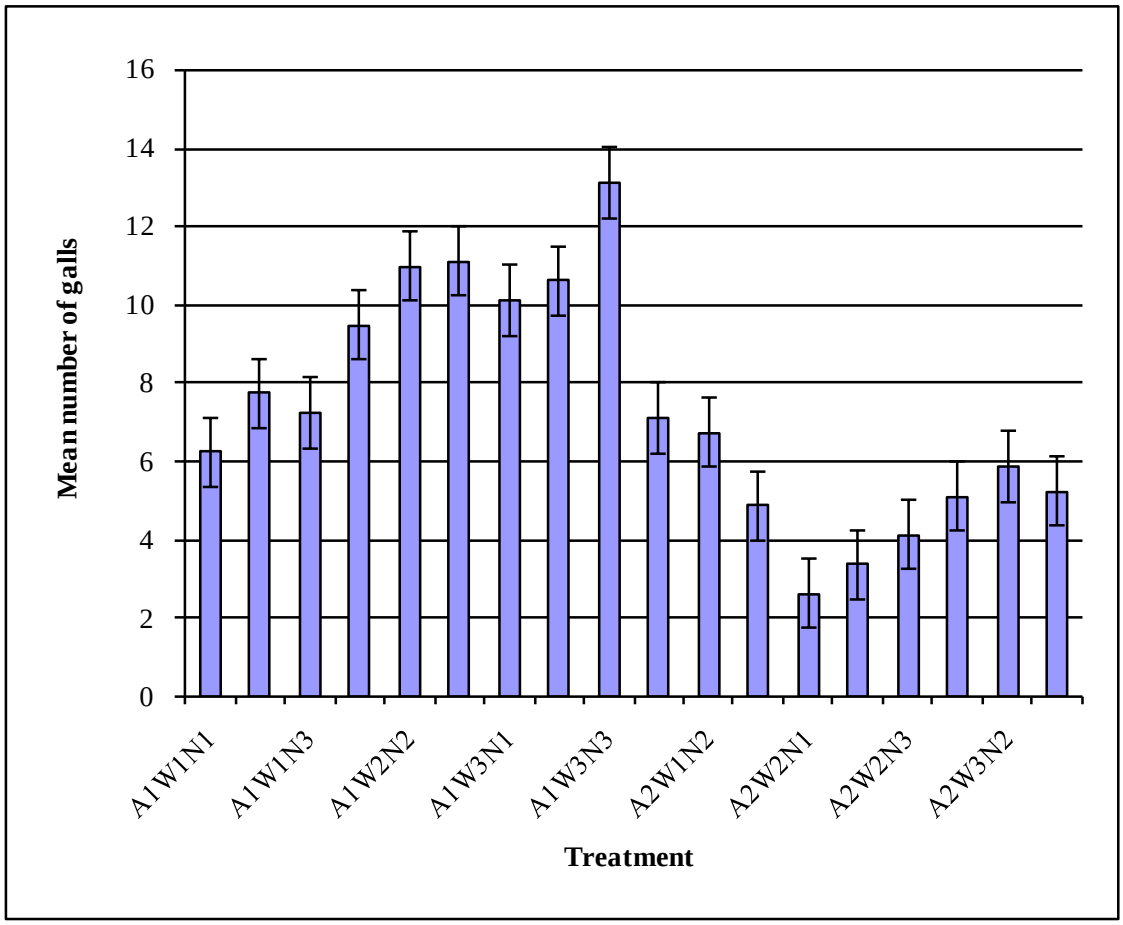


Figure 11: Mean gall counts per seedling in relation to *E. saligna* host condition for successful attack by *L. invasa*

4.4. The potential of selected herbaceous plant species as Eucalyptus IPM components against *L. invasa*

Data on the potential of a few selected plant species acting as components of a Eucalyptus IPM strategy against *L. invasa* is presented in Figure 12 and Appendix 8. The herbaceous species studied were *Leonotis nepetifolia*, *Schkuria pinnata* and *Tagetes erecta*. Considering mean gall counts on *E. saligana* seedlings, which was 10.8 ± 1.7 galls when grown alone, those grown together with *T. erecta* had the least number of galls (4.2± 0.8), followed by those grown together with *S. pinnata* (6.0 ± 1.3), and *L. nepetifolia* (7.4 ±1.8) (Figure 12). The differences in gall count among the four treatments were significant ($p < 0.05$; Table 10). Leaf mid-rib was the most preferred position of attack, followed by petioles and twigs (Figure 13). These investigations showed on the overall that planting of *E. saligna* seedlings with *T. erecta* provided maximum protection to the seedlings against *L. invasa* pointing to its higher potential than other herbaceous plant species tested for use as components in an IPM strategy fashioned against the pest. *Eucalyptus saligna* seedlings grown with *T. erecta* as companion plants showed good growth compared to those grown without *T. erecta* (Plate 8).

The respective mean height (Ht) and root collar diameter (RCD) growth of *E. saligna* seedlings after twenty (20) weeks under different treatments were significantly different ($p < 0.05$). These were as follows: *E. saligna* with *Leonotis nepetifolia* (Ht: 126 ± 4; RCD: 2.7 ± 0.1); *E. saligna* with *Schkuria pinnata* (Ht: 124 ± 1RCD: 4.3 ± 0.1); *E. saligna* with *Tagetes recta* (Ht: 56 ± 2; RCD: 3.7 ± 0); *E. saligna* that were enclosed alone (Ht: 85 ± 2; RCD: 26 ± 0); and control *E. saligna* seedlings (Ht: 140 ± 1; RCD: 5.0 ± 0.1) (Figure 14 and 15; Table 11).

From the data presented in on height and root collar measurements it was further shown that seedlings of *E. saligna* planted together with herbaceous plants studied were healthier than those grown in pure stands; thus there is potential of the herbaceous species studied as components of an IPM control strategy for *Eucalyptus* against the blue gum chalcid.

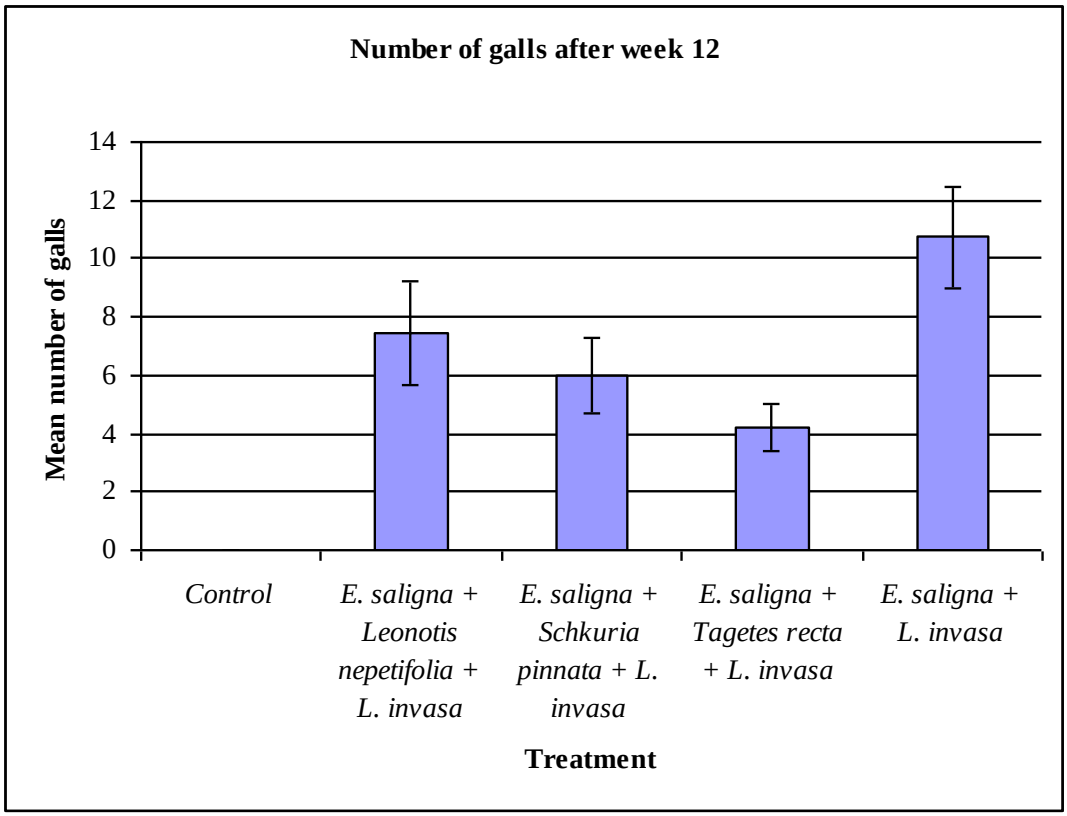


Figure 12: Mean gall count on *E. saligna* grown together with different herbaceous plants

Table 10: Tukey HSD on mean number of galls induced by *L. invasa* (Hymenoptera: Eulophidae) on *E. saligna* grown together with different herbaceous plants after week twelve

Treatment	Mean number of galls
Control	0 ± 0^{a}
E. saligna + Leonotis nepetifolia + L. invasa	7.4 ± 1.8^{b}
E. saligna + Schkuria pinnata + L. invasa	6.0 ± 1.3^{c}
E. saligna + Tagetes recta + L. invasa	4.2 ± 0.8^{d}
E. saligna + L. invasa	10.8 ± 1.7^{e}

Means with similar superscript are not significantly different at 95% CI

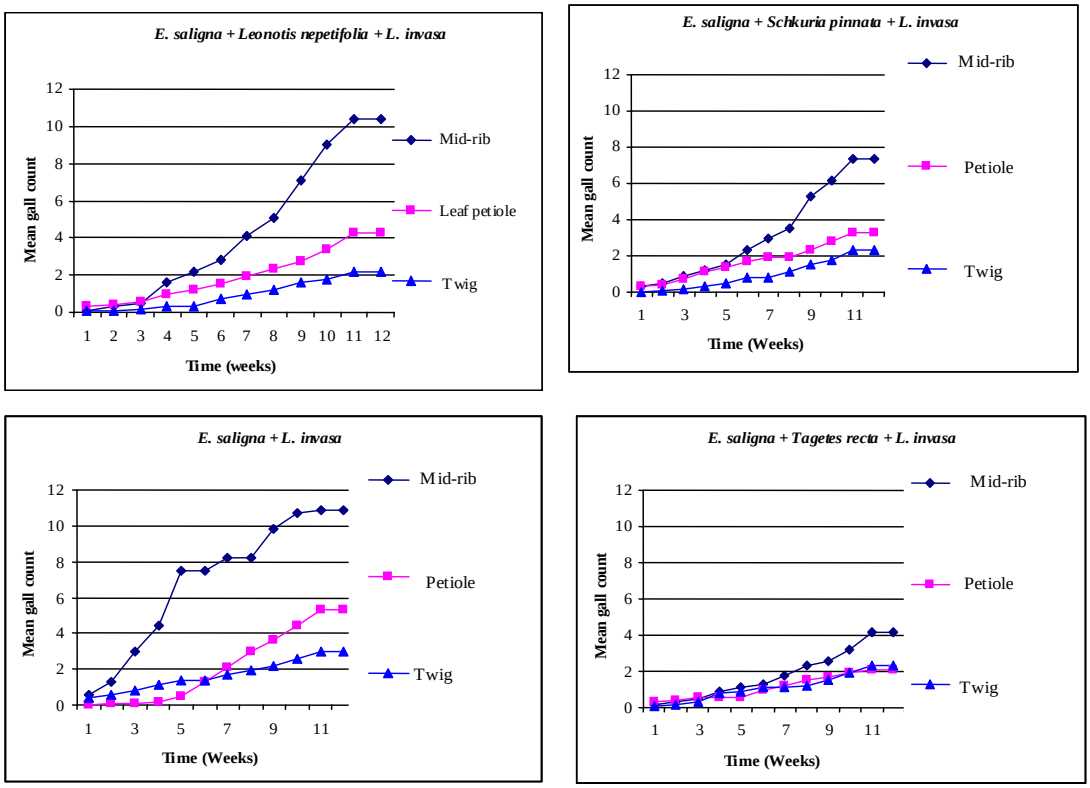


Figure 13: Mean gall count on mid-rib, petiole and twigs of *E. saligna* when grown together with different herbaceous plants

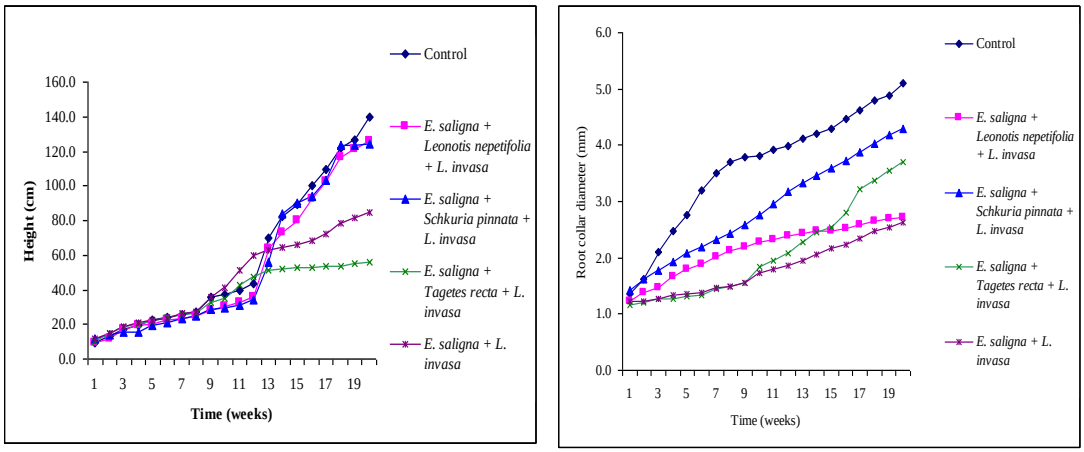


Figure 14: Mean height (cm) and root collar diameter of *E. saligna* when grown together with different herbaceous plants

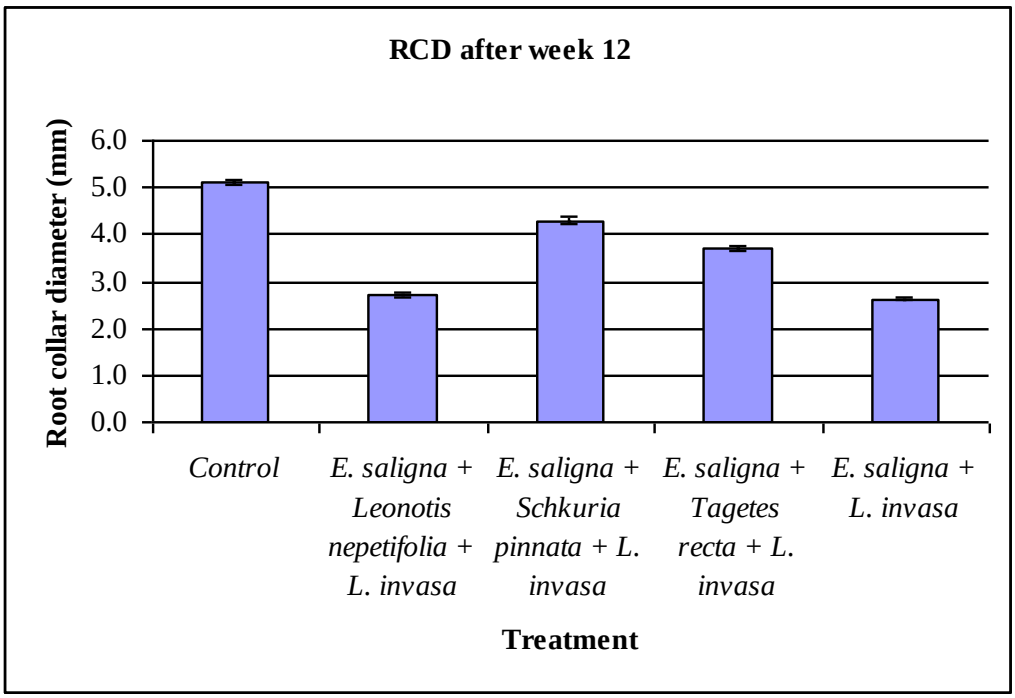


Figure 15: Mean root collar diameter of *E. saligna* when grown together with different herbaceous plants after week twelve

Table 11: Tukey HSD on mean root collar diameter of *E. saligna* grown together with different herbaceous plants after week twelve

Treatment	Mean root collar diameter (mm)
Control	5.1 ± 0.1^{a}
E. saligna + *Leonotis nepetifolia* + *L. invasa*	2.7 ± 0.1^{b}
E. saligna + *Schkuria pinnata* + *L. invasa*	4.3 ± 0.1^{c}
E. saligna + *Tagetes recta* + *L. invasa*	3.7 ± 0.0^{c}
E. saligna + *L. invasa*	2.6 ± 0.0^{b}

Means with similar superscript are not significantly different at 95% CI.

Plate 8: Appearance of samples of *E. saligna* seedlings grown with (tall seedlings) and without *T. erecta* (short seedlings) as companion plant after three months of gall induction by *Leptocybe invasa.*

4.5. Variability in *L. invasa* attack among five major eucalyptus species

Data, based on gall counts, on susceptibility of five *Eucalyptus spp*. studied is presented in appendix 9 and depicted in Figure 16. The data indicated that *E. saligna* was the most susceptible species to *L. invasa* attack (15.43± 0.29 galls per seedling) while *E. globulus* and *E. citriodora* seemed to tolerate *L. invasa* attack by > 150%, having only 0.86±0.07 and 0.94±0.07 galls per seedling respectively (Figure 16; Appendices 9a). Whereas *E. camaldulensis* seemed resistant in the presence of *E. saligna*, the species also appeared slightly susceptible to *L. invasa* attack when exposed to the insect alone. In the presence of *E. saligna*, gall count per seedling on *E. camaldulensis* were 3.21±0.33 while 7.11±0.24 galls per seedling was recorded when the species was alone (Appendices 9c and 9d). The variability in *L. invasa* attack between the major *Eucalyptus species* was significant ($p < 0.05$; Appendix 9b). It was concluded from these investigations that there was potential for use of host plant resistence as a tool in an IPM control strategy against *L. invasa*.

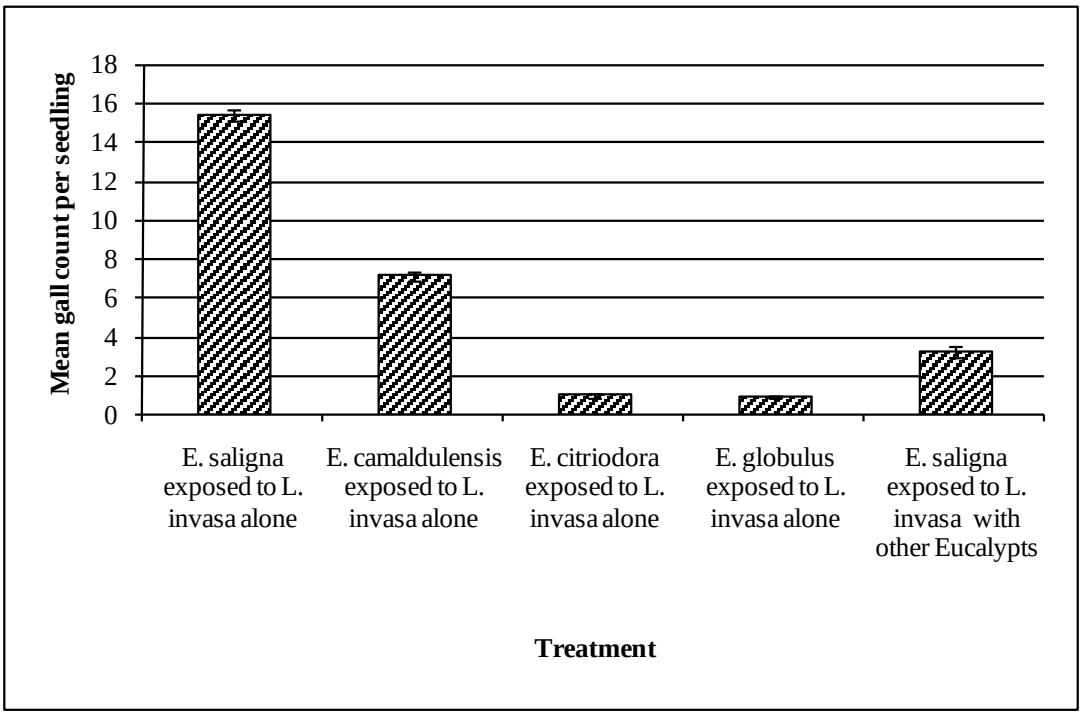


Figure 16: Mean gall count on different species of *Eucalyptus* seedlings following exposure to *L. invasa* attack alone or together with other *Eucalyptus* species

CHAPTER FIVE

DISCUSSION

5.1. Colonization and rearing procedure for *Leptocybe invasa*

Development period for a generation of *L. invasa* spans over a duration of four months although actual emergence seemed to be staggered and occurred highest in the ninth week from the start of adult emergence. This means that three generations can succed one another in an entire year in Kenya.

The survival was brisk, normally less than one week but can be extended to fifteen days when 10% sucrose solution is used as supplementary diet over and above the insect's natural food. Eucalyptus as a host is replete with the nutritive value of *L. invasa*. When these characterises an insect pest then studying it becomes difficult especially for an invasive species like *L. invasa* that survives for 3 days (Mendel *et al*, 2004) universally, yet invariably depending on how it is cultured can be enhanced by a factor of five. An extended survival duration would enable many appropriate studies of the pest to be undertaken especially in situations where environmental temperature can be maintained at 25.5 ^{o}C.

The fact that no mating behaviour of *Leptocybe invasa* was observed suggested that either the insect was hermaphroditic or it reproduced parthenogenetically, either completely or at certain times of the year or at some time intervals. The insect's likely mode or reproduction is thelytokous parthenogenesis, where female seem to dominate.

5.2. Biology and Ecology of *Leptocybe invasa* (Hymenoptera: Eulophidae)

The current study has brought forth more insight regarding the life history of *L. invasa,* some of the finding concurring with other workers results. *L. invasa* appears to have two to three generations in a year, with higher rate of reproduction occurring during warmer or hotter seasons of the year. While this study revealed that the insect takes 128 – 131 days from oviposition to adult emergence, a report by Kumari *et al*. (2010) indicated that it could take much shorter time (54 – 65 days). This shorter developmental duration, according to Kumari *et al.* (2010) is attributed to modified conditions of polythene enclosures.

Some of the aspects of the biology of *L. invasa* emanating from this study are that *L. invasa* has thelytokous parthenogenetic reproduction with its larvae undergoing four instars. Other aspects includes egg and adult measurements, which can provide bases for further taxonomic studies of the insect.

Animals usually require information about the state of their environment to take adaptive decisions (Tentelier and Fauvergue, 2007). A forager may asses current habitat profitability, based on cues it has perceived in the past, through a learning process (Ollasson, 1980; McNamara & Houston, 1985; Berstein *et al*., 1988; McNamara *et al*., 2006; Valone, 2006). In these studies (section 4.3.1), the number of eggs laid by *L. invasa* in response to cues for oviposition in relation to visual and olfaction stimuli suggested that the insect relied more on olfaction than visual stimuli in finding suitable host plant. How the insect did this was not studied. There is also evidence that visual stimuli played a role during host finding, although whether it played a role in host recognition was not clear but a gall former has to integrate many

selection processes if it has to oviposit in a host that will sustain its survival (Pyke, 1984).

Volatile plant metabolites, produced normally or in response to herbivore activities are known to attract or repel different groups of insects (Tentelier & Fauvergue, 2007). The fact that smell may be stronger than sight regarding *L. invasa* finding suitable Eucalyptus host (Section 4.3.1) can be exploited in the pest control by planting susceptible Eucalypts in polycultures as opposed to monocultures, particularly with strongly aromatic and resistant or repellent species like *Eucalyptus citriodora*.

Travel time from patch to patch was 4 - 6 min longer when *L. invasa* landed on plants other than *E. saligna* than when the insect landed on *E. saligna* in 1-2 minutes only for both vertical and horizontal habitat structures (Section 4.3.2). This indicated that the insect have strongly coevolved in its host finding, detection and recognition mechanisms suggesting semiochemicals participated in infestations when they occurred naturally. Although many studies on foraging by insects have been more on parasitoids (Turlings et al., 1993; Turlings & Wäckers, 2004; Thiel et al., 2006; Tentelier & Fauvergue, 2007) than on phytophagous insects, the phytophages too have adapted mechanisms, coevolved or otherwise, of foraging and that are most beneficial to them in an environment presenting both host and non-host plant species, a fete that occurs when eucalyptus trees are grown under farmer fields..

Other than finding and recognizing a suitable host, the host condition in relation to successful attack is an important factor in severity of attack by a phytophagous insect.

In this study low nitrogen fertilization and moderate watering regime seem to lower the severity of attack by the gall wasp, *L. invasa* (Hymenoptera: Eulophidae). This has implications on tree nursery practices where *L. invasa* infestation is common. High nitrogen levels in plant tissues promote succulence and luxuriant growth of plant tissues, providing suitable oviposition sites for *L. invasa*, so is nursery husbandry to be modified.

5.3. Potential of selected herbaceous plant species as IPM components against blue-gum chalcid pest, *L. invasa* (Hymenoptera: Eulophidae)

These studies showed that three local herbaceous plant species, namely lion's ear, *Leonotis nepetifolia* (Family Labiatae or Lamiaceae), dwarf marigold, *Schkuria pinnata* Kuntz ex Thell (Compositae or Asteraceae) and marigold, *Tagetes erecta* L. (Family Asteraceae or Compositae), when grown together with *E. saligna* as companion plants seemed to protect *Eucalyptuis species* from damage by *L. invasa,* either by being lethal, repellent or disruptive to the insect pest, to an extent that oviposition on the host plant was avoided or minimised. They pushed attack to be minimal when grown together with *E. saligna*.

However, although *T. erecta* reduced pest damage by 52 %, it also adversely affected growth of *E. saligna* seedlings. *Schkuria pinnata*, which reduced damage by 44 %, had little effect on growth of the seedlings. Although *L. nepetifolia* also reduced pest damage by 31 %, it had adverse effect on growth of *E. saligna* seedlings. As much as it is desirable to reduce or eliminate *L. invasa* pest prevalence in a tree nursery, vigorous seedling growth should not be compromised as this can hinder the establishment of the seedling after planting out. Therefore of the three herbaceous plants studied, *Schkuria pinnata* stood out as a good companion plant for planting with *E. saligna* to control *L. invasa.*

5.4. Variability in *L. invasa* attack between major eucalyptus germplasm.

Whereas only four *Eucalyptus species* were studied due to their being major plantation and farm forestry tree species in Kenya, there are several other Eucalyptus elsewhere whose susceptibility to *L. invasa* attack have either been studied or not (Mutitu *et al*., 2004; Kulkarni, 2010; Kulkarni, *et al*., 2010). In the context of the current studies *Eucaluyptus citriodora* and *E. camaldulensis* seemed resistant to *L. invasa* attack while *E. saligna* appeared highly susceptible to *Leptocybe* attack. In a separate work by Kulkarni *et al*., 2010, *Eucalyptus tereticocornis*, *E. camaldulensis*, *E. grandis* and their hybrids were severely affected by the gall wasp, *Leptocybe invasa*(Hymenoptera: Eulophidae) while *E. alba, E. urophyla, E. citriodora* and *E. torelliana* were gall free. From these studies, however, *E. camaldulensis* appere resistant only in the presence of *E. saligna*, but is equally susceptible to *L. invasa* attack when it is grown in monocultures. An IPM strategy therefore is possible when *E. saligna* and *E. camaldulensis* are grown in mixed stands in light of these findings. Further, the growing of companion plant, *S. pinnata*, with eucalyptus in woodlots or plantations can be practiced as as *L. invasa* control strategy.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1. Conclusion

Leptocybe invasa takes nineteen weeks (128-131 days) from oviposition date to adult emergence at a room temperature of 25.5 ^{o}C with ten infested and caged *Eucalyptus* seedlings giving an output of 30± 6 insects per 10 individual per day. Egg incubation takes 8.2±5.1 days, larval stage, 89.1±10.2 days and pupal stage 28.4±1.2 days.

Diet application method influences the survival of the insects and the longest survival occurs when diet solution is supplied in a ball of cotton wool placed on cloth covering the beaker containing the insects. 5% - 15% sucrose solution as diet results in the longest survival of the insect (sixteen days).

Newly emerged adult *L. invasa* take a few minutes to begin laying eggs. The egg morphology has been described in these studies together with that of other immature stages. *L. invasa* larvae undergo four instars before pupation.

The insect doesn't exhibit any mating behaviour, hence could be reproducing by thelytokous parthenogenesis.

Leonotis nepetifolia, dwarf marigold, *S. pinnata* Kuntz ex Thell (Compositae or Asteraceae) and marigold, *T. erecta* L. (Family Asteraceae or Compositae) contain volatile chemical compounds that are repellent to *L. invasa*. They may be planted as companion plants to *E. saligan* for *L. invasa* control and produce plausible results in reducing infestation.

Unlike the dwarf marigold, *S. pinnata* Kuntz ex Thell (Compositae or Asteraceae), the planting of *L.nepetifolia* (Family Labiatae or Lamiaceae) and marigold, *T. erecta* L. (Family Asteraceae or Compositae) as companion plants with *E. saligna* have adverse effects on height growth of the latter.

E. globulus and *E. citriodora* seemed tolerant, almost resistant to *L. invasa* attack, having only 0.86±0.07 and 0.94±0.07 galls per seedling respectively which was nearly 18 times less attack compared to *E. saligna*.

Whereas *E. camaldulensis* seemed not preferred by the insect pest in the presence of *E. saligna*, the plant species also was susceptible to *L. invasa* attack when exposed to the insects alone. In the presence of *E. saligna*, gall count per seedling on *E. camaldulensis* was 50% less than when the species was alone.

6.2. Recommendation

1. This study recommends the use of *S. pinnata* in particular and other herbaceous hosts studied as companion plants to *E. saligna* as part of IPM strategy against L. *invasa*.
2. Further research should focus on genetic modification of susceptible Eucalyptus germplasm for resistance against *Leptocybe invasa*. Species such as *E. citriodora*,

which showed appreciable levels of resistance, can be used as sources of desirable genes.

3. Research geared towards elucidating the potential of biological control agents against the pest is recommended.

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APPENDICES

Appendix 1: Procedure for colonisation and rearing of blue-gum chalcid pest, *L. invasa*)

The following procedure has been recommended for colonisation and rearing of blue-gum chalcid pest, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) is recommended.

1. Make ventilated glass cages measuring 1 – 1.5 m^3 in a well-lit room or laboratory, e.g. near a window. Ventilations can be made by having windows (15 cm diameter or 15 cm long) covered by cotton cloth on walls and roofs of the cages.
2. Collect infected seedlings from the field and spray them with contact insecticide followed by water then place them in cloth bags. The seedlings should have galls already but the galls should be green in colour and showing no emergent holes. Transfer the seedlings to the laboratory within 1 – 2 days and put them in clean cages. Each cage can hold a maximum of 10 seedlings. Centrally hang a thermometer from the roof of each cage and another one outside the cage. Environmental temperature should be maintained at 25.5^oC.
3. Carry out daily cage management: noting and recording daily diurnal temperature, weeding the seedlings, mopping excess water on cage floor or wet cage walls, removal of dry fallen leaves, adding about 10 pellets of urea fertilizer near root collar of each seedling once after a month, e.t.c. Fumigation of the cages for fungal control may be necessary although thorough maintenance of cage hygiene would minimize the need for fumigation.

Appendix 1 continued

4. Prepare for the experiment(s) in which emergent insects are to be used. This involves arrangements for rearing the insects on artificial diet (5 - 15% sucrose).
5. Emergent insects will frequently be seen crawling on the wall of the cages, especially the side that receives much light. The insects can be picked using moist cotton wool tied at the end of a glass rod and gently placed in 250-ml beakers covered by cotton cloth. A rubber band can be used to fasten the cloth on the beaker.
6. If continuous supply of the insects is required several cages can be set, as is convenient, at an interval of seven weeks. Management of seedlings of each cage can also be continued beyond the emergence period of one generation of the insects since they will always contain younger galls that would provide insects later and the emergent insects are also likely to be collected after they have oviposited in the seedlings.

Appendix 2: Observations on oviposition, gall development and emergence of *L. invasa*

(a) Experimental Set I

Date	Observation	Cumulative no. of days
14-11-2005	Exposure date	0
15-11-2005	Appearance of white/ yellowish substance on different parts of midrib, leaf petiole and stems of seedlings	1
16-11-2005	=do=	2
17-11-2005	=do=	3
18-11-2005	White/ yellowish substance decrease in number on different parts of midrib, leaf petiole and stems of seedlings	4
19-11-2005	=do=	5
20-11-2005	The white substances disappear	6
21-11-2005	No white/ yellowish substance on plant surface	7
22-11-2005 to 26-11-2005	Green, shinny galls appear on some seedlings (12 out of 20 seedlings)	12
27-11-2005	Number of green, shinny galls on seedlings increase (18 out of 20 seedlings)	13
28-11-2005	All the 20 seedlings have green, glossy galls	14
29-11-2005 to 7-12-2005	No observable change	23
8-12-2005	Typical bump-shaped galls appear affected parts of seedling	24
13-12-2005	Gall colours begin to turn pinkish	29
18-12-2005 to 16-2-2006	All galls are pinkish and glossy	94
17-2-2006 to 18-3-2006	Galls lose their shininess	124
19-3-2006	Adult *L. invasa* emergence begins	125
20-3-2006 to 22-3-2006	Adult emergence continues with increasing intensity of occurrence	128
23-3-2006 to 24-3-2006	Adult emergence decrease in intensity of occurrence	130
25-3-2006	Very few adult *L. invasa* insects emerge	131
26-3-2006	No emergence of adult *L. invasa* takes place	132

(b) Experimental Set II

Date	Observation	Cumulative no. of days
27-3-2006	Exposure date	0
28-3-2006 to 29-3-2006	Appearance of white/ yellowish substance on different parts of midrib, leaf petiole and stems of seedlings	2
30-3-2006	White/ yellowish substance decrease in number on different parts of midrib, leaf petiole and stems of seedlings	3
31-3-2006	=do=	4
1-4-2006	The white substances disappear completely	5
9-4-2006	Green, shinny galls appear on seedlings	13
21-4-2006	Typical bump-shaped galls appear	25
27-4-2006	Gall colour begins to turn pink	31
25-7-2006	Galls lose their shininess	120
5-8-2006 to 6-8-2006	Adult *L. invasa* insects begin to emerge	131 – 132
7-8-2006	End of *L. invasa* emergence	133

(c) Experimental Set III

Date	Observation	Cumulative no. of days
6-11-2006	Exposure date	0
8-11-2006	Appearance of white/ yellowish substance on different parts of midrib, leaf petiole and stems of seedlings	2
18-11-2006	Galls appear.	12
26-11-2006	Bump-shaped galls	27
4-3-2007	Green, shinny galls begin to turn pinkish	35
7-3-2007	Glossy galls lose their shininess	128
10-3-2007	Adult *L. invasa* insects begin to emerge	131
11-3-2007 to 13-3-2007	Emergence of adult *L. invasa* continues	134
14-3-2007	No adult *L. invasa* emergence occurs	135

(d) Experimental Set IV

Date	**Observation**	**Cumulative no. of days**
10-3-2008	Exposure date	0
11-3-2006	Appearance of white/ yellowish substance on different parts of midrib, leaf petiole and stems of seedlings	1
23-3-2008	Galls appear.	13
4-4-2008	Bump-shaped galls	25
17-4-2008	Green, shinny galls begin to turn pinkish	38
12-7-2008	Glossy galls lose their shininess	126
15-7-2008	Adult *L. invasa* insects begin to emerge	129
16-7-2008	Emergence of adult *L. invasa* continues	130
17-7-2008 to 24-7-2008	No adult *L. invasa* emergence occurs	138

Appendix 3: *Leptocybe invasa* egg measurements (diameter in mm)

(a) Egg diameter frequency

Egg diameter (mm)	Frequency	Percent
0.08	1	1.3
0.09	24	30.0
0.10	14	17.5
0.11	20	25.0
0.12	19	23.8
0.14	2	2.5
Total	**80**	**100.0**

(b) Mean egg diameter (mm) of *Leptocybe invasa* (Hymenoptera: Eulophidae)

Replicate	Mean	N	Std. Deviation	Std. Error of Mean
Replicate 1	0.1035	20	0.011	0.002
Replicate 2	0.1070	20	0.017	0.003
Replicate 3	0.1030	20	0.011	0.002
Replicate 4	0.1065	20	0.011	0.002
Total	**0.1050**	**80**	**0.013**	**0.001**

Appendix 4: *Leptocybe invasa* larva measurements (mm): BL= Body length, HCW = Head capsule width.

(a) Body length and head capsule width (mm) of *L. invasa* larva

S/No.	Set I		Set II		Set III		Set III	
	BL	**HCW**	**BL**	**HCW**	**BL**	**HCW**	**BL**	**HCW**
1	0.14	0.09	0.13	0.09	0.14	0.09	0.14	0.09
2	0.14	0.09	0.14	0.09	0.14	0.09	0.14	0.09
3	0.14	0.1	0.14	0.09	0.15	0.1	0.15	0.1
4	0.15	0.1	0.15	0.1	0.15	0.1	0.15	0.1
5	0.15	0.1	0.15	0.1	0.15	0.1	0.15	0.1
6	0.15	0.11	0.16	0.1	0.16	0.1	0.15	0.11
7	0.16	0.12	0.16	0.11	0.16	0.11	0.16	0.11
8	0.16	0.12	0.16	0.11	0.16	0.11	0.16	0.11
9	0.16	0.12	0.16	0.12	0.16	0.11	0.16	0.12
10	0.16	0.12	0.16	0.12	0.17	0.11	0.16	0.12
11	0.17	0.13	0.16	0.12	0.17	0.12	0.16	0.12
12	0.17	0.13	0.16	0.13	0.17	0.12	0.16	0.12
13	0.17	0.13	0.17	0.13	0.17	0.12	0.17	0.13
14	0.18	0.13	0.17	0.13	0.18	0.12	0.17	0.13
15	0.18	0.14	0.17	0.13	0.18	0.13	0.17	0.13
16	0.19	0.14	0.17	0.14	0.18	0.13	0.17	0.14
17	0.19	0.14	0.18	0.14	0.18	0.14	0.18	0.14
18	0.19	0.14	0.18	0.14	0.19	0.14	0.19	0.14
19	0.19	0.14	0.19	0.14	0.19	0.14	0.19	0.14
20	0.19	0.14	0.19	0.14	0.19	0.14	0.19	0.14
Mean	**0.17**	**0.12**	**0.16**	**0.12**	**0.17**	**0.12**	**0.16**	**0.12**
SD	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**

(b) Body length of *L. invasa* larva (mm)

S/No.	Set I	Set II	Set III	Set IV		
	BL	BL	BL	BL	Mean	SD
1	0.14	0.13	0.14	0.14	0.14	0.01
2	0.14	0.14	0.14	0.14	0.14	0.00
3	0.14	0.14	0.15	0.15	0.15	0.01
4	0.15	0.15	0.15	0.15	0.15	0.00
5	0.15	0.15	0.15	0.15	0.15	0.00
6	0.15	0.16	0.16	0.15	0.16	0.01
7	0.16	0.16	0.16	0.16	0.16	0.00
8	0.16	0.16	0.16	0.16	0.16	0.00
9	0.16	0.16	0.16	0.16	0.16	0.00
10	0.16	0.16	0.17	0.16	0.16	0.01
11	0.17	0.16	0.17	0.16	0.17	0.01
12	0.17	0.16	0.17	0.16	0.17	0.01
13	0.17	0.17	0.17	0.17	0.17	0.00
14	0.18	0.17	0.18	0.17	0.18	0.01
15	0.18	0.17	0.18	0.17	0.18	0.01
16	0.19	0.17	0.18	0.17	0.18	0.01
17	0.19	0.18	0.18	0.18	0.18	0.01
18	0.19	0.18	0.19	0.19	0.19	0.01
19	0.19	0.19	0.19	0.19	0.19	0.00
20	0.19	0.19	0.19	0.19	0.19	0.00
Mean	**0.17**	**0.16**	**0.17**	**0.16**	**0.16**	
SD	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	

(c) Ratio of increase of head capsule width of *Leptocybe invasa*

S/No.	Set I	Set II	Set III	Set IV	Mean	SD	Ratio	Instars
1	0.09	0.08	0.09	0.09	0.09	0.01	0.10	1st
2	0.09	0.09	0.09	0.09	0.09	0.00	0.10	
3	0.09	0.09	0.09	0.09	0.09	0.00	0.10	
4	0.09	0.10	0.09	0.09	0.09	0.01	0.10	
5	0.09	0.10	0.09	0.09	0.09	0.01	0.10	
6	0.11	0.10	0.10	0.10	0.10	0.01	0.11	2nd
7	0.11	0.10	0.10	0.10	0.10	0.01	0.11	
8	0.11	0.10	0.10	0.10	0.10	0.01	0.11	
9	0.12	0.11	0.11	0.11	0.11	0.01	0.12	3rd
10	0.12	0.11	0.11	0.11	0.11	0.01	0.12	
11	0.12	0.11	0.11	0.11	0.11	0.01	0.12	
12	0.12	0.11	0.11	0.11	0.11	0.01	0.12	
13	0.12	0.12	0.11	0.13	0.12	0.01	0.13	
14	0.12	0.12	0.11	0.13	0.12	0.01	0.13	
15	0.13	0.13	0.13	0.13	0.13	0.00	0.14	
16	0.14	0.14	0.14	0.14	0.14	0.00	0.16	4th
17	0.14	0.14	0.14	0.14	0.14	0.00	0.16	
18	0.14	0.14	0.14	0.14	0.14	0.00	0.16	
19	0.14	0.14	0.14	0.14	0.14	0.00	0.16	
20	0.14	0.14	0.14	0.14	0.14	0.00	0.16	
Mean	**0.12**	**0.11**	**0.11**	**0.11**	**0.11**			
SD	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**			

(d) Measurements of adult *Leptocybe invasa* (Hymenoptera: Eulophidae)

Note: Measurements are in micrometer units (10 units = mm). L = Length, S = No. of segments, W = width

Day	Date	S/N	Body length	Head capsule (eye to eye)	Antenna		Wing length	Abdomen	
					L	S		L	W
1	15-12-05	1	11.5	2.5	2.5	2.0	6.8	3.5	3.0
2	16-12-05	1	11.5	2.5	2.5	2.0	7.0	4.0	2.8
		2	12.0	2.6	2.5	6.0	8.0	5.0	3.0
		3	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		4	11.4	2.5	2.5	2.0	6.8	3.5	3.0
		5	11.5	2.6	2.5	6.0	8.0	5.0	3.0
3	17-12-05	1	10.0	2.4	2.5	2.0	6.8	3.5	2.8
4	18-12-05	1	12.0	2.5	2.5	6.0	7.0	4.0	3.0
		2	10.0	2.4	2.5	6.0	9.0	5.0	3.0
		3	11.5	2.6	2.5	6.0	8.5	5.0	2.8
		4	12.0	2.5	2.5	6.0	8.5	4.5	3.0
5	19-12-05	1	11.5	2.5	2.5	6.0	8.0	5.0	3.0
6	20-12-05	1	11.5	2.6	2.5	2.0	7.0	3.5	2.8
7	21-12-05	1	10.0	2.6	2.5	2.0	6.8	3.5	3.0
8	22-12-05	1	12.0	2.5	2.5	2.0	7.0	3.5	3.0
		2	11.5	2.5	2.5	6.0	9.0	5.0	2.8
		3	10.5	2.5	2.5	2.0	6.9	4.0	3.0
9	23-12-05	1	10.0	2.4	2.5	2.0	6.8	3.5	3.0
10	24-12-05	1	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		2	11.5	2.5	2.5	2.0	6.8	4.0	3.0
		3	11.5	2.6	2.5	2.0	6.8	3.5	2.8
		4	10.0	2.5	2.5	6.0	8.4	5.0	3.0
		5	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		6	12.0	2.4	2.5	2.0	7.0	3.5	3.0
		7	10.0	2.5	2.5	6.0	9.0	5.0	2.8
		8	10.5	2.5	2.5	2.0	7.0	4.0	3.0
		9	12.0	2.5	2.5	6.0	9.0	5.0	3.0
		10	11.5	2.5	2.5	6.0	9.0	4.5	3.0
		11	11.5	2.4	2.5	2.0	7.0	3.5	2.8
		12	11.5	2.5	2.5	2.0	6.8	3.5	2.8
11	25-12-05	1	12.0	2.4	2.5	2.0	7.0	4.0	2.8
		2	10.0	2.5	2.5	2.0	7.0	3.5	3.0
		3	11.5	2.5	2.5	6.0	9.0	5.0	3.0
12	26-12-05	1	12.0	2.5	2.5	2.0	7.0	3.5	2.8
		2	11.4	2.5	2.5	6.0	8.5	5.0	3.0
13	27-12-05								
14	28-12-05	1	11.5	2.5	2.5	6.0	8.0	5.0	3.0

		2	12.0	2.5	2.5	2.0	6.9	3.5	3.0
		3	11.5	2.5	2.5	2.0	7.0	3.5	2.5
15	29-12-05	1	10.0	2.4	2.5	2.0	7.0	4.0	3.0
16	30-12-05	1	10.0	2.5	2.5	2.0	6.8	3.5	2.8
		2	11.5	2.5	2.5	6.0	8.0	5.0	3.0
		3	11.5	2.5	2.5	2.0	6.8	3.5	3.0
		4	10.4	2.6	2.5	2.0	6.8	3.5	2.8
		5	11.5	2.5	2.5	6.0	9.0	5.0	3.0
		6	11.4	2.5	2.5	6.0	8.5	4.5	3.0
		7	12.0	2.5	2.5	6.0	8.6	5.0	3.0
		8	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		9	10.0	2.5	2.5	6.0	9.0	5.0	3.0
		10	12.0	2.4	2.5	2.0	7.0	3.5	2.8
		11	11.5	2.5	2.5	2.0	7.0	4.0	3.0
		12	12.0	2.5	2.5	6.0	8.0	4.5	2.8
		13	10.0	2.5	2.5	6.0	9.0	4.5	3.0
		14	12.0	2.4	2.5	6.0	8.0	5.0	3.0
		15	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		16	10.4	2.5	2.5	2.0	7.0	4.0	2.8
		17	10.4	2.6	2.5	6.0	9.0	4.5	3.0
		18	12.0	2.4	2.5	2.0	7.0	3.5	3.0
17	31-12-05	1	11.5	2.6	2.5	2.0	7.0	3.5	3.0
		2	11.5	2.5	2.5	2.0	7.0	3.5	2.8
		3	10.0	2.5	2.5	6.0	8.5	5.0	3.0
		4	11.5	2.5	2.5	2.0	7.0	3.5	2.8
		5	12.0	2.4	2.5	2.0	6.8	3.5	2.8
		6	11.5	2.5	2.5	6.0	9.0	5.0	3.0
		7	10.5	2.5	2.5	6.0	9.0	5.0	3.0
		8	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		9	11.5	2.5	2.5	2.0	7.0	4.0	2.8
		10	10.0	2.6	2.5	2.0	6.8	3.5	3.0
		11	12.0	2.5	2.5	6.0	9.0	5.0	3.0
		12	11.5	2.5	2.5	2.0	7.0	3.5	2.8
		13	11.5	2.5	2.5	2.0	6.8	3.5	3.0
		14	12.0	2.6	2.5	6.0	8.9	5.0	3.0
		15	10.0	2.5	2.5	6.0	9.0	5.0	3.0
		16	11.5	2.5	2.5	2.0	7.0	4.0	2.8
		17	12.0	2.5	2.5	2.0	7.0	3.5	3.0
		18	10.4	2.4	2.5	6.0	9.0	4.5	3.0
		19	11.5	2.5	2.5	2.0	7.0	3.5	3.0
18	01-01-06	1	11.5	2.5	2.5	6.0	8.5	5.0	3.0
		2	12.0	2.5	2.5	2.0	6.9	3.5	3.0
		3	10.0	2.5	2.5	2.0	7.0	3.5	2.8
		4	11.5	2.5	2.5	2.0	7.0	4.0	3.0
		5	11.5	2.5	2.5	6.0	9.0	5.0	3.0

		6	12.0	2.5	2.5	6.0	9.0	5.0	3.0
		7	10.0	2.5	2.5	2.0	7.0	3.5	2.8
		8	11.5	2.5	2.5	2.0	7.0	4.0	3.0
		9	10.0	2.5	2.5	6.0	8.0	5.0	3.0
		10	11.5	2.5	2.5	6.0	8.0	4.5	3.0
		11	11.5	2.5	2.5	6.0	9.0	5.0	2.8
		12	10.5	2.5	2.5	6.0	8.0	5.0	3.0
		13	11.5	2.6	2.5	2.0	7.0	3.5	3.0
		14	12.0	2.7	2.5	2.0	7.0	3.5	3.0
		15	12.0	2.5	2.5	2.0	7.0	3.5	3.0
19	02-01-06	1	11.5	2.5	2.5	2.0	6.8	3.5	3.0
		2	11.5	2.5	2.5	2.0	7.0	4.0	3.0
		3	11.5	2.5	2.5	6.0	8.0	5.0	3.0
		4	12.0	2.5	2.5	2.0	7.0	3.5	3.0
		5	11.5	2.4	2.5	6.0	9.0	5.0	3.0
		6	11.5	2.5	2.5	6.0	9.0	5.0	3.0
		7	12.0	2.6	2.5	6.0	9.0	5.0	3.0
		8	11.5	2.5	2.5	6.0	9.0	4.5	2.8
		9	10.5	2.5	2.5	2.0	7.0	3.5	3.0
		10	11.5	2.5	2.5	6.0	8.0	5.0	2.8
		11	12.0	2.5	2.5	2.0	7.0	4.0	3.0
		12	11.5	2.6	2.5	2.0	7.0	3.5	3.0
		13	12.0	2.6	2.5	2.0	6.8	4.0	2.8
		14	12.0	2.7	2.5	6.0	9.0	4.5	3.0
		15	11.5	2.5	2.5	2.0	7.0	3.5	3.0
20	03-01-06	1	11.5	2.5	2.5	2.0	6.8	3.5	3.0
		2	10.0	2.5	2.5	2.0	7.0	4.0	2.8
		3	12.0	2.5	2.5	6.0	9.0	5.0	3.0
		4	10.0	2.5	2.5	2.0	7.0	3.5	3.0
		5	11.5	2.4	2.5	2.0	7.0	3.5	3.0
		6	11.5	2.5	2.5	6.0	8.0	5.0	3.0
		7	12.0	2.5	2.5	6.0	8.0	4.5	2.8
		8	12.0	2.5	2.5	6.0	9.0	5.0	3.0
		9	11.5	2.5	2.5	6.0	8.0	5.0	3.0
		10	12.0	2.5	2.5	6.0	8.0	5.0	3.0
		11	11.5	2.5	2.5	6.0	9.0	4.5	3.0
		12	11.5	2.5	2.5	6.0	8.0	5.0	2.8
		13	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		14	11.5	2.5	2.5	6.0	8.0	5.0	3.0
		15	11.0	2.5	2.5	2.0	7.0	3.5	3.0
		16	11.5	2.6	2.5	2.0	6.8	4.0	3.0
		17	12.0	2.5	2.5	2.0	7.0	3.5	3.0
		18	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		19	12.0	2.5	2.5	6.0	8.0	5.0	3.0
21	04-01-06	1	11.5	2.4	2.5	2.0	6.8	4.0	3.0

		2	11.5	2.5	2.5	2.0	7.0	3.5	2.8
		3	11.5	2.5	2.5	2.0	6.8	3.5	3.0
		4	11.5	2.5	2.5	2.0	6.8	3.5	3.0
		5	10.5	2.4	2.5	6.0	9.0	5.0	3.0
		6	12.0	2.5	2.5	6.0	8.5	5.0	2.8
		7	10.0	2.5	2.5	2.0	7.0	3.5	3.0
		8	11.4	2.6	2.5	6.0	8.0	4.5	3.0
		9	10.0	2.5	2.5	6.0	9.0	4.5	3.0
		10	11.5	2.4	2.5	6.0	9.0	5.0	3.0
		11	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		12	10.0	2.5	2.5	6.0	8.5	5.0	2.8
		13	11.5	2.5	2.5	2.0	6.8	3.5	3.0
22	05-01-06	1	11.5	2.5	2.5	6.0	9.0	5.0	3.0
		2	12.0	2.7	2.5	2.0	7.0	3.5	3.0
		3	11.4	2.5	2.5	2.0	7.0	3.5	2.8
		4	11.5	2.5	2.5	2.0	7.0	4.0	3.0
23	06-01-06	1	11.5	2.5	2.5	2.0	7.0	3.5	3.0
24	07-01-06	1	12.0	2.5	2.5	2.0	7.0	3.5	3.0
		2	11.4	2.7	2.5	2.0	6.8	4.0	3.0
		3	11.4	2.5	2.5	6.0	9.0	5.0	2.8
		4	10.0	2.4	2.5	2.0	7.0	3.5	3.0
25	08-01-06	1	12.0	2.5	2.5	6.0	8.0	5.0	3.0
26	09-01-06	1	11.5	2.5	2.5	2.0	6.8	3.5	2.8
27	10-01-06								
28	11-01-06								
29	12-01-06	1	10.5	2.5	2.5	6.0	8.0	4.5	3.0
		2	12.0	2.5	2.5	2.0	7.0	3.5	3.0
		3	10.0	2.5	2.5	2.0	6.8	3.5	2.8
		4	11.4	2.5	2.5	6.0	9.0	4.5	3.0
30	13-01-06	1	11.5	2.5	2.5	2.0	7.0	3.5	3.0
		2	10.0	2.5	2.5	6.0	9.0	4.5	3.0
		3	12.0	2.5	2.5	6.0	9.0	4.5	3.0
31	14-01-06	1	11.4	2.6	2.5	2.0	7.0	3.5	2.8
		2	10.0	2.5	2.5	2.0	7.0	3.5	3.0
32	15-01-06	1	11.5	2.5	2.5	6.0	9.0	5.0	2.8
33	16-01-06	-	-	-	-	-	-	-	-
34	17-01-06	-	-	-	-	-	-	-	-
35	18-01-06	-	-	-	-	-	-	-	-
36	19-01-06	-	-	-	-	-	-	-	-
37	20-01-06	-	-	-	-	-	-	-	-
Mean			**11.3**	**2.5**	**2.5**	**3.7**	**7.7**	**4.2**	**2.9**
SD			**0.7**	**0.1**	**0.0**	**2.0**	**0.9**	**0.7**	**0.1**

n = 159

Appendix 5(a): Number of eggs laid by *Leptocybe invasa* (Hymenoptera: Eulophidae) in response to cues for oviposition in relation to visual and olfaction stimuli

Note: Each insect was presented with the following (treatments) : **A:** piece of *E. saligna* leaf, **B:** piece of vanished *E. saligna* leaf, **C:** piece of *E. saligna* leaf presented together with piece of filter paper soaked in *E. saligna* leaf extract (i.e. C_1 and C_2 respectively), **D:** piece of vanished *E. saligna* leaf presented together with piece of filter paper soaked in *E. saligna* leaf extract (i.e. D_1 and D_2 respectively), **E:** piece of *E. saligna* leaf presented together with piece of filter paper not soaked in *E. saligna* leaf extract (i.e. E_1 and E_2 respectively), **F:** piece of vanished *E. saligna* leaf presented together with piece of filter paper not soaked in *E. saligna* leaf extract (i.e. F_1 and F_2 respectively), **G:** piece of filter paper soaked in *E. saligna* leaf extract, **H:** piece of filter paper not soaked in *E. saligna* leaf extract

Treatment	**A**			**B**			**C_1**			**C_2**			**D_1**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Trial															
1	4	4	7	2	2	3	0	7	6	3	3	4	0	2	0
2	4	6	6	2	2	2	1	5	5	2	4	4	1	1	2
3	6	4	4	3	2	3	2	6	5	3	3	3	1	0	2
4	4	7	4	0	2	0	5	4	5	2	4	3	0	2	2
5	7	5	4	3	3	0	5	7	6	4	3	4	1	2	0
6	7	4	7	0	2	1	6	7	0	2	4	3	1	2	1
7	4	6	5	0	3	2	7	7	7	2	3	3	2	1	1
8	5	7	5	0	2	3	7	6	7	4	3	3	0	2	0
9	4	4	6	3	2	3	4	7	1	3	4	3	2	1	2
10	6	4	4	0	3	2	6	5	7	3	3	4	1	0	1
11	4	6	5	1	2	2	5	6	6	2	3	3	2	2	2
12	7	5	6	1	3	1	6	7	5	2	3	4	2	1	2
13	5	6	4	2	3	0	6	6	7	3	4	3	2	1	2
14	6	5	6	3	2	1	7	1	5	4	4	4	0	2	1
15	4	5	6	0	3	3	0	6	5	3	3	3	1	1	2
16	7	4	5	2	3	2	7	5	5	4	4	4	1	2	1
17	4	5	4	1	1	0	2	0	0	4	4	3	2	2	0
18	7	6	6	3	0	1	5	7	5	3	3	3	2	2	1
19	6	7	6	0	0	2	6	0	5	2	4	4	2	1	0
20	6	4	7	3	3	0	7	0	6	3	3	3	2	1	2

Appendix 5 continued

Treatment	D_2			E_1			E_2			F_1			F_2		
Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Trial															
1	6	5	6	8	7	7	0	2	1	4	2	4	0	1	0
2	4	5	5	7	7	8	0	1	2	4	1	4	1	0	0
3	5	4	5	7	7	7	0	1	2	3	3	3	0	1	0
4	5	4	5	8	8	0	1	2	1	4	4	4	0	1	1
5	6	6	5	7	7	1	1	2	1	3	4	4	0	0	1
6	6	5	4	6	8	8	2	2	2	4	3	3	1	0	1
7	5	5	5	7	1	7	0	1	1	3	1	3	0	0	1
8	4	6	6	8	0	4	2	2	2	3	4	0	1	1	1
9	6	5	6	7	7	7	2	1	1	3	3	1	0	1	0
10	5	6	5	8	8	5	1	1	2	3	4	2	1	1	0
11	4	6	5	7	7	8	1	1	2	3	1	3	1	0	0
12	6	5	6	8	8	6	1	2	1	0	4	4	1	0	0
13	5	5	4	7	7	5	2	2	2	0	4	3	0	0	0
14	6	4	5	7	8	0	1	1	1	3	4	3	1	0	0
15	5	5	6	7	6	7	2	2	2	3	3	4	0	1	0
16	4	5	6	8	7	7	2	1	1	4	3	4	1	1	1
17	6	5	5	8	6	4	3	1	2	3	3	4	1	0	1
18	5	5	6	8	7	8	2	2	1	1	2	3	0	0	0
19	5	6	6	6	7	3	1	1	1	2	1	3	1	0	1
20	6	5	5	8	7	1	1	2	1	4	3	2	1	1	0

Appendix 5 continued

Treatment	G			H		
Replicate	1	2	3	1	2	3
Trial						
1	4	6	4	0	1	0
2	6	4	5	0	2	2
3	6	5	6	1	2	1
4	4	4	6	0	2	1
5	3	4	6	1	1	2
6	0	4	6	0	1	2
7	4	6	7	2	1	1
8	4	4	0	0	0	0
9	7	5	3	1	1	2
10	6	6	2	1	0	1
11	4	2	4	0	1	0
12	6	2	4	1	0	2
13	4	4	7	2	2	2
14	6	5	2	2	1	1
15	6	6	3	1	1	2
16	5	6	2	1	2	2
17	7	7	6	2	0	1
18	4	2	4	0	1	0
19	6	3	4	1	2	2
20	5	6	6	2	2	2

Appendix 5b: Mean *Leptocybe invasa* egg count following four days of exposure to different treatments

S/NO.	Treatment	N	Mean	SD	SE
1	Unvanished *E. saligna* leaf	60	5.30	1.14	0.15
2	Vanished *E. saligna* leaf	60	1.72	1.15	0.15
3	Unvanished *E. saligna* leaf with soaked filter paper	60	4.85	2.33	0.30
4	Soaked filter paper with unvanished *E. saligna* leaf	60	3.25	0.65	0.08
5	Vanished *E. saligna* with soaked filter paper	60	1.28	0.76	0.09
6	Soaked filter paper with vanished *E. saligna* leaf	60	5.20	0.68	0.08
7	Unvanished *E. saligna* leaf with unsoaked filter paper	60	6.33	2.23	0.29
8	Unsoaked filter paper with unvanished *E. saligna* leaf	60	1.40	0.64	0.08
9	Vanished *E. saligna* leaf with unsoaked filter paper	60	2.92	1.14	0.15
10	Unsoaked filter paper with vanished *E. saligna* leaf	60	0.47	0.50	0.06
11	Soaked filter paper	60	4.58	1.68	0.22
12	Unsoaked filter paper	60	1.12	0.78	0.10
	Total	**720**	**3.20**	**2.30**	**0.08**

Appendix 5c: ANOVA of mean *Leptocybe invasa* egg count following four days of exposure to different treatments

SV	Sum of Squares	df	Mean Square	*F*	*p*
Between Groups	2632.315	11	239.301	144.378	.000
Within Groups	1173.483	708	1.657		
Total	3805.799	719			

Appendix 5d: Multiple comparison (by Tukey HSD* test) of different treatment effects on mean *Leptocybe invasa* egg count

Multiple Comparisons (Tukey HSD)
Dependent Variable: Egg count

(I) Treatment	**(J) Treatment**	**Mean Difference (I-J) ± Std. Error**	***p***
Unvanished *E. saligna* leaf	Vanished *E. saligna* leaf	3.58 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	0.45 ± 0.24	0.750
	Soaked filter paper with unvanished *E. saligna* leaf	2.05 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	4.02 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	0.01 ± 0.24	1.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.03 ± 0.24*	0.001
	Unsoaked filter paper with unvanished *E. saligna* leaf	3.90 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	2.38 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.83 ± 0.24*	0.000
	Soaked filter paper	0.72 ± 0.24	0.095
	Unsoaked filter paper	4.18 ± 0.24*	0.000
Vanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-3.58 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-3.13 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	-1.53 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	0.43 ± 0.24	0.794
	Soaked filter paper with vanished *E. saligna* leaf	-3.48 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-4.62 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	0.32 ± 0.24	0.973
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.20 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	1.25 ± 0.24*	0.000
	Soaked filter paper	-2.87 ± 0.24*	0.000
	Unsoaked filter paper	0.60 ± 0.24	0.307

Appendix 5d continued

Unvanished *E. saligna* leaf with soaked filter paper	Unvanished *E. saligna* leaf	-0.45 ± 0.24	0.750
	Vanished *E. saligna* leaf	3.13 ± 0.24*	0.000
	Soaked filter paper with unvarnished *E. saligna* leaf	1.60 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	3.57 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-0.35 ± 0.24	0.944
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.48 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	3.45 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	1.93 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.38 ± 0.24*	0.000
	Soaked filter paper	0.27 ± 0.24	0.993
	Unsoaked filter paper	3.73 ± 0.24*	0.000
Soaked filter paper with unvarnished *E. saligna* leaf	Unvanished *E. saligna* leaf	-2.05 ± 0.24*	0.000
	Vanished *E. saligna* leaf	1.53 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-1.60 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	1.97 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-1.95 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-3.08 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	1.85 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	0.33 ± 0.24	0.960
	Unsoaked filter paper with vanished *E. saligna* leaf	2.78 ± 0.24*	0.000
	Soaked filter paper	-1.33 ± 0.24*	0.000
	Unsoaked filter paper	2.13 ± 0.24*	0.000
Vanished *E. saligna* with soaked filter paper	Unvanished *E. saligna* leaf	-4.02 ± 0.24*	0.000
	Vanished *E. saligna* leaf	-0.43 ± 0.24	0.794
	Unvanished *E. saligna* leaf with soaked filter paper	-3.57 ± 0.24*	0.000
	Soaked filter paper with unvarnished *E. saligna* leaf	-1.97 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-3.92 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-5.05 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	-0.12 ± 0.24	1.000
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.63 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	0.82 ± 0.24*	0.026
	Soaked filter paper	-3.30 ± 0.24*	0.000

Appendix 5d continued

	Unsoaked filter paper	0.17 ± 0.24	1.000
Soaked filter paper with vanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-0.01 ± 0.24	1.000
	Vanished E. saligna leaf	3.48 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	0.35 ± 0.24	0.944
	Soaked filter paper with unvanished *E. saligna* leaf	1.95 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	3.92 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.13 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	3.80 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	2.28 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.73 ± 0.24*	0.000
	Soaked filter paper	0.62 ± 0.24	0.266
	Unsoaked filter paper	4.08 ± 0.24*	0.000
Unvanished *E. saligna* leaf with unsoaked filter paper	Unvanished *E. saligna* leaf	1.03 ± 0.24*	0.001
	Vanished *E. saligna* leaf	4.62 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	1.48 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	3.08 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	5.05 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	1.13 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	4.93 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	3.42 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	5.87 ± 0.24*	0.000
	Soaked filter paper	1.75 ± 0.24*	0.000
	Unsoaked filter paper	5.22 ± 0.24*	0.000
Unsoaked filter paper with unvanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-3.90 ± 0.24*	0.000
	Vanished *E. saligna* leaf	-0.32 ± 0.24	0.973
	Unvanished *E. saligna* leaf with soaked filter paper	-3.45 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	-1.85 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	0.12 ± 0.24	1.000
	Soaked filter paper with vanished *E. saligna* leaf	-3.80 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-4.93 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.52 ± 0.24*	0.000

Appendix 5d continued

	Unsoaked filter paper with vanished *E. saligna* leaf	0.93 ± 0.24*	0.004
	Soaked filter paper	-3. 18 ± 0.24*	0.000
	Unsoaked filter paper	0.28 ± 0.24	0.989
Vanished *E. saligna* leaf with unsoaked filter paper	Unvanished *E. saligna* leaf	-2.38 ± 0.24*	0.000
	Vanished *E. saligna* leaf	1.20 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-1.93 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	-0.33 ± 0.24	0.960
	Vanished *E. saligna* with soaked filter paper	1.63 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-2.28 ± 0.24*	00.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	3.42 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	1.52 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	2.45 ± 0.24*	0.000
	Soaked filter paper	-1.67 ± 0.24*	0.000
	Unsoaked filter paper	1.80 ± 0.24*	0.000
Unsoaked filter paper with vanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-4.83 ± 0.24*	0.000
	Vanished E. saligna leaf	-1.25 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-4.38 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	-2.78 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	-0.82 ± 0.24*	0.026
	Soaked filter paper with vanished *E. saligna* leaf	-4.73 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-5.87 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	-0.93 ± 0.24*	0.004
	Vanished *E. saligna* leaf with unsoaked filter paper	-2.45 ± 0.24*	0.000
	Soaked filter paper	-4.12 ± 0.24*	0.000
	Unsoaked filter paper	-0.65 ± 0.24	0.195
Soaked filter paper	Unvanished *E. saligna* leaf	-0.72 ± 0.24	0.095
	Vanished *E. saligna* leaf	2.87 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-0.27 ± 0.24	0.993
	Soaked filter paper with unvanished *E. saligna* leaf	1.33 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	3.30 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-0.62 ± 0.24	0.266
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.75 ± 0.24*	0.000

Appendix 5d continued

	Unsoaked filter paper with unvanished *E. saligna* leaf	3.18 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	1.67 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.12 ± 0.24*	0.000
	Unsoaked filter paper	3.47 ± 0.24*	0.000
Unsoaked filter paper	Unvanished *E. saligna* leaf	-4.18 ± 0.24*	0.000
	Vanished *E. saligna* leaf	-0.60 ± 0.24	0.307
	Unvanished *E. saligna* leaf with soaked filter paper	-3.73 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	-2.13 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	-0.17 ± 0.24	1.000
	Soaked filter paper with vanished *E. saligna* leaf	-4.08 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-5.22 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	-0.28 ± 0.24	0.989
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.80 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	0.65 ± 0.24	0.195
	Soaked filter paper	-3.47 ± 0.24*	0.000
Unvanished *E. saligna* leaf	Vanished *E. saligna* leaf	3.58 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	0.45 ± 0.24	0.056
	Soaked filter paper with unvanished *E. saligna* leaf	2.05 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	4.02 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	0.01 ± 0.24	0.671
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.03 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	3.90 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	2.38 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.83 ± 0.24*	0.000
	Soaked filter paper	0.72 ± 0.24*	0.002
	Unsoaked filter paper	4.18 ± 0.24*	0.000

Appendix 5d continued

Vanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-3.58 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-3.13 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	-1.53 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	0.43 ± 0.24	0.066
	Soaked filter paper with vanished *E. saligna* leaf	-3.48 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-4.62 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	0.32 ± 0.24	0.178
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.20 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	1.25 ± 0.24*	0.000
	Soaked filter paper	-2.87 ± 0.24*	0.000
	Unsoaked filter paper	0.60 ± 0.24*	0.011
Unvanished *E. saligna* leaf with soaked filter paper	Unvanished *E. saligna* leaf	-0.45 ± 0.24	0.056
	Vanished *E. saligna* leaf	3.13 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	1.60 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	3.57 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-0.35 ± 0.24	0.137
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.48 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	3.45 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	1.93 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.38 ± 0.24*	0.000
	Soaked filter paper	0.27 ± 0.24	0.257
	Unsoaked filter paper	3.73 ± 0.24*	0.000
Soaked filter paper with unvanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-2.05 ± 0.24*	0.000
	Vanished *E. saligna* leaf	1.53 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-1.60 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	1.97 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-1.95 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-3.08 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	1.85 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	0.33 ± 0.24	0.157
	Unsoaked filter paper with vanished *E. saligna* leaf	2.78 ± 0.24*	0.000
	Soaked filter paper	-1.33 ± 0.24*	0.000

Appendix 5d continued

	Unsoaked filter paper	2.13 ± 0.24*	0.000
Vanished *E. saligna* with soaked filter paper	Unvanished *E. saligna* leaf	-4.02 ± 0.24*	0.000
	Vanished *E. saligna* leaf	-0.43 ± 0.24	0.066
	Unvanished *E. saligna* leaf with soaked filter paper	-3.57 ± 0.24*	0.000
	Soaked filter paper with unvarnished *E. saligna* leaf	-1.97 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-3.92 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-5.05 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	-0.12 ± 0.24	0.620
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.63 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	0.82 ± 0.24*	0.001
	Soaked filter paper	-3.30 ± 0.24*	0.000
	Unsoaked filter paper	0.17 ± 0.24	0.479
Soaked filter paper with vanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-0.01 ± 0.24	0.671
	Vanished *E. saligna* leaf	3.48 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	0.35 ± 0.24	0.137
	Soaked filter paper with unvarnished *E. saligna* leaf	1.95 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	3.92 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.13 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	3.80 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	2.28 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.73 ± 0.24*	0.000
	Soaked filter paper	0.62 ± 0.24*	0.009
	Unsoaked filter paper	4.08 ± 0.24*	0.000
Unvanished *E. saligna* leaf with unsoaked filter paper	Unvanished *E. saligna* leaf	1.03 ± 0.24*	0.000
	Vanished *E. saligna* leaf	4.62 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	1.48 ± 0.24*	0.000
	Soaked filter paper with unvarnished *E. saligna* leaf	3.08 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	5.05 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	1.13 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	4.93 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	3.42 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	5.87 ± 0.24*	0.000

Appendix 5d continued

	Soaked filter paper	1.75 ± 0.24*	0.000
	Unsoaked filter paper	5.22 ± 0.24*	0.000
Unsoaked filter paper with unvarnished *E. saligna* leaf	Unvanished *E. saligna* leaf	-3.90 ± 0.24*	0.000
	Vanished *E. saligna* leaf	-0.32 ± 0.24	0.178
	Unvanished *E. saligna* leaf with soaked filter paper	-3.45 ± 0.24*	0.000
	Soaked filter paper with unvarnished *E. saligna* leaf	-1.85 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	0.12 ± 0.24	0.620
	Soaked filter paper with vanished *E. saligna* leaf	-3.80 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-4.93 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.52 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	0.93 ± 0.24*	0.000
	Soaked filter paper	-3.18 ± 0.24*	0.000
	Unsoaked filter paper	0.28 ± 0.24	0.228
Vanished *E. saligna* leaf with unsoaked filter paper	Unvanished *E. saligna* leaf	-2.38 ± 0.24*	0.000
	Vanished *E. saligna* leaf	1.20 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-1.93 ± 0.24*	0.000
	Soaked filter paper with unvarnished *E. saligna* leaf	-0.33 ± 0.24	0.157
	Vanished *E. saligna* with soaked filter paper	1.63 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-2.28 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-3.42 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	1.52 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	2.45 ± 0.24*	0.000
	Soaked filter paper	-1.67 ± 0.24*	0.000
	Unsoaked filter paper	1.80 ± 0.24*	0.000
Unsoaked filter paper with vanished *E. saligna* leaf	Unvanished *E. saligna* leaf	-4.83 ± 0.24*	0.000
	Vanished *E. saligna* leaf	-1.25 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-4.38 ± 0.24*	0.000
	Soaked filter paper with unvarnished *E. saligna* leaf	-2.78 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	-0.82 ± 0.24*	0.001
	Soaked filter paper with vanished *E. saligna* leaf	-4.73 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-5.87 ± 0.24*	0.000
	Unsoaked filter paper with unvarnished *E. saligna* leaf	-0.93 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	-2.45 ± 0.24*	0.000

Appendix 5d continued

	Soaked filter paper	-4.12 ± 0.24*	0.000
	Unsoaked filter paper	-0.65 ± 0.24*	0.006
`Soaked filter paper	Unvanished *E. saligna* leaf	-0.72 ± 0.24*	0.002
	Vanished *E. saligna* leaf	2.87 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with soaked filter paper	-0.27 ± 0.24	0.257
	Soaked filter paper with unvanished *E. saligna* leaf	1.33 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	3.30 ± 0.24*	0.000
	Soaked filter paper with vanished *E. saligna* leaf	-0.62 ± 0.24*	0.009
	Unvanished *E. saligna* leaf with unsoaked filter paper	-1.75 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	3.18 ± 0.24*	0.000
	Vanished *E. saligna* leaf with unsoaked filter paper	1.67 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	4.12 ± 0.24*	0.000
	Unsoaked filter paper	3.47 ± 0.24*	0.000
Unsoaked filter paper	Unvanished *E. saligna* leaf	-4.18 ± 0.24*	0.000
	Vanished *E. saligna* leaf	-0.60 ± 0.24*	0.011
	Unvanished *E. saligna* leaf with soaked filter paper	-3.73 ± 0.24*	0.000
	Soaked filter paper with unvanished *E. saligna* leaf	-2.13 ± 0.24*	0.000
	Vanished *E. saligna* with soaked filter paper	-0.17 ± 0.24	0.479
	Soaked filter paper with vanished *E. saligna* leaf	-4.08 ± 0.24*	0.000
	Unvanished *E. saligna* leaf with unsoaked filter paper	-5.22 ± 0.24*	0.000
	Unsoaked filter paper with unvanished *E. saligna* leaf	-0.28 ± 0.24	0.228
	Vanished *E. saligna* leaf with unsoaked filter paper	-1.80 ± 0.24*	0.000
	Unsoaked filter paper with vanished *E. saligna* leaf	0.65 ± 0.24*	0.006
	Soaked filter paper	-3.47 ± 0.24*	0.000

* The mean difference is significant at the 0.05 level

Appendix 5e: Tukey HSD* homogenous subsets of mean *Leptocybe invasa* egg count following four days of exposure to different treatments

			Subset for alpha = 0.05				
S/ NO.	**Treatment**	**n**	**1**	**2**	**3**	**4**	**5**
1	Unsoaked filter paper with vanished *E. saligna* leaf	60	0.47a				
2	Unsoaked filter paper	60	1.12a	1.12b			
3	Vanished *E. saligna* with soaked filter paper	60		1.28b			
4	Unsoaked filter paper with unvanished *E. saligna* leaf	60		1.40b			
5	Vanished *E. saligna* leaf	60		1.72b			
6	Vanished *E. saligna* leaf with unsoaked filter paper	60			2.92c		
7	Soaked filter paper with unvanished *E. saligna* leaf	60			3.25c		
8	Soaked filter paper	60				4.58c	
9	Unvanished *E. saligna* leaf with soaked filter paper	60				4.85c	
10	Soaked filter paper with vanished *E. saligna* leaf	60				5.20c	
11	Unvanished *E. saligna* leaf	60				5.30c	
12	Unvanished *E. saligna* leaf with unsoaked filter paper	60					6.33d
	P		0.195	0.307	0.960	0.095	1.000

Means for groups in homogeneous subsets are displayed. Those with same letters are not significantly different.

* Uses Harmonic Mean Sample Size = 60.000.

Appendix 6: Patch use by *L. invasa* indicated by duration of time spent at a patch and time spent moving from patch to patch. ***(For patch type: 1⇒ landing at a patch; 0⇒ no landing at a patch)***

(a) Vertical habitat structure

	Patch type			Time taken	Time taken from
Trial	***E. saligna***	***C. lusitanica***	***G. robusta***	**at patch**	**patch to patch**
1	1	0	0	5	6
2	0	1	0	1	4
3	0	0	1	1	2
4	1	0	0	10	2
5	1	0	0	11	2
6	0	1	0	2	1
7	0	0	1	1	1
8	0	0	1	1	1
9	1	0	0	15	1
10	1	0	0	16	1
11	1	0	0	17	1
12	1	0	0	17	1
13	1	0	0	16	2
14	0	1	0	3	4
15	0	0	1	2	5
16	1	0	0	10	3
17	1	0	0	12	2
18	1	0	0	16	1
19	1	0	0	18	1
20	1	0	0	17	2

Appendix 6 continued

(b) Horizontal habitat structure

Trial	Patch type					Time taken at patch	Time taken from patch to patch
	Dry Paper	Moist Paper	Paper soaked in *E. saligna* leaf extract	Paper soaked in *E. saligna* leaf extract	Paper soaked in *G. robusta* leaf extract		
1	0	1	0	0	0	3	5
2	0	0	0	1	0	1	5
3	0	0	0	0	1	3	2
4	1	0	0	0	0	2	6
5	0	1	0	0	0	6	3
6	0	0	1	0	0	12	1
7	0	0	1	0	0	18	1
8	0	1	0	0	0	10	2
9	0	1	0	0	0	11	1
10	0	0	1	0	0	16	1
11	0	0	1	0	0	17	1
12	0	0	1	0	0	18	1
13	0	0	1	0	0	14	1
14	0	0	0	1	0	2	4
15	1	0	0	0	0	2	3
16	0	1	0	0	0	1	4
17	0	0	1	0	0	12	1
18	0	0	1	0	0	16	2
19	0	0	1	0	0	17	1
20	0	0	1	0	0	18	1

Appendix 7a: Factors under consideration in determining *E. saligna* host condition in relation to successful attack by *Leptocybe invasa* (Hymenoptera: Eulophidae)

Factor	**Level**
Age*	One week old *E. saligna* seedlings (A1)
	Six weeks old *E. saligna* seedlings (A2)
Watering	No watering (W1)
	10 cm^3 of water added once a week (W2)
	10 cm^3 of water added thrice a week (W3)
Nitrogen fertilization	No fertilization (N1)
	1g CAN fertilizer added every after 6 weeks (N2)
	1g CAN fertilizer added every after 2 weeks (N3)

***Age after pricking out (transplanting in polythene tubes)**

Appendix 7b: Treatments given to *E. saligna* seedling before being exposed to *L. invasa* attack

A1W1N1 one week old seedling + no watering + no fertilizer

A1W1N2 one week old seedling + no watering + 1g CAN fertilizer applied after every 6 weeks

A1W1N3 one week old seedling + no watering + 1g CAN fertilizer applied after every 2 weeks

A1W2N1 one week old seedling + 10 cm^3 water added once a week + no fertilizer

A1W2N2 one week old seedling + 10 cm^3 of water added once a week + 1g CAN fertilizer applied after every 6 weeks

A1W2N3 one week old seedling + 10 cm^3 of water added once a week + 1g CAN fertilizer applied after every 2 weeks

A1W3N1 one week old seedling + 10 cm^3 of water added thrice a week + no fertilizer

A1W3N2 one week old seedling + 10 cm^3 of water added thrice a week + 1g CAN fertilizer applied after every 6 weeks

A1W3N3 one week old seedling + 10 cm^3 of water added thrice a week + 1g CAN fertilizer applied after every 2 weeks

A2W1N1 six weeks old seedling + no watering + no fertilizer

A2W1N2 six weeks old seedling + no watering + 1g CAN fertilizer applied after every 6 weeks

A2W1N3 six weeks old seedling + no watering + 1g CAN fertilizer applied after every 2 weeks

A2W2N1 six weeks old seedling + 10 cm^3 of water added once a week + no fertilizer

A2W2N2 six weeks old seedling + 10 cm^3 of water added once a week + 1g CAN fertilizer applied after every 6 weeks

A2W2N3 six weeks old seedling + 10 cm^3 of water added once a week + 1g CAN fertilizer applied after every 2 weeks

A2W3N1 six weeks old seedling + 10 cm^3 of water added thrice a week + no fertilizer

A2W3N2 six weeks old seedling + 10 cm^3 of water added thrice a week + 1g CAN fertilizer applied after every 6 weeks

Appendix 7c: Univariate ANOVA of mean number of galls per seedling as a response to *E. saligna* attack by *Leptocybe invasa* (Hymenoptera: Eulophidae) following various treatments given to the seedlings prior to exposure to insect attack.

Tests of Between-Subjects Effects
Dependent Variable: Number of galls

Source	Type III Sum of Squares	df	Mean Square	*F*	*p*	Eta Squared
Corrected Model	1215.535[a]	17	71.502	11.232	0.000	0.602
Intercept	7729.340	1	7729.340	1214.146	0.000	0.906
TREAT	1215.535	17	71.502	11.232	0.000	0.602
Error	802.125	126	6.366			
Total	9747.000	144				
Corrected Total	2017.660	143				

a R Squared = 0.602 (Adjusted R Squared = 0.549)

Appendix 7d: Mean gall numbers per seedling in relation to *E. saligna* host condition for successful attack by *L. invasa* (Hymenoptera: Eulophidae): (*key to treatments listed in appendix 7b*)

Number of galls
Tukey HSD [a,b]

Treatment	N	Subset						
		1	2	3	4	5	6	7
A2W2N1	8	2.62						
A2W2N2	8	3.38	3.38					
A2W2N3	8	4.13	4.13					
A2W1N3	8	4.88	4.88					
A2W3N1	8	5.13	5.13	5.13				
A2W2N3	8	5.25	5.25	5.25				
A2W3N2	8	5.88	5.88	5.88	5.88			
A1W1N1	8	6.25	6.25	6.25	6.25	6.25		
A2W1N2	8	6.75	6.75	6.75	6.75	6.75	6.75	
A2W1N1	8		7.13	7.13	7.13	7.13	7.13	
A1W1N3	8		7.25	7.25	7.25	7.25	7.25	
A1W1N2	8		7.75	7.75	7.75	7.75	7.75	
A1W2N1	8			9.50	9.50	9.50	9.50	9.50
A1W3N1	8				10.13	10.13	10.13	10.13
A1W3N2	8					10.63	10.63	10.63
A1W2N2	8						11.00	11.00
A1W2N3	8						11.13	11.13
A1W3N3	8							13.13
P		0.098	0.053	0.053	0.073	0.053	0.053	0.268

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square (Error) = 6.366.
a Uses Harmonic Mean Sample Size = 8.000.
b Alpha =0 .05.

Appendix 8: Height (cm) and root collar diameter (mm) measurements of *E. saligna* seedlings grown together with selected plant species acting as a *Eucalyptus* IPM strategy against *L. invasa*

Month	Week	Treatment	N	Height (cm)			RCD (mm)		
				Sum	Mean	SE	Sum	Mean	SE
1	1	Control	10	94.9	9.5	0.6	13.6	1.4	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	94.3	9.4	0.4	12.3	1.2	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	118.9	11.9	0.4	14.3	1.4	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	104.1	10.4	0.3	11.6	1.2	0.0
		E. saligna + *L. invasa*	10	113.4	11.3	0.3	12.2	1.2	0.0
		Total	50	525.6	10.5	0.2	64.0	1.3	0.0
	2	Control	10	131.9	13.2	0.2	16.2	1.6	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	116.6	11.7	0.7	13.8	1.4	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	142.9	14.3	0.2	16.2	1.6	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	144.1	14.4	0.5	12.1	1.2	0.0
		E. saligna + *L. invasa*	10	151.3	15.1	0.2	12.2	1.2	0.0
		Total	50	686.8	13.7	0.2	70.5	1.4	0.0
	3	Control	10	168.7	16.9	0.2	21.1	2.1	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	168.2	16.8	1.5	14.6	1.5	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	152.6	15.3	0.3	17.8	1.8	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	185.1	18.5	0.6	12.7	1.3	0.0
		E. saligna + *L. invasa*	10	183.0	18.3	0.3	12.8	1.3	0.0
		Total	50	857.6	17.2	0.4	79.0	1.6	0.1
	4	Control	10	197.3	19.7	0.4	24.7	2.5	0.0
		E. saligna + *Leonotis*	10	190.3	19.0	1.3	16.6	1.7	0.1

		nepetifolia + L. invasa							
		E. saligna + Schkuria pinnata + L. invasa	10	157.2	15.7	0.3	19.2	1.9	0.1
		E. saligna + Tagetes recta + L. invasa	10	203.6	20.4	0.8	12.8	1.3	0.0
		E. saligna + L. invasa	10	208.0	20.8	0.4	13.3	1.3	0.0
		Total	50	956.4	19.1	0.4	86.6	1.7	0.1
	Total	Control	40	592.8	14.8	0.6	75.6	1.9	0.1
		E. saligna + Leonotis nepetifolia + L. invasa	40	569.4	14.2	0.8	57.3	1.4	0.0
		E. saligna + Schkuria pinnata + L. invasa	40	571.6	14.3	0.3	67.5	1.7	0.0
		E. saligna + Tagetes recta + L. invasa	40	636.9	15.9	0.7	49.2	1.2	0.0
		E. saligna + L. invasa	40	655.7	16.4	0.6	50.5	1.3	0.0
		Total	200	3026.4	15.1	0.3	300.1	1.5	0.0
2	1	Control	10	227.3	22.7	0.1	27.6	2.8	0.0
		E. saligna + Leonotis nepetifolia + L. invasa	10	203.1	20.3	0.5	17.9	1.8	0.0
		E. saligna + Schkuria pinnata + L. invasa	10	194.6	19.5	0.8	20.9	2.1	0.1
		E. saligna + Tagetes recta + L. invasa	10	218.4	21.8	0.8	13.2	1.3	0.0
		E. saligna + L. invasa	10	223.3	22.3	0.3	13.5	1.4	0.0
		Total	50	1066.7	21.3	0.3	93.1	1.9	0.1
	2	Control	10	237.6	23.8	0.2	32.0	3.2	0.0
		E. saligna + Leonotis nepetifolia + L. invasa	10	223.9	22.4	0.4	18.9	1.9	0.0
		E. saligna + Schkuria pinnata + L. invasa	10	211.1	21.1	0.7	22.0	2.2	0.1
		E. saligna + Tagetes recta + L. invasa	10	238.9	23.9	0.6	13.3	1.3	0.0
		E. saligna + L. invasa	10	234.6	23.5	0.2	13.8	1.4	0.0
		Total	50	1146.1	22.9	0.3	100.0	2.0	0.1
	3	Control	10	256.2	25.6	0.2	35.1	3.5	0.0

		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	236.7	23.7	0.3	20.1	2.0	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	232.0	23.2	0.5	23.3	2.3	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	258.3	25.8	0.5	14.4	1.4	0.0
		E. saligna + *L. invasa*	10	262.8	26.3	0.2	14.7	1.5	0.0
		Total	50	1246.0	24.9	0.2	107.6	2.2	0.1
	4	Control	10	274.8	27.5	0.3	37.1	3.7	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	244.8	24.5	0.2	21.3	2.1	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	247.9	24.8	0.6	24.4	2.4	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	262.7	26.3	0.5	14.9	1.5	0.0
		E. saligna + *L. invasa*	10	272.1	27.2	0.3	14.9	1.5	0.0
		Total	50	1302.3	26.0	0.2	112.6	2.3	0.1
	Total	Control	40	995.9	24.9	0.3	131.8	3.3	0.1
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	40	908.5	22.7	0.3	78.2	2.0	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	40	885.6	22.1	0.5	90.6	2.3	0.0
		E. saligna + *Tagetes recta* + *L. invasa*	40	978.3	24.5	0.4	55.8	1.4	0.0
		E. saligna + *L. invasa*	40	992.8	24.8	0.3	56.9	1.4	0.0
		Total	200	4761.1	23.8	0.2	413.3	2.1	0.1
3	1	Control	10	360.2	36.0	0.5	37.8	3.8	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	278.1	27.8	0.7	22.0	2.2	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	285.8	28.6	0.9	25.9	2.6	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	325.1	32.5	0.5	15.6	1.6	0.1
		E. saligna + *L. invasa*	10	355.0	35.5	0.9	15.6	1.6	0.0
		Total	50	1604.2	32.1	0.6	116.9	2.3	0.1

	2	Control	10	369.3	36.9	0.4	38.0	3.8	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	301.2	30.1	0.4	22.7	2.3	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	297.4	29.7	0.8	27.7	2.8	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	346.0	34.6	0.7	18.3	1.8	0.0
		E. saligna + *L. invasa*	10	412.9	41.3	1.3	17.2	1.7	0.0
		Total	50	1726.8	34.5	0.7	123.9	2.5	0.1
	3	Control	10	394.1	39.4	0.5	39.3	3.9	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	329.6	33.0	0.4	23.2	2.3	0.1
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	313.5	31.4	0.8	29.5	3.0	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	430.4	43.0	1.4	19.5	2.0	0.0
		E. saligna + *L. invasa*	10	515.8	51.6	1.7	18.0	1.8	0.0
		Total	50	1983.4	39.7	1.1	129.5	2.6	0.1
	4	Control	10	431.3	43.1	0.9	39.9	4.0	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	354.4	35.4	0.5	23.8	2.4	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	341.5	34.2	1.0	31.8	3.2	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	473.0	47.3	1.9	20.9	2.1	0.0
		E. saligna + *L. invasa*	10	594.6	59.5	2.3	18.7	1.9	0.0
		Total	50	2194.8	43.9	1.5	135.1	2.7	0.1
	Total	Control	40	1554.9	38.9	0.5	155.0	3.9	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	40	1263.3	31.6	0.5	91.7	2.3	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	40	1238.2	31.0	0.5	114.9	2.9	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	40	1574.5	39.4	1.1	74.3	1.9	0.0
		E. saligna + *L. invasa*	40	1878.3	47.0	1.7	69.5	1.7	0.0

		Total	200	7509.2	37.5	0.6	505.4	2.5	0.1
4	1	Control	10	698.7	69.9	2.9	41.2	4.1	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	634.5	63.5	3.5	24.4	2.4	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	561.7	56.2	2.0	33.2	3.3	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	515.7	51.6	2.2	22.8	2.3	0.0
		E. saligna + *L. invasa*	10	629.1	62.9	2.6	19.5	2.0	0.0
		Total	50	3039.7	60.8	1.5	141.1	2.8	0.1
	2	Control	10	821.3	82.1	2.1	42.0	4.2	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	732.8	73.3	3.2	24.7	2.5	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	836.2	83.6	2.3	34.5	3.5	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	523.4	52.3	2.2	24.5	2.5	0.0
		E. saligna + *L. invasa*	10	642.6	64.3	2.4	20.6	2.1	0.0
		Total	50	3556.3	71.1	2.0	146.3	2.9	0.1
	3	Control	10	891.7	89.2	1.8	43.0	4.3	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	802.8	80.3	2.5	24.8	2.5	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	903.1	90.3	1.6	36.0	3.6	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	526.4	52.6	2.2	25.5	2.6	0.0
		E. saligna + *L. invasa*	10	657.1	65.7	2.3	21.6	2.2	0.0
		Total	50	3781.1	75.6	2.3	150.9	3.0	0.1
	4	Control	10	1002.5	100.3	1.2	44.6	4.5	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	920.4	92.0	1.7	25.2	2.5	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	936.2	93.6	2.2	37.2	3.7	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	531.0	53.1	2.2	28.1	2.8	0.0
		E. saligna +	10	680.0	68.0	2.2	22.4	2.2	0.0

		L. invasa							
		Total	50	4070.1	81.4	2.7	157.5	3.2	0.1
	Total	Control	40	3414.2	85.4	2.0	170.8	4.3	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	40	3090.5	77.3	2.2	99.1	2.5	0.0
	1	*E. saligna* + *Schkuria pinnata* + *L. invasa*	40	3237.2	80.9	2.6	140.9	3.5	0.0
		E. saligna + *Tagetes recta* + *L. invasa*	40	2096.5	52.4	1.1	100.9	2.5	0.0
		E. saligna + *L. invasa*	40	2608.8	65.2	1.2	84.1	2.1	0.0
		Total	200	14447.2	72.2	1.2	595.8	3.0	0.1
5		Control	10	1097.7	109.8	2.3	46.1	4.6	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	1027.1	102.7	1.9	25.9	2.6	0.1
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	1029.7	103.0	2.5	38.8	3.9	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	534.5	53.5	2.0	32.1	3.2	0.0
		E. saligna + *L. invasa*	10	722.3	72.2	1.8	23.4	2.3	0.0
		Total	50	4411.3	88.2	3.2	166.3	3.3	0.1
	2	Control	10	1223.1	122.3	1.4	47.9	4.8	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	1168.5	116.9	2.8	26.5	2.7	0.1
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	1231.9	123.2	0.6	40.2	4.0	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	535.9	53.6	2.0	33.8	3.4	0.1
		E. saligna + *L. invasa*	10	787.1	78.7	1.2	24.7	2.5	0.0
		Total	50	4946.5	98.9	4.1	173.1	3.5	0.1
	3	Control	10	1266.7	126.7	2.1	48.9	4.9	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	1209.0	120.9	1.5	26.9	2.7	0.1
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	1238.6	123.9	0.5	41.9	4.2	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	549.0	54.9	2.1	35.4	3.5	0.0

		E. saligna + *L. invasa*	10	812.0	81.2	1.2	25.5	2.6	0.0
		Total	50	5075.3	101.5	4.1	178.6	3.6	0.1
	4	Control	10	1396.0	139.6	1.3	51.0	5.1	0.1
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	10	1256.9	125.7	0.4	27.2	2.7	0.1
		E. saligna + *Schkuria pinnata* + *L. invasa*	10	1242.2	124.2	0.5	43.0	4.3	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	10	557.9	55.8	1.7	37.0	3.7	0.0
		E. saligna + *L. invasa*	10	846.2	84.6	1.9	26.3	2.6	0.0
		Total	50	5299.2	106.0	4.5	184.5	3.7	0.1
	Total	Control	40	4983.5	124.6	1.9	193.9	4.8	0.0
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	40	4661.5	116.5	1.6	106.5	2.7	0.0
		E. saligna + *Schkuria pinnata* + *L. invasa*	40	4742.4	118.6	1.6	163.9	4.1	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	40	2177.3	54.4	1.0	138.3	3.5	0.0
		E. saligna + *L. invasa*	40	3167.6	79.2	1.1	99.9	2.5	0.0
		Total	200	19732.3	98.7	2.0	702.5	3.5	0.1
Total	1	Control	50	2478.8	49.6	5.2	166.3	3.3	0.2
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	50	2237.1	44.7	4.9	102.5	2.1	0.1
		E. saligna + *Schkuria pinnata* + *L. invasa*	50	2190.7	43.8	4.8	133.1	2.7	0.1
		E. saligna + *Tagetes recta* + *L. invasa*	50	1697.8	34.0	2.5	95.3	1.9	0.1
		E. saligna + *L. invasa*	50	2043.1	40.9	3.4	84.2	1.7	0.1
		Total	250	10647.5	42.6	1.9	581.4	2.3	0.1
	2	Control	50	2783.2	55.7	5.8	176.1	3.5	0.2
		E. saligna + *Leonotis nepetifolia* + *L. invasa*	50	2543.0	50.9	5.6	106.6	2.1	0.1
		E. saligna + *Schkuria pinnata* + *L. invasa*	50	2719.5	54.4	6.1	140.6	2.8	0.1
		E. saligna + *Tagetes recta*	50	1788.3	35.8	2.3	102.0	2.0	0.1

		+ L. invasa							
		E. saligna + L. invasa	50	2228.5	44.6	3.5	88.5	1.8	0.1
		Total	250	12062.5	48.3	2.2	613.8	2.5	0.1
	3	Control	50	2977.4	59.5	6.0	187.4	3.7	0.1
		E. saligna + Leonotis nepetifolia + L. invasa	50	2746.3	54.9	5.7	109.6	2.2	0.1
		E. saligna + Schkuria pinnata + L. invasa	50	2839.8	56.8	6.1	148.5	3.0	0.1
		E. saligna + Tagetes recta + L. invasa	50	1949.2	39.0	2.2	107.5	2.2	0.1
		E. saligna + L. invasa	50	2430.7	48.6	3.4	92.6	1.9	0.1
		Total	250	12943.4	51.8	2.2	645.6	2.6	0.1
	4	Control	50	3301.9	66.0	6.6	197.3	3.9	0.1
		E. saligna + Leonotis nepetifolia + L. invasa	50	2966.8	59.3	6.0	114.1	2.3	0.1
		E. saligna + Schkuria pinnata + L. invasa	50	2925.0	58.5	6.1	155.6	3.1	0.1
		E. saligna + Tagetes recta + L. invasa	50	2028.2	40.6	2.2	113.7	2.3	0.1
		E. saligna + L. invasa	50	2600.9	52.0	3.6	95.6	1.9	0.1
		Total	250	13822.8	55.3	2.4	676.3	2.7	0.1
	Total	Control	200	11541.3	57.7	3.0	727.1	3.6	0.1
		E. saligna + Leonotis nepetifolia + L. invasa	200	10493.2	52.5	2.8	432.8	2.2	0.0
		E. saligna + Schkuria pinnata + L. invasa	200	10675.0	53.4	2.9	577.8	2.9	0.1
		E. saligna + Tagetes recta + L. invasa	200	7463.5	37.3	1.1	418.5	2.1	0.1
		E. saligna + L. invasa	200	9303.2	46.5	1.7	360.9	1.8	0.0
		Total	1000	49476.2	49.5	1.1	2517.1	2.5	0.0

Appendix 9a: Mean gall count on different *Eucalyptus* species following attack by *L. invasa*

Descriptives
Number of galls

	N	Mean	SD	SE
E. saligna exposed to *L. invasa* alone	160	15.43	3.68	0.29
E. camaldulensis exposed to *L. invasa* alone	160	7.11	3.04	0.24
E. citriodora exposed to *L. invasa* alone	160	0.94	0.91	0.07
E. globulus exposed to *L. invasa* alone	160	0.86	.89	0.07
E. saligna exposed to *L. invasa* with other Eucalypts	160	3.21	4.23	0.33
Total	800	5.51	6.18	0.22

Appendix 9b: ANOVA of mean gall count on different *Eucalyptus* species following attack by *L. invasa*

ANOVA
Number of galls

SV	Sum of Squares	df	Mean Square	*F*	*p*
Between Groups	23797.820	4	5949.455	703.413	.000
Within Groups	6724.100	795	8.458		
Total	30521.920	799			

Appendix 9c: Multiple comparisons by tukey test on mean gall count on different *Eucalyptus* species following attack by *L. invasa*

(I) Treatment	(J) Treatment	Mean Difference (I-J)	SE	Sig.
E. saligna exposed to *L. invasa* alone	*E. camaldulensis* exposed to *L. invasa* alone	8.32*	0.33	0.000
	E. citriodora exposed to *L. invasa* alone	14.49*	0.33	0.000
	E. globulus exposed to *L. invasa* alone	14.57*	0.33	0.000
	E. saligna exposed to *L. invasa* with other Eucalypts	12.23*	0.33	0.000
E. camaldulensis exposed to *L. invasa* alone	*E. saligna* exposed to *L. invasa* alone	-8.32*	0.33	0.000
	E. citriodora exposed to *L. invasa* alone	6.16*	0.33	0.000
	E. globulus exposed to *L. invasa* alone	6.24*	0.33	0.000
	E. saligna exposed to *L. invasa* with other Eucalypts	3.90*	0.33	0.000
E. citriodora exposed to *L. invasa* alone	*E. saligna* exposed to *L. invasa* alone	-14.49*	0.33	0.000
	E. camaldulensis exposed to *L. invasa* alone	-6.16*	0.33	0.000
	E. globulus exposed to *L. invasa* alone	8.12E-02	0.33	0.999
	E. saligna exposed to *L. invasa* with other Eucalypts	-2.26*	0.33	0.000
E. globulus exposed to *L. invasa* alone	*E. saligna* exposed to *L. invasa* alone	-14.57*	0.33	0.000
	E. camaldulensis exposed to *L. invasa* alone	-6.24*	0.33	0.000
	E. citriodora exposed to *L. invasa* alone	-8.12E-02	0.33	0.999
	E. saligna exposed to *L. invasa* with other Eucalypts	-2.34*	0.33	0.000
E. saligna exposed to *L. invasa* with other Eucalypts	*E. saligna* exposed to *L. invasa* alone	-12.23*	0.33	0.000
	E. camaldulensis exposed to *L. invasa* alone	-3.90*	0.33	0.000
	E. citriodora exposed to *L. invasa* alone	2.26*	0.33	0.000
	E. globulus exposed to *L. invasa* alone	2.34*	0.33	0.000

* The mean difference is significant at the .05 level.

Appendix 9d: Tukey's homogenous subsets of mean gall count per seedling of *Eucalyptus* species following attack by *L. invasa*

Number of galls
Tukey HSD*

Treatment	n	Subset for alpha = .05			
		1	2	3	4
E. globulus exposed to *L. invasa* alone	160	0.86a			
E. citriodora exposed to *L. invasa* alone	160	0.94a			
E. saligna exposed to *L. invasa* with other Eucalypts	160		3.21b		
E. camaldulensis exposed to *L. invasa* alone	160			7.11c	
E. saligna exposed to *L. invasa* alone	160				15.43d
P		0.999	1.000	1.000	1.000

Means for groups in homogeneous subsets (with similar letters) are displayed.
*Uses Harmonic Mean Sample Size = 160.000.

Appendix 10: Definition of terms as used in this study

- **Adnotaular setae** (with reference to hymenopterous insect morphology) refer to one or more rows of differentiated setae on the mesoscutal midlobe adjacent to each notaulus.
- **Antibiosis** refers to the preventive, injurious, or destructive effects on the insect life history which results from the insect's use of a resistant host variety or species for food.
- **Biological control (of insect pests)** refers to the manipulation or use of natural enemies of pests to reduce their populations to levels where economic losses due to them are tolerable.
- **Callus** or callosity): (a) is an especially toughened area of skin (i.e. integument) which has become relatively thick and hard in response to repeated friction, pressure, or other irritation; or (b) Overgrowth of tissues at the margins of wounds and diseased tissues.
- **Cecidogenesis** is a process leading to the formation of galls.
- **Companion plant** is a plant planted together with the main crop plant in order to confer benefits to the main crop plant in terms of (for example) insect pest repellence, improved water and nutrient uptake, e.t.c.
- **Cultural control (or Silvicultural control) of insect pests** refers to the modification of methods of growing or harvesting tree crop in order to reduce insect-caused damage by avoiding conditions that favour the pests.
- **Ectoparasitoid** is an insect parasite which develops externally on its arthropod host.
- **Endoparasitoid** is an insect parasite which develops within the body of of its arthropod host.

- **Gall** (e.g. plant galls) refer to swellings or deformities on plant tissues or organs due to plant-insect interactions.
- **Gallicolous** (e.g. gallicolous insect) refers to gall-forming or gall-inducing insects.
- **Glycerolipids** are organic compounds formed by chemical combination of glycerols and fatty acids.
- **Gregarious** (insects) refers to the tendency of organisms staying, living or migrating together (usually in large numbers) with other organisms of the same species.
- **Host plant resistance** refers to the collective heritable characteristics by which a plant species, race, clone, or individual may reduce the possibility of successful utilization of the plant as a host by an insect species, race, biotype or individual.
- **Idiobiont** (parasitoids) are those which prevent any further development of the host after initial parasitization; this typically involves a host life stage which is immobile (e.g., an egg or pupa), and almost without exception, they live outside the host.(*see koinobiont*).
- **Infection** is a process by which an insect or a pathogen establishes contact with susceptible cells or tissues of a host to get nutrients.
- **Insect colonisation** refers to the establishment of a laboratory culture of an insect using material collected from the field or the bringing of insects from their natural ecosystem into the laboratory for propagation (rearing)
- **Integrated pest management (IPM)** is a pest management system that uses a number of alternative or complementary control procedures in a co-coordinated way to reduce and maintain pest populations below economic injury level.
- **Koinobiont** (parasitoids) are those that allow the host to continue its development and often do not kill or consume the host until the host is about to either pupate or

become an adult; this therefore typically involves living within an active, mobile host. Koinobionts can be further subdivided into endoparasitoids, which develop inside of the prey, and ectoparasitoids, which develop outside the host body, though they are frequently attached or embedded in the host's tissues.(*see idiobiont*)

- **Mesoscutum** is the scutum or dorsal plate of the middle thorasic segment of an insect. The anterior portion of mesonotum is also called the mesoscutum, or simply "scutum".
- **Mesothorax** is the middle of the three segments in the thorax of an insect, and bears the second pair of legs. Its principal sclerites (exoskeletal plates) are the mesonotum (dorsal), the mesosternum (ventral), and the mesopleuron (lateral) on each side.
- **Notaulus** (with reference to hymenopterous insect morphology) is a line that extends submedially along the mesoscutum and corresponds to the median border of the site of origin of the first mesopleuro-mesonotal muscle.
- **Olfaction** is the ability to interpret information and surroundings from the effects of smell (scent).
- **Oviposition** refers to laying of eggs.
- **Parasite** is an animal species which lives on or in a larger animal, the host, feeding upon it, and frequently destroying it. A parasite needs only one or part of one host to reach maturity.
- **Parasitism** is a qualitative term referring to a kind of symbiosis in which one party (the parasite) lives at the expense of the other (the host), contributing nothing to the relationship, and frequently destroying the host in the process.

- **Parasitization** is a quantitative term referring to the proportion of a host population attacked by parasites.
- **Parasitoid** is an insect parasite of arthropod: parasitic only in its immature stages, destroying its host in the process of its development, and free-living as an adult.
- **Pest** (e.g. insect pest) refers to an organism that competes with man for valuable resource and by doing so damages or lowers the quantity, quality and/ or aesthetic value of the resource.
- **Phospholglycerides** are esters of only two fatty acids, phosphoric acid and a trifunctional alcohol - glycerol (IUPAC name is 1, 2, 3-propantriol). The fatty acids are attached to the glycerol at the 1 and 2 positions on glycerol through ester bonds. There may be a variety of fatty acids, both saturated and unsatured, in the phospholipids.
- **Phytophagous** (insects) are those that feed on plants, i.e. herbivorous.
- Predator is an animal which feeds upon animal (prey) that is usually smaller and weaker than it, frequently devouring it completely and rapidly. A predator most often is required to seek out and attack more than one prey to reach maturity.
- **Propodeum** is the first abdominal segment in Apocrita Hymenoptera (wasps, bees and ants). It is fused with the thorax to form the mesosoma. It is a single large sclerite, not subdivided, and bears a pair of spiracles. It is strongly constricted posteriorly to form the articulation of the petiole, and gives apocritans their distinctive shape.
- **Secondary parasitoid or hyperparasitoid:** is a parasitoid whose host is also a parasitoid. The term hyperparasite is commonly used, but this term is slightly misleading, as both the host and the primary parasitoid are killed. A better term is

secondary parasitoid, or hyperparasitoid; most such species known are in the insect order Hymenoptera.

- **Seta or setum (plural: setae)** is a biological term derived from the Latin word for "bristle". It refers to a number of different bristle- or hair-like structures on living organisms.
- **Solitary** (insects) refers to the tendency of organisms staying, living or migrating alone (i.e. as single individuals).
- **Spiracle** refers to pore on an insect's body wall through which gaseous exchange takes place.
- **Stimulus** (pl: stimuli) is a detectable change in the internal or external environment. The ability of an organism or organ to respond to external stimuli is called sensitivity.
- **Thelyotoky** is a type of parthenogenetic reproduction in which only female progeny are produced.
- **Tumourigenesis** is a process leading to the formation of tumours.
- **Visual** (perception) is the ability to interpret information and surroundings from the effects of visible light reaching the eye. The resulting perception is also known as eyesight, sight, or vision (adjectival form: visual, optical, or ocular).