# DISTRIBUTION MODELLING OF *Leptocybe invasa* (HYMENOPTERA: EULOPHIDAE), AND AN ASSESSMENT OF HOST SUSCEPTIBILITY AND RELATIVE EFFICACIES OF SYSTEMIC INSECTICIDES FOR GUM SEEDLING PROTECTION IN ZIMBABWE


**BY**

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## DECLARATION

I hereby declare that this thesis is my own original work and has not been submitted for a degree in any other university.

______________________________________ __________________________

Kudzai Mafuwe Date

I, as the supervisor, confirm that the work reported in this thesis was carried out by the student under my supervision. The thesis was examined and I approved it for final submission.

______________________________________ __________________________

Dr. P. Chinwada Date

# DEDICATION

Dedicated to family, friends and all those who believed in me

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## ABSTRACT

Studies were conducted from February 2015 to December 2015 to predict the potential distribution of the blue gum chalcid or Eucalyptus gall wasp, *Leptocybe invasa* Fisher and La Salle in Zimbabwe, to screen locally available *Eucalyptus* species for tolerance and susceptibility to the pest and to test the efficacy of imidacloprid (Imidacloprid® 20% SL) and thiamethoxam (Thiamethoxam® 25% WG) in protecting gum tree seedlings. The Maximum Entropy method (MaxEnt) was used to model the extent of distribution of *L. invasa* using environmental variables, namely elevation, temperature and rainfall. Data for the environmental variables were obtained from the Wordclim database while occurrence records for *L. invasa* in Zimbabwe were obtained from forest research centres, forestry companies and district forestry offices from cases reported between December 2008 and August 2012 as well as countrywide surveys conducted between February and April 2015. Experiments were also conducted at Chesa Forest Research Station to screen *Eucalyptus tereticornis*, *E. camadulenisis*, *E. grandis* and *E. propinqua* seedlings for susceptibility to *L. invasa* and to test the efficacy of the systemic insecticides in protecting *E. tereticornis* seedlings against *L. invasa* attack. Seedlings were exposed to natural infestation and after a month of exposure, assessments were carried out at 2 week intervals to assess for susceptibility and severity of damage. A positive relationship was observed between altitude and the occurrence of *L. invasa* as well as between rainfall and the occurrence of the pest. Temperature was showed to have a negative relationship with the probability of occurrence of *L. invasa*. There is a high probability of finding *L. invasa* in areas where annual temperatures range between $10^{o}$ and $26^{o}$C. The *Eucalyptus* seedlings screened for susceptibility to *L. invasa* attack were all equally damaged by the pest. The insecticides used in this study were not effective in protecting *E. tereticornis* seedlings against *L. invasa* attack during the period of assessment. The preliminary results of this study together with data from other related distribution surveys of *L. invasa* may aid in focusing control in areas that have a high risk of invasion by *L. invasa*. It is recommended that further studies to determine the susceptibility of different *Eucalyptus* species and hybrids to blue gum chalcid attack as well as screen locally available insecticides for use in protecting seedlings from *L. invasa* attack be conducted.

## TABLE OF CONTENTS

DECLARATION ..... ii
DEDICATION ..... iii
ACKNOWLEDGEMENTS ..... iv
ABSTRACT ..... v
TABLE OF CONTENTS ..... vi
LIST OF FIGURES ..... x
LIST OF APPENDICES ..... xi
CHAPTER 1 ..... 1
INTRODUCTION ..... 1
1.1 Overview ..... 1
1.2 Statement of the Problem and Justification ..... 2
1.3 Objectives ..... 3
1.3.1 Main objective ..... 3
1.3.2 Specific objectives ..... 3
1.4 Hypotheses ..... 4
CHAPTER 2 ..... 5
LITERATURE REVIEW ..... 5
2.1 Introduction ..... 5
2.2 Taxonomic Placement of Leptocybe invasa Fisher and La Salle ..... 6
2.3 Geographical Distribution of Leptocybe invasa ..... 6
2.4 Morphology of Leptocybe invasa ..... 7
2.5 Leptocybe invasa Life History ..... 8
2.6 Leptocybe invasa Host Plants ..... 9
2.7 Symptoms and Damage by Leptocybe invasa ..... 11

2.8 Economic, Social and/or Environmental Significance of Leptocybe invasa on Host Plants 12
2.9 Management of Leptocybe invasa ........ 15
2.9.1 Host plant resistance ........ 15
2.9.2 Cultural Control ........ 16
2.9.3 Biological control ........ 16
2.9.4 Chemical control ........ 18
2.10 Conclusion ........ 19
CHAPTER 3 ........ 20
GENERAL MATERIALS AND METHODS ........ 20
3.1 Study Areas ........ 20
3.1.1 Chesa Forest Research Station ........ 21
3.2 General Methods ........ 22
CHAPTER 4 ........ 23
THE POTENTIAL DISTRIBUTION OF *Leptocybe invasa* IN ZIMBABWE ........ 23
4.1 Introduction ........ 23
4.2 Materials and Methods ........ 23
4.2.1 *Leptocybe invasa* occurrence data ........ 23
4.2.2 Environmental variables ........ 24
4.2.3 Collinearity ........ 24
4.2.4 Modelling of *Leptocybe invasa* distribution ........ 25
4.2.5 Model performance and evaluation ........ 25
4.3 Results ........ 25
4.4 Discussion ........ 28
CHAPTER 5 ........ 31

THE RELATIVE SUSCEPTIBILITY AND SEVERITY OF *Leptocybe invasa* DAMAGE IN SELECTED *Eucalyptus* SPECIES IN ZIMBABWE ........ 31

5.1 Introduction ........ 31

5.2 Materials and Methods ........ 31

5.2.3 Study site ........ 31

5.2.4 Experimental design ........ 31

5.2.5 Data analysis ........ 32

5.3 Results ........ 32

5.3.1 Incidence ........ 32

5.3.2 Severity ........ 33

5.4 Discussion ........ 34

CHAPTER 6 ........ 37

THE RELATIVE EFFICACY OF ROOT SYSTEMIC INSECTICIDES FOR PROTECTION OF *Eucalyptus tereticornis* SEEDLINGS AGAINST *Leptocybe invasa* IN ZIMBABWE ........ 37

6.1 Introduction ........ 37

6.2 Materials and Methods ........ 38

6. 2.1 Study area ........ 38

6.2.2 Experimental design ........ 38

6.3 Results ........ 39

6.3.1 Incidence ........ 39

6.3.2 Severity ........ 41

6.4 Discussion ........ 43

CHAPTER 7 ........ 45

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS ........ 45

7.1 General Discussion ........ 45

7.2 Conclusion ........ 48

7.3 Recommendations ........................................................................................................ 48
REFERENCES ........................................................................................................................ 50
APPENDICES ......................................................................................................................... 65

## LIST OF FIGURES

Figure 1. Study area showing the Natural Farming Regions of Zimbabwe and the location of Chesa Forest Research Centre ........................................................................................... 21
Figure 2. Area Under Curve (AUC) of the receiver operating characteristic curve (ROC) for the model indicating model performance ............................................................................. 26
Figure 3. Response curve showing how a) altitude b) rainfall c) temperature affects the Maxent model in predicting *Leptocybe invasa* occurrence ............................................................ 27
Figure 4. The probabilistic predictive map of *Leptocybe invasa* ................................................... 28
Figure 5. Incidence of *L. invasa* induced galls on four *Eucalyptus* seedlings ............................... 33
Figure 6. Severity indices of galls induced by *Leptocybe invasa* among four selected *Eucalyptus* species ........................................................................................................................ 34
Figure 7. Incidence of *L. invasa* induced galls on *E. tereticornis* seedlings after treatment with systemic insecticides compared against an untreated control. ...................................................... 40
Figure 8. Efficacy of systemic insecticides on the incidence of *Leptocybe invasa* induced galls weeks after treatment [1 = control, 2 = imidacloprid and 3 = thiamethoxam]. ............................. 41
Figure 9. Severity indices of galls induced by *Leptocybe invasa* on *Eucalyptus tereticornis* seedlings after treatment with systemic insecticides compared against an untreated control ....... 42
Figure 10. Severity indices of galls induced by *Leptocybe invasa* on *Eucalyptus tereticornis* seedlings after treatment with systemic insecticides [1= control, 2= imidacloprid and 3= thiamethoxam] ........................................................................................................................ 43

## LIST OF APPENDICES

Appendix A. List of localities in where *Leptocybe invasa* was recorded in Zimbabwe................65

# CHAPTER 1

# INTRODUCTION

## 1.1 Overview

A previously unknown eucalyptus pest, commonly known as the blue gum chalcid and recently described to science as *Leptocybe invasa* Fisher and La Salle (Mendel *et al.*, 2004) has been reported to be causing damage to young *Eucalyptus* (gum tree) plantations and nurseries worldwide (Luo *et al.*, 2014; Roychoudhury *et al.*, 2015; Kumar *et al.*, 2015). The wasp was first recorded in the Middle East in the year 2000 and is believed to be native to Australia, where its distribution is still unknown (Fisher *et al.*, 2014). It has so far spread outside its native range to most eucalypt-growing countries in Africa, Asia and the Pacific, Europe, North America and the near East (APFISN, 2009; Quang *et al.*, 2009; Nyeko *et al.*, 2010; Mutitu and Mwangi, 2013; Udagedara and Karunaratne, 2014; Luo *et al.*, 2014; de Souza *et al.*, 2014).

*Leptocybe invasa* is a stenophagous feeder which attacks the leaves and stems of eucalyptus trees of all ages, from nursery stock to mature trees, though the damage is most severe on the younger trees, coppices as well as nursery seedlings (Mendel *et al.*, 2004; Dittrich-Schröder *et al.*, 2012). The adult female wasp oviposits inside plant tissue on the mid-ribs, petioles and stems of the leaves or stems; consequently, abnormal outgrowths of the plant tissues known as galls are induced by the developing larvae (Dittrich-Schröder *et al.*, 2012; de Souza *et al.*, 2014; Chakrabarti, 2015). Severely affected trees show leaf fall, gnarled appearance, and loss of vigour, stunted growth, dieback and eventually tree death. In an outbreak situation, wasp pressure is intensive and all new growth may be damaged (Mendel *et al.*, 2004; Paine *et al.*, 2011). The wasp has a relatively narrow host range and has been reported only on *Eucalyptus* species including *E. saligna*, *E. grandis, E. deanei*, *E. globulus* ssp. *globulus*, *E. nitens*, *E. botryoides*, *E. camaldulensis*, *E. gunnii*, *E. robusta*, *E. bridgesiana*, *E. viminalis* and *E. tereticornis* (Mendel *et al.*, 2004; Quang *et al.*, 2009; Kulkarni *et al.*, 2010; YaLing *et al.*, 2014).

In context, eucalyptus plantations are the major source of timber, firewood and honeybee foraging, and are used as recreational areas, as shelterbelts from drifting sands, and as windbreaks surrounding cultivated and residential areas (Spodek, 2007). Gum is considered as the ideal tree to address demand for wood as it is fast-growing and adaptable to a range of sites (Gominho *et al*., 2001; Mendel, 2004; Leach and Mearns, 2013). However, the massive presence of *L. invasa*-induced galls in young tree plantations and nurseries draws attention not only to the severe damage inflicted on infested trees but also to the potential of causing serious economic damage to the eucalyptus forests in the affected countries.

In Zimbabwe, gum trees are ideal for addressing the demand for fuel wood and construction timber that continues to grow. It is estimated that more than 90% of Zimbabweans use firewood for cooking, while all construction uses poles for support purposes and scaffolding (Hansen *et al*., 2010). Furthermore, the need for timber for carpentry, telephone poles and other applications is also evident. More importantly and of particular concern of late is the continued annual increase in the amount of firewood needed by newly resettled farmers for tobacco curing (Campbell *et al*., 1997; Kaimowitz, 2003; Gogo, 2014). *Leptocybe invasa* has already been reported to be causing serious damage to eucalyptus stands around Zimbabwe since 2007 (CABI, 2007; Jimu *et al*., 2015). The pest is threatening to reduce the wood production industry if it is not controlled and the severities of its impacts are not addressed.

### 1.2 Statement of the Problem and Justification

At present, the *Eucalyptus* plantation industry in Zimbabwe is reliant on only a few species which may all be seriously damaged by *L. invasa* in many nurseries and young plantations (Matimaire, 2015). It is becoming increasingly difficult to find seedlings to establish new plantations in many areas. There is no understanding of the driving forces and factors that are influencing the distribution and spread of *L. invasa* in Zimbabwe. Despite strategies such as the planting of resistant or tolerant species and hybrids being utilised to manage the pest, no effective control measures currently exist for the wasp and no chemical products are currently registered for its control in any country (SAIF, 2012; Nahrung, 2012). Although several chemical products have been tested internationally both in the nursery and in the field in countries such as Kenya, Tanzania and India (Mutitu *et al*., 2007; Jiang *et al*., 2009; Jhala *et al*., 2009; Petro *et al*., 2015), the most effective one is yet to be identified.

*Leptocybe invasa* was first recorded in Zimbabwe by The Forest Commission of Zimbabwe in December 2008 in Masvingo, Chakari, Norton, Headlands, Gutu and Rusape. In January 2009, the pest was again recorded in the Harare, Mvurwi, Guruve, Dande and Bindura areas, (Forestry Commission of Zimbabwe, 2015). The pest has since been recorded from a number of other eucalyptus stands at commercial plantations, schools, community groups, rural woodlots, and other various localities, scattered throughout Zimbabwe (see Appendix A). However there is no understanding of mechanisms or factors that shape and influence the spread and distribution of the pest. There is therefore a need to understand the ecological requirements of *L. invasa* so as to determine the areas that are at high risk of invasions by the pest in Zimbabwe. There is also the need to screen a wide range of *Eucalyptus* species and hybrids for resistance or tolerance to *L. invasa* and to find the most effective and affordable chemicals that can be used to protect seedlings in the nursery and at plantation establishment. This study therefore seeks to fill these critical gaps by understanding the ecological requirements of *L. invasa* in order to predict its potential distribution in Zimbabwe, to screen locally available *Eucalyptus* species for tolerance and susceptibility to its attack and to test the efficacy of some locally registered systemic insecticides that could potentially control the chalcid. The study will therefore help to identify the high risk areas that *L. invasa* could occupy in Zimbabwe if it were to spread to where conditions are suitable. It will also help to identify the *Eucalyptus* species that are tolerant or susceptible to *L. invasa* attack and lastly it will help to identify the most effective and affordable chemicals available in Zimbabwe for controlling *L. invasa*. The study will help to fill in the knowledge gap that is required in developing an integrated management approach that might be more efficient in controlling *L. invasa* and will help optimize and target the management strategies in the high risk areas. This research project will also benefit smallholder eucalyptus farmers who risk losing most of their crop if nothing is done to manage the pest.

## 1.3 Objectives

### 1.3.1 Main objective

The main objective of the study was to identify the factors that influence the distribution of *L. invasa* and the most effective means for controlling it in Zimbabwe.

### 1.3.2 Specific objectives

1) To predict the potential spatial distribution of *L. invasa* based on elevation, rainfall and temperature in Zimbabwe.

2) To determine the relative susceptibility and severity of damage caused by *L. invasa* in selected *Eucalyptus* species in Zimbabwe.
3) To determine the relative efficacies of the systemic insecticides imidacloprid and thiamethoxam for protection of *E. tereticornis* seedlings in Zimbabwe.

**1.4 Hypotheses**

1) The distribution of *L. invasa* is not significantly explained by elevation, rainfall and temperature.
2) There are no significant differences in the relative susceptibilities and severity of damage caused by *L. invasa* in selected *Eucalyptus* species in Zimbabwe.
3) Root systemic insecticides imidacloprid and thiamethoxam are not effective for the protection of *E. tereticornis* seedlings against *L. invasa* attack.
4) The protective effects of imidacloprid and thiamethoxam against *L. invasa* are similar.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

Until recent years, eucalypt trees were considered virtually free of serious insect pests (Mendel *et al*., 2004). In California, the first insect pest to be recorded on *Eucalyptus* was the eucalyptus long horned borer (*Phoracantha semipunctata*) and this was discovered in Orange County in 1984 (Hanks *et al*., 1995). The eucalypt borer was also considered the sole minor pest attacking mainly drought-weakened trees in the Middle East and the Mediterranean (Mendel, 1985).

*Eucalyptus* trees that have been introduced in new habitats have now become plagued by a growing number of serious new phytophagous insect pests originating from their native habitats in Australia (Landis *et al*., 2000; Paine *et al*., 2011). In 1990, several gall-inducing wasps invaded and established themselves in a number of habitats outside of Australia, including *Quadrastichodella nova* (Hymenoptera: Eulophidae), *Epichrysocharis burwelli* (Hymenoptera: Eulophidae and *Ophelimus eucalypti* (Hymenoptera: Eulophidae) (Mendel *et al*., 2004; Protasov *et al*., 2007). The continued use of *Eucalyptus* trees and the survival of certain species in the urban landscape have become threatened.

Recently, a previously unknown *Eucalyptus* pest, the blue gum chalcid, a newly described gall-inducing wasp known scientifically as *Leptocybe invasa* Fisher and La Salle, was reported to be now present in many plantations worldwide and currently spreading in many countries and causing damage to young eucalyptus trees and nurseries (Nyeko *et al*., 2009; Sujay *et al*., 2010; Paine *et al*., 2011; Luo *et al*., 2014). In this review, findings from various researches and studies carried out over the past years are integrated to provide an understanding of the origins, biology and impacts of the pest. The review also aims to provide an understanding of the driving forces and factors that are influencing the distribution and spread of *L. invasa* in different countries and to highlight the different options available for its control.

## 2.2 Taxonomic Placement of *Leptocybe invasa* Fisher and La Salle

Leaves with the typical bump-shaped galls induced by what was then only known as an invasive gall inducer were collected between 2001 and 2004 from several *Eucalyptus* species in the Middle East and Mediterranean Regions (Mendel *et al.*, 2004). In 2004, the wasp was then described to science as *L. invasa* (Nugnes *et al.*, 2015). The wasp was described from the female sex only based on materials obtained from Israel and Syria as type specimens, and from other material which are not type specimens obtained from Algeria, Kenya and Spain. Diagnosis was based on the weak head with a distinctive grove and weakened area around ocellar triangle typical of the genus *Leptocybe* (Mendel *et al.*, 2004). *Leptocybe invasa* is the only species inside the genus *Leptocybe*, which belongs to the sub-family Tetrastichinae (Durand *et al.*, 2011). The common names for *L. invasa* are blue gum chalcid and eucalyptus gall wasp.

## 2.3 Geographical Distribution of *Leptocybe invasa*

The blue gum chalcid was first discovered in the Middle East and Mediterranean basin in 2000 when river red gums (*E. camaldulensis*) began developing disfiguring galls. The damage became severe enough to cause crop losses in tree plantations (Mendel *et al.*, 2004). The infestation initiated a description on the new species of the chalcid wasp and a study of its biology.

Believed to be a native of Australia, where its distribution is still unknown (Fisher *et al.*, 2014), the wasp has spread to most eucalypt-growing countries in world. In Europe, it was detected in Italy in 2000, in Portugal and Spain in 2003, and in 2005 in Turkey and the south of France (Hesami *et al.*, 2005; EPPO, 2006; Branco *et al.*, 2009; Dhahri *et al.*, 2010). In Africa, *L. invasa* was first recorded in a number of countries including Uganda and Kenya in 2002, in Tanzania in 2005, Algerian in 2006, South Africa in 2007 and Zimbabwe in 2010 (Nyeko *et al.*, 2009; Dhahri *et al.*, 2010). In the Asia–Pacific region, the pest is a threat in countries like Cambodia, Thailand, Vietnam, India and New Zealand. The pest has also been detected in North America and the Near East (APFISN, 2009).

The rate at which *L. invasa* establishes in a region gives an indication of the adequacy of phytosanitary procedures which are aimed at preventing its spread. Indications are that new introductions are taking place at an alarming rate pointing to deficiencies in the regulatory procedures and constraints faced by regulatory systems in a number of these countries (Giliomee, 2011). Krishnakumar (2010) reported that likely source of introduction of this pest insect to India could be through exchange of vegetative materials of *Eucalyptus*.

The distribution of *L. invasa* was studied from 2005 to 2006 in detail in Turkey. The gall wasp was determined almost everywhere in the Mediterranean and Aegean coastal regions of Turkey. However, the vertical distribution limit of *L. invasa* was observed to be 682 m above sea level in Turkey (Aytar, 2006).

A study by Nyeko *et al.* (2009) examined *L. invasa* infestation on different *Eucalyptus* germplasms in different agro-ecological zones of Uganda. The results showed a direct negative relationship between altitude and *L. invasa* infestation and no infestation were observed on *Eucalyptus* stands at altitudes ranging from 1,938 to 2,452 m above sea level. An attempt was also made by Maddumage *et al.* (2012) to map the distribution of gall wasp threat in Badulla and Nuwara Eliya districts of Kenya using GIS techniques and to analyze the relationship between altitude and pest infestation levels. A negative relationship was observed between altitude and the gall wasp infestations. Infestations were not observed on *Eucalyptus* plantations at altitudes greater than 1,200 m above sea level. Conversely, the blue gum chalcid incidence was observed to decrease with a decrease in altitude by Mutitu *et al.* (2014) in Kenya. Similarly, PengFei *et al.* (2012) in their study showed that the pest has extreme low temperature tolerance and has the ability to survive in higher altitudes and colder climatic conditions. The results suggest that this pest has the potential of spreading further up to the north Chinese provinces were the temperatures are lower.

Thompson (2013) mentions that the incidence of *L. invasa* infestation is probably worse in the Lowveld in South Africa because that is where a highly susceptible species, *E. grandis*, is almost solely grown thereby suggesting that the distribution of the host plant could also have influence in the distribution and incidence of the pest.

### 2.4 Morphology of *Leptocybe invasa*

The adult female is described as a small wasp with an average length of 1.2 mm. The body of the female is brownish in colour with a blue to green metallic shine. The fore coxae appear to be yellow while mid and hind coxae are more brown in colour (Mendel *et al.*, 2004; FAO, 2009; APFISN, 2009). Eyidozehi *et al.* (2014) also describes the legs and tarsi as yellow with the last tarsal segment brown apically. On the female, the antennae are mostly brown and the scape of the antennae is yellowish in colour.

Like the female wasp, the head and mesosoma on the male wasp are brown with a distinct blue to green metallic shine. However, the metasoma is brown with a slightly metallic tinge. The legs of the male are pale yellow. The mid and hind coxae, however, also have a metallic shine. The antennae are also yellow in colour (Doganlar, 2005).

Adult males are on average 1.28 mm long (Kumari *et al.*, 2010). The head is about 0.22-0.33 mm long and 0.24-0.36 mm wide. The thorax is 0.48-0.55 mm long and 0.28-0.31 mm wide. The abdomen is on averages 0.51 mm long and 0.32 mm wide. Male antennae are about 0.49 mm long and each consists of three claval, four funicular and three ring segments. The antenna of the female is about 1.2-1.4 mm long and consists of four anelli and three funicular segments.

## 2.5 *Leptocybe invasa* Life History

*Leptocybe invasa* displays thelytokous reproduction which is the ability to produce female offspring from unfertilized eggs (Mendel *et al.*, 2004; Lu *et al.*, 2014; Mutitu *et al.*, 2007; Kumari *et al.*, 2010). A small number of males have, however, been recorded in China and India (Chen *et al.*, 2009; Kumari *et al.*, 2010; Akhtar *et al.*, 2012; Eyidozehi *et al.*, 2014). It is believed that the diverse modes of reproduction, that is thelytokous and sexual reproduction, are one of the advantages for rapid colonization and outbreak of this species (Zheng *et al.*, 2014).

*Leptocybe invasa* females usually attack *Eucalyptus* trees by ovipositing on newly developed leaves and twigs within 1 to 2 weeks of bud break (Mendel *et al.*, 2004; Protasov *et al.*, 2007). However, in a study carried out in Iran, egg-laying was noticed even on buds within three day of bud burst (Eyidozehi *et al.*, 2014). Studies carried out in India on the biology of *L. invasa* both in the field and in the laboratory showed that the female adults readily lay their eggs on mid ribs, petiole and tender stem of the seedling (Kumari *et al.*, 2010). Fecundity is close to 60 eggs and the females will lay the eggs in clusters, in the epidermis on the upper sides of the new leaves, on both sides of the midrib, in the petioles or in the parenchyma of twigs (Mendel *et al.*, 2004; Zheng *et al.*, 2014).

White minute, legless larvae that develop within the host plant have five instars and a developmental period of about 20 days at temperatures between 17 and 20°C. Five stages of gall development have also been recorded on *E. camaldulensis* in Israel (ICFR, 2005). The larva grows by feeding on tender portions of the plant. The larva then changes into a pupa after about a fortnight. Pupation takes place within the gall itself.

Egg-adult developmental durations of *L. invasa* reported from different studies have been variable. In India, Kumari (2010) reported an egg-adult duration of 54-65 days while in South Africa this was reported to take 72 days (ICFR, 2005). Mendel *et al.* (2004) reported a mean developmental time from oviposition to emergence of 132.6 days at room temperature and adults survived for 6.5 days when provided with honey and water, and for three days without food. In a study carried out by Hesami *et al.* (2005), the developmental period was 126.2 and 138.3 days under laboratory and field conditions, respectively. Two to three overlapping generations per year have been observed in Iran, Israel and Turkey (FAO, 2009).

### 2.6 *Leptocybe invasa* Host Plants

*Leptocybe invasa* has a relatively narrow host range. Suitable hosts for this species include *E. camaldulensis*, *E. globulus*, *E. gunii*, *E. grandis*, some *E. grandis* × *camaldulensis clones*, *E. botryoides*, *E. saligna*, *E. robusta*, *E. bridgesiana*, *E. viminalis* and *E. tereticornis* (Aytar, 2003; Mendel *et al.*, 2004; Aytar, 2006;; Wiley and Skelley, 2008; Branco *et al.*, 2009). Work undertaken by the Tree Protection Co-operative Program (TPCP) at the University of Pretoria has shown that most of *Eucalyptus* plants screened showed signs of oviposition by *L. invasa*. For some plants, however, there was no further development and these plants were then defined as resistant. Tolerant species were defined as those that were infested by the wasp but showed only limited gall formation. Susceptible species appear to be readily infested by the wasp with severe gall development (ICFR, 2005).

Mendel *et al.* (2004) investigated host susceptibility by exposing seedlings of 36 species to *L. invasa* and observing whether galls were formed. Ten species were found to be suitable hosts: *E.*

*camaldulensis*, *E. tereticornis*, *E. botryoides*, *E. grandis*, *E. robusta*, *E. saligna*, *E. bridgesiana*, *E. globulus*, *E. gunii* and *E. viminalis*. In Florida, *L. invasa* damage was confirmed on *E. camaldulensis*, *E. grandis*, *E. propinqua*, *E. rudis* and *E. Dunnii* (Brown, 2014). Ten species were found to be suitable hosts in Israel: *E. botryoides*, *E. bridgesiana E. camaldulensis*, *E. globules*, *E. gunii*, *E. grandis*, *E. robusta*, *E. saligna*, *E. tereticornis* and *E. Viminalis* (Mendel, 2004). In tests, three species—*E. erythrocorys*, *E. gomphocephala* and *E. occidentalis*—were tested more rigorously for host suitability and preference.

Under both nursery and field conditions, the most susceptible hosts for *L. invasa* were found to be *E. rudis* and *E. Dunnii* in Florida (Wiley and Skelley, 2008) and *E. camaldulensis*, *E. grandis and E. tereticornis* in Vietnam (reference). Variation in the susceptibility of provenances of *E. camaldulensis*, *E. urophylla* and *E. grandis* were observed (Quang *et al.*, 2009).

In Turkey, Kulkarni *et al.* (2010) screened *Eucalyptus* species and clones at both nursery and plantations level for resistance and susceptibility to the *L. invasa*. The results showed that *E. tereticornis*, *E. camaldulensis*, *E. grandis* and their hybrids were severely affected by the gall wasp while *E. alba*, *E. urophylla*, *E citriodora* and *E. torelliana* were gall-free. In a separate study, Durand *et al.* (2011) also reported differences in susceptibility between *E. camaldulensis* and *E. globules*. Differences in susceptibility were also found within the hybrid populations and between families of *E. globules*, demonstrating inter and intra-specific susceptibility variations.

Out of 35 *Eucalyptus* species and hybrids evaluated for *L. invasa* infestations in Uganda and Kenya by Nyeko *et al.* (2009), only *E. henryi* and the clonal hybrids GC 578 and GC581 were found to be resistant to the pest. Most of the species and hybrids that were evaluated were ranked as either tolerant or moderately susceptible to wasp attack. Highly susceptible germplasms included *E. camaldulensis*, GC540 and GC784 in Tororo, Uganda, and MAU1, GC14, GC15 and GC10 in Busia, Kenya. However, some variations on the extent of damage caused in different species by *L. invasa* have been recorded in other studies. For example, YaLing *et al.* (2014) found that *E. camaldulensis* and *E. grandis* were more severely affected compared to *E. tereticornis* and *E. propinqua* amongst other species they tested. Further tests on known hybrid strains also showed that strains from *E. grandis* and *E. camaldulensis* had higher infection rates by *L. invasa* than the other species.

### 2.7 Symptoms and Damage by *Leptocybe invasa*

The first symptoms of *L. invasa* attack on *Eucalyptus* species is appearance of corky tissue at the egg insertion point. Spots begin to appear one to two weeks after oviposition. After hatching out of eggs, the larvae remain in a cavity formed within the plant tissues (Wylie and Speight, 2012). As the larvae develop and feed on the plant tissues, they release oxalic acid which results in the formation of cancerous growth on the plant in the form of galls (Narendran *et al.*, 2007; Petro and Madoffe, 2014). The green colour of the leaves containing the galls turns glossy pink. The glossiness then declines and the galls turn from pink to red (Wiley and Skelley, 2008).

The typical bump shape of the galls reaches their maximum size of about 2.7 mm wide. According to Mendel *et al.* (2004), the mean length of a gall containing a single wasp was 2.1 mm and the intensively grown trees carried over 50 galls per leave. The pupae are non-feeding and, therefore, non-damaging. Upon emergence of the adult wasps, the galls on the leaves turn light brown while the galls on the stems turn reddish brown (Wiley and Skelley, 2008). Mendel *et al.* (2004) reported white secretions coming out of the oviposition wounds three days after egg-laying. The early stages of gall formation could be observed after three weeks from oviposition and mature galls at 6-7 weeks after oviposition.

In a study carried out by Kumari *et al.* (2010) in India, the gall development stages included scarring tissue as the first stage within 7-8 days of oviposition. The scarring then turned into the typical bump-shaped green-coloured gall of 2.7 mm diameter 6-8 days later. The green-coloured galls gradually turned glossy pink 12-13 days later and dull pink within 15-17 days. At the last stage, exit holes were noticed 9-12 days later. However, in the clones that were used in India, Jayaraj *et al.* (2014), it appeared that colouration may not be a good indicator of gall development stage as in some of the clones the galls remained green throughout. There were also clones that had galls that turned red, but without emergence holes.

Galls caused by this wasp can result in substantial injury to young trees and can seriously weaken or kill the tree. All new growth is susceptible to damage when large concentrations of the wasps are present. Photosynthetic activity of the plant is greatly affected. Galling also causes the leaves to curl and may stunt growth and weaken the trees (Jhala *et al.*, 2009; Dell *et al.*, 2012).

Although *L. invasa* has been observed to attack new growth of all ages of eucalyptus trees, damage has been observed on young trees (Quang *et al.*, 2009; Nyeko, 2009; Jhala *et al.*, 2010; Kumari *et al.*, 2010) Very little is, however, known about the impact of the wasp on adult trees (Wiley and Skelley, 2008). Similarly, Rajpoot *et al.* (2014) reported in their study that trees up to two years of age were found to be more prone to the pest.

### 2.8 Economic, Social and/or Environmental Significance of *Leptocybe invasa* on Host Plants

Several *Eucalyptus* species have been reported to have serious adverse consequences on the environment due to competition for water with other plant species and an increased incidence of allelopathy. Gum trees release compounds which inhibit other plant species from growing nearby (Santos, 1997; Bassam, 2013*)*. Consequently, there is the risk that natural forests might be cleared to make way for forest plantations. The tropical forests are disappearing at a rapid rate not for timber harvest but actually for forestry development aimed at supplying the high demand for fuel and pulp wood (Madeley, 1999). McMichael (1993) reported that the forest around Blixen`s Kenyan coffee plantation was cleared to be replaced by the eucalypts (Carr, 1997). Carr (1997) also reports that large areas of the rainforests in the Amazon are being cleared, with serious consequences. The negative impacts of monoculture in eucalyptus plantations have lead to their suspension in some countries, for instance in the State of Sao Paulo in Brazil (WRM, 2010). It has been argued that pests of eucalyptus trees are beneficial to regulate the spread of the invasive trees into native forests.

*Leptocybe invasa* has the potential of becoming a problematic pest of eucalyptus plantations if the pest is not controlled. It may become severe in all the eucalypt-growing areas and may lead to heavy economic loss to farmers and other planting agencies (Jhala *et al.*, 2009). Because eucalypts are widely grown for forestry and ornamental purposes, possible risks include heavy losses to nurseries and to the paper and construction industries (Oballa *et al.*, 2010). There are, however, some positive aspects of eucalyptus trees. They have been reported to be a good source of fuel wood and have also helped to combat the effects of deforestation that has resulted in many countries due to this high demand for wood (Pohjonen and Pukkala 1990; Sedjo, 1999; Lampietti *et al.*, 2007). In many countries, several *Eucalyptus* species have been a prime choice for wood production because of their high growth rates (Carle *et al.*, 2002). In Central American countries, seven eucalypt species have been identified as ideal for firewood production. In northern India and parts of Nepal, *E. tereticornis* and *E. camaldulensis* attain the desired girth at eight years whereas the indigenous *Shorea robusta* would take one hundred years to reach this size (Khanal, 1993). *Dalbergia sissoo*, another timber species, in the region would need about 22 years to reach the desired width (Sunder, 1995).

In Kenya, eucalyptus is a significant part of the country's forest cover and is a vital contributor to the national supply of wood. Some estimate that it contributes about KSH 1 billion to the economy each year. Therefore a widespread mortality of eucalyptus in the country would result in significant economic losses (Hunt, 2010). In countries like Israel, Thailand and China, planting of *E. camaldulensis* was stopped because of extensive attacks by *L. invasa* (Petro *et al.*, 2015) and in India, the wasp poses a threat to an estimated 8 million ha of eucalypt plantations (Ramanagouda *et al.*, 2010).

*Eucalyptus* species have also been vastly used for the production of charcoal for domestic and industrial use. In Brazil, plantations of *E. grandis*, *E. saligna*, *E. urophylla* and *E. deglupta* are raised specifically for the production of charcoal for iron smelting (Chauhan *et al.*, 2006). A number of *Eucalyptus* species are also economically significant for producing poles which are used in the construction of huts, for scaffolding, as well as for power and telecommunication lines (Sunder, 1995; Dessie, 2011). In the dry zone of India, small-sized poles of eucalypt hybrids are now meeting the high demand for poles and the coal mines that have previously been using sal and Casuarina poles are now switching over to poles of *E. tereticornis* (Turnbull, 2000). In West Bengal, India, sale of eucalypts as poles and firewood fetch 65% better returns than as pulpwood (Sunder, 1995).

Many species of eucalypts are ideal for production of pulp with suitable fibre quality, density, colour and homogeneity (Sunder, 1995). *Eucalyptus grandis* and *E. globules* are the most used varieties in papermaking (Hiwale, 2015). *Eucalyptus globulus* is also the principal source of eucalyptus oil which is used in deodorizing and is also used in very small quantities as food supplements, especially sweets, cough drops and toothpaste (Panga, 2014). Eucalyptus has insect repellent properties and is used as an active ingredient in some commercial mosquito repellents (Batish *et al.*, 2008; Nerio *et al.*, 2010).

Eucalypt plantations have also helped to raise people's living standards and livelihoods in many countries over the years. This has been achieved through the increased job opportunities both in the plantations and in the processing industries (Catton *et al.*, 2004; McDonald and Spong, 2009). Eucalyptus plantations have also helped improve the incomes of many poor farmers that have settled on land unsuitable for sustainable agriculture in many developing countries (Sunder, 1995).

Studies carried out in Ethiopia showed that planting eucalyptus trees significantly increased household incomes as compared to agriculture in those areas where the land was degraded and not very productive for agricultural crops (Gebre-Markos, 1998; Jagger and Pender, 2000; Holden *et al.*, 2003). A study also showed that at least 20% of the total income for most families came from eucalyptus plantations (Gustavsson and Kimeu, 1992).

In urban areas, eucalypts also have recreational functions and aesthetic values associated with the presence of forests. In the 19$^{th}$ century, East Bay residents in San Francisco viewed eucalyptus trees to be more aesthetically pleasing than the native ones. The perceived aesthetic value was the major force of bringing then to the East Bay in the first place (Hennessy, 2012; Nance, 2014). Several studies also show that eucalyptus plantations have a significant forest recreation value (Turnbull, 2000, Zhao, 2011; Lopes and Cunha-e-Sá, 2013).

Commercial eucalyptus plantations are scattered throughout Zimbabwe with the exception of Masvingo and Matebeleland North provinces. They occupied more than 29,314 ha (25%) of the total 119,130 ha of plantation area in the country in 2001. Of this total, 4,000 hectares are at the Forest Commission's Mtao Forest Reserve in the Midlands province, centrally situated outside the town of Mvuma (Mabugu and Chitiga, 2002). The major species planted in Zimbabwe are *E. grandis, E. cloeziana*, *E. tereticornis*, *E. globulus*, *E. macarthurii*, *E. microcorys*, *E. paniculata*, *E. robusta* (Matowanyika, 1998; Mullin, 1998). More than 2,000 woodlots of eucalyptus can also be found in Forest Commission research centres, schools, community groups and individuals homesteads in rural areas (Bradley and Dewees, 1993). Most of these woodlots range from 1 to 10 ha, with *E. camaldulensis* and *E. tereticornis* primarily planted due to their drought tolerance and adaptability to a wide range of soil types (Gwaze *et al.*, 2000; Tyynelä, 2001; Jimu *et al.*, 2015).

In Zimbabwe, due to the power challenges, it is estimated that more than 90% of the people use firewood for cooking and a majority of the firewood is sourced illegally from the indigenous forests (Dube *et al*., 2014; Gogo, 2014; Joshi, 2015). This high demand for firewood has increased the rate and scale of deforestation in the country (Chirara, 2015). Of particular concern is the continued increase annually of firewood for tobacco curing by the newly resettled farmers engaged in tobacco production. According to Chirara (2015), the massive increase in tobacco farmers in the past six years has seen the country suffering one of the worst deforestation periods in its history as farmers continuously cut down trees for tobacco curing. Well managed planted forests will therefore help to reduce the logging pressure on native forests and conserve biodiversity within them. *Leptocybe invasa* therefore has the potential of becoming a problematic pest of eucalyptus plantations if the problem is not attended to as a matter of urgency. It may become severe in all the eucalyptus-growing areas and may lead to heavy economic losses to farmers and other planting agencies (Jhala *et al*., 2009). Because eucalypts are widely grown for forestry and ornamental purposes, possible risks include heavy losses to nurseries, to the paper industry and to construction industry (Oballa *et al*., 2010). This section was too long. Four paragraphs would have been enough.

### 2.9 Management of *Leptocybe invasa*

A number of potential methods are available to control *L. invasa* (Dittrich-Schröder*et al*., 2012; Dhahri *et al*., 2010). These include host plant resistance, cultural control, biological control and chemical control.

#### 2.9.1 Host plant resistance

Where ever possible, *Eucalyptus* species or clones that have demonstrated resistance or tolerance to *L. invasa*, should be used to establish plantations. Several resistant *Eucalyptus* species or clones have been identified in Uganda, Kenya, Portugal and India (Nyeko *et al*., 2010; Prabhu, 2010; Durand *et al*., 2011) and are available for planting.

An active screening program for resistant *Eucalyptus* species or clones was carried out in South Africa for *L. invasa* (Dittrich-Schröder *et al*., 2012). The study investigated 50 different *Eucalyptus* varieties against *L. invasa*. One variety, GC540, which is known to be very susceptible to the wasp, was used as a control against and the results of all the other screened 50 Eucalyptus varieties were compared to rank the level of tolerance or resistance. The results identified significant differences between the levels of galling of the *Eucalyptus* varieties. Most of the 35 *Eucalyptus* accessions evaluated for *L. invasa* infestations by Nyeko *et al.* (2010) in Uganda and Kenya were also ranked as tolerant or moderately susceptible to wasp attack. In another study by Dittrich-Schröder *et al.* (2012), the variety most susceptible to most galling was the hybrid *E. nitens* × *E. grandis* followed by *E. grandis* × *E. camaldulensis*. The hybrids *E. grandis* × *E. urophylla* and *E. saligna* × *E. urophylla* were less susceptible to *L. invasa* than the above-mentioned two hybrids. The studies therefore show that resistance breeding amongst these varieties is clearly a viable objective in the control of *L. invasa*. The selection and planting of resistant/less susceptible genotypes will therefore be an important management strategy for damage caused by *L. invasa* invasion.

### 2.9.2 Cultural Control

Although cultural control and mechanical methods are yet to be examined on their effectiveness, they have been suggested in the control of *L. invasa* outbreaks (Baran, 2011; Zheng *et al*., 2014). Cultural control can help to lower populations of *L. invasa* during the early stages of an invasion in a particular area. Examples of such methods include periodic examinations of infestations in nurseries and young plantations and the setting of yellow sticky traps to attract the adults.

Physically removing and burning of infested plant parts and the avoidance of susceptible *Eucalyptus* species or clones have also be been suggested as possible cultural control methods for the pest (APFISN, 2009). However, they are inefficient during high infestations when it is impossible to remove all infested material over a wide area. Moreover, in warmer regions, these cultural practices are less effective due to the year–round presence and overlapping generations of *L. invasa* making it impossible to schedule cultural control methods for periods of pest absence (ICFR, 2011).

### 2.9.3 Biological control

The possible release of biological control agents arguably offers the best chance of long-term control of *L. invasa* (Garnas *et al*., 2012; Wingfield *et al*., 2013; Verghese *et al*., 2013). In its native home, *L. invasa* is not considered detrimental to the eucalyptus plantations because the natural enemies are able to control the increase in populations (Mutitu *et al.*, 2007; Mutitu and Mwangi, 2013).

Two parasitoids which show potential for classical biological control of *L. invasa* are *Quadrastichus mendeli* Kim and LaSalle and *Selitrichodes kryceri* Kim and LaSalle (Hymenoptera: Eulophidae: Tetrastichinae) (Kim *et al.*, 2008). The parasitoids were successfully established in Israel (Mendel, 2004). Another eulophid parasitoid, *Selitrichodes neseri* Kelly and La Salle, was released in a number of countries including South Africa and Kenya (Kim *et al.*, 2008; Hurley, 2012; Dittrich-Schröder *et al.*, 2014; Zheng *et al.*, 2014).

The study by Dittrich-Schröder *et al.* (2014) also showed that *S. neseri* has considerable potential to act as a biological control agent of *L. invasa* due to its relatively short developmental time, the long adult life span, and its ability to utilize a range of gall ages. *Selitrichodes neseri* also showed a high level of host specificity as the galls of native insects most closely related to *L. invasa* and galls of similar morphology to *L. invasa*-induced galls were found not suitable for oviposition. Despite the potential highlighted above, biological control is, however, not an effective method at fighting pests that can cause very large scale damage. Nonetheless, positive results were recorded a year after the release of approximately 6,000 female *S. neseri* wasps at over 300 sites across the most highly infested areas in KwaZulu-Natal, Mpumulanga and Limpopo provinces in 2003. *Selitrichodes neseri* was recaptured from all the release sites included in the post-release study, and parasitism levels of up to 9% were obtained at some sites just five months after the original release, It is, however, not known how long it will take before there is noticeable impact on the populations of *L. invasa*, but expectations are that, in time, parasitism rates will increase to over 70%, as obtained contained release sites in Pretoria (FABI, 2013).

*Aprostocetus causalis* (Hymenoptera: Eulophidae) La Salle and Wu, was also identified to be a parasitoid of *L. invasa* outside of its native range in China and Thailand (Yang *et al.*, 2014). Several other Torymidae known to parasitize the blue gum chalcid were *Megastigmus zvimendeli* and *M. lawsoni* (Nahrung *et al.*, 2012; Doğanlar, 2015).

In their study, Garnas *et al.* (2012) argue that even though biological control does represent a major component of a strategy to mitigate the damages of *L. invasa* invasions, the current effort and scope for developing such control are woefully inadequate for dealing with the increasing rates of spread of the pest. They suggest that the biological control strategies would benefit enormously from an international, collaborative focus.

### 2.9.4 Chemical control

Application of granular and botanical insecticides in eucalyptus nurseries has also been suggested for the management of this pest (Chakrabarti, 2015). Several chemical products have been tested internationally for their effectiveness in controlling *L. invasa* both in the nursery and field with varying levels of success (ICFR, 2011). For instance, Jhala *et al.* (2010) undertook some studies to evaluate some granular insecticides against blue gum chalcid. The data on per cent damaged leaves and number of galls per leaf in their study revealed that all the insecticidal treatments resulted in significantly lower damage after first and second sprays as compared to the control. Application of Rogor® (dimethoate) or Metacid® 50 (methyl parathion) (at 2 ml per litre of water) on foliage at fortnightly intervals was found to be effective for controlling *L. invasa* (APFISN, 2009). Confidor® (imidaloprid) and Methomex® (methomyl) were also found to be very effective systemic pesticides on nursery seedlings in Kenya (Nyeko *et al.*, 2010). Aregowda *et al.* (2010) also tested Carbofuran® 3G (carbofuran), Methyl parathion® 50 EC and Imidacloprid 17.8® SL and the botanic insecticides Multineem® and 5% soapnut (*Sapindus* spp.), as systemic insecticides against *L. invasa* at fortnightly intervals. They found out that the insecticides were more effective in reducing gall formation than the foliar-based treatments. Imidacloprid was the most effective of the treatments.

Botanic extracts have also been assessed for the control of *L. invasa* in Zimbabwe (Paringira, 2014). The results showed that the best botanical extract for reducing *L. invasa* induced galls was a mixture of tobacco and neem. This mixture significantly reduced the mean gall numbers by 99% when compared against the control.

Due to the fact that the developing stages of *L. invasa* are within the gall and the pest has overlapping generations, it is difficult to determine the optimum period for pesticide applications in order to obtain effective chemical control. Another challenge faced by most countries affected by the blue gum chalcid is the lack of effective chemicals on the local markets (FAO, 2009). As such, there currently are no chemical products registered for use against *L. invasa* in several countries including South Africa (ICFR, 2011) and Zimbabwe (Mushongahande, 2014).

## 2.10 Conclusion

Significant progress has been made in recent years in trying to recognize the impacts of *L. invasa* and in trying to identify resistant *Eucalyptus* clones and effective chemicals for controlling the pest. However, there are notable gaps in literature. For example, the probability of the pest emerging if plantations are established in new geographical locations given suitable biotic conditions is yet to be determined for many areas and the most efficient control mitigations are yet to be found. The objectives of the subsequent chapters are to investigate whether the spatial distribution of *L. invasa* can be predicted using elevation, rainfall and temperature in Zimbabwe, to investigate the relative susceptibility and severity of damage by *L. invasa* on selected *Eucalyptus* species and to determine the relative efficacies of locally available systemic insecticides for protecting *E. tereticornis* seedlings in Zimbabwe.

# CHAPTER 3

# GENERAL MATERIALS AND METHODS

## 3.1 Study Areas

The potential distribution of *L. invasa* was determined for the whole of Zimbabwe. Presence of *L. invasa* infestations was determined from sampling conducted in nurseries and plantation stands randomly selected from various locations within each of the six Natural Farming Regions of Zimbabwe (Mungandani *et al.*, 2012), i.e. Natural Farming Regions I, IIa, IIb, III, IV and V (Fig. 1). These agro-ecological zones where chosen because they represent a combination of homogenous agro-climate, ecology, soil units and agricultural activities (FAO, 1978). The coordinates of each sampling/study location were recorded using a GPS unit. Studies for determining host susceptibility and the efficacy of systemic insecticides were conducted at Chesa Forest Research Station in Bulawayo.

Natural Farming Region I lies in the east of the country and it is characterized by rainfall of more than 1,000 mm/year, low temperatures, high altitude and steep slopes. Natural Farming Region II is located in the middle of the northern part of the country. The region receives fairly reliable rains (750 to 1,000 mm/year) between November and April. Natural Farming Region II is characterized by mean maximum temperature of 19-23ºC, mean minimum temperature of 10-13ºC, mean annual temperature of 16-19ºC and annual rainfall of 700-1,000 mm. Natural Farming Region III is located mainly in the mid-altitude areas of the country. It is characterized by annual rainfall of 500-750 mm, mid-season dry spells and high temperatures. Natural Farming Region IV is located in the low-lying areas in the north and south of the country. The characteristics of the region are: annual rainfall of 450-650 mm, severe dry spells during the rainy season and frequent seasonal droughts. This region also has a mean minimum temperature of 11-20ºC, mean maximum temperature of 19-26ºC and mean annual temperature range of 18-24ºC (Mungandani *et al.*, 2012). Natural Farming Region V covers the lowland areas below 900 m above sea level in both the north and south of the country. The region is characterized by erratic rains of less than 650 mm/year. The country has three major regions distinguished on the basis of elevation: the Lowveld (below 600 m above sea level), Middleveld (600-1,200 m) and Highveld (above 1,200 m).

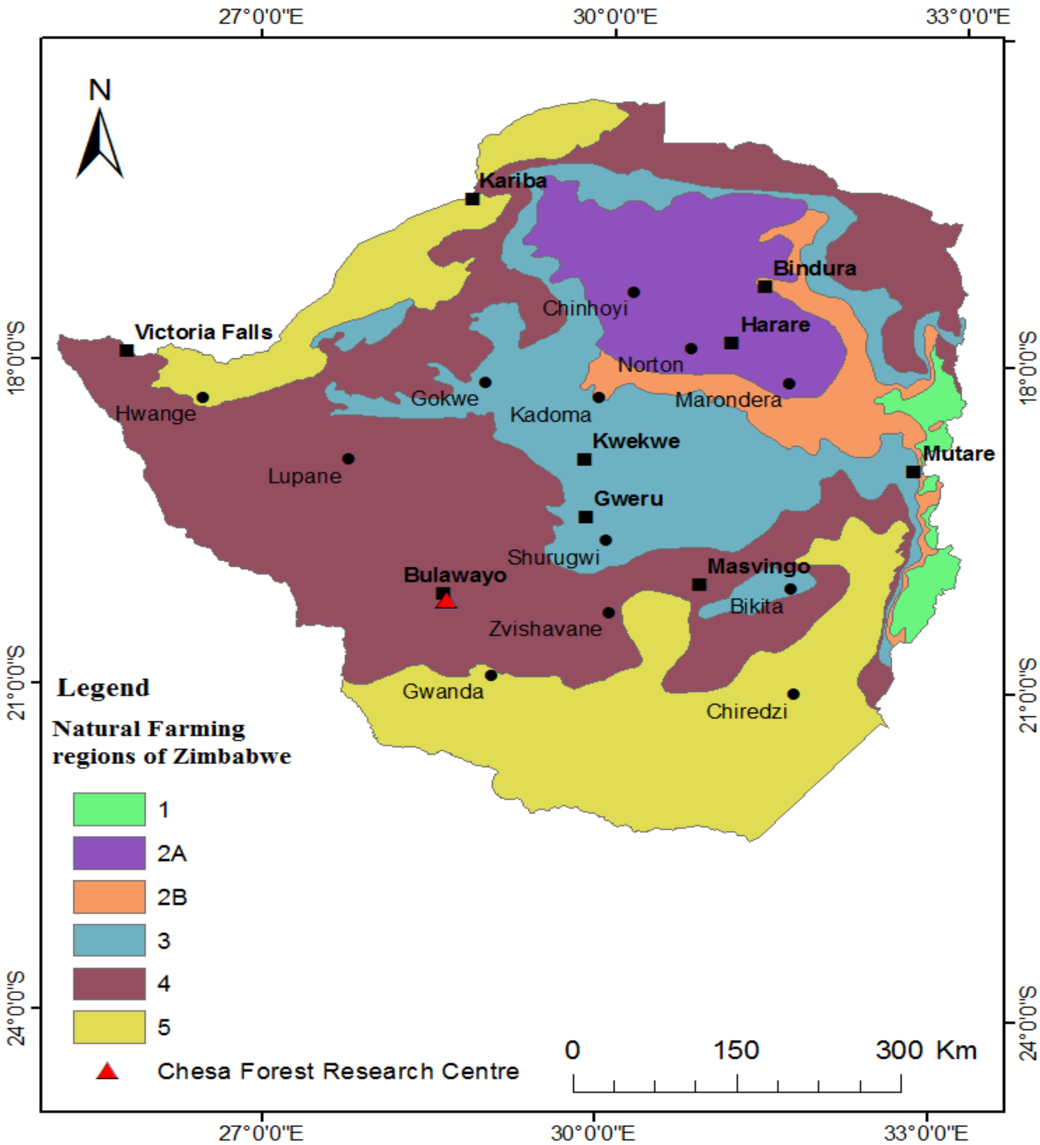


Figure 1. Map showing the Natural Farming Regions of Zimbabwe and the location of Chesa Forest Research Centre

### 3.1.1 Chesa Forest Research Station

Chesa Forest Research Station is located in Bulawayo at latitude 20°0'34.02"S and longitude 28°12'36.79"E in Natural Farming Region IV (Fig. 1). The estimate terrain elevation above sea level is 1,246 m. The research station owns a nursery where seedlings are raised for sale and for donation. There are several provenance trial plots for various *Eucalyptus* species located around the research station.

## 3.2 General Methods

To predict the potential spatial distribution of *L. invasa* in Zimbabwe, the Maximum Entropy method (MaxEnt) was used following the methods outlined by Elith *et al.* (2011), Barnhart and Gillam (2014), Bosch *et al.* (2014) and Feng and Papeş (2015). Data for the predictors, elevation, temperature and rainfall were extracted from the Worldclim database (http://www.worldclim.org) and Collinearity tests were applied on the predictors to test those that were perfectly correlated.

Occurrence records on the location of *L. invasa* were obtained from forest research centres, forestry companies and district forestry officers from cases reported between December 2008 and August 2012. Additional records were collected from surveys conducted between February 2015 and April 2015 to collect data from poorly-sampled and under-represented areas of the country, following the literature survey records that had been obtained. Sampling points were chosen at random across each of the Natural Farming Regions from government and privately-owned plantations, forest research centres, schools, community groups and rural woodlots in Zimbabwe. Belt transects were used to visually check for signs of *L. invasa* infestation and the coordinates were marked with the aid of a hand-held Global Positioning System (GPS) unit.

The methods used by Nyeko *et al.* (2010) to evaluate *Eucalyptus* germplasms for infestations by *L. invasa* in Uganda and Kenya were adopted to achieve the second and third objectives of determining host susceptibility and efficacy of root systemic insecticides in preventing gall induction by *L. invasa*. The second objective for determining host susceptibility was carried out using seedlings of four species of *Eucalyptus* namely, *E. tereticornis*, *E. camadulenisis*, *E. grandis* and *E. propinqua*).

The data from the host susceptibility and insecticide efficacy studies were tested for normality and analyzed using a non-parametric test for the significant differences in mean severity ranks and incidence among the treatments. Where significant differences were observed, the Tamhane Post Hoc test was used to establish where the significant differences lie between the groups for the severity indices. The Bonferroni correction test was used to test for significance differences between the groups for *L. invasa* incidence.

# CHAPTER 4

## THE POTENTIAL DISTRIBUTION OF *Leptocybe invasa* IN ZIMBABWE

### 4.1 Introduction

The demand for timber in Zimbabwe continues to grow, for purposes such as firewood and construction (Gogo, 2014). With the recent increase in the amount of firewood needed by newly resettled farmers for tobacco curing, considerable pressure has now been placed on the indigenous forests (Offat, 2014). *Eucalyptus* species are considered ideal to address this demand as they are fast-growing and adaptable to a range of sites. Eucalyptus plantations require good management to be successful and this will depend on knowledge of growth constraints of the gum trees, including pests and diseases.

*Leptocybe invasa* Fisher and La Salle, a newly described gall-inducing wasp, has been reported to be present in Zimbabwe`s gum tree plantations and causing damage to young trees and nurseries since the year 2008 (Forest Commission of Zimbabwe, 2015). The pest is native to Australia where its distribution is still unknown. The driving forces and factors that are influencing the distribution and spread of *L. invasa* in Zimbabwe are currently unknown (Forest Commission of Zimbabwe, 2015). This study sought to model the potential geographical distribution of *L. invasa* in Zimbabwe using the Maximum Entropy (MaxEnt) niche modelling approach (Phillips *et al.*, 2006). Results from the study would be used to identify areas that have a high probability of occurrence of *L. invasa* as well as showing those areas that the chalcid could occupy if it were to spread to where conditions are suitable. This would help to optimize on management strategies such as concentrating efforts in high-risk locations during active detection programs.

### 4.2 Materials and Methods

#### 4.2.1 *Leptocybe invasa* occurrence data

A total of 194 presence records were used in this study. One hundred and sixty occurrence records from 49 localities were obtained from forest research centres, forestry companies and district forestry officers from cases reported between December 2008 and August 2012 (see Appendix A). To ensure consistency in the presence data points, geo-referencing was done using the GeoLocate Software (http://www.museum.tulane.edu/geolocate/) and the point radius method. Additional presence records from 10 localities were collected from field surveys conducted between February 2015 and April 2015 (see Appendix A). These field surveys targeted areas that were either poorly sampled or under-represented in the databases described in earlier sections. Data collection was based on a stratified random sampling procedure where the six Natural Farming regions of Zimbabwe were used to stratify the area. These agro-ecological zones were chosen because they represent a combination of homogenous agro-climate, ecology, soil units and agricultural activities (FAO, 1978). A minimum of five sampling points across each of these regions were chosen at random from plantations, forest research centres, schools, community groups and rural woodlots in Zimbabwe. Age of stands (< 5 years) and accessibility of these sites was also considered as criteria for selecting the points. At each point, a belt transect measuring 5 m by 500 m was marked on the ground. Evidence of *L. invasa* within each belt transect was recorded as presence while lack of evidence was recorded as absence. A GPS handheld unit was then used to mark the location of the sites where evidence of the pest was observed in the field.

### 4.2.2 Environmental variables

The predictors used were elevation, mean annual temperature and rainfall acquired from the Worldclim database. Elevation data was acquired at 30 m spatial resolution while data on mean annual temperature and rainfall were acquired at 1 km spatial resolution. The variables where selected for their potential importance and relevance in predicting the geographical distribution of an invasive species based on knowledge from previous studies of *L. invasa* and other invasive species (Baker *et al.*, 2000; Peterson and Vieglais, 2001; Mutitu *et al.*, 2007; Petro *et al.*, 2015; Bebber *et al.*, 2014; Battisti and Larsson, 2015). The data were clipped to restrict prediction to the study site. These data sets were also resampled to match the geo-references from the elevation data.

### 4.2.3 Collinearity

Collinearity for the predictors was tested using both correlation analysis and the Variance Inflation Factor (VIF). Using the threshold of VIF >10 and following the methods described by Botkin *et al.* (2007) and Dormann *et al.* (2013), the calculated VIF for temperature (6.8540), elevation (5.3107) and rainfall (1.917) yielded no evidence of excessive Collinearity among the predictors, therefore all predictors were used for the model.

### 4.2.4 Modelling of *Leptocybe invasa* distribution

The spatial distribution of *L. invasa* was predicted using the Maxium Entropy niche modelling approach, MaxEnt version 3.3.3k (http://www.cs.princeton.edu/~schapire/maxent/). The program was selected because it has been proven to outperform other available methods and requires a limited number of presence data (> 30) to produce a good model (Phillips *et al.*, 2006). Seventy five percent of the presence data was randomly selected and used to calibrate the model and the remaining 25% was set aside for model evaluation. Data splitting for calibration and validation followed the example described by Du *et al.* (2014). Maps showing the probability of presence of *L. invasa* obtained from the MaxEnt software were imported into a GIS to produce a map showing areas predicted as habitat for the pest.

### 4.2.5 Model performance and evaluation

The importance of individual predictor variables was inferred from the variable contribution output made available in MaxEnt. The ability of the final model to accurately predict presence/absence of the pest was tested using the Area Under Curve (AUC) of the receiver operating characteristic curve (ROC). The AUC has values that range from 0.5 to 1.0. Values close to 0.5 indicate a fit no better than expected by random, while a value of 1.0 indicates a perfect fit. An AUC greater than 0.9 denotes very good predictive ability of the model while an AUC of 0.7-0.9 denotes a good fit and AUC less than 0.7 denotes an uninformative model (Baldwin, 2009).

## 4.3 Results

The final model successfully predicted the distribution of *L. invasa* (test AUC = 0.79) (Fig. 2). This shows that temperature, altitude and rainfall can be used to predict the potential distribution of *L. invasa* is Zimbabwe.

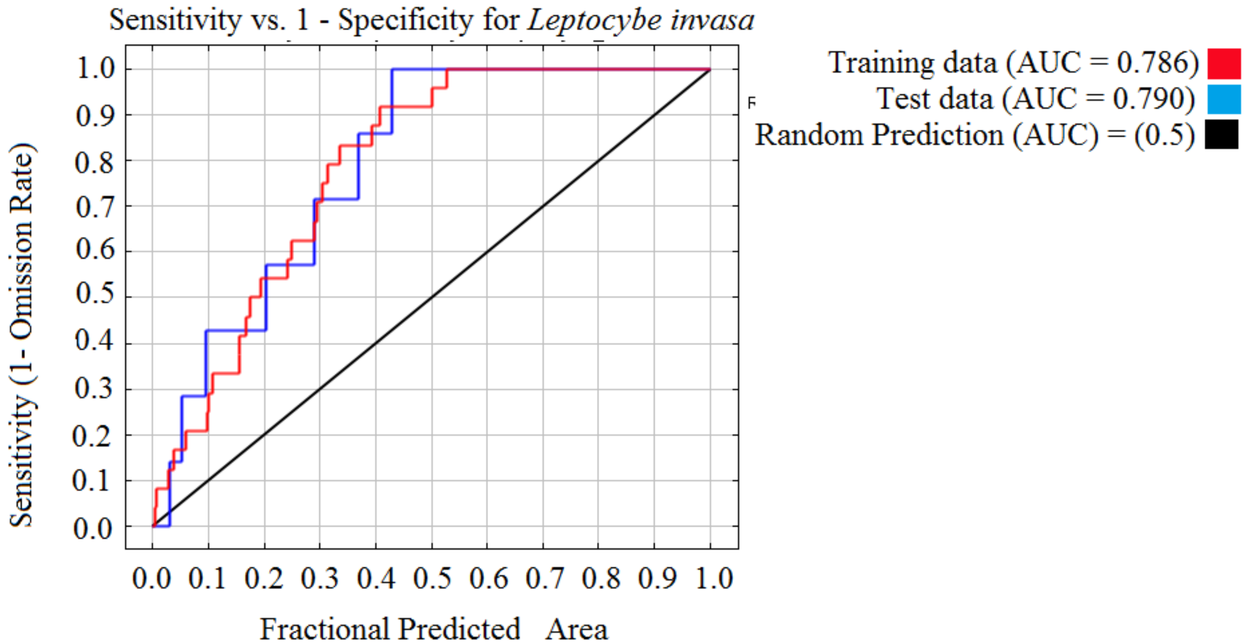


Figure 2. Area Under Curve (AUC) of the receiver operating characteristic curve (ROC) for the model indicating model performance

Temperature had the highest contribution (83.9%) to the model, indicating that it is the environmental factor that exerts the most influence in determining *L. invasa* occurrence in Zimbabwe. Rainfall had a contribution of 16% while elevation had the least contribution to the model (0.1%).

A positive relationship was observed between altitude and the occurrence of *L. invasa* (Fig. 3a). The highest probability of 0.95 was found at altitudes greater than 2,400 m. At elevations of less than 500 m, the probability of finding the pest was less than 0.2. Rainfall also showed a positive relationship with the probability of occurrence of *L. invasa* (Fig. 3b). The probability of finding of *L. invasa* increased from 0.15 in areas that receive annual rainfall of 300 mm to 0.47 in areas that have annual rainfall of 600 mm. The potential occurrence of *L. invasa* was predicted to be highest for areas that have an annual precipitation of more than 2,400 mm, Temperature showed a negative relationship with the probability of occurrence of *L. invasa* (Fig. 3c). The highest probability (i.e. 0.9) of finding *L. invasa* was observed for areas with annual temperatures between 10.5°C and 12°C. The probability of finding the blue gum chalcid decreased from a probability of 0.9 in areas that have annual temperatures of 12°C to zero in areas that have an annual temperature of 26°C (Fig. 3c).

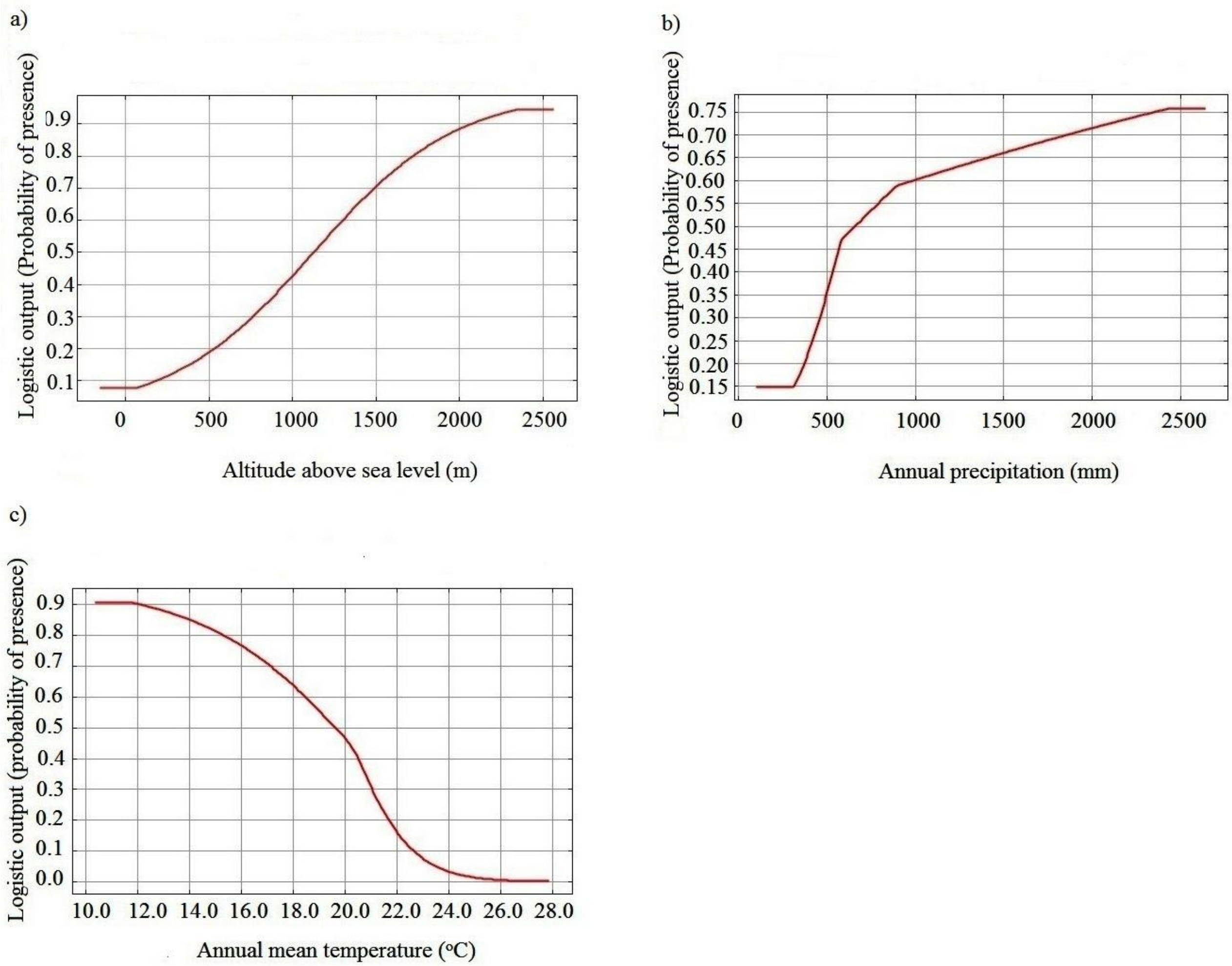


Figure 3. Response curve showing how a) altitude b) rainfall c) temperature affects the MaxEnt model in predicting *Leptocybe invasa* occurrence

The present results indicate that areas with the highest probability of occurrence of *L. invasa* are in the Eastern Highlands, the Highveld and Middleveld regions of Zimbabwe (Fig. 4). These regions also encompass Natural Farming Regions I, IIa, IIb and significant portions of Natural Farming Regions III and IV. The major cities where *L. invasa* has a high probability of being encountered include Mutare, Bindura, Harare, Masvingo, Kwekwe, Gweru and Bulawayo. The results also show that there is a low probability of finding *L. invasa* in the low-lying areas of

Zimbabwe particularly in the Kalahari Sandveld, Zambezi Valley and the South-eastern Lowveld and Middle Save Valley.

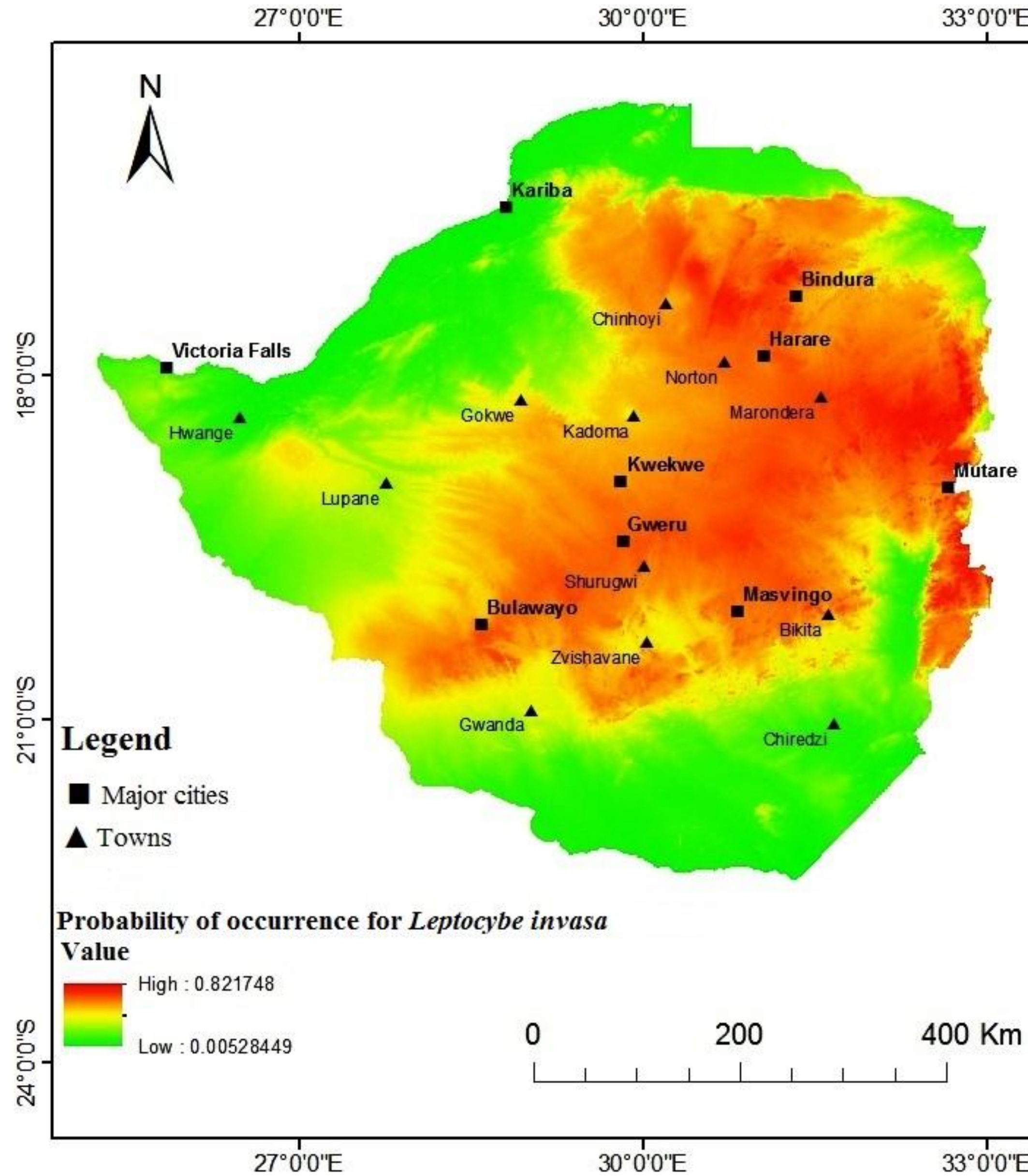


Figure 4. The probabilistic predictive map of *Leptocybe invasa*

## 4.4 Discussion

The results of this study showed that altitude, temperature and rainfall have an influence on the distribution of *L. invasa* and can be used to determine its occurrence. The highest probability of occurrence of *L. invasa* was modelled to increase with an increase in altitude. The current finding are similar to those by Mutitu *et al.* (2014) which showed the blue gum chalcid incidence to decrease with a decrease in altitudes areas of East Africa. In contrast, observations made by Nyeko *et al.* (2009) showed that *L. invasa* had a higher probability of occurring in the lowland areas and in the hotter and drier agro-ecological zones than in the cooler, wetter and highland areas of Uganda. Similarly, another study by Maddumage *et al.* (2012) in Kenya also predicted the probability of occurrence of *L. invasa* to increase with a decrease in altitude areas as compared to those areas of higher elevation. However, the studies by Nyeko *et al.* (2009), Maddumage *et al.* (2012) and Mutitu *et al.* (2014) did not include rainfall and temperature in their predictive models. The three studies only used altitude as a predictor to determine the distributions of *L. invasa* in East Africa and it is possible that the additional variables included in the current study contributed to the contrasting results. The study by Cushman *et al.* (2010) states there is an addition of valuable information describing the habitat characteristics not covered by one variable when additional variables are included.

It can also be deduced from the results of this study that altitude is not a good choice of a predictor for determining the distribution of *L. invasa* because the percentage contributions made by altitude in the model were very negligible as compared to the contributions made by the other two variables. Temperature was shown to be the most important predictor and exerts a much stronger influence in predicting where *L. invasa* is likely to be found in Zimbabwe. This can be explained by the fact that most insects have limited control over body temperature, and therefore suitable outside temperatures are important for most activities and metabolic processes such as reproduction to occur. When temperature is too low, the metabolic processes in insects slow down and when temperatures are extremely high, metabolic reactions become unbalanced and enzymatic activity is weakened or even destroyed (Savage *et al.*, 2004; Régnière *et al.*, 2012; Wrigglesworth and Wrigglesworth, 2015). A study by Lu *et al.* (2014) found out that when the metabolism of *L. invasa* was inhibited, its longevity decreased gradually from 360 hours with an increase in temperature, to zero at 25$^{o}$C. This could also explain why the results from the current study showed that the chance of finding the chalcid gradually decreases from a probability of 0.9 at 10$^{o}$C to a probability of zero at 26$^{o}$C. Similarly a study by Mutitu *et al.* (2007) found high infestations of *L. invasa* occurring in areas that have a temperature range of 10-25$^{o}$C.

Results from this study also showed higher probability of *L invasa* presence in areas of higher annual rainfall. In Zimbabwe, these are the regions where a majority of commercial plantations and trial plots for forest research centres have been established and more than 10 host species and hybrids for *L. invasa* are found (Matowanyika, 1998). The probability of finding *L. invasa* was also very low for landscapes that have an annual rainfall of less than 400 mm. Similarly, Oballa *et al*. (2010) in Kenya, also mentions that *Eucalyptus* should not be grown in areas with less than 400 mm of rainfall as the gum trees do not do well in these areas. Likewise, there are fewer and smaller eucalyptus stands in schools, community groups and rural woodlots in the low-lying areas (Bradley and Dewees, 1993) where the probability was predicted to be low. A few host species such as *E. camaldulensis* and *E. tereticornis* are the most widely-grown species in areas that receive less than 400 mm of annual rainfall in Zimbabwe (Mullin, 1998). The distribution of *L. invasa* in Zimbabwe can however be attributed to the differences in the availability of host plants and silvicultural practices in the different regions of the country. The large quantities of coppices and re-growths found in plantations and research trial plots after harvest could also be increasing the resource availability for oviposition thus accounting for the higher probabilities of occurrence in the Eastern Highlands, in the Highveld and in the Middleveld of Zimbabwe. It can therefore be presumed that variations in resource availability and carrying capacity are important determinants of fluctuations in the populations of *L. invasa* and require further investigation in combination with the ecological requirement of the pest.

# CHAPTER 5

## THE RELATIVE SUSCEPTIBILITY AND SEVERITY OF *Leptocybe invasa* DAMAGE IN SELECTED *Eucalyptus* SPECIES IN ZIMBABWE

### 5.1 Introduction

The eucalypt plantation industry in Zimbabwe is currently reliant on only a few species that are adapted to semi-arid conditions. These species have all been reported to be infested by *L. invasa* in many nurseries, young plantations and woodlots throughout the country since the year 2008 (Forest Commission of Zimbabwe, 2015). It is therefore increasingly becoming difficult to find seedlings to establish new plantations (CABI, 2007; Matimaire, 2015).

Various strategies are being pursued for the management of *L. invasa* in its introduced range (Dittrich-Schroder, 2012). In addition to biological and chemical control measures, planting eucalyptus material resistant to *L. invasa* and avoiding the production and planting of highly susceptible clones offers an additional option for an integrated management strategy. The list of susceptible and resistant genotypes has since been developed from studies done in many countries (e.g. Nyeko *et al.*, 2009; Prabhu, 2010). There is therefore an urgent need to screen a wide range of *Eucalyptus* species, clones and hybrids for resistance, tolerance and susceptibility to *L. invasa* in Zimbabwe.

### 5.2 Materials and Methods

#### 5.2.3 Study site

This study was conducted at the Chesa Forestry Research Station in Bulawayo.

#### 5.2.4 Experimental design

The experiment was carried out using seedlings of four species of *Eucalyptus*: *E. tereticornis*, *E. camaldulensis*, *E. grandis* and *E. propinqua*. Following the methods from Nyeko *et al.* (2010), the eucalyptus seeds were directly sown into pots (5 × 12 cm) containing soil and granular fertilizer (2% by weight) (Nitrogen, Phosphorus and Potassium), and placed under hail netting in the nursery for protection against pest infestation.

Upon germination one week later, the seedlings were thinned out until one seedling remained per pot. Pots were watered 2–3 times per week for three months (March-May 2015) in the nursery. When the seedlings had reached an average height of 15 cm with approximately 16–17 leaves or more (depending on the species as some had few but larger leaves), 40 seedlings of each species were randomly selected and set up as a Randomized Complete Block Design (R.C.B.D) of four treatments (*Eucalyptus* species) in four blocks/replications. Each treatment plot comprised of 10 plants. Four different sites, treated as blocks, were randomly selected where the seedlings were exposed to natural *L. invasa* infestation for a period of 72 days from June 2015 until August 2015 at the Chesa Forest Research Station. Four seedlings were selected at random from each treatment and scored for pest incidence and severity at 4, 6, 8 and 10 weeks after treatment. Incidence was recorded as the presence or absence of *L. invasa*-induced symptoms in each seedling. Severity of infestation was scored visually as a percentage according to the following scale:

- 0: no *L. invasa*-induced gall observed on any leaf or twig on each seedling,
- 1: < 25% of the leaves and twigs bearing *L. invasa*-induced galls on each seedling,
- 2: 25–50% of the leaves and twigs bearing galls on each seedling, and
- 3: > 50% of the leaves and twigs bearing galls on each seedling (Nyeko *et al.*, 2010).

### 5.2.5 Data analysis

After testing for normality using the Kolmogorov-Smirnov normality test and the Shapiro test, the data were found to deviate significantly from a normal distribution. The data were then analyzed by chi-squared in order to test for significant differences in the proportions of seedlings infested with *L. invasa*. Krustal Wallis test was used to test for differences in the mean severity ranks among the treatments.

## 5.3 Results

### 5.3.1 Incidence

More than 50% of the seedlings were recorded with signs of *L. invasa* infestation for all the *Eucalyptus* species that were tested in the study (Fig. 5). Overall, 72% of *E. tereticornis* and 72% of *E. camaldulensis* seedlings were infested by *L. invasa* galls, while 65.52% of *E. propinqua* seedlings and 62.86% of *E. grandis* seedlings showed signs of infestation. The incidences recorded were not statistically different amongst the four *Eucalyptus* species ($X^2$ = 1.0953, d.f. = 3, $P > 0.05$).

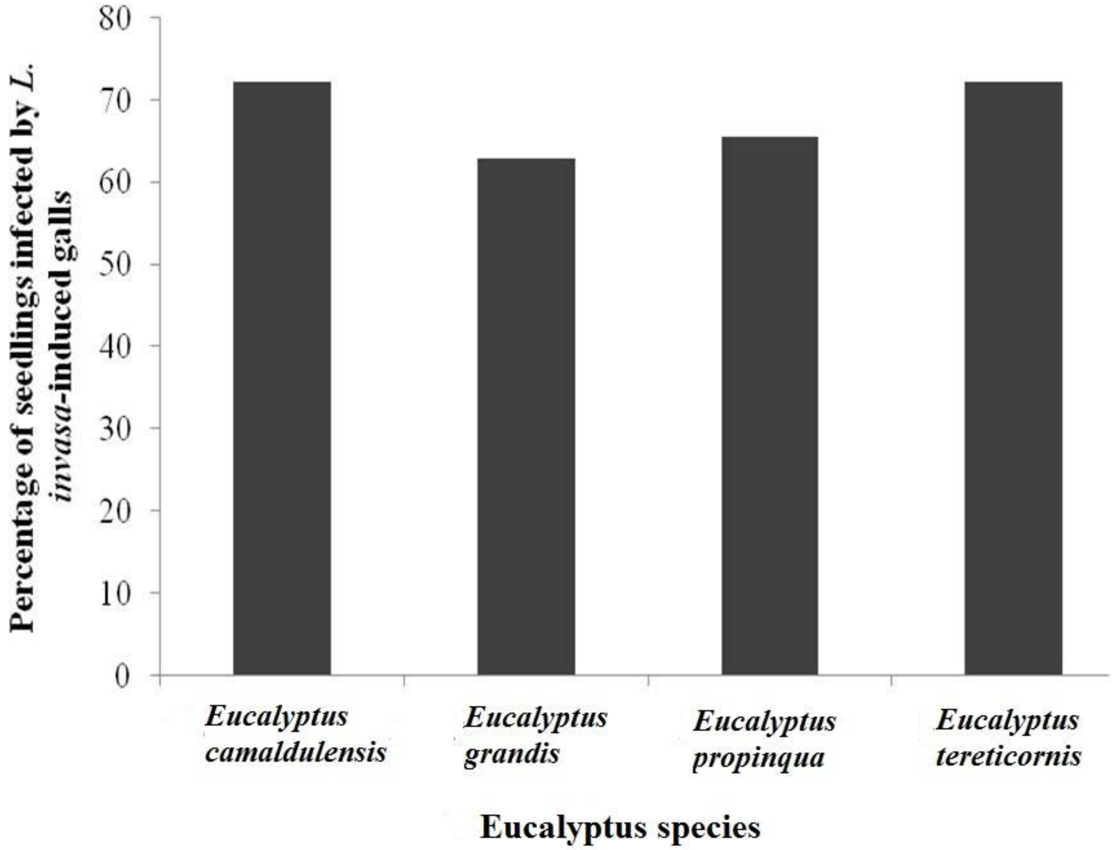


Figure 5. Incidence of *Leptocybe invasa*-induced galls on four *Eucalyptus* seedlings

### 5.3.2 Severity

There were no significant differences ($\chi^2$ = 2.358, d.f. = 3, $P > 0.05$) in the severity of galls induced by *L. invasa* among the four species of *Eucalyptus*. *Eucalyptus camaldulensis* seedlings had a mean rank of 77.21, while *E. grandis*, *E. propinqua* and *E. tereticornis* seedlings had mean ranks of 84.93, 76.18 and 88.96, respectively (Fig. 6).

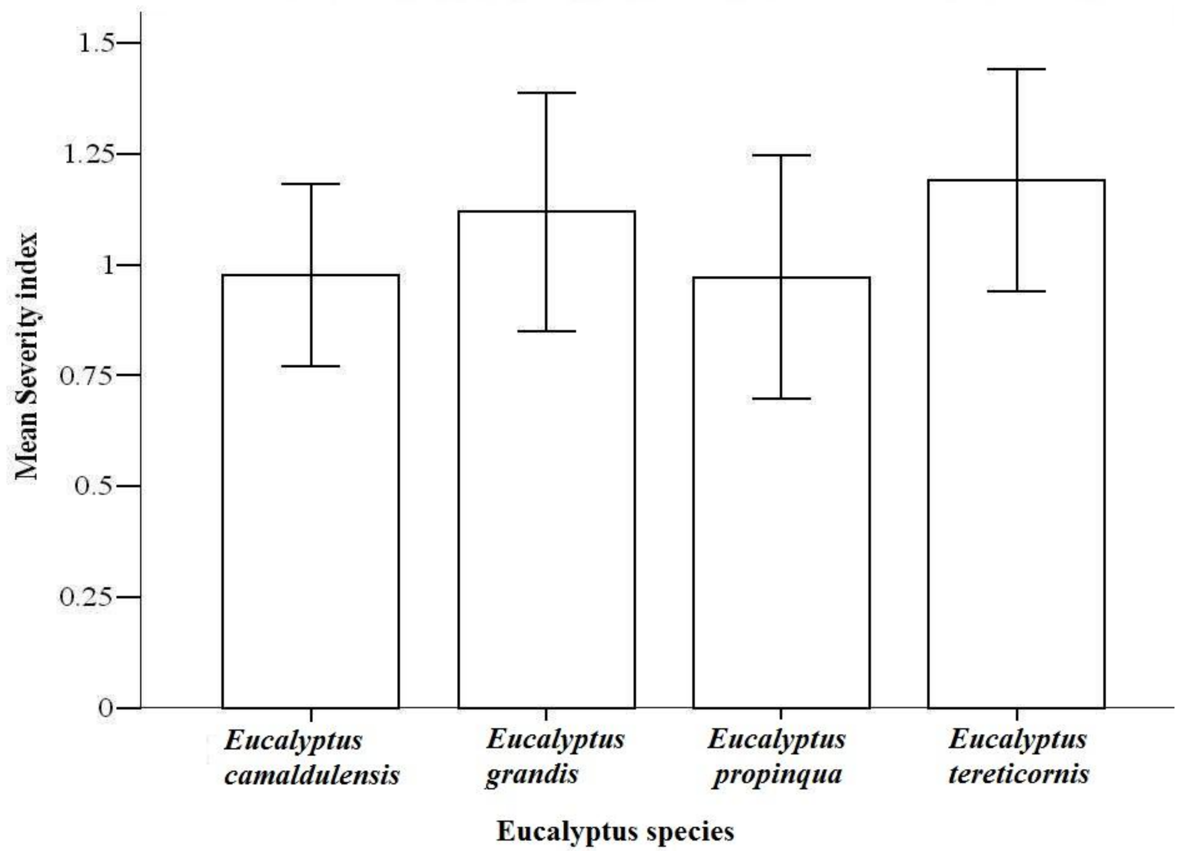


Figure 6. Severity indices of galls induced by *Leptocybe invasa* among four selected *Eucalyptus* species

## 5.4 Discussion

The findings from the study showed that *E. grandis*, *E. tereticornis*, *E. camaldulensis* and *E. propinqua* in Zimbabwe are all susceptible to attack by *L. invasa* and do not differ in the severity of damage caused by the pest. The current findings also corroborate the results from studies that have been conducted with the four species in China (Zhu *et al.*, 2012), Kenya (Nyeko *et al.*, 2010), South Africa (ICFR, 2011) and Taiwan (YaLing *et al.*, 2014). *Leptocybe invasa* was also found on *E. camaldulensis* and *E. grandis* in Uganda (Nyeko *et al.*, 2009) and Brazil (Fernandes *et al.*, 2014). In Florida, *E. camaldulensis*, *E. grandis* and *E. propinqua* were all confirmed to be susceptible to *L. invasa* (Wiley and Skelley, 2008). However, these findings differ from other studies that have reported some variations in the extent of damage caused by *L. invasa* on the tested species. For example, Zhu *et al.* (2012) found that *L. invasa* attacks where most severe on

*E. propinqua* compared to *E. grandis*, *E. tereticornis* and *E. camaldulensis* while YaLing *et al.* (2014) found *E. camaldulensis* and *E. grandis* to be more severely affected by *L. invasa* compared to *E. tereticornis* and *E. propinqua* amongst other species that were tested.

The differences in observations may be attributed to the different methods that were used to assess for severity of damage by Zhu *et al.* (2012). Zhu *et al.*'s (2012) study assessed for severity of *L. invasa* damage using the number of galls that were found on each plant. In comparison, the present study used the proportion of the leaves that were affected to assess for susceptibility and severity. It is therefore possible that the methods used in the present study may have limited the outcome of the study by not taking into consideration pest behaviour. The methods used in the current study also did not give an assessment of the host's reaction and as well as the variations in structural, chemical and nutritional properties that are required to develop a better understanding of the general mechanisms of resistance that are at work as argued by Jayaraj *et al.* (2014). However, the same methods of assessment were also used by Nyeko *et al.* (2010) and YaLing *et al.* (2014) who observed some significant differences in the severity of damage among some of the species.

The findings of the present study also differ from those observed by YaLing *et al.* (2014) who reported some significant differences in the severity of damage by *L. invasa* on *E. grandis* and *E. camaldulensis*. However, the study by YaLing *et al.* (2014) artificially infested each seedling using an equal number of female adult wasps. On the other hand, the present study exposed the seedlings to natural *L. invasa* infestation. It is therefore likely that exposing the seedlings to natural infestation could have influenced the outcome of the study as seedlings were placed in an environment where resource availability for oviposition by *L. invasa* was higher and where other, possibly more susceptible species could have been present from the other trees and seedlings that were present in the field.

The results from the present study are also in contrast to the observations that were made by Nyeko *et al.* (2010). The evaluations carried out by Nyeko *et al.* (2010) in Uganda were conducted during the hot and wet season and were carried out for a period of nine months. In the same study by Nyeko *et al.* (2010), the plots for the evaluations that were done in Kenya were also established in the wet season and the assessments were carried out for an even longer period of two years. In the study by Nyeko *et al*. (2010) in Kenya, significant differences were observed in the susceptibility and severity of *L. invasa*-induced galls in *E. tereticornis* and *E. camaldulensis*. However, the current study was only carried out for a period of 10 weeks and during the cold dry season. Although *L. invasa* has been reported to be active throughout the year, its activities have been noted to be highest during the warm season (Karunaratne *et al*., 2010; Eyidozehi *et al*., 2014). It is therefore possible that the findings of the present study may also be indicative of the season of planting when the pest is not very active, as well as the duration of seedlings' exposure to the pest as the seedlings may not have been exposed long enough to account for any significant differences. A longer assessment period under different seasons could provide more reliable results in Zimbabwe. A comparison of seedling and hybrids established from other different countries may also need to be carried out.

# CHAPTER 6

# THE RELATIVE EFFICACY OF ROOT SYSTEMIC INSECTICIDES FOR PROTECTION OF *Eucalyptus tereticornis* SEEDLINGS AGAINST *Leptocybe invasa* IN ZIMBABWE

## 6.1 Introduction

*Eucalyptus* trees are native to Australia and are highly favoured in many exotic plantations across the world because of their relatively fast growth rate and high productivity per unit area (Turnbull, 1999). They are being used to meet the ever increasing demands in wood products such as paper, furniture and construction. Several species of *Eucalyptus* are thus being planted in many countries to meet the increasing wood demand (Evans, 1992). However, *E. camaldulensis* and *E. tereticornis* are two species that are more extensively grown and adapted to semi-arid conditions (Zahid *et al.*, 2010).

The occurrence of *L. invasa*-induced galls on a number of *Eucalyptus* species is creating a huge concern for both the forestry and agricultural sectors. Serious damage to young plantations and nursery seedlings has been reported in several countries around the world and it is becoming increasingly difficult to establish new plantations (Doganlar 2005; Wiley and Skelley, 2008; Jimu *et al.*, 2015). Since the blue gum chalcid develops within plant tissue and is thus protected during development, effective control methods are still very limited (Nyeko *et al.*, 2009; Blanche, 2012). However, the endoparasitic insects can be reached when systemic insecticides are used to control the pest (Blanche, 2012). Therefore, the use of systemic insecticides as seed dressings is considered one of the most effective components in an Integrated Pest Management (IPM) program targeting cryptically-feeding insects or sap-suckers. Neonicotinoid insecticides represent the fastest growing class of insecticides introduced to the market since the launch of pyrethroids (Banerjee and Banerjee, 2012; Paramasivam, 2012).

Currently, no insecticides are registered for *L. invasa* control in Zimbabwe. There is therefore need to investigate the effectiveness of systemic insecticides for managing the pest in the country. If the insecticides are found to be effective, they could possibly be used to manage *L. invasa* in nurseries. This study therefore aimed to determine the relative efficacy of the systemic insecticides imidacloprid and thiamethoxam for protection of gum tree seedlings in Zimbabwe.

## 6.2 Materials and Methods

### 6. 2.1 Study area

This study was conducted at the Chesa Forestry Research Station in Bulawayo.

### 6.2.2 Experimental design

The efficacy of imidacloprid and thiamethoxam was tested on one hybrid of *E. tereticornis*. The experiment was set up as a Complete Randomized Design (CRB) of three treatments (Imidacloprid 20% SL, Thiamethoxam 25% WG and Untreated Control). The test plants were randomly selected from seedlings that had been sown into potting medium (5 × 12 cm) containing soil and granular NPK (2% by weight) and placed under hail netting in the nursery for protection against pest infestation. Forty plants were randomly selected and treated with Imidacloprid 20% SL using an overall rate of 220 ml/100 litres water and applying one 30-ml cup of mix in each planting hole immediately after filling it prior to transplanting. Another 40 plants were also randomly selected and treated with Thiamethoxam 25% WG using a rate of 125 g/100 litres water then applying one 30-ml cup of mix per plant just before transplanting. Application rates were adopted from the Zimbabwe Crop Chemicals Handbook (CropLife, 2010) for tobacco aphid control since there are currently no pesticides registered for the control of *L. invasa* in Zimbabwe to date. Another 40 randomly selected plants remained untreated and were used as the control.

The seedlings were exposed to natural *L. invasa* infestation from June 2015 until August 2015. This time period was specifically chosen to ensure that *L. invasa* would complete its life cycle based on the 72 days observed in South Africa (ICFR, 2011). After exposure to *L. invasa*, 10 seedlings from each treatment where picked at random and scored for incidence of *L. invasa* at 4, 6, 8 and 10 weeks after treatment. Each seedling was treated as an experimental unit. Following the methods by Nyeko *et al*. (2010), incidence was recorded as the presence or absence of *L. invasa*-induced symptoms for each seedlings and severity of infestation was scored visually as a percentage out of all the total leaves on each seedling according to the following scale:

- 0: no *L. invasa*-induced gall observed on any leaf or twig in each seedling,
- 1: < 25% of the leaves and twigs bearing *L. invasa*-induced galls on each seedling,
- 2: 25–50% of the leaves and twigs bearing galls on each seedling, and

- 3: > 50% of the leaves and twigs bearing galls on each seedling (Nyeko et al., 2010).

After testing for normality using the Kolmogorov-Smirnov normality test and the Shapiro test, the data for all treatment significantly deviated from a normal distribution. The data were then analyzed using chi-square so as to test for differences in the proportions of seedlings infested with *L. invasa*. Krustal Wallis test was used to test for differences in the mean severity ranks among the treatments. Where significant differences were observed, the Tamhane Post Hoc test was used to establish where the significant differences lay between groups for the severity indices while the Bonferroni correction test was used to test for the significance between the groups for the pest incidence.

## 6.3 Results

### 6.3.1 Incidence

No significant differences were observed ($\chi^2$ = 5.8195, d.f. = 2, $P > 0.05$) in the percentage of seedlings infested by *L. invasa* among all the treatments. Sixty-eight percent of the seedlings in the control, 57% of the seedling treated with imidacloprid and 40% of the seedlings treated with thiamethoxam were observed to have *L. invasa*-induced galls (Fig. 7).

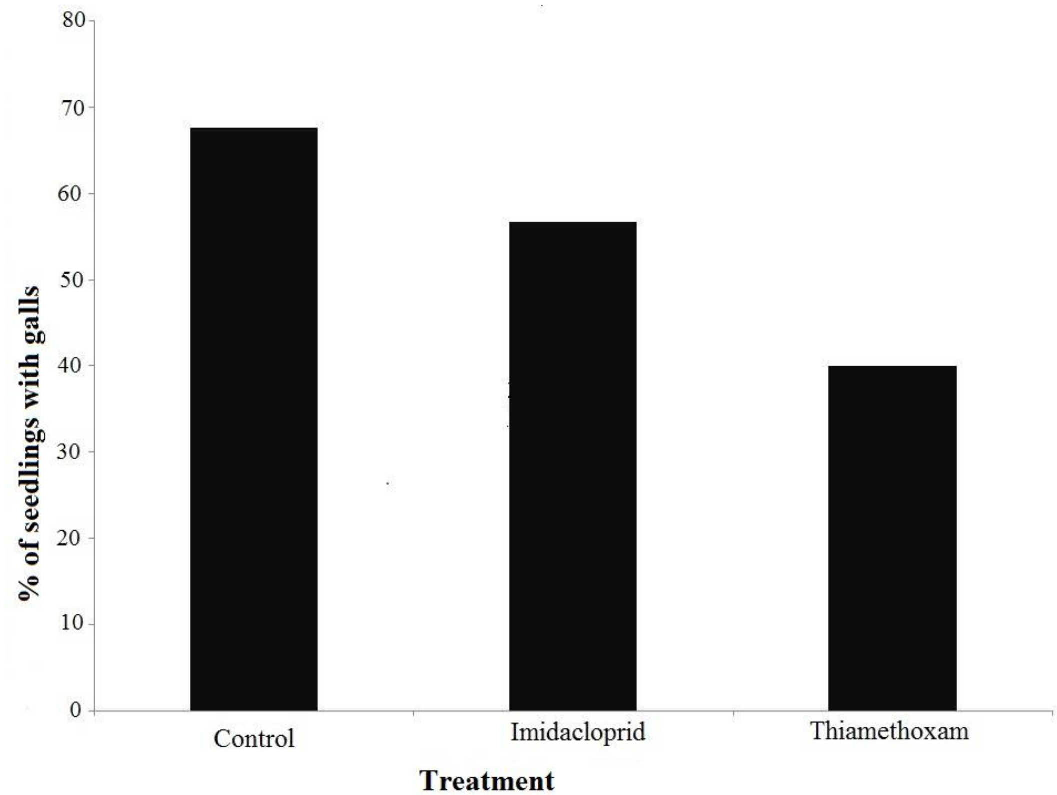


Figure 7. Incidence of *Leptocybe invasa* induced galls on *Eucalyptus tereticornis* seedlings after treatment with systemic insecticides compared against an untreated control.

No pest incidence was recorded four weeks after applying the chemicals. However, significant differences ($\chi^2$ = 14.4563, d.f. = 2, $P < 0.05$) were observed in the percentage of seedlings infested by *L. invasa* six weeks after applying the chemicals. The Bonferroni Post Hoc test showed that there were significant differences between the control and the thiamethoxam treatment ($P < 0.01$). No significant differences were observed in the incidence of galls induced by *L. invasa* among all the treatments after eight weeks ($\chi^2$ = 5.8195, d.f. = 2, $P > 0.05$) and after 10 weeks ($\chi^2$ = 5.8195, d.f. = 2, $P > 0.05$) of applying the chemicals (Fig. 8).

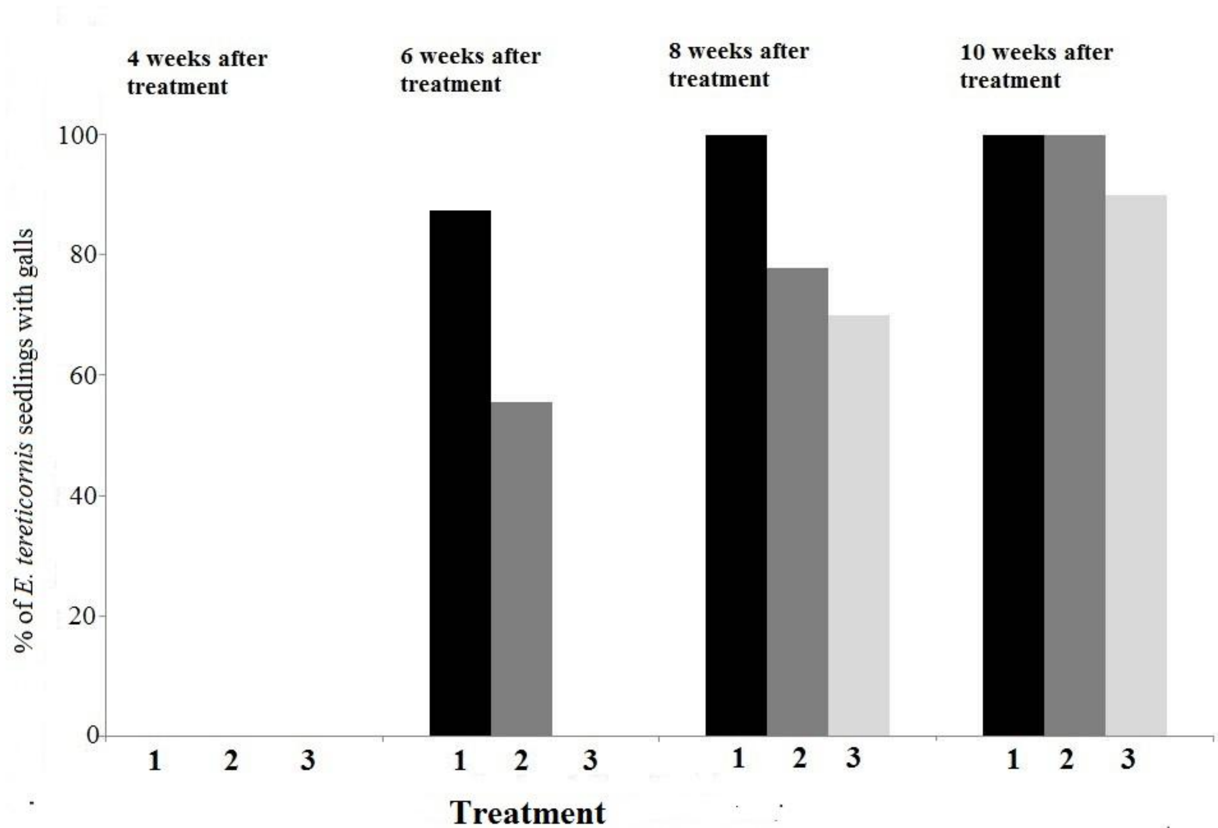


Figure 8. Efficacy of systemic insecticides on the incidence of *Leptocybe invasa*-induced galls weeks after treatment [1 = control, 2 = imidacloprid and 3 = thiamethoxam].

### 6.3.2 Severity

There were significant differences in the severity of galls induced by *L. invasa* among the three treatments ($\chi^2 = 7.7$; d.f. = 2; $P < 0.05$) with a mean rank of 64.99 for the control and 58.26 for the seedlings treated with imidacloprid. The lowest severity was recorded for the thiamethoxam-treated seedlings which had a mean rank of 46.28 (Fig. 9). The post hoc test showed that there were significant differences between the control and the thiamethoxam-treated seedlings. However, no significant differences were observed in mean severity ranks between the control and imidacloprid as well as between thiamethoxam and imidacloprid.

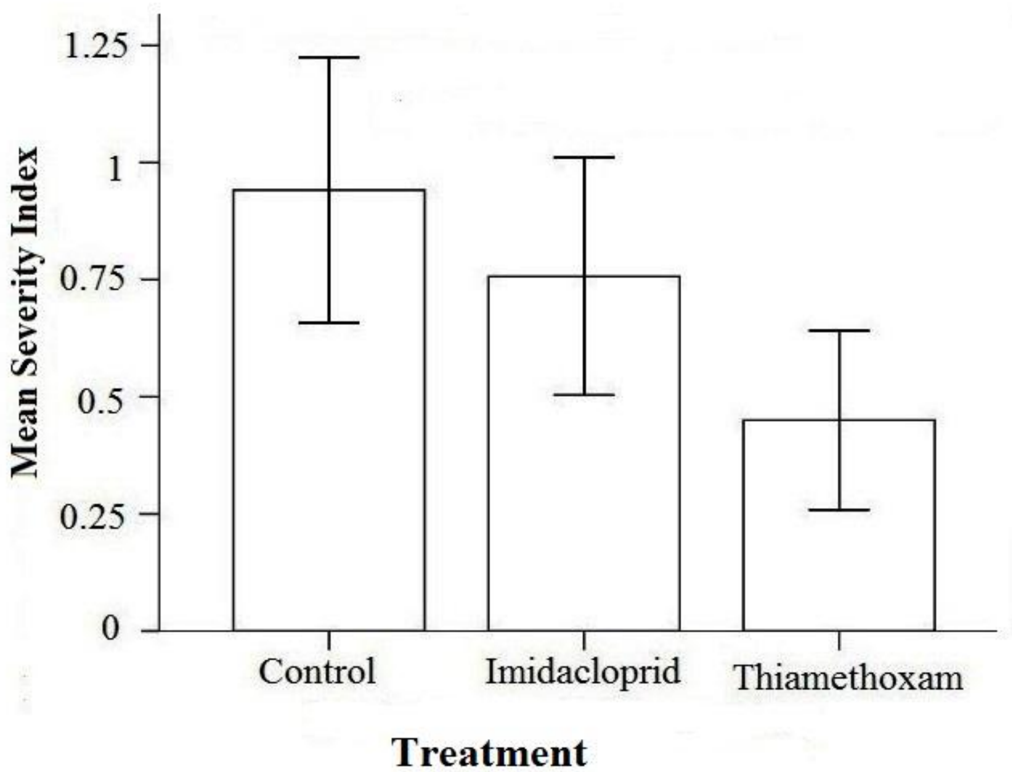


Figure 9. Severity indices of galls induced by *Leptocybe invasa* on *Eucalyptus tereticornis* seedlings after treatment with systemic insecticides compared against an untreated control

No galls where reported in all the treatments during the first assessments, that is at 4 weeks after treatments (Fig. 10.). Significant differences ($\chi^2$ = 12.578; d.f. = 2; $P < 0.05$) were then observed at 6 weeks after application. Post hoc tests showed significant differences between the control and the thiamethoxam-treated seedlings. No significant differences were observed in the severity of galls among all the treatments at 8 weeks ($\chi^2$ = 2.563; d.f = 2; $P$ = 0.278) and at 10 weeks ($\chi^2$ =4.151; d.f = 2; $P$ = 0.125) after applying the treatments.

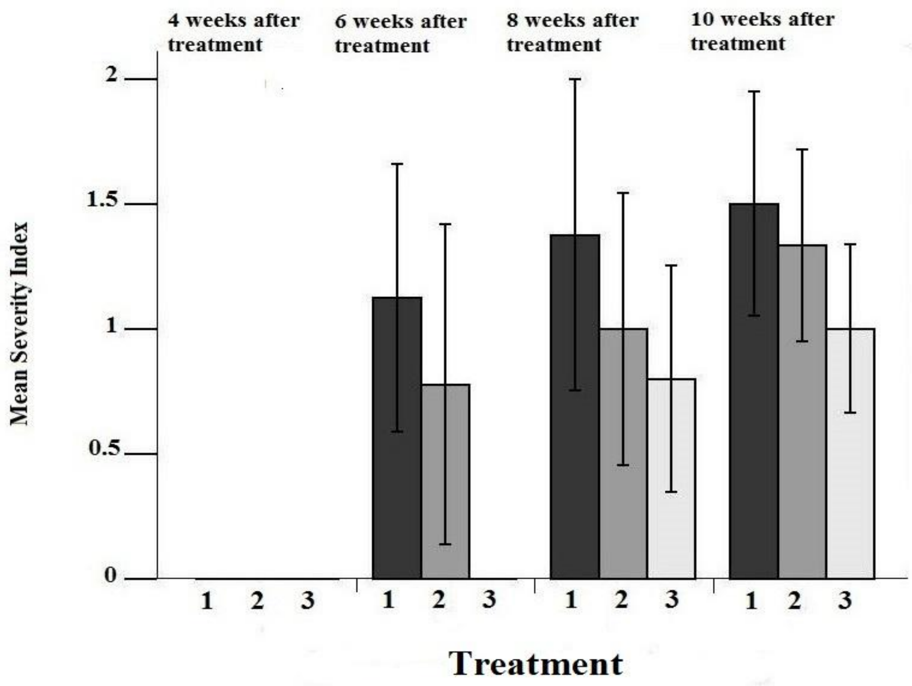


Figure 10. Severity indices of galls induced by *Leptocybe invasa* on *Eucalyptus tereticornis* seedlings after treatment with systemic insecticides [1 = control, 2 = imidacloprid and 3 = thiamethoxam]

### 6.4 Discussion

The results of the study showed that there were no significant differences in the proportion of seedlings infested by *L. invasa* among and between the treatments. This shows that that Imidacloprid 20% SL and Thiamethoxam 25% WG were not effective in protecting *E. tereticornis* seedlings against *L. invasa* attacks for the period that they were assessed. However, thiamethoxam did seem to be providing protection against *L. invasa* attack at 6 weeks after treatment when no galls were recorded on any seedling and when some significant differences were observed compared against the control. The findings from the current study also showed that the protective effects of imidacloprid and thiamethoxam against *L. invasa* are not similar. Thiamethoxam significantly protected *E. tereticornis* against severity of damage after attack by *L. invasa* while imidacloprid was not effective. Where significant protective effects by the two insecticides has been observed, this has been against other pests for example up to 14 days against aphids on cotton in China (Torres and Roberson, 2004) and against *Bemisia tabaci* on cotton in Brazil. However, it has been noted that as the plants grows and ages, the active ingredients in the systemic insecticides are gradually reduced. This could explain why the insecticides were ineffective in protecting the seedlings after the period of assessment. Thiamethoxam has been reported to increase plant vigour (Afifi *et al.*, 2015). This could also explain why it was observed to be more effective at protecting *E. tereticornis* seedlings against *L. invasa* damage and attacks at 6 weeks after treatment.

The dosages of the insecticides that were used in the present study were adopted from the control of aphids and may not have been well suited for the control of *L. invasa*. In Torres and Roberson's (2004) study, the insecticides did significantly protect seedlings against aphids on cotton. However, all the life stages of aphids are known to damage the plants through their feeding habits as they are phytophagus feeders (Ali and Agrawal, 2012). On the other hand, adult *L. invasa* wasps only cause damage to eucalyptus seedlings through laying of the eggs on the leaves (Fernandes *et al.*, 2014) meaning that the adults are only affected by the insecticide through residual contact. However, both imidacloprid and thiamethoxam are known to be more toxic via ingestion than residual contact (Torres and Ruberson, 2004). It is therefore likely that the control of adult *L. invasa* using the systemic insecticides may require different dosages of the chemicals or may require the use of chemicals that have contact action. Thus, the investigation of various dosages of thiamethoxam and imidacloprid requires further testing for the control of internally-feeding *L. invasa* larvae whilst residual contact insecticides should be assessed for adult wasp control. For the efficacy parameters, gall development should also be considered in combination with the percentage of damaged leaves.

# CHAPTER 7

# GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 7.1 General Discussion

The present study demonstrated that it is possible to determine the distribution of *L. invasa* through modelling which makes use of variables such as altitude, rainfall and temperature. The results differ from findings by Nyeko *et al.* (2010) and Maddumage *et al.* (2012) that showed that there was a decrease in the probability of occurrence of *L. invasa* with an increase in altitude. However, the results of the present study also suggested that altitude is not a very important predictor in determining the distribution of *L. invasa*. When it was used with other related environmental factors to model the distribution of *L. invasa* in the present study, it had negligible contributions in the model. Temperature seems to play a major role in determining the distribution *L. invasa* distribution. It has been demonstrated that most insects have limited control over body temperature and therefore suitable outside temperatures are most important for activities and metabolic processes such as reproduction to occur (Atkinson, 1994). Temperature and relative humidity have also been reported to be the most fundamental environmental factors for insect growth (Child, 2007).

The regions where the occurrence of *L. invasa* was predicted to be high in Zimbabwe are also the areas where most commercial plantations and forest research centres have been established (Suttie and Reynolds, 2006). It is likely that in these areas, resource availability is much higher for *L. invasa* oviposition from the larger quantities of coppices and re-growths found in the commercial plantations after harvests are carried out (Sunder, 1995) compared to the low-altitude regions where commercial plantations are not found. More species of *Eucalyptu*s are also grown in the Highveld and Middleveld regions of Zimbabwe as compared to the low-lying areas where only a few species are preferred because of their adaptability to the climate (Mullin, 1998). It is therefore possible that the occurrence of *L. invasa* in Zimbabwe may also be indicative of resource availability and the distribution of host species for oviposition by *L. invasa* female wasps. However, other studies in Uganda (Nyeko *et al.*, 2009) and in Brazil (Fernandes *et al.*, 2014) demonstrated that *E. maidenii*, a species known to be highly susceptible to *L. invasa* (Petro *et al*., 2015), did not show any signs of susceptibility outside the ecological range of the pest in those countries. The variations in resource availability and carrying capacity therefore require further investigation in combination with the ecological requirement of the pest to effectively predict the areas it might occur or invade in Zimbabwe.

Results on host susceptibility showed that *E. tereticornis*, *E. camaldulensis*, *E. grandis* and *E. propinqua* are all attacked by *L. invasa* and they do not differ in their susceptibilities and severity of attack by the pest. All four species have also been reported to be prone to attack by *L. invasa* in China (Zhu *et al*., 2001), East Africa (Nyeko *et al*., 2010), South Africa (ICFR, 2011) and Taiwan (YaLing *et al*., 2014). Other studies have, however, reported significant differences and variations in the severity of damage with *E. propinqua* suffering more damage compared to *E. grandis*, *E. tereticornis* and *E. camaldulensis* in China (Zhu *et al*., 2012), and *E. camaldulensis* and *E. grandis* being more severely affected compared to *E. tereticornis* and *E. propinqua* amongst other species tested in Taiwan (YaLing *et al*., 2014). The different results may be attributed to different methodologies and parameters that were used to assess the severity of damage. The study by Zhu *et al*. (2012) used gall volume to assess damage while the present study only used the proportion of damage. The same method of considering proportion of damage was used by YaLing *et al*. (2014); however, significant differences in the severity of damage were observed for the species. Jayaraj *et al*. (2014) does argue that the method of considering proportion of damage has limitations as it does not take into consideration the behaviour of the pest nor does it give an assessment of host reaction and variation in structural, chemical and nutritional properties of the plant that are required to develop a better understanding of the general mechanisms of resistance that are at work.

Origins of progeny for host species, time of planting and duration of the assessment may also explain the outcome of this study. In a study by Nyeko *et al*. (2010) in Kenya and Uganda, plots were established during the hot and wet season and assessments were carried out for longer periods. Significant differences were observed in the severity of damage in *E. tereticornis* and *E. camaldulensis* compared to other species. It is therefore probable that the results of the present study may be indicative of the time of planting and the duration of study since the plots were established and the assessments were carried out only in the cold-dry season. Although *L. invasa* has been reported to occur throughout the year, it was found to be highly active during the warmer season (Karunaratne *et al.*, 2010; Eyidozehi *et al.*, 2014). Therefore the data that was collected during the cold-dry season for a short period of time may not have been sufficient to bring out significant differences among the species.

Efficacy studies of systemic insecticides revealed that Imidacloprid 20% SL and Thiamethoxam 25% WG were not effective in protecting *E. tereticornis* seedlings against *L. invasa* attacks for the period that they were assessed. However, the study showed that the protective effects of imidacloprid and thiamethoxam against *L. invasa* are not similar as thiamethoxam did significantly protect *E. tereticornis* seedlings against the severity of damages caused by *L. invasa* than imidacloprid. Thiamethoxam did significantly protect the *E. tereticornis* seedlings from *L. invasa* attacks after 6 weeks of treatment and no galls were recorded compared to imidacloprid and the control seedlings. However, at 8 and 10 weeks after treatment, no significant differences in seedling susceptibility were observed. Severity index data also seemed to portray a similar trend. This demonstrates that the toxicity of the two systemic insecticides gradually declines as the plants age (Zhang *et al.*, 2011).

Female blue gum chalcid wasps can only be affected by the toxins through residual contact as they oviposit on the leaves. Systemic insecticides therefore, may not be efficient in protecting eucalyptus seedlings against attacks by female adult wasps since the systemic insecticides are known to be more toxic via ingestion than residual contact (Torres and Ruberson, 2004). Instead, contact insecticides might be more effective at protecting seedlings against attacks by female adult wasps. On the same note, the systemic insecticides are ingested by the developing larvae that live and feed inside the leaves and twigs, therefore gall development should also be considered in combination with the proportion of damaged leaves so as to fully assess the effectiveness of the systemic insecticides against the severity of *L. invasa*-induced damage. It should also be noted that the dosages that were used in the present study were adopted from those used for tobacco aphid (*Myzus persicae nicotianae*) control in Zimbabwe (CropLife, 2010). It is therefore likely that the control of *L. invasa* using the two systemic insecticides may require different dosages of the two chemicals.

### 7.2 Conclusion

Results from the current study show that altitude, rainfall and temperature can be used to predict the potential distribution of *L. invasa* and other areas where it is likely to invade. However, temperature seems to be the pivotal predictor when used in combination with other environmental variables. The probability of finding *L. invasa* in Zimbabwe increases with an increase in altitude and mean annual rainfall. There is lowest probability of finding *L. invasa* at elevations of less than 500 m above sea level and in areas that receive annual rainfall of less than 300 mm. *Leptocybe invasa* has a high probability of being found in areas where annual mean temperatures are between 10$^{o}$C and 26$^{o}$C in Zimbabwe. There is no likelihood of finding *L. invasa* in areas were annual mean temperatures are greater 26$^{o}$C in Zimbabwe. *Eucalyptus* seedlings tested in this study were all prone to *L. invasa* attack and were all equally damaged by the pest. The insecticides used in this study were not effective in protecting *E. tereticornis* seedlings against *L. invasa* attacks during the 10-week period of assessment. Thiamethoxam was, however, effective at protecting the seedlings up to 6 weeks after treatment. The preliminary results of this study together with data from other related distribution surveys of *L. invasa* may aid to target control tactics in areas that have a high probability of occurrence for *L. invasa*. Growing least susceptible *Eucalyptus* seedlings in high risk areas or growing susceptible species in regions outside the ecological requirements of *L. invasa* as well as using effective chemicals provide a potential integrated management strategy for controlling *L. invasa*. The results of this study together with data from related past studies carried out in Zimbabwe and beyond therefore show the need to further assess the susceptibility of *Eucalyptus* species and hybrids and to test for the most effective and locally available chemicals so as to develop an effective integrated management approach for *L. invasa*.

### 7.3 Recommendations

From the results of the study, several recommendations can be made going forward.

- Variations in resource availability and carrying capacity require further investigation in combination with the ecological requirement of *L. invasa* to effectively predict the areas it might occur or invade in Zimbabwe.

- There is need to further investigate the susceptibility of other *Eucalyptus* species found in Zimbabwe as well as undertaking such a study over several years under different seasons.
- Further assessment of other locally available insecticides (both systemic and contact) need to be carried out and should take into consideration gall development in combination with the proportion of damaged leaves so as to fully assess the effectiveness of the insecticides against *L. invasa*.
- The application of various dosages of imidacloprid and thiamethoxam for *L. invasa* control on *Eucalyptus* species require further investigation in Zimbabwe.
- Areas with annual temperatures between 10.5$^{o}$C and 12$^{o}$C should be monitored for *L. invasa* and control strategies should be intensified in these areas.
- An integrated management strategy should be adopted for controlling *L. invasa* such as not growing susceptible *Eucalyptus* seedlings, as well as using effective chemicals to protect susceptible seedlings grown in high risk areas.

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**APPENDICES**

Appendix A. List of localities where *Leptocybe invasa* was recorded in Zimbabwe

| Natural Farming Regions of Zimbabwe | Locality | Observation Month and Year |
|---|---|---|
| I | John Meikles Forest Research Station | July 2009 |
| | Meguro | July 2009 |
| | Juliasdale | October 2009 |
| | Scrutiny area | October 2009 |
| | Gungunyana Forest, Chirinda | April 2015 |
| | Stapleford | April 2015 |
| IIA | Norton | December 2008 |
| | Dande | January 2009 |
| | Guruve | January 2009 |
| | Mvurwi | January 2009 |
| | Norton Hunyani | March 2009 |
| | Beatrice | June 2009 |
| | Makoholi Forest | June 2009 |
| | Concession | September 2009 |
| | Glendale | September 2009 |
| | Mazoe | September 2009 |
| | Mvurwi | September 2009 |
| | Mutorashanga | November 2009 |
| | Matoranhembe Primary School, Zvimba | May 2010 |
| | Marondera | July 2010 |
| | Chiweshe | January 2011 |
| | B and K Estates Marondera | February 2012 |
| | Gwebi College | August 2009 |
| | Forest Research Centre | February 2015 |
| | Chitungiwza | February 2015 |
| | Green acres estate, Norton | April 2015 |
| IIB | Headlands | December 2008 |
| | Rusape, Juliasdale Eden Forest | December 2008 |
| | Bindura | January 2009 |
| | Chegutu | October 2009 |
| | Macheke | July 2010 |

Appendix A. (continued)

| NATURAL FARMING REGIONS OF ZIMBABWE | LOCALITY | OBSERVATION MONTH AND YEAR |
|---|---|---|
| III | Chakari | December 2008 |
| | Gutu | December 2008 |
| | Jerera | December 2008 |
| | Chaka School | June 2009 |
| | Furtherstone | June 2009 |
| | Mtao Forest | June 2009 |
| | Connemara | October 2009 |
| | Gweru | October 2009 |
| | Kwekwe | October 2009 |
| | Bhora | May 2010 |
| | Murehwa | May 2010 |
| | Musani turnoff | May 2010 |
| | Pagejo | May 2010 |
| | Shamva turnoff | May 2010 |
| | Zaranyika School | May 2010 |
| | Sea Forth Farm | April 2015 |
| IV | Masvingo, Nkundu road | December 2008 |
| | Mushangashi | June 2009 |
| | Masvingo | April 2010 |
| | Matapa | April 2010 |
| | Musume High School | April 2010 |
| | Neshuro | April 2010 |
| | Zvishavane | April 2010 |
| | Mutawatawa | May 2010 |
| | Lonchard Farm along Gwanda road | August 2012 |
| | Chesa Forest Research Center | February 2015 |
| | Matobo High School | February 2015 |
| | Tohwe Secondary School | February 2015 |
| | Whitewaters High School | February 2015 |