UNIVERSITY OF NAIROBI

SPATIAL DISTRIBUTION OF *Psyllaephagus bliteus* AND ITS IMPACT ON *Glycaspis brimblecombei* POPULATION IN EASTERN AND WESTERN KENYA

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree. Where other people's work or not my own work has been used, this has been properly acknowledged and referenced.

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DEDICATION

This thesis is dedicated to my husband and two sons, may God bless you immensely for being the joy that lightens up my days.

And to my mother Fraciah Wambui and my late father Gathogo Macharia, I dedicate this thesis to you for being by my side and believing in me throughout the journey to completion of my MSc. Study. Dad, may your soul rest in eternal peace.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ARC-GIS	Arc-Geographic Information System
BGC	Blue Gum Chalcid
EC	Ecosystem Conservator
EPPO	European and Mediterranean Plant Protection Organization
FABI	Forest and Agriculture Biotechnology Institute
FAO	Food and Agriculture Organization
FRC	Forest Research Centre
FRIM	Forest Research Institute Malawi
GPS	Global Positioning System
IPM	Integrated Pest Management
IRC	Insect Reference Collection
IUCN	International Union for Conservation of Nature
KEFRI	Kenya Forestry Research Institute
KFS	Kenya Forest Service
KTDA	Kenya Tea Development Agency
NaFORRI	National Forestry Resources Research Institute
PR	Parasitism rate
RGLP	Red Gum Lerp Psyllid
TAFORI	Tanzania Forestry Research Institute
TPCP	Tree Production Cooperative Program

ABSTRACT

Glycaspis brimblecombei Moore (Hemiptera: Psyllidae), a sap-sucking psyllid is known to cause 20-30% defoliation, crown thinning and death of eucalypts. The main natural enemy that has proved effective against this pest in Australia is a parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae). This parasitoid reportedly spread and established unaided in areas where the G. *brimblecombei* is found. In 2007, Forestry Resources Research Institute (NaFORRI) released *P. bliteus* in *Eucalyptus camaldulensis* plantations in Uganda bordering Western Kenya under the assumption that it will spread and contain populations of *G. brimblecombei*. The current study was designed to evaluate the extent of distribution of *P. bliteus* and determine parasitism rate on *G. brimblecombei* in Western and Eastern, Kenya. During the study, three (3) *E. camaldulensis* plantations in Western Kenya (Busia, Bungoma and Siaya counties) and two (2) in Eastern Kenya (Ndufa and Kiamuringa Kenya Tea Development factory plantations in Embu County) were assessed. Global Positioning System Coordinates of identified plantations were recorded and ten (10) *E. camaldulensis* trees were randomly selected from each plantation and assessed. Four different damage categories by *G. brimblecombei* were assigned to sample trees through visual observation. Adult population of *P. bliteus* was assessed by placing two yellow sticky traps on each sample tree. Three assessments were carried out in three different months and generate data analysed was carried out using packages in R Environment. Distribution maps were drawn using ArcGis to indicate the intensity of spread of the pest in the regions of study. Tree infestations by *G. brimblecombei* were highly varied across the four counties ($F_{3,1492}$= 7.44; $p < 0.001$) and time of assessment ($F_{2,1492}$= 91.91; $p < 0.001$). Embu County had the highest *G. brimblecombei* populations (40.67±1.58), recorded in the month of February with damage ranging between 50–75%. This was followed by Busia (28.79±1.19), Siaya (25.47±1.86) and Bungoma (20.80±1.29) Bungoma recorded the highest parasitism rate of *P. bliteus* (11.61%), followed by Siaya (7.92%), Embu (5.08%) and Busia (3.54%), indicating that *P. bliteus* found its way into Kenya. This study provides important information on the establishment of *P. bliteus* in Kenya and this would guide enable initiation of implementation of Bio-control program for sustainable management of *G. brimblecombei* in Kenya.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General introduction

The influx of alien species has increased exponentially in Africa and other parts of the Globe (Sharma *et al.*, 2013). This is attributed to the increase in travel and trade, and challenges in implementing suitable quarantine regulations. When introduced, invasive species establish and spread sometimes becoming pests that cause a threat to biodiversity and natural ecosystems (Mack *et al*, 2000). Invasive species are considered by International Union for Conservation of Nature (IUCN) as the second source of habitat destruction (IUCN, 2000).

Economically, *Eucalyptus* is the most commonly distributed of all tree species and widely planted tree genus globally. *Eucalyptus camaldulensis* is the most extensively distributed of all *Eucalyptus* species and planted worldwide in arid and semiarid lands (Eldridge *et al.*, 1995). As at 2010, the approximate value of *Eucalyptus* in Kenya was around Ksh.30 Billion which covers about 100,000 ha (Oballa *et al.*, 2010). Currently, the approximate value of *Eucalyptus* in Kenya has increased to about Ksh.90 Billion covering around 140,000 ha both in plantations and onfarm (Cheboiwo *pers comm*, 2020). The rapid growth of *Eucalyptus* and ability to adapt to different ecological conditions favours their extensive cultivation, especially in the tropics, providing global resources such as paper pulp, structural timber, and fuel-wood (Flynn, 2010). Growing of Eucalypts is expanding as a result of the increased demand for fuel-wood, for carbon sequestration, renewable energy, and easing effects of climate change (Stape *et al.*, 2004). In Kenya, exotic phytophagous insects causing economic losses to *Eucalyptus* include *Goniptera scutellatus* (Coleoptera: Cerambycidae) (*Eucalyptus* Snout Beetle) reported in 1940's, *Leptocybe invasa* (Hymenoptera: Eulophidae) (Blue Gum Chalcid) reported in 2004, *Thaumastocoris peregrinus* (*Eucalyptus* Bronze bug) and *Glycaspis brimblecombei*. These pests have lately been reported in all major *Eucalyptus* growing zones of Kenya. In Kenya, Red Gum Lerp Psyllid (RGLP) was first reported in October 2014 according to the Forestry Research Institute of Malawi newsletter (FRIM, 2015). Currently, the pest has been detected attacking *E. camaldulensis* in Central and Western parts of Kenya (Unpublished, KEFRI).

Glycaspis brimblecombei Moore (Hemiptera: Psyllidae), a lerp forming Psylloidea originating from Australia is found to attack Eucalypts (Nadel *et al*., 2010). Nymphal stages of RGLP produce honeydews during initial feeding by manipulating toughening anal exudates in species-specific scale-like plates (Sharma *et al.*, 2013), and uses wax secretions to form tapered white shelters called lerps on leaf surfaces for defence (Sharma *et al.*, 2013). Formation of lerps is an evolutionary mechanism to allow the insect overcome desiccation in the environment of inland Australia and furthermore, these lerps shelter the nymphal stages from their parasitoids and predators (Sullivan *et al.*, 2006).

Glycaspis brimblecombei female adult deposits 6-45 eggs for each eucalypt leaf, commonly on the young foliage (Firmino - Winckler *et al*., 2009). The life cycle takes 15-34 days with multiple generations in a year (2-4 in Australia) depending on temperature (Laudonia *et al*., 2014, Tuller *et al*., 2017). Adults are 4-5 mm, about 1/8 inch long from head to wing tip, slender, light green or brown with some orange and yellow blotches. The adult stage occur on foliage and do not live under lerp shelters (http://ipm.ucanr.edu/PMG/PESTNOTES/pn 7460. html). In Australia, RGLP prefers feeding on *E. camaldulensis* (Myrtacaea) and associated natural enemies, for instance parasitoids and predators apply sturdy management of the psyllid populations (Collett, 2001). Dense infestations result in leaf chlorosis, leaf drop and twig dieback which may result to plant death (Tuller *et al*., 2017).

The principal natural control agent from Australia, a parasitoid known as *Psyllaephagus bliteus* Reik (Hymenoptera: Encyrtidae), has been studied to control *G. brimblecombei* (Daane *et al.,* 2002). *Psyllaephagus bliteus* exists as a koinobiont parasitoid; female adults like to deposit eggs on the third instar stage of *G. brimblecombei*, but progeny development is deferred till the psyllid hatches into the fifth nymphal stage (Daane *et al.*, 2005). White and dark parasitoid bodies are seen through the transparent membrane of mummified psyllid nymphal stage (Daane et al., 2002). As a strategy to manage *G. brimblecombei* on Eucalypts, Forestry Research Center, Zimbabwe involved expertise of the Tree Production Cooperative Program (TPCP), Forest and Agriculture Biotechnology Institute (FABI) in the University of Pretoria, South Africa to undertake studies on the utilization of *P. bliteus* as a parasitoid to manage *G. brimblecombei*. The parasitoid was released in some parts of South Africa over the Limpopo Province near Zimbabwean boarder (FAO, 2016). Ugandan NaFORRI released *P. bliteus* in *Eucalyptus* plantations neighbouring Western Kenya with an intention to manage spread of *G. brimblecombei*. This was as a result of the Eucalypts pest distribution to Kabale District according to FAO (2016).

The foremost objective of this work entailed studying the status on distribution of *G. brimblecombei* and parasitism rates of *P. bliteus* in Western and Eastern Kenya. This study will inform the implementation of biological control as a management option. A sustainable Integrated Pest Management strategy would therefore be enhanced to ensure improvement of livelihood of different communities in Kenya that depend on *Eucalyptus* for their income and as a source of energy to fuel production process in fuel-wood dependant companies in Kenya.

1.2 Problem statement and justification

Glycaspis brimblecombei attack on Eucalypts result in leaf chlorosis, leaf drop and twig dieback, however, heavy infestations may result to death of trees. In Kenya, the Lerp Psyllid was first reported in October 2014, in Kiambu County and is currently reported in many *Eucalyptus* plantations and on-farm trees in the country. The drier parts of Eastern Kenya are known to have heavy *G. brimblecombei* infestations, especially in Kenya Tea Development Agency (KTDA) *Eucalyptus* planted areas. *Glycaspis brimblecombei* infestation can result in crown thinning, 20% - 30% defoliation and *Eucalyptus* death (Wilcken *et al.,* 2003; Queiroz-Santana and Burckhardt, 2007). The lerps formed on the adaxial lamina reduces the photosynthetic area hence a drop in nutrients synthesis in developing Eucalypts. There is an exigent need to develop strategies intended to sustainably control this pest in Kenya.

An Integrated Pest Management (IPM) approach known to combine different management methods has formerly been used in other countries to reduce the effect of *G. brimblecombei* to levels below economic injury. In Uganda, NaFORRI released *P. bliteus* in their *E. camaldulensis* plantations neighbouring Western Kenya between 2017 and 2019 with an intention to manage *G. brimblecombei*. Determination of whether *P. bliteus* has found its way in Kenya would provide important information for implementation of Biological control program against RGLP. However, despite the economic injury it causes in terms of loss of quality and quantity of timber and other products of *Eucalyptus*, there is limited literature on the spread and distribution of Lerp Psyllid and its parasitoid *P. bliteus* in Kenyan forest systems.

1.3 Objectives

1.3.1 General objective

To determine distribution of *Psyllaephagus bliteus* in Eastern and Western Kenya, and determine its potential in management of *Glycaspis brimblecombei*

1.3.2. Specific objectives

i. To assess the distribution of *G. brimblecombei* and *P. bliteus* in Eastern and Western Kenya

ii. To determine parasitism rates of *P. bliteus* in *G. brimblecombei* attacking *E. camaldulensis* plantations in Eastern and Western Kenya.

1.4 Research hypothesis

Since the release of *P. bliteus* in Uganda in 2017, the parasitoid has spread to Eastern and Western parts of Kenya and significantly affected populations of *G. brimblecombei*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Major invasive pests of *Eucalyptus* species in Kenya

The emergence of invasive pests has become one of the biggest threats to growth of *Eucalyptus* (Myrtaceae) in various parts of the world. In Kenya, there are four major invasive insect pests of *Eucalyptus* which are considered to be the most destructive. The Red Gum Lerp Psyllid (RGLP), *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), is the most destructive *Eucalyptus* pest (Mendel *et al.,* 2004), which was registered for the first time in a certain region in Italy in 2010 (Laudonia and Garonna, 2010). The eggs are laid on the leaves, and here, nymphs develop inside shielding white conical coverings called lerps. Adults and the nymphal stages are phloem feeders which produce high amounts of honey-dew that act as substrate for sooty mold development (Mendel *et al*., 2004). The damage as a result of leaf feeding include foliar discoloration, dieback, early leaf fall and reduced plant growth (Daane *et al*., 2005). Defoliation in consecutive years can lead to the death of young plants or susceptible clones (Laudonia and Garonna, 2010). *Glycaspis brimblecombei* has spread to most of the *Eucalyptus* growing areas of Kenya.

The specific koinobiont parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) has effectively been used to manage RGLP in classical biological control programs in Mediterranean-climate regions (Mendel *et al*., 2004). Parasitoid oviposit preferentially on third and fourth instar nymphs and the egg develops inside the fourth and fifth instar (Daane *et al*., 2005).The parasitoid was initially released in Uganda (FAO, 2016)and no study has been undertaken to determine if the parasitoid found its way into Kenya.

Winter Bronze Bug, *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae) is a sap-sucking pest infesting *Eucalyptus* plantations. It originates from Australia (Noack & Rose, 2007; Nadel *et al.,* 2010), where it has been reported as a harmful species since 2002. The Bronze bug is now widespread in many other areas since its escape from the endemic area in 2003 (Jacobs & Kirkaldy, 2005). The Bronze bug is known to attack at least 30 *Eucalyptus* species and three common commercial hybrids (Noack & Coviella 2006). Since its invasion in Kenya in Nov. 2009, it has currently been reported in all major *Eucalyptus* growing areas (Mutitu *et al*, 2013). Classical biological control programs have been implemented through the introduction of egg parasitoid *Cleruchoides noackae* where effective management has been achieved (Mutitu *et al*, 2013).

Eucalyptus Snout Beetle, *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae) is also native to Australia. Different species in closely related to this genus *Gonipterus* have been inadvertently introduced in Africa, Europe, America and New Zealand (Mapondera *et al*., 2012). *Gonipterus scutellatus* was first recorded on *Eucalyptus* in Italy in 1975 (Arzone, 1976).

Blue Gum Chalcid (BGC), *Leptocybe invasa*, Fisher & La Salle is capable of causing severe injury to young foliage of *Eucalyptus.* This is by causing galls on nursery and in fields on young saplings, in *Eucalyptus* plantations, on shoots, and even on the new shoots of old trees. Mendel *et al*. (2004) reported that the wasps lay eggs in the petiole and midrib of leaves and stems of the young shoots that leads to galling. Gall formation by BGC damages growing shoots tips and leaves resulting in quicker abscission of leaves and drying up of shoots. The gall wasps cause adversative effect on their host by depriving them of nutrients and galling the plant tissue (Stone & Schonrogge, 2003), which prevents further growth of the infested shoots.

Eucalypts are native to Australia and are grown in many areas as exotics in the world. In Kenya, *Eucalyptus* were introduced before 1902 (Oballa, 2010). About 100 species/hybrid clones have since been introduced and planted at KEFRI Arboretum, Muguga (Gottneid & Thogo, 1975). The reason of the first introduction was to identify fast growing *Eucalyptus* species to supply wood fuel for the Kenya-Uganda railway (Oballa, 2010). The usages of *Eucalyptus* have since increased and now include; pulp, plywood, timber, transmission poles, fencing posts, building materials, ornamentals, windbreaks, rails and enhancement of the environment. Eucalypts provide products that would otherwise be sourced from the natural forests (Speight & Wainhouse, 1989). They are preferred because of their rapid growth and are easy to cultivate and acclimatize to a wide range of growing conditions (Oballa, 2010). The low levels of pest epidemics in mixed tropical forests are often cited as proof for the importance of biodiversity in stabilizing different plant communities (Speight & Wainhouse, 1989). Use of exotics in forest plantation programs, have been linked with outbreak of invasive insect infestations and diseases (Mutitu *et al*., 2013).

In Kenya, on a conventional potential monetary value, grown *Eucalyptus* trees are valued at 30 billion Kenya Shillings, arising from the estimate of 100,000 ha under *Eucalyptus* owned by famers and local authorities (Oballa *et al*., 2010). With such high monetary value, it is necessary to protect farmers from economic losses that may arise from pest damage. In so doing, Integrated Pest Management (IPM) program which entails biocontrol programs, which are environmentally friendly pest control strategies, are put in place. This will enhance sustainable intervention measures to keep pests below economic injury levels.

2.2 *Glycaspis brimblecombei*

2.2.1 Taxonomy of *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae)

Glycaspis brimblecombei belongs to order Hemiptera, family Psyllidae commonly called jumping plant lice or psyllids. Genus Glycaspis contains approximately 140 species (Moore 1970a). Notably, *G. brimblecombei* is an invasive pest of *E. camaldulensis* commonly known as the Red Gum lerp psyllid, RGLP. Red Gum denotes the most favoured hosts, *Eucalyptus sp.* in the River Red Gum comprising *E. camaldulensis,* and a lerp, is an indigenous word for the cone-shaped cover comprised of excreted honeydew and other chemical components secreted by malpighian tubules of nymphal stages. Not all *Glycaspis sp.* produce lerps, and shapes and the general appearance of lerps can be species-specific (Moore, 1964).

2.2.2 Distribution of *G. brimblecombei*

Glycaspis brimblecombei was first reported in SãoPaulo State, Brazil in 2003 (Wilcken *et al*., 2003, Queiroz-Santana and Burckhardt, 2007) and quickly spread all over the country (Pereira *et al*., 2013). In Italy, *G. brimblecombei* was recorded for the first time in 2010 (Laudonia and Garonna, 2010). *Glycaspis brimblecombei* is believed to have found its way to South Africa through mediums like tourism, trade and transport (Mutitu *Pers comm*). It spread to neighbouring countries including Uganda and Kenya through porous borders of Mozambique. In Kenya, *G. brimblecombei* was recorded in 2014 and has been observed to spread quickly with its damage symptoms easily visible on affected crowns.

2.2.3 Biology of *G. brimblecombei*

Eggs are laid indiscriminately on leaves or in 50-75 egg clusters, at an angle perpendicular to the host surface. Eggs are yellow, spindle-shaped whose size is slightly less than 1mm. The eggs hatch within 10 to 35 days and this depends on temperature and also other environmental conditions (FAO, 2012).

There are five instar stages and their sizes vary to the last instar which is about 1.5-2.0 mm in length. The nymphs are yellow to orange, with brown wing pads, antennae, legs, last abdominal segments and on the blotches found on the dorsal area of thorax and head (Collet, 2001). Nymphs increase in size and move to form a new lerp (Olivares *et al*., 2004).

Crawlers and young nymphs are located entirely on hid mature leaves (Halbert *et al.*, 2001). Nymphs use the lerps covering as a source of protection from predators and shelter from external harsh environmental conditions. Usually, lerps with nymphs occupying have tendrils of wax protuberant from the upper surface (Collet, 2001). Nymphs are sap suckers and are known to cause damage on host foliage (Olivares *et al*., 2004).

Adults are about 4-5 mm long from the wing tips to the head. They range from yellow to light green with conspicuous dark eyes (Sullivian *et al*., 2006), and dorsally flat thorax (Moore, 1964). The exposed genal cones used to identify these psyllids morphologically consists a pair of cone shaped extensions, which are extremely long, even longer than the head (Tuller, 2017). Adults are not sap suckers like in the case of nymphal stages, hence not destructive (Olivares *et al*., 2004).

2.2.4 Identification of damage symptoms of *G. brimblecombei*

Infested leaves are evident due to numerous white lerps on surfaces of leaves. High populations of *G. brimblecombei* on infested leaves produce high quantities of waxy secretions and honeydew which forms media for fungal growth, hence sooty moulds formation and premature leaf drop (Moore, 1964). The honey dews and wax secretions are used to form lerps. Lerps can be 1-4 mm long depending on development stage of the instar, white in appearance, which changes from grey to black coloration if sooty mould start to form on the lerp (Tuller, 2017). Lerps are large enough to allow nymph move within and avoid natural enemies ovipositing through the thin walled lerp of third nymph (Sullivan *et al*., 2006).

Heavy and prolonged infestations cause extensive defoliation which weakens trees leading to untimely death of predisposed species, especially *E. camaldulensis* (Moore, 1964). Honeydew encourages the unappealing development of some black sooty moulds observed on branches and leaves, while honeydew sopping from infested trees tarnishes sidewalks and concrete walls (Olivares *et al*., 2004).

2.2.5 Economic significance of *G. brimblecombei*

Glycaspis brimblecombei is considered a serious pest that causes defoliation as well as tree mortality on some Eucalypts, and has been included in the EPPO list of quarantine species since 2002 (Laudonia *et al*., 2014). In Southern California, thousands of established *E. camaldulensis* were killed in about 2-3 years by unmanaged populations of *G. brimblecombei* (Wilcken *et al*., 2003). High density *G. brimblecombei* populations secrete abundant honey-dew and pressure from excessive feeding can cause premature leaf drop. Honey dew excretion from heavily infested trees promotes growth of sooty moulds on foliage and branches and staining of concrete walls and sidewalks (Olivares *et al*., 2004). This destructive sap-sucking insect result to reduced photosynthetic area and crown die back, stunted growth of young trees and severe attacks may kill trees (Wilcken *et al*., 2003). A survey carried out by KEFRI indicated that farmers have lost timber from attacked trees which have stunted growth and delayed realization of tree products due to attack of trees by *G. brimblecombei* (Mutitu *pers comm*)*.* The dropped white lerps and stained leaves cover the ground and understorey giving a hail appearance.

2.2.6 Management of *G. brimblecombei*

In the recent past, *G. brimblecombei* was managed by use of different parasitoids including lady beetles, *Harmonia axyridis* and *Chilocorus bipustulatus* which were not effectively keeping the populations of *G. brimblecombei* below economic injury level (Dahlsten *et al*., 2005). Cultural control measures according to Dreistadt *et al*. 2004 include minimizing stress in trees by providing silvicultural practices since establishment of saplings and protecting trees from severe injuries. Supplemental water during periods of prolonged drought once a month is encouraged. Too much watering promotes new growth and increased Nitrogen content on the new flashy leaves which encourages establishment of *G. brimblecombei* (Tuller *et al*., 2017). Fertilizing Eucalypts in high-risk areas is detrimental but use of systemic foliar spray chemicals with caution on high value crop like ornamental trees can save a tree (Dreistadt *et al*., 2004).

2.2.7 Host range of *G. brimblecombei*

Glycaspis brimblecombei is a major threat in *Eucalyptus* plantations in the Latin America and countries in Africa. According to Moore 1970a, common hosts of *G. brimblecombei* include; tumble-down red gum (*E. dealbeta*), River red gum *(E. camaldulensis)*, Blakely's red gum *(E. blakelyi)*, Forest red gum *(E. tereticornis)* and Shining gum *(E. nitens).* Other hosts include; Tasmanian blue gum (*E. globulus)* and Karri (*E. diversicolor*) (Brennan *et al*., 2001a). The degree of resistance to *G. brimblecombei* attack by different Eucalypts was studied which showed *E. camaldulensis, E. tereticornis* and Flooded gum (*E. rudis)* having the lowest resistance to the psyllid attack (Brennan & Weinbaum 2001).

2.2.8 Classical biological control of *G. brimblecombei*

Classical biocontrol program involves studying the parasites, predators and pathogens that may be used in controlling a pest in a native habitat. Once the natural enemies expected to be effective are identified, screening process in a quarantine facility is conducted in order to determine whether the natural enemies can be introduced safely (Dahlsten *et al*., 2003). This is followed by mass rearing of the natural enemies and release.

A natural control program against *G. brimblecombei* used a parasitoid *P. bliteus,* native to Australia (Paine *et al*., 2000). This parasitoid was released widely in California from 2000 to 2002 after quarantine screening studies showed no significant risks posed to other species (Daane *et al.,* 2012). Parasitism entails female parasitoids puncturing nymphs using an ovipositor and ovipositing inside the host (Paine *et al*., 2000). These studies also showed that *P. bliteus* lays eggs in third and fourth nymphal stages (Daane *et al*., 2012). The egg hatches and *P. bliteus* larva feed on the host visceral organs and hence killing it. On completion of development, the parasitoid pupates and using its mandibles, it chews a circular exit hole which is an indicator of presence of *P. bliteus* attacking *G. brimblecombei* (Daane *et al.,* 2012). Other methods that the female parasitoid kill *G. brimblecombei* is through; host-feeding and feeding on lerps to boost their nutrition (Dahlsten *et al*., 2003).

The control program of *G. brimblecombei* using *P. bliteus* has been successful in California's coastal regions but only sporadic control has been provided in the hot and dry regions (Dhahir *et al*., 2014). Biological control program is influenced by pesticide use and cultural practices and hence integration of these activities makes it an effective pest management option (Daane *et al*., 2012)

2.3 The parasitoid, *Psyllaephagus bliteus*

2.3.1 Taxonomy of *P. bliteus*

Psyllaephagus bliteus belongs to Order Hymenoptera and family Encyrtidae. Psyllaephagus is a diverse genus comprising over 200 described species as described by Noyes & Hanson, 1996. Their utmost taxonomic divergence occurs in Australia where prevalent Psyllaephagus species attack instars of Psylloidea, while a small number is reported as hyper-parasitoids that attack other species of Psyllaephagus (Noyes & Hanson, 1996; Riek, 1962). The specificity and efficacy of this parasitoid has made it a suitable candidate in classical biological control programs targeting *Eucalyptus* psyllid-pests. This parasitoid is native to Australia which was released in California between 2000 and 2002 to control

RGLP, after rigorous studies in quarantine showed that it caused no major risk to untargeted organisms.

2.3.2 Life cycle of *P. bliteus*

Psyllaephagus bliteus shows preference to third and fourth instars of Red Gum Lerp Psyllid (Dahlsten *et al.,* 2003). However, the parasitoid is capable of parasitizing all stages, sometimes physically killing nymphs by stabbing them with ovipositors and sucking the liquids from nymphs (Dahlsten *et al*., 2005).The adult female wasp uses the ovipositor to insert one egg into lerps (Daane *et al*., 2002). Eggs develop inside the nymphs and time of development depends on weather conditions. The adult parasitoids emerge from the lerps through a characteristic circular exit hole.

2.3.3 Host range and specificity of *P. bliteus*

Sometimes bio-control programs have failed due to paucity of literature on biology of natural enemies on target and non-target pests and host plant populations (Erbilgin *et al*., 2004). *Psyllaephagus bliteus* is a specific parasitoid of the *Eucalyptus* pest *G brimblecombei* (Laudonia *et al*., 2014). Host specificity studies were done on important parasitoids in the American Agro-Industry and were found ineffective (Dahlsten *et al*., 2003).This shows that *P. bliteus* is host-specific to *G. brimblecombei* and will only attack the psyllid. The intrigued predation, a phenomenon in which predators lessen the usefulness of natural control agent can negatively impact the progress of the bio-control agent program (Laudonia *et al*., 2014). Studies have shown that a bug *Anthocoris nemoralis* (Fabricius) (Heteroptera: Anthocoridae) predates on *P. bliteus* and the mummified *G. brimblecombei*, hence reducing the effectiveness of the parasitoid (Erbilgin *et al*., 2004)

2.3.4 Parasitism mode of *P. bliteus*

Female parasitoids use their ovipositors to rupture nymphs and lay an egg into the body of the instar. Laboratory bioassays have shown that *P. bliteus* can oviposit in psyllid instars of any age, but females prefer the third and fourth instar (Dahlsten *et al*., 2003). The egg of the parasitoid hatches and the *P. bliteus* larva feed and kill its host. The dead parasitized host exoskeleton forms a mummy, and the nymphal stage is used up by the developing parasitoid. Through the *G. brimblecombei* mummified exoskeleton, which becomes transparent, a black maturing wasp is detected during the stages of development (Sullivian *et al*., 2006).To escape from the body of the host, the parasitoid uses mandibles to make a distinctive exit hole. The occurrence of these exit holes indicates that *P. bliteus* is present and parasitizing *G. brimblecombei* nymphs (Dahlsten *et al*., 2003).

2.3.5 Known distribution of *P. bliteus*

According to literature, *P. bliteus* is distributed in Australian Capital Territory, South Australia, New South Wales, Queensland, New Zealand (Withers, 2008), North America, Spain, Morocco (Bami, 2011). *Psyllaephagus bliteus* has also been reared and released in Mexico and Chile (Ide *et al.,* 2006). Forestry Research Center (FRC), Zimbabwe engaged expertise of Tree Protection Co-operative Program (TPCP), Forestry and Agriculture Biotechnology Institute (FABI), and the University of Pretoria in South Africa to conduct studies on the use of *P. bliteus* as a parasitoid in Ugandan plantations neighbouring Western Kenya (FAO, 2010).

2.3.6 Other natural enemies against *G. brimblecombei*

Lady beetles *(Harmonia axyridis)* is one of the natural enemies which occur in large numbers in Ardenwood, California. Other predators include spiders, mites, syrphid fly larvae; C*occinella californica, Hippodamia convergens* and *Zelus renardii* have been found attacking *G. brimblecombei* nymphal stages (Dhahir *et al.,* 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was carried out in five sites: three sites in Bungoma, Busia and Siaya Counties (one site per County) representing the Western region, and two sites in Embu County, Eastern Kenya (Fig. 3.1). *Glycaspis brimblecombei* and *P. bliteus* were assessed on *E. camaldulensis* plantations in Bungoma, Busia and Siaya Counties with close proximity to release sites by NAFORRI while Embu County has vast Kenya Tea Development (KTDA) *E. camaldulensis* plantations with a history of *G. brimblecombei* invasion.

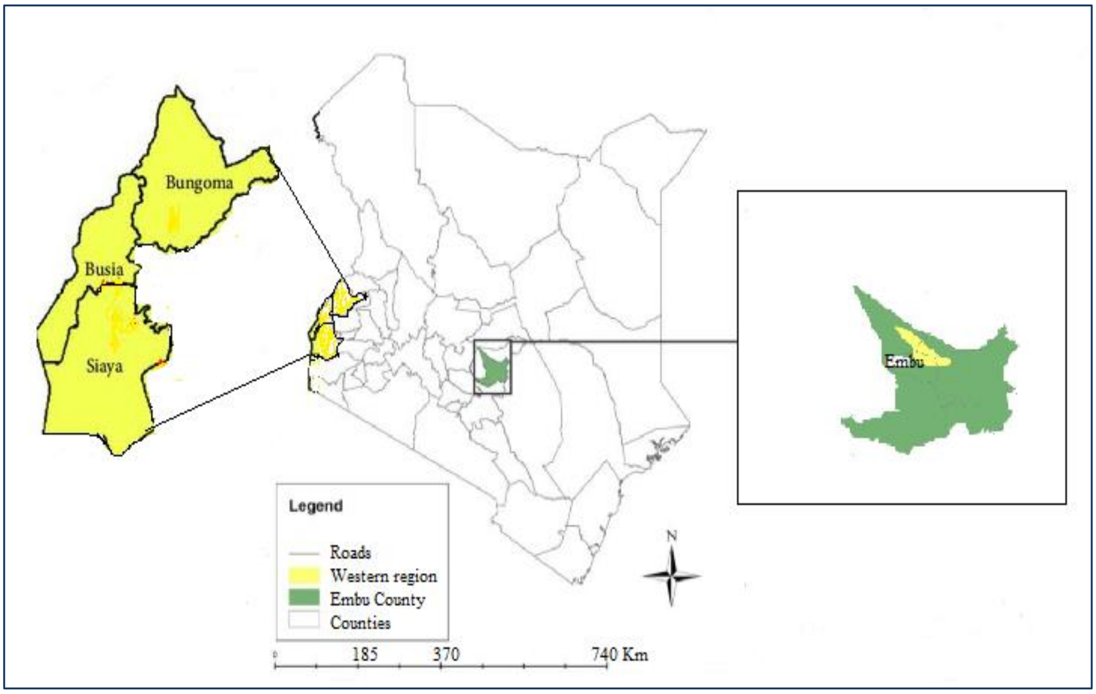

Figure 3.1: The study area: Bungoma, Busia, Siaya and Embu Counties

3.2 Assessment of current distribution of *G. brimblecombei* and *P. bliteus* in Eastern and Western Kenya

This entailed identification of sample trees, determination of incidence and severity of *G. brimblecombei* and assessment for *P. bliteus* presence / absence on sample trees.

3.2.1 Identification of sample trees

Identification of the sample sites with *E. camaldulensis* plantations was done in consultation with Kenya Forest Service (KFS) and regional Ecosystem Conservators (EC). A total of Five (5) *Eucalyptus* plantations located in the study sites were assessed. Three *E. camaldulensis* plantations were identified in Western region as follows; Bumtiru in Busia

County, Bosio in Bungoma County and Nyaranga Primary School, Siaya County. Two *E. camaldulensis* plantation sites were identified in Kiamuringa and Ndufa, belonging to KTDA in Embu County, Eastern Kenya. Each plantation consisted of at least 50 trees aged between 2 and 3 years.

While considering the edge effect, ten sample trees were randomly selected from each plantation and Global Positioning System (GPS) Coordinates of the sample trees recorded using My Handy GPS mobile phone application. The GPS readings were collected about 2m from the edge of the plot and this information was used to generate distribution maps as described by Mutitu *et al*. (2013). This provided information on the incidence and severity patterns of *G. brimblecombei* and distribution of *P. bliteus* within the regions of study.

3.2.2 Time of assessment

In Western region, three assessments were carried out in the months of April, June and December while the months of assessment were February, October and December in Eastern region. This was dependent on availability of facilities to undertake the study. Assessment was carried out in months with unique climate characteristics; from hot and dry, a transitional month and a wet month. In Western Kenya April represents month of highest rainfall and lowest temperature, December represents month of lowest rainfall and highest temperature while June is transitional month with moderate rainfall and temperature. In Eastern Kenya (Embu County), October represents month of highest rainfall and lowest temperature, February represents month of lowest rainfall and highest temperature while December is transitional month with moderate rainfall and temperature.

Table 3.1 indicates average annual temperature, average annual rainfall and periods of rainfall seasons of the four selected counties (https://weatherspark.com/y/98118).

Table 3.1: Mean temperature and rainfall conditions in surveyed counties

County	Time of assessment	Mean monthly parameters	
		Temperature (°C)	Rainfall (mm)
Bungoma	December/2021	21.1	533
	April/2022	20.5	2,057
	June/2022	21.0	1016
Busia	December/2021	22.8	711
	April/2022	21.7	2,159
	June/2022	21.1	940
Siaya	December/2021	22.8	889
	April/2022	22.7	2,413
	June/2022	21.1	914
Embu	October/2021	20.6	635
	December/2021	20.0	584
	February/2021	21.7	203

3.2.3 Incidence and severity of *G. brimblecombei* on *E. camaldulensis*

The infestation of *G. brimblecombei* (i.e. the lerps abundance) was determined through direct observation of sample trees and examining presence/absence of white lerps and darkened sooty mold on the tree foliage canopy as described by Alejandro *et al.,* (2016).

During this study, *G. brimblecombei* psyllids were distinguished from other psyllids through long cephalic projections below the eyes, which are indicated as genal processes according to Laudonia and Garonna, 2010. Lerps in this study were identified as honeydew built white cover on sample trees (Firmino-Winckler *et al*., 2009), with a maximum size of 3.0 × 2.0 mm according to Ide *et al*. (2006).

Severity of *G. brimblecombei* on *E. camaldulensis* sample trees was assessed by assigning tree damage category levels. The level of damage on a four-point scale was determined by percentage of white lerps and darkened sooty mold symptoms on the sample tree canopy foliage. The four-point scale damage categories were as stated below: (i) Category 1: No (0) canopy damage (= No infestation by target pest), (ii) Category 2: between 0.1-25.0% canopy damage (= slight infestation), (iii) Category 3: Between 25.1 – 50.0% canopy damage (= moderate infestation), (iv) Category 4: Greater than 50.1% canopy damage (= high infestation). The near neighbours of experimental sites were assessed for presence of *G.*

brimblecombei, a requirement during development of the pest distribution map in Eastern and Western Kenya (Appendix 2).

3.2.4 Presence/absence of *P. bliteus* on the sample trees and in lerps on sample leaves

The adult *P. bliteus* population was sampled by setting two yellow sticky-traps (10 cm x 15 cm) on branches about 1.5m above the ground level on. Through visual observation, leaves in each sample tree were assessed for presence of *G. brimblecombei* and a yellow sticky trap set on a randomly selected branch to sample mature *P. bliteus.* The yellow sticky traps were changed during each assessment period and the number of captured adults counted. Using hand lenses, the number of lerps with distinct parasitoid escape holes on randomly selected ten (10) leaves from the sample trees (Dahlsten *et al*., 2005: Ferreira *et al*., 2008) was recorded. Parasitized psyllid nymphal stages (those made by fourth and fifth nymphs) were identified by visual observation of lerps on sample leaves placed on a white sheet background and opening lerps for viewing under a hand lens. The near neighbours of experimental sites were assessed for presence of *P. bliteus*, which was required during development of distribution maps (Appendix 2). The insects collected on the traps were carried to Kenya Forestry Research Institute (KEFRI) Insect Reference Collection (IRC) for identification.

3.3 Establishment and parasitism rates of *P. bliteus* in *G. brimblecombei* attacking *E. camaldulensis*

Field and laboratory assessment of parasitized lerps presence/absence was carried out using hand lenses and microscopy.

3.3.1 Assessment of lerps for parasitism rates determination

Unparasitized juveniles (nymphs) lerps, lerps with exit holes and mummified lerps (the parasitized nymphs) were recorded (from sample leaves collected in objective 1), giving the total number of lerps counted. Counting of lerps was carried out on both abaxial and adaxial sides of the 10 sampled leaves using a hand lens. Parasitism rate (PR) was calculated as the percentage of parasitized psyllid (lerps with exit holes and mummies/ total number of lerps).

$$PR = \frac{No.of\ lerps\ with\ exit\ holes + No.of\ parasitoid\ with\ immature\ stages\ (Mummies)}{Total\ number\ of\ counted\ lerps} \times 100$$

3.3.2 Laboratory assessment of parasitized lerps presence

Five *E. camaldulensis* infested leaves from each of the ten sample trees were carefully cut at about 1.5-2m high using a pair of sharp scissors and placed in separate plastic zip-lock bags. Sampled leaves were packed in plastic insect rearing containers labelled with information on collection point. The containers were placed in cooler boxes and ferried to KEFRI Quarantine facility. In the quarantine facility, sampled leaves were put in insect rearing cages and monitored for 5 days for *P. bliteus* emergence.

3.3.3 Microscopic observation of the nymphs

The lerps without holes were opened and nymphal instars extricated gently with a fine needle. Nymphal instars were obtained using fine-tipped camel hair brush and gently transferred into vials with 70% alcohol for observation. Nymphs were placed on a Whatman® filter paper and observed under a dissecting microscope to determine presence/absence of *P. bliteus* and parasitism. Field and laboratory studies were carried out simultaneously during the assessment period.

3.4 Data analysis

Data was recorded on customised data capture sheets and managed using MS Excel. Species distribution maps were generated using Geographical Information System (GIS) software, ARC-GIS (Corbett *et al*., 2001) to inform the intensity of spread of *G. brimblecombei* and *P. bliteus* in Embu, Siaya, Bungoma and Busia counties. Statistical analyses were performed in R environment, a Statistical software Version 4.2.1 (R Core Team, 2011). Proportions of sampled trees per damage category levels were obtained for the different counties, during the different assessment months. Parasitism rates were expressed as percentages. Homogeneity of variances was determined using Bartlett test. Shapiro Wilks test was carried out to assess normal distribution of data. Log transformation ($\log_{10}[X+1]$) was undertaken to normalize the data before subjecting them to parametric tests.

Damage category and percentage parasitism data that did not meet assumption of parametric statistics after log transformation (Shapiro Wilks test; $p < 0.0001$) were subjected to Generalized Linear Model (GLM). Count data on the number of lerps per leaf, number of lerps with exit holes, number of mummies had zero counts and were analyzed using Poisson regression model. In this study, significance level was set at a critical value of $\alpha=0.05$. Means were separated using the Tukeys' HSD (Honest Significant Difference) post-hoc test where significant differences were determined among the tested parameters.

CHAPTER FOUR

4.0 RESULTS

4.1 Observed parameters of the study sites

Parameters in the surveyed counties varied immensely with Embu and Siaya being the driest during the entire assessment period while Busia and Bungoma were characterized by some showers in the months of April and December. In all assessment sites, the month of December was hot and dry with a high number of lerps observed on *Eucalyptus* foliage. Multiple generations observed on the sample leaves displayed the five nymphal instars and adults laying eggs during the same assessment period. As the nymphal stages tend to 5th instar, the newly formed lerp becomes more elaborate taking a bigger surface area on the leaf. More whole and well-formed lerps were an indication of maturing nymphal stages of *G. brimblecombei*. In some other lerps, sooty mould formed on the honeydew constructed lerps which eventually covered the entire lerp. Some lerps formed along the midrib of sample leaves while others were evenly distributed on the adaxial and abaxial lamina. Concentration of the lerps along the midrib indicated location of resources which were readily available for sucking from the translocation structures (Fig. 4.1).

Figure 4.1: Observed parameters of the study sites
Generations of *G. brimblecombei* from young lerps to older lerps covered in sooty moulds (A); Heavy infestation of *G. brimblecombei* under damage category 4 (B); An old attack of *E. camaldulensis* by *G. brimblecombei* (C); Hardening of leaves after *G. brimblecombei* attack (D); A young sapling showing *G. brimblecombei* damage category 3 (E); A new *G. brimblecombei* infestation vs. an old attack (F)

4.2 Distribution and tree damage intensity by *G. brimblecombei* in Western and Eastern Kenya

The current distribution of *G. brimblecombei* in Western and Eastern Kenya is demonstrated through a distribution map in Fig. 4.2. Different damage levels indicated the level of severity in spread.

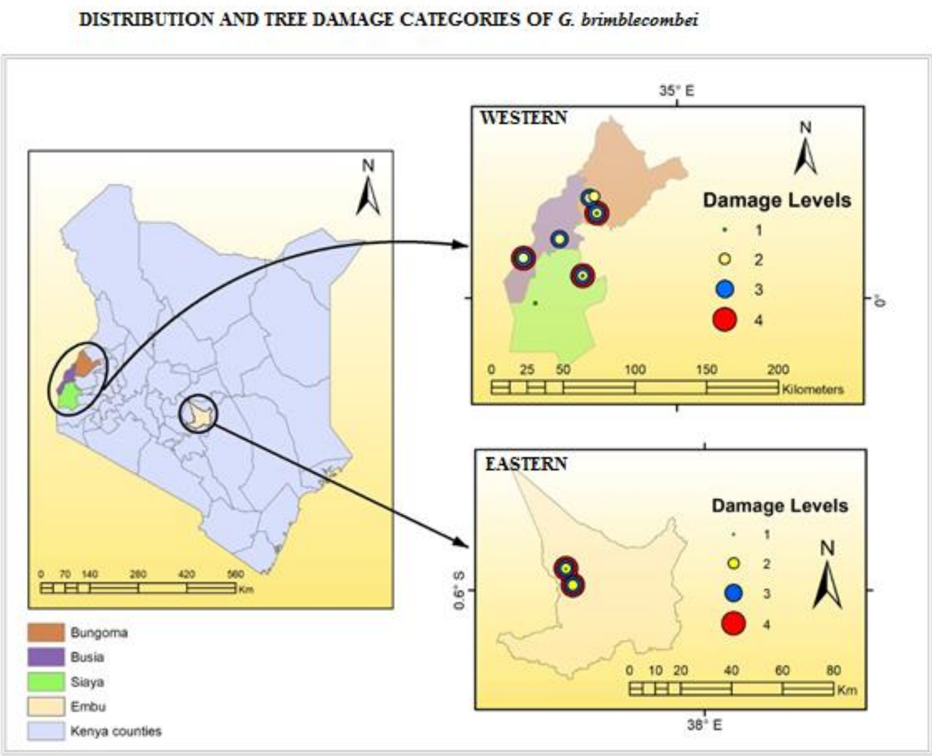

Figure 4.2: Current distribution and tree damage categories by *G. brimblecombei* in Western and Eastern Kenya

The number of lerps per sample Eucaplytus tree as indication of *G. brimblecombei* spread varied significantly among the four counties ($F_{3,994} = 8.31$; $p < 0.001$) and between time of assessment ($F_{2,994} = 74.58$; $p < 0.001$). The highest abundance of *G. brimblecombei* was recorded in Embu County (16.44±0.95), followed by Busia County (10.83±0.99) and Bungoma County (10.11±1.26) and lowest in Siaya County (7.26±0.63) (Table 4.1).

Table 4.1: The abundance of *G. brimblecombei* lerps of psyllids in *Eucalyptus* trees in four counties of Kenya

County	Assessment time (month/year)	Mean (Mean ± SE) of lerps per tree	Overall means (Mean ± SE)
Bungoma	December/2021	12.30±1.33^{b}	
	April/2022	0.52±0.26^{a}	10.11±1.26^{b}
	June/2022	17.50±2.19^{c}	
Busia	December/2021	5.24±0.71^{b}	
	April/2022	0.08±0.04^{a}	10.83±0.99^{b}
	June/2022	27.16±2.21^{c}	
Siaya	December/2021	4.60±0.40^{b}	
	April/2022	0.10±0.10^{a}	7.26±0.63^{a}
	June/2022	17.07±1.39^{c}	
Embu	October/2021	17.43±1.17^{b}	
	December/2021	9.17±0.69^{a}	16.44±0.95^{c}
	February/2022	22.72±0.99^{c}	

Different small letters following the mean and standard error (SE) indicate significant differences within different counties during different months of assessment in column 3 (mean of lerps per tree) while in column 4 (overall mean), the different small letters indicate significant differences between different counties during different months of assessment. The significance was determined at p =0.05 using Tukey test

4.3 Severity of *G. brimblecombei* on *E. camaldulensis* trees

Tree damage by *G. brimblecombei* were highly varied across the four counties ($F_{3,1492}$=7.44; p < 0.001) and time of assessment ($F_{2,1492}$=91.91; p < 0.001). Generally, Bungoma had lowest infestation (20.80 ± 1.29). Moderate infestations of 28.79 ± 1.19 and 25.47 ± 1.86 were recorded in Busia and Siaya Counties respectively while Embu County had the highest severity at 40.67 ± 1.58. In Western Kenya, high levels of *G. brimblecombei* infestations were evident during hot and dry month (December) and transitional month (June). In Eastern Kenya (Embu County), the *brimblecombei* infestations were relatively high in the three months of assessment, with highest infestations recorded in February, followed by October and December (Fig. 4.3).

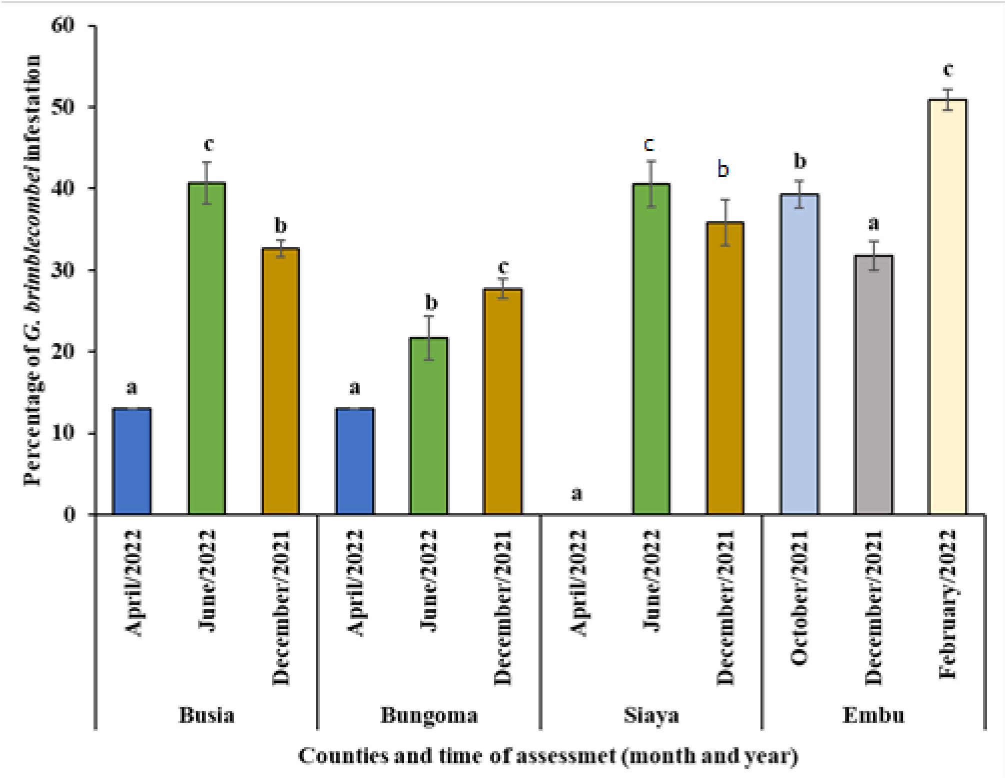

Figure 4.3: Proportion of *G. brimblecombei* infestation on *E. camaldulensis* trees among sampling time within counties.

Different small letters above error bars indicate significant difference across the times of assessment at $p = 0.05$ according to the Tukey test.

4.4 Variations among tree damage categories in different counties

Tree damages varied significantly among different tree damage categories ($F_{3,990} = 8.11$; $p < 0.001$), counties ($F_{3,990} = 18.36$; $p < 0.001$) and time of assessment ($F_{2,990} = 29.76$; $p < 0.001$). Damage category 4 was highest in Embu County (36.07±8.98), followed by Siaya County (26.67±8.21) and least in Busia County (13.33±6.31) and Bungoma County (6.67±4.63). However, category 3 tree damage was the highest in Busia County (33.33±8.75) and Embu County (33.87±9.15), followed by Bungoma County (23.33±7.85) and least in Siaya County (10.00±5.57) (Table 4.2).

Table 4.2: Percentage of *Eucalyptus* trees under different *G. brimblecombei* damage categories

County	Mean percentage tree damage in different categories ($\bar{x} \pm se$)			
	Category 1	Category 2	Category 3	Category 4
Bungoma	13.33 ± 6.31^{cA}	56.67 ± 9.20^{bB}	23.33 ± 7.85^{bB}	6.67 ± 4.63^{aA}
Busia	0.00 ± 0.00^{aA}	53.33 ± 9.26^{bD}	33.33 ± 8.75^{cC}	13.33 ± 6.31^{aB}
Siaya	40.00 ± 9.10^{dC}	23.33 ± 7.85^{aB}	10.00 ± 5.57^{aA}	26.67 ± 8.21^{abB}
Embu	8.02 ± 6.31^{bA}	22.04 ± 8.95^{aB}	33.87 ± 9.15^{cBC}	36.07 ± 8.98^{bcC}

Different lowercase letters following the mean and standard error across columns indicate significant differences in tree damage categories among counties. Different uppercase letters following the mean and SE across rows indicate significant differences in tree damage categories among different categories. The significance was determined at $p = 0.05$ using Tukey test.

4.5 Damage categories variations within *G. brimblecombei* assessment months

In Bungoma County, the highest attack of *G. brimblecombei* was recorded in the December 2021 with 60% of trees reported in damage Category 3. In Busia County, *G. brimblecombei* infestations were higher in December during which 80% of the trees were in damage Category 3. In Siaya, damage Category 1 was highest in April (100%) with none of its cases observed in December, while category 4 was highest (35-40%) in June and December. Generally, Embu had the highest infestation across all the months of assessment, with categories 2, 3 and 4 generally high (50-70%) (Fig. 4.4).

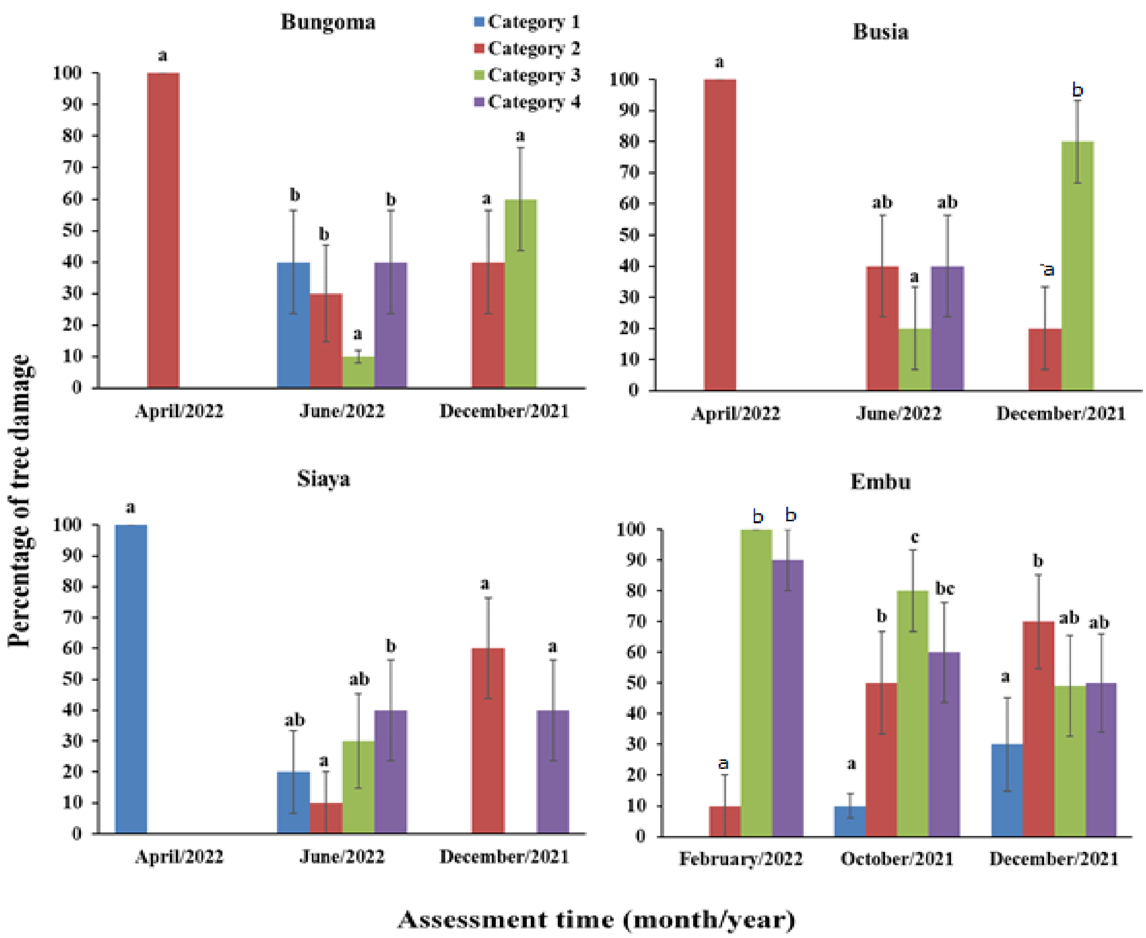

Figure 4.4: Percentage damage category by *G. brimblecombei* in different counties over the assessment months

4.6 Distribution of *P. bliteus* in Western and Eastern Kenya

Psyllaephagus bliteus was recorded in all counties assessed with populations being higher in Bungoma County compared to other study counties (Fig. 4.5 and Fig. 4.6).

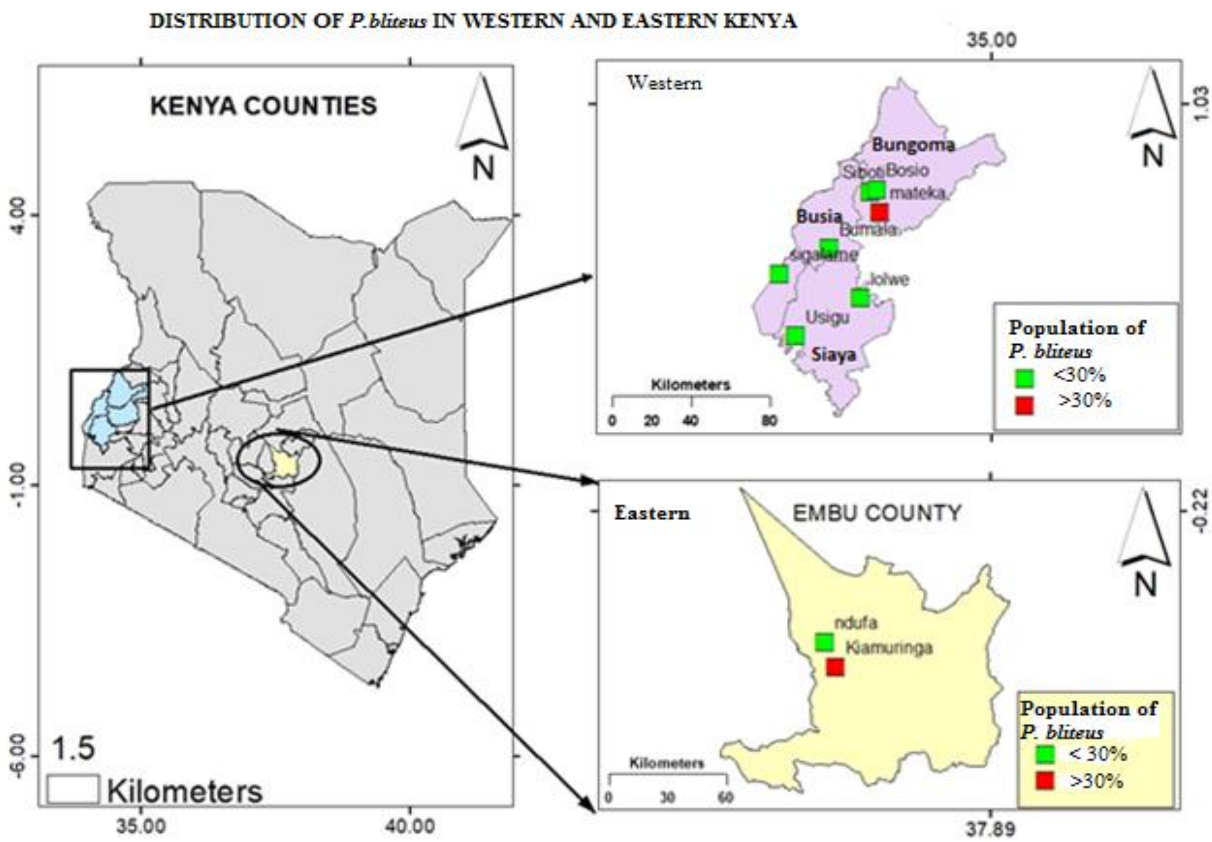

Figure 4.5: Distribution of *P. bliteus* in Western and Eastern Kenya

On opening lerps in the field, developing parasitoids were evident in mature lerps while adult parasitoids were observed ovipositing in the lerps of 3rd, 4th and 5th instar stages. Fig. 4.6 shows images taken in the field indicating presence of *P. bliteus* in Western and Eastern regions.

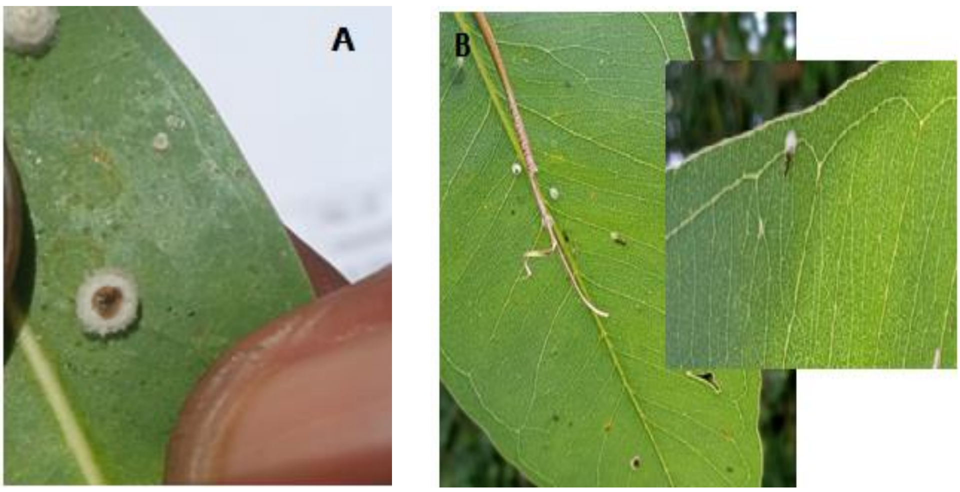

Figure 4.6: Observations indicating presence of *P. bliteus* during assessment. Open lerp with mummified nymph (A), *P. bliteus* ovipositing into mature lerp (B).

Besides *P. bliteus*, different insects were captured in the yellow sticky traps. They included various Ichniumoid wasps and Cochineal beetles. Trapped wasps had distorted morphology which interfered with identification to species level. However, other natural enemies identified on the yellow traps include black ants and Syrphid flies (Fig. 4.7). Curculionidae beetles and ladybirds collected on the yellow traps were identified as possible predators of *G. brimblecombei.*

Figure 4.7: Field assessment. Yellow stick trap assessment (A), assorted insects on a sticky card (B)

4.7 Parasitism rates of *P. bliteus* in *G. brimblecombei*

Variations in parasitism rates were recorded for both assessment months and among different damage categories.

4.7.1 Parasitism rates of *P. bliteus* in different counties within assessment months

Bungoma recorded the highest parasitism rate of *P. bliteus* (11.61%), followed by Siaya (7.92%), and least in Busia (3.54%) and Embu (5.08%). There were significant variations in the number of exit holes among the counties ($F_{3,994}$=72.72; p<0.0001) and time of assessment ($F_{2,994}$= 5.76; p = 0.0032). The number of exit holes differed significantly among the counties ($F_{3,994}$= 34.54; p<0.0001) and time of assessment ($F_{2,994}$= 5.38; p = 0.0047). The number of lerps with exit holes was highest in both December (1.81±0.21) and June (1.59±0.34) in Bungoma County. The lowest numbers of lerps with exit holes (0.4-0.5) were observed in the month of December. Embu County had considerably low number of lerps with exit holes in both October (0.27±0.05) and February (0.31±0.05). Evidently, all the leaves with lerps with exit holes had mummies. The highest number of lerps (1.22±0.25) was observed in Bungoma County in June (Table 4.3).

Table 4.3: The mean abundance of different lerps per *Eucalyptus* tree in four counties of Kenya

County	Assessment time	Mean number exit holes	Mean number mummies on leaves	Mean parasitism rate (%)	Overall mean Parasitism (%)
Bungoma	Dec-21	1.81 ± 0.21b	0.44 ± 0.09b	21.65 ± 2.48c	
	Apr-22	0.06 ± 0.04a	0.04 ± 0.03a	2.06 ± 1.31a	11.61
	Jun-22	1.59 ± 0.34b	1.22 ± 0.25c	11.12 ± 1.95b	
Busia	Dec-21	0.40 ± 0.08b	0.12 ± 0.05b	10.63 ± 2.31b	
	Apr-22	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	3.54
	Jun-22	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	
Siaya	Dec-21	0.5 ± 0.11b	0.57 ± 0.10b	23.77 ± 3.12b	
	Apr-22	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	7.92
	Jun-22	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	
Embu	Oct-21	0.27 ± 0.05b	0.31 ± 0.05a	5.75 ± 1.08a	
	Dec-21	0.04 ± 0.01a	0.36 ± 0.04b	5.10 ± 0.81a	5.08
	Feb-22	0.31 ± 0.05b	0.66 ± 0.08c	4.40 ± 0.54a	

Different small letters following the mean and standard error (SE) indicate significant differences between time of assessment in each county at p = 0.05 according to Tukey test.

The parasitism rates of *P. bliteus* varied significantly across the study counties ($F_{3,488}$ = 52.50; p<0.0001) as well as among the tree damage categories ($F_{3,1488}$= 9.16; p<0.0001) and assessment months ($F_{2,1488}$= 48.09; p<0.0001).

4.7.2 Variations of parasitism rates among assessment months and damage categories

Across the assessment months, counties of study registered high parasitism especially in December (Fig. 4.8). In Bungoma, parasitism rate was highest in the month of December (22%) at damage category 2 and category 3 followed by June at 20 % for damage category 4. In Busia County, parasitism was only reported in December for damage category 3 (12%) and damage category 2 (5%). Siaya County registered a parasitism of 36% in the month of December for damage category 2 and 8% for category 3. Except for damage category 1, parasitism rate at Embu County were recorded in other three categories. The parasitism rates in Embu County for damage category 2, 3 and 4 ranged from 2-10%.

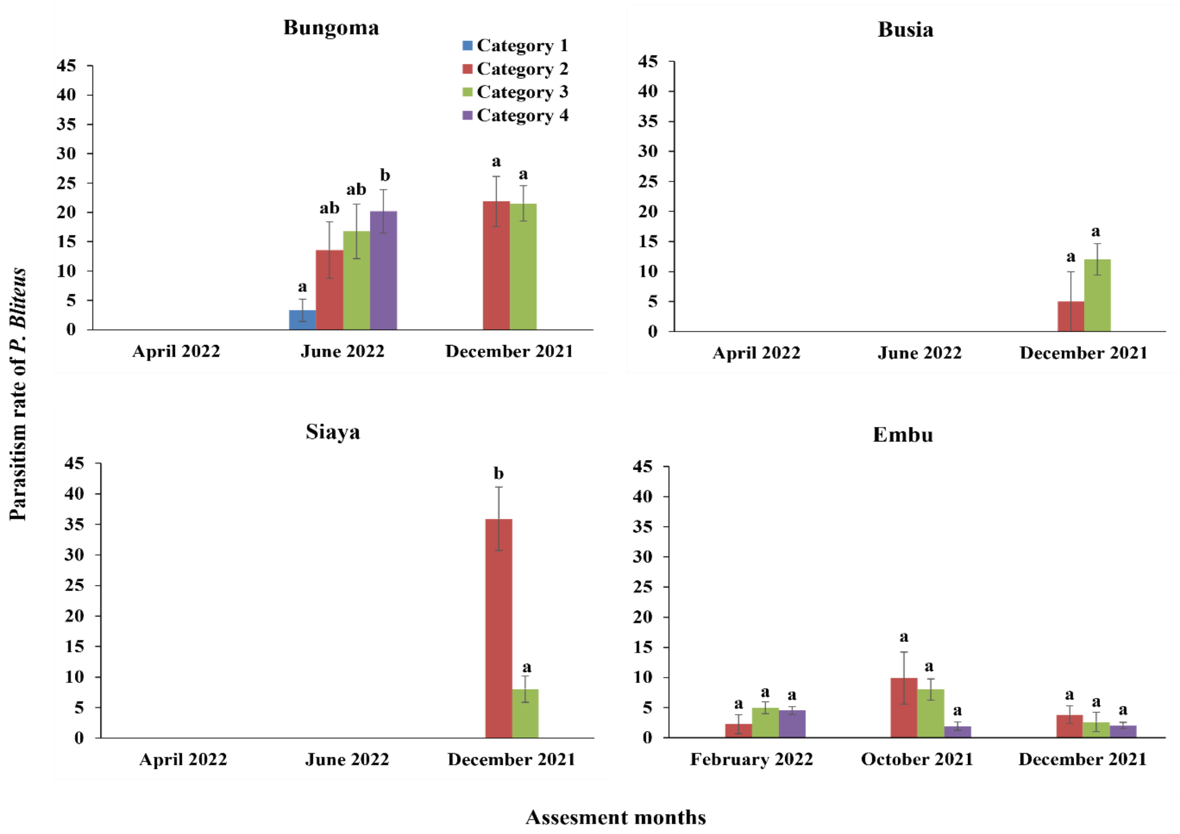

Figure 4.8: Parasitism rates at different damage category levels over the assessment period

4.7.3 Laboratory assessment for parasitized lerps presence/absence

Different stages of development of *P. bliteus* were identified from the lerps from the leaves samples. However, it was not possible to retain the lerps in position on the leaves for longer than three days due to desiccation of the field samples and loss of grip by the lerps. The honey dews dried up and the lerps dislodged from the leaves which interfered with the lab experiments. Microscopy results indicated that on average, 5 dead and live parasitoids from samples of each County were collected which was a clear indication of presence of *P. bliteus* in the fields where the samples were collected. During microscopic observation of the mature *P. bliteus*, the larval stages of development and the mummified forms concurred with the morphological features of mature and immature stages of *P. bliteus* as shown in Fig. 4.9. Inside the lerps of parasitized nymphal stages were mummies of seized or completely consumed dead hosts as the parasitoid completed development.

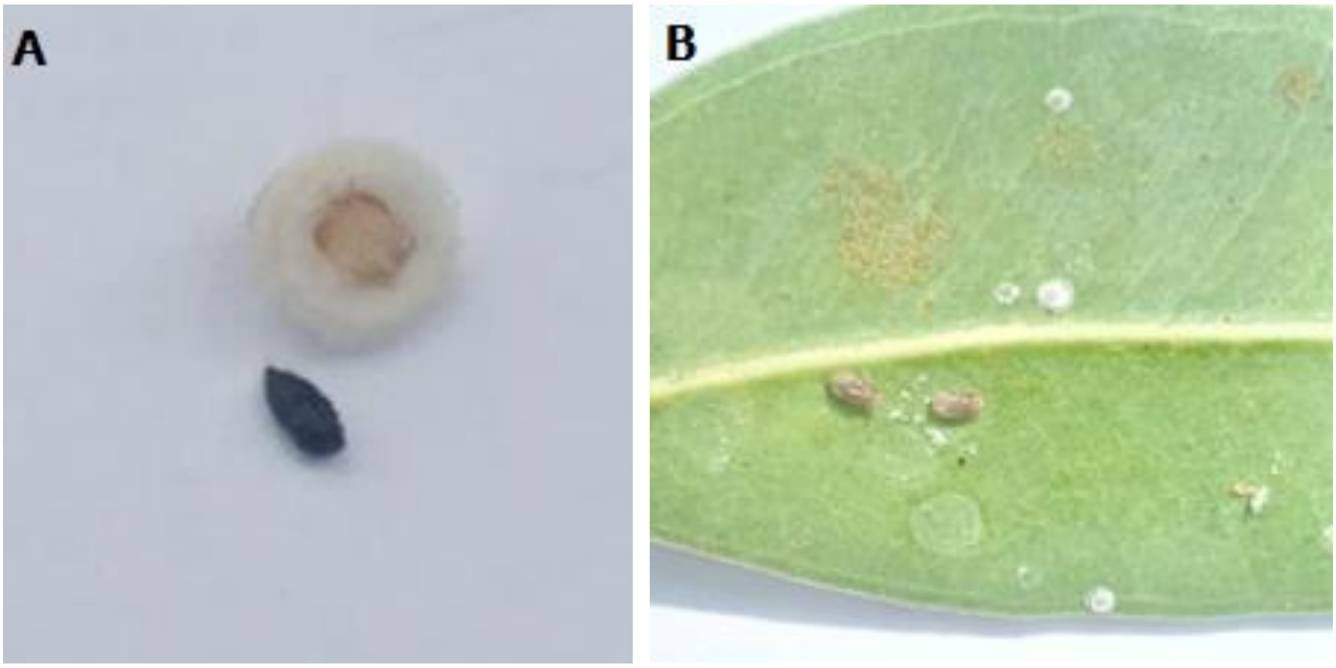

Figure 4.9: Laboratory assessment of lerps. Immature parasitoid and lerp (A), Seized mummified nymphs (B)

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Originating from Australia**,** *Eucalyptus* is one of the most used tree for forest plantations, to obtain products like wood, pulp, biomass and for soil conservation (Dhahir *et al*., 2014). In the last 50 years, the number of alien insect pests has increased steadily (Kenis & Branco, 2010). The emerging invasive pests threaten Eucalypts especially *E. camaldulensis* Dehn (Myrtaceae) plantations (Deidda *et al*., 2016). The insect pests of *Eucalyptus* are known to follow their host, and in most cases leave behind the natural control agents which regulate them below economic injury levels (Ndlela *et al*., 2018). Understanding the possible pathways, susceptibility of Eucalypts and management options to deal with invasive pests has become a major concern (Brennan *et al*., 1999).

Glycaspis brimblecombei (Hemiptera: Psyllidae) which is an invasive, sap sucking *Eucalyptus* pest is considered the most serious pest of Eucalypts in the Mediterranean area, and *E. camaldulensis* being the most susceptible to infestation (Deidda *et al*., 2016). In Tanzania, *G. brimblecombei* was first registered in October of 2016, on *E. camaldulensis* and on *Eucalyptus* clones on the Tanzania Forestry Research Institute (TAFORI) experimental sample plots in Mbizi forest plantation (Revocatus *et al*., 2018).

The present work focussed on determining spatial distribution of *P. bliteus* (Hymenoptera: Ancyrtidae) in Busia, Bungoma and Siaya Counties in Western Kenya and Embu County in Eastern Kenya. During this study, *G. brimblecombei* psyllids were distinguished from other psyllids through the long cephalic projections below the eyes, which are indicated as genal processes according to Laudonia & Garonna, (2010). Lerps in this study were identified as honeydew built white cover on sample trees (Firmino-Winckler *et al*., 2009), with a maximum size of 3.0 by 2.0 mm according to Ide *et al*. (2006).

The higher distribution of *G. brimblecombei* recorded in Embu County is attributed to the dry conditions in this region which are known to favour its establishment (Dhahir *et al.*, 2014) Lower populations of *G. brimblecombei* was registered in Busia and Bungoma counties, the counties that are known to receive higher amounts of rainfall that is known to disfavour establishment of *G. brimblecombei* (Dhahir *et al*., 2014; Revocatus *et al*., 2018).

This study underlined peak nymph population density of *G. brimblecombei* on the host, *E. camaldulensis* during the month of December where damage categories 3 and 4 were recorded. This is during the dry season in the counties under study which corroborates other studies (Wilcken *et al*., 2003). In Bungoma and Busia, the month of December registered the highest population of *G. brimblecombei,* while Embu County registered the highest population in February. These were hot and dry months in these counties during the assessment period. Various studies conducted on *G. brimblecombei* in other areas indicate temperature as the main factor involved in this psyllid population dynamics and distribution (Laudonia *et al*., 2014), which agrees with the findings of this study.

This study highlighted the effect of rainfall in distribution and population dynamics of *G. brimblecombei.* In the counties of study, the month of April presented low populations of *G. brimblecombei* (damage categories 1 and 2) compared to other months of assessment. This was attributed to heavy rainfall recorded in the counties during the April assessment. Results from other studies reveal a strong effect of rainfall on nymph density as described in studies carried out in Mexico (Ramierez *et al*., 2003) and also in Mauritius Island according to Sookar *et al*. (2003). Further, a controlled experiment involving rainfall simulation indicated that mechanical removal of the psyllid lerps by water droplets and solubilisation of lerps by leaf moisture produced through metabolic activities may decrease populations of *G. brimblecombei* (Oliveira *et al*., 2012). According to Ramierez *et al*. (2003), the higher humidity during the rainy season increases the population of entomopathogenic fungi which feed on psyllid lerps and nymphs killing them. Ferreira-Filho *et al*. 2018 indicated that the populations of *G. brimblecombei* and its parasitoid *P. bliteus* were inversely correlated with temperature and rainfall, according to season with peak in the winter, and decreases in the summer when rainfall increases.

The yellow sticky traps assessed in the field indicated that besides *P. bliteus*, other generalists like various Ichniumoid wasps, black ants, Syrphid flies and Cochineal beetles are actively involved in management of *G. brimblecombei.* However, generalists are not species specific and hence not able to sustainably manage a specific pest. These generalists have been observed sucking honey dews from the lerps which exposes the nymphs to predators. Others are predators which were observed feeding on the nymphs' soft tissues directly hence killing them. According to Valente & Hodkinson (2009), entomophagus species recorded attacking *G. brimblecombei* include lady beetles, birds, green lacewings, pirate bugs and spiders. *Anthocoris nemoralis* F. (Hemiptera: Athocoridae) is a potentially effective predator found attacking *G. brimblecombei* according to literature. In spite of that, predators do not provide a comprehensive biocontrol to reduce psyllid abundance.

In this study, female *G. brimblecombei* are found to prefer ovipositing on the abaxial surface of *Eucalyptus* leaves, an observation that agrees with findings in previous study by Tuller *et al.* (2017). Similar finding were made by Dal Pagettoa *et al.* (2021) where they noted that *G. brimblecombei* prefers ovipositing on the abaxial surface of *E. camaldulensis*. These observations were attributed to the ability of *G. brimblecombei* to tolerate physical and chemical defences due to the long coexistence with *E. camaldulensis* in Australia. Further, the greater flow of nutrients and low chances of desiccation on the abaxial surface relates to the same (Firmino-Winckler *et al*., 2009; Tuller *et al*., 2017).

Multiple generations were observed on the sample leaves in which the five nymphal instars and adults laying eggs were recorded during the same assessment period. This could be explained by the adult females ovipositing eggs at different times on the same leaf (Tuller *et al*., 2017). Young nymphs are known to form lerps for protection and as the nymphal stages tend to 5th instar, the newly formed lerp becomes more elaborate taking a bigger surface area on the leaf. More whole and well-formed lerps were an indication of maturing nymphal stages of *G. brimblecombei*. In some other lerps, sooty moulds formed on the lerps, an indication of presence of fungus on the honeydews. Some lerps formed along the midrib of sample leaves while others were evenly distributed on the adaxial and abaxial lamina, which indicated location of resources, readily available for sucking by *G. brimblecombei* nymphs.

On washing away of old lerps from the leaves by rainfall and before inception of a new attack, hardened round rust brown to dark brown patches were left on the leaf epidermis. On comparing the old and new attacks, this study underlined that *G. brimblecombei* prefers to attack the flashy young leaves that sprout after the rains. This agreed with results of a study by Firmino-Winckler *et al*. (2009) which underscore that *G. brimblecombei* reproduces sexually, and lays six to 45 eggs per leaf. However, Pereira *et al*. (2013) indicated that this psyllid oviposits and completes its life cycle on both sides of expanded and fully mature leaves.

Among hymenopteran parasitoids, *P. bliteus* develops only in *G. brimblecombei* (Paine *et al*., 2000) and has been employed in classical bio-control programs. This parasitoid was accidentally introduced into Brazil and reported almost immediately after *G. brimblecombei* in 2003 (Berti-Filho *et al*., 2003). The current distribution of *P. bliteus* indicated higher population in Bungoma County compared to other counties in question. This could be attributed to proximity to release site by Uganda NAFORRI, according to Nyeko *pers comm*. 2018. The parasitoid may have found its way from the Kenya-Uganda border plantations into Kenya plantations through Bungoma County. However, *P. bliteus* is known to follow its host and has been found in other countries where it was not imported or released like Portugal and Tunisia (Daane *et al*., 2012). This could be attributed to the presence of the parasitoid in Eastern Kenya. The parasitoid was first released as a biocontrol agent against *G. brimblecombei* in California in the year 2000 and was later reported in various countries including S. America and South Africa (Ferreira-Filho *et al*., 2015). A relatively low parasitism rate recorded in Embu, a hot and dry County, corroborates findings by Dhahir *et al*. (2014) who reported a 1.2% parasitism rate in similar dry and hot conditions in Tunisia and Portugal. Additionally, from other studies, *G. brimblecombei* is identified to be under good control by the parasitoid, but it does not persist well under hot, dry conditions (Ferreira-Filho *et al*., 2015).

In this study, parasitism rates varied seasonally throughout the year with lowest and highest recorded in April and December respectively, an observation attributed to the collapse and build-up period of *G. brimblecombei.* Similar observations were made by Dhahir *et al*. (2014) during their study in Tunisia and Portugal where they observed that parasitism rates varied through time and season as per the host population dynamics, showing a density-dependent relationship, marginally delayed in time. Consequently, the observed parasitism rates were lowest at the beginning of the season when *G. brimblecombei* population density was highest with parasitoid population under development. On the other hand, parasitism rates were highest as the season came to an end, leading to the collapse of psyllid population.

Parasitism of *G. brimblecombei* was generally low, an observation that agrees with results from Brazil (Ferreira-Filho *et al*., 2018), Italy (Caleca *et al*., 2011), Tunisia and Portugal (Dhahir *et al*., 2014) and United States of America (Daane *et al*., 2012). Nevertheless, rearing followed by mass release of *P. bliteus* showed some promising results which was found to increase parasitism rates in the field up to about 94% (Huerta *et al*., 2010). Other studies have shown that in spite of augmentation and conservation of available *P. bliteus* populations, the parasitoid failed to develop into viable populations in warm climate regions according to Daane *et al*. (2012) and in Brazil according to Ferreira- Filho *et al*. (2015). This calls for periodic mass release to augment existing populations of the parasitoid, which would increase the cost of sustainable management of *G. brimblecombei*. However, the long term efficiency of the parasitoid to control its host through mass release show that IPM approach which combines various control methods such as chemical, physical and cultural control methods to reduce pest damage is paramount, while use of resistant *Eucalyptus* genotye can act as a substitute for insect management.

Observations from this study indicated that *P. bliteus* oviposited in nymphal instar stages 3 and 4 but not 5. This observation agrees with results of a study conducted by Sime (2004) that entailed dissection of *G. brimblecombei* exposed to *P. bliteus.* The latter study demonstrates that 5^{th} stage has more free space within the lerp to escape the parasitoid and increased handling time hence the observed non parasitism. In addition to increased handling time, Moore (1964) reported that wasp's ovipositor is not long enough to effectively reach nymphs with larger lerps.

Parasitism rates were highest in trees under damage categories 2 and 3 with seasonal variability which tallied with results from other studies. Dhahir *et al.* 2014 highlighted high seasonal variability and a density-dependence pattern in *P. bliteus* parasitism. The evident temporal disparity on the populations of *G. brimblecombei* underlines the vulnerability of this insect to variations in temperature and rainfall. This susceptibility coupled with detected parasitism rates by *P. bliteus* can be used to improve IPM strategies for an effective management of *G. brimblecombei* in *E. camaldulensis* plantations (Tuller, 2017).

5.2 Conclusions

The pest of Eucalypts, *G. brimblecombei*, reported in Embu, Siaya, Bungoma and Busia, present a serious challenge to forest plantation. With infestation varying between 25 and 50%, there is urgent need to initiate management to minimise threats. Considering the high demand of fuel wood from *Eucalyptus* by KTDA and other wood-dependent companies in Kenya, and the ecosystem impact of this tree, there is need to manage *G. brimblecombei* and enhance productivity of this tree.

Fortunately, the parasitoid, *P. bliteus* that was released in Uganda in 2017 by NaFORRI, to manage *G. brimblecombei* has spread and established in Eucalypt plantations in Embu, Siaya, Bungoma and Busia. Establishment of *P. bliteus* is reflected in parasitism rates recorded in the assessed counties, including Embu County which is distal to the release site in Uganda. The presence of parasitoid in Kenya can be attributed to its biological behaviour of naturally following its host, *G. brimblecombei* on *E. camaldulensis* plantations. *P. bliteus* has the potential to spread to other *E. camaldulensis* growing areas in Kenya. Though observed parasitism levels were low, the parasitoid have potential to increase pest mortality when mass reared and released.

Positive impacts were recorded in other areas where the parasitoid was released as a biological control agent including California, Portugal and Tunisia. Contrary to report on impacts of ecological conditions on the establishment of the parasitoid, this study showed that the drier parts of Embu in Eastern Kenya where *E. camaldulensis* is a suitable species are still viable for *P. bliteus* establishment.

5.3 Recommendations

Implementation of classical biological control programme is the most efficient way to ensure *G. brimblecombei* infestations fall below economic injury level. The parasitoid efficiency relies on understanding *G. brimblecombei* susceptibility to environmental conditions, lifecycle, female oviposition preference, population dynamics and mortality from natural enemies.

Collection of *P. bliteus* from the field, mass rearing and release for augmentation of the existing population and identification of suitable ecological conditions for its spread are prerequisites towards implementation of classical biological control of *G. brimblecombei*. Conservation of *P. bliteus* in infested areas is paramount.

Further studies to confirm the temporal pattern indicated in this study and other potential mechanisms and physiological forces driving the abundance of *G. brimblecombei* in drier areas of Kenya are paramount. A trytophic study will inform the suitable ecological and behavioural conditions of *P. bliteus* before mass release. The susceptibility to climatic variations coupled with low parasitism rates of *P. bliteus* detected in this study may deter effective management of *G. brimblecombei* in *E. camaldulensis* plantations. However, IPM strategy which combines methods such as physical, chemical and cultural control methods to reduce pest damage is paramount in enabling sustainable management of *G. brimblecombei* below economic injury level.

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APPENDICES

Appendix 1: Data collection sheet

SPATIAL DISTRIBUTION OF *Psyllaephagus bliteus* IN WESTERN AND EASTERN KENYA AND ITS IMPACT ON *Glycaspis brimblecombei* POPULATION

Region ………………………….......... Assessment date ………………………

County …………………………….... Assessors ……………………………...

Compartment name ………………… Tree number……………………………

Tree damage category………………. Longitude ……………………………..

Latitude …..…………………………. Elevation……………………………… M asl

Leaf Number	No. of Lerps on the upper side	No. of Lerps on the lower side	Presence/ Absence of exit hole	No. of Lerps with exit holes	Presence/ Absence of mummies	No. of mummies on the leaves
1.						
2.						
3.						

Definition of tree damage category for RGLP

Category 1: No canopy damage
Category 2: Below 0 - 25% canopy damage
Category 3: Between 25%-50% canopy damage
Category 4: Greater than 50% canopy damage

Yellow traps assessment

Tree number	Yellow trap number	Presence/ absence of parasitoid	Number of parasitoids per yellow trap	Other natural enemies recorded
1	**1**			

Appendix 2: Geographical positions and altitude of study sites used for distribution maps development

Region	County	Locality	Longitude	Latitude	Altitude
Western	Busia	Bumtiru	34.26346	0.369365	1292m
		Sigalame	34.0363	0.25215	1289m
	Bungoma	Mateka	34.4981	0.535	1441m

		Siboti/ Musakasa	34.33	0.3553	1452m
		Bosio	34.481	0.63931	1431m
	Siaya	Usigu	34.1117	-0.0315	1260m
		Nyaranga Pry	34.163	0.6162 S	1258m
		Lolwe	34.4083	0.1399	1251m
Eastern	Embu	Kiamuringa	37.32.4.2E	0.34.54 S	1241m
		Ndufa	37.30.37 E	0.31.24 S	1367m

Appendix 3: Comparison of damage levels within months of assessment

Months of assessment	**Tree damage category (%)**			
	Category 1	**Category 2**	**Category 3**	**Category 4**
Feb. 2022	0.00 ± 0.00	5.00 ± 0.00	50.00 ± 8.16	45.00 ± 8.16
April 2022	33.3 ± 0.00	66.67 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
June 2022	20.0 ± 9.78	26.67 ± 13.87	20.00 ± 12.87	33.33 ± 15.33
October 2021	4.76 ± 4.00	28.57 ± 8.16	38.10 ± 8.16	28.57 ± 8.16
Dec. 2021	7.54 ± 3.82	37.59 ± 11.48	35.64 ± 9.03	19.23 ± 6.65

Appendix 4: Comparison of parasitism rates under different damage levels within months of assessment

Months	**Tree damage category (%)**			
	Category 1	**Category 2**	**Category 3**	**Category 4**
February 2022	0.00 ± 0.00	2.26 ± 1.57	4.97 ± 0.96	4.50 ± 0.69
April 2022	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
June 2022	1.11 ± 0.64	4.52 ± 1.60	5.59 ± 1.55	6.72 ± 1.23
October 2021	0.00 ± 0.00	9.88 ± 4.34	8.01 ± 1.78	1.90 ± 0.69
Dec. 2021	0.00 ± 0.00	16.65 ± 3.98	11.05 ± 2.34	0.51 ± 0.12