Parasitism Rates of *Psyllaephagus bliteus* in Eastern and Western Kenya for Biocontrol of *Glycaspis brimblecombei*

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Abstract

The principal natural enemy of *Glycaspis brimblecombei* is a parasitoid *Psyllaephagus bliteus* Reik which has been studied to control its host. The foremost objective of this work entails studying the parasitism rates of *P. bliteus* in Western and Eastern Kenya after the National Forestry Resources Research Institute (NaFORRI) released *P. bliteus* in their *Eucalyptus camaldulensis* plantations bordering Western Kenya. Plantations in Busia, Bungoma and Siaya counties in Western Kenya and; Ndufa and Kiamuringa Kenya Tea Development factory in Embu County were assessed. Ten (10) *E. camaldulensis* trees were randomly selected from each plantation. Adult *P. bliteus* population was sampled by placing two yellow sticky traps on each sample tree while immature stages were assessed by opening mature lerps and observing for the presence of developing parasitoid forms, mummified nymphs or exit holes. Three assessments were carried out in three different months and generated data analyses were performed using R statistical software. Distribution maps were drawn using ArcGis. The number of exit holes differed significantly among the counties and months of assessment. 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. 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 parasitism rates of *P. bliteus* in Kenya, and this would guide the implementation of biocontrol program for sustainable management of *G. brimblecombei* in Kenya.

Keywords: Classical biocontrol, Eucalypts, integrated pest management, red gum lerp psyllid

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1. Introduction

Red Gum Lerp Psyllid (RGLP) *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), as well as using 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 to overcome desiccation in the environment of inland Australia and these lerps shelter the nymphal stages from their parasitoids and predators (Sullivan *et al*., 2006).

According to Dreistadt *et al*. (2004) applicable cultural control measures include minimizing stress in trees through silvicultural practices and protection of trees from severe injuries, rendering them immune-compromised (Mutitu *pers comm*). Other methods include supplemental watering during periods of prolonged drought. Nonetheless, too much watering is known to promote new foliar growth and increased Nitrogen content on the new flashy leaves (Tuller *et al*., 2017). Fertilizing trees in high-risk areas is detrimental. The use of systemic foliar spray chemicals with caution on high-value crops like ornamentals can save a tree (Dreistadt *et al*., 2004).

Recently, *G. brimblecombei* was managed via use of different parasitoids which includes lady beetles like, *Harmonia axyridis* and *Chilocorus bipustulatus.* These generalists are not able to effectively keep the populations of *G. brimblecombei* below economic injury level (Dahlsten *et al*., 2005). This called for use of a classical biocontrol program.

A classical biocontrol program implicates studying parasites, predators and pathogens that may be used in effectively controlling a pest in its native habitat. Once the natural enemies are identified, a screening process in a quarantine facility is conducted to determine whether the natural enemies can be introduced safely (Dahlsten *et al*., 2003).

A natural control program against *G. brimblecombei* used a parasitoid *Psyllaephagus bliteus* Reik (Hymenoptera: Encyrtidae) 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 the third and fourth nymphal stages (Daane *et al*., 2012). The egg hatches and *P. bliteus* larva feeds on the host's visceral organs, 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 the presence of *P. bliteus* attacking *G. brimblecombei* (Daane *et al*., 2012). Other methods that the female parasitoid kills *G. brimblecombei* are 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). The biological control program is influenced by pesticide use and cultural practices and hence the integration of these activities makes it an effective pest management option (Daane *et al*., 2012).

Psyllaephagus bliteus belongs to the Order Hymenoptera and the 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 are reported as hyper-parasitoids that attack other species of *Psyllaephagus* (Noyes & Hanson, 1996; Riek, 1962). The specificity and efficacy of this parasitoid have made it a suitable candidate in classical biological control programs targeting *Eucalyptus* psyllid pests (Daane *et al*., 2012). This parasitoid is native to Australia and 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 (Ferreira-Filho *et al*., 2015).

Psyllaephagus bliteus shows a preference for third and fourth instars of RGLP (Dahlsten *et al*., 2003). However, the parasitoid can parasitize 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 the time of development depends on weather conditions. The adult parasitoids emerge from the lerps through a characteristic circular exit hole.

Sometimes biocontrol programs have failed due to the paucity of literature on the 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 agents can negatively impact the progress of the bio-control agent program (Laudonia *et al*., 2014). Studies have shown that the 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).

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 (Sullivan *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).

There is paucity of information regarding impact of *P. bliteus* on *G. brimblecombei* spread in Kenya after release of the parasitoid by the National Forestry Resources Research Institute (NaFORRI) near Uganda- Kenya border. Therefore, this study provides important information on the parasitism rates of *P. bliteus*, which would guide the implementation of biocontrol program for sustainable management of *G. brimblecombei* in Kenya.

2. Materials and methods

2.1 Study Area

Data was collected in five sites; three in Bungoma, Busia and Siaya Counties (one site per County) representing the Western region of Kenya, and two sites in Embu County, Eastern Kenya (Figure 1). *Glycaspis brimblecombei* and *P. bliteus* were assessed on *Eucalyptus camaldulensis* plantations considering the proximity to *P. bliteus* release sites by NAFORRI along the Uganda-Kenya border near Bungoma, Busia and Siaya Counties. Embu County has vast Kenya Tea Development Agency (KTDA) *E. camaldulensis* plantations with a history of *G. brimblecombei* invasion.

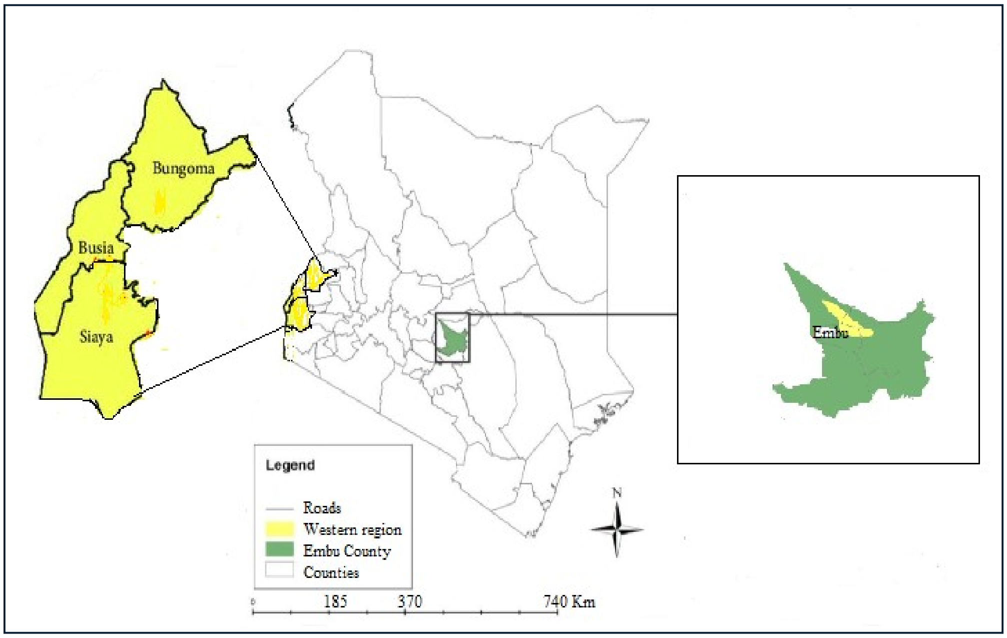

Figure 1. The study area: Bungoma, Busia, Siaya and Embu Counties

2.2 Identification of Sampling Sites and Trees

Kenya Forest Service (KFS) and regional Ecosystem Conservators (EC) were consulted during identification of the sample sites with *E. camaldulensis* plantations. Five (5) *Eucalyptus* plantations located in the study sites were assessed as follows; Bumtiru in Busia County, Bosio in Bungoma County and Nyaranga Primary School, Siaya County. Two plantation sites were assessed in Kiamuringa and Ndufa, Embu County, Eastern Kenya. Each plantation consisted of at least 50 trees aged between 2 and 3 years.

Ten (10) 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.

In Western Kenya, three (3) assessments were carried out in the months of April, June and December while the Eastern region was assessed in February, October and December, subject to availability of facilities to undertake the study. The months of assessment have unique climatic characteristics ranging from hot and dry, a transitional month and a wet month. In Western region, April represents the month of highest rainfall and lowest temperature, December represents the month of lowest rainfall and highest temperature while June is the transitional month with moderate rainfall and temperature. In Eastern region, October represents the month of highest rainfall and lowest temperature, February represents the month of lowest rainfall and highest temperature while December is the transitional month.

2.3 Assessment of Psyllaephagus bliteus and Lerps on the Sample Trees

Through visual observation, leaves in each sample tree were assessed for the presence of *G. brimblecombei.* On identifying presence of *G. brimblecombei*, two yellow sticky traps (10 cm x 15 cm) were dangled on branches about 1.5m above the ground level for adult parasitoid sampling. The number of captured adults counted on the yellow sticky traps, and replaced with new ones in every assessment. Immature parasitoids and lerps with exit holes were sampled using a hand lens on ten (10) leaves, randomly selected from the sample trees (Dahlsten *et al*., 2005: Ferreira *et al*., 2008). Parasitized nymphal stages were identified through visual observation of lerps on sample leaves on a white sheet background and carefully opening lerps for viewing under a hand lens. The experimental sites near neighbours were assessed for the presence of *P. bliteus*, a prerequisite during the development of distribution maps. The insects collected on the traps were ferried to the Kenya Forestry Research Institute (KEFRI) Insect Reference Collection (IRC) for identification to species level.

2.4 Field Observation of Lerps for Parasitism Rates

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).

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

2.5 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 the collection point. The containers were placed in cooler boxes and ferried to the KEFRI Quarantine facility. In the quarantine facility, sampled leaves were put in insect-rearing cages and monitored for 5 days for *P. bliteus* emergence.

2.6 Microscopic Observation of the Glycaspis brimblecombei Nymphs

The lerps without holes were opened and nymphal instars were extricated gently with a fine needle. Nymphal instars were obtained using a fine-tipped camel hairbrush 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.

2.7 Data Analysis

Data was recorded on customized data capture sheets and managed using MS Excel. Parasitism rates were expressed as percentages. The homogeneity of variances was determined using the Bartlett test. Shapiro Wilks test was carried out to assess the normal distribution of data. Log transformation (log10[X+1]) was undertaken to normalize the data before subjecting them to parametric tests. Percentage parasitism data that did not meet the assumption of parametric statistics after log transformation (Shapiro Wilks test; $p < 0.0001$) were subjected to the Generalized Linear Model (GLM). Count data on the number of lerps per leaf, number of lerps with exit holes, and number of mummies had zero counts and were analyzed using the Poisson regression model. In this study, the 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. Data analysis was performed in R Statistical software version 4.0.3 (R Core Team, 2020). Parasitism rate maps were generated using Geographical Information System (GIS) software, ARC-GIS (Corbett *et al*., 2001).

3. Results

3.1 Field Observations

On opening lerps in the field, developing parasitoids were evident in mature lerps while adult parasitoids were observed ovipositing in the lerps of the 3rd, 4th and 5th instar stages. Figure 2 shows images taken in the field indicating the presence of *P. bliteus* in Western and Eastern regions.

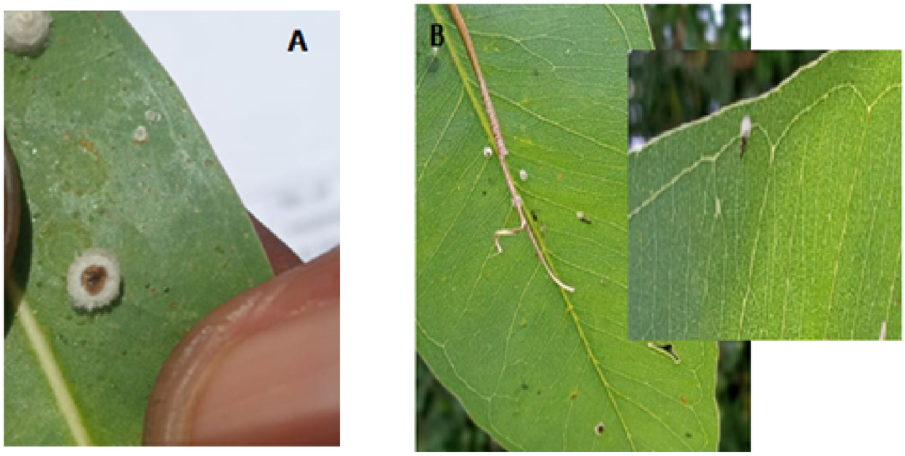

Figure 2. Observations indicating the presence of *P. bliteus* during the assessment. Open lerp with a mummified nymph (A), *P. bliteus* ovipositing into mature lerp (B)

3.2 Insects Trapped on the Yellow Cards

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 at the species level. However, other natural enemies identified on the yellow traps include black ants and Syrphid flies (Figure 3). Curculionidae beetles and ladybirds collected on the yellow traps were identified as possible predators of *G. brimblecombei*:

Figure 3. Field assessment. Yellow stick trap assessment (A), assorted insects on a sticky card (B)

3.3 Parasitism Rates of P. bliteus in Different Counties within Assessment Months

Variations in parasitism rates were recorded for both assessment months and among different Counties as shown in the distribution map (Figure 4).

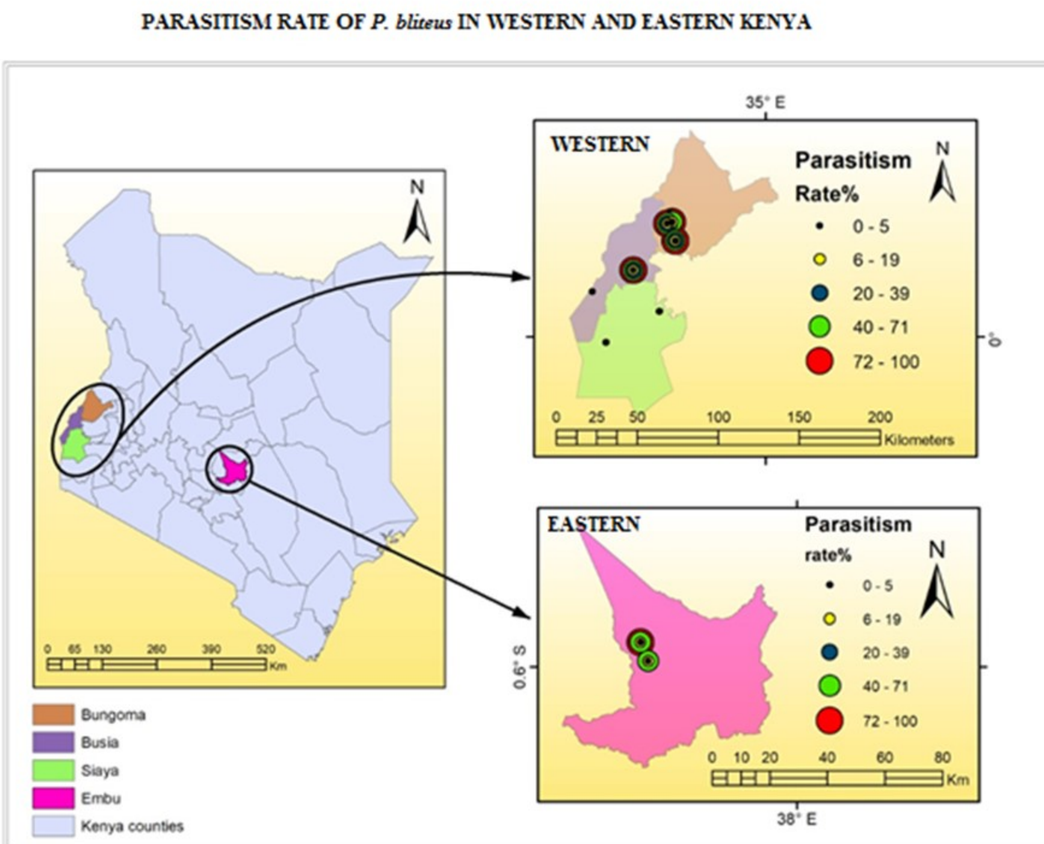

Figure 4. Distribution of parasitism rates of *Psyllaephagus bliteus* in Eastern and Western Kenya

The parasitism rates of *P. bliteus* varied significantly across the study counties ($F_{3,488}$ = 52.50; p<0.0001) and assessment months ($F_{2,488}$= 48.09; p<0.0001). Further to this, 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 a considerably low number of lerps with exit holes in both October (0.27±0.05) and February (0.31±0.05). 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 1).

Table 1. The mean number of exit holes, mummies on leaves, and parasitism rates of *P. bliteus* across different assessment months in four counties of study

County	Assessment time	Mean number of exit holes	Mean number of 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 the time of assessment in each county at p = 0.05 according to the HSD test.

3.4 Variations of Parasitism Rates across Assessment Months

Across the assessment months, counties of study registered high parasitism, especially in December. In Bungoma, the parasitism rate was highest in the month of December (22%) followed by June at 20%. In Busia County, parasitism was only reported in December (12%). Siaya County registered a parasitism of 36% in the month of December.

3.5 Laboratory Assessment of Parasitized Lerps for Psyllaephagus bliteus Presence/Absence

Different stages of development of *P. bliteus* were identified from the lerps from the leaf 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, five dead and live parasitoids from samples of each County were collected which was a clear indication of the 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.5. Inside the lerps of parasitized nymphal stages were mummies of seized or completely consumed dead hosts as the parasitoid completed development.

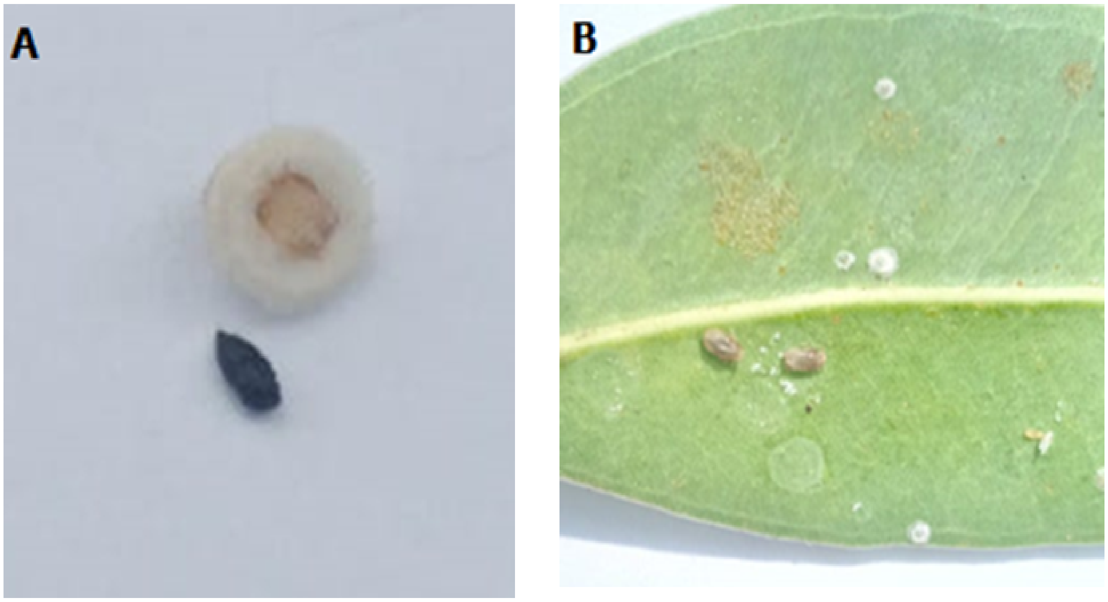

Figure 5. Laboratory assessment of lerps. Immature parasitoid and lerp (A), Seized mummified nymphs (B)

4. Discussion

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 the 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 expose 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), entomophagous 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 the literature. Despite that, predators do not provide a comprehensive biocontrol to reduce psyllid abundance.

Among hymenopteran parasitoids, *P. bliteus* develops only in *G. brimblecombei* (Paine *et al*., 2000) and has been employed in classical biocontrol 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 a higher population in Bungoma County compared to other counties in question. This could be attributed to the proximity to the release site by Uganda NAFORRI, according to Nyeko (2008). 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 the 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 the parasitoid population under development. On the other hand, parasitism rates were highest as the season came to an end, leading to the collapse of the 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 the United States of America (Daane *et al*., 2012). Nevertheless, rearing followed by mass release of *P. bliteus* showed some promising results which were found to increase parasitism rates in the field up to about 94% (Huerta *et al*., 2010). Other studies have shown that despite 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 shows that the integrated pest management (IPM) approach which combines various control methods such as chemical, physical and cultural control methods to reduce pest damage is paramount, while the use of resistant *Eucalyptus* genotypes can act as a substitute for insect management.

5. Conclusion

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 for the 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 the 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.

Declaration of competing interest

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

E Mutitu conceived the study. E Mutitu and M Gathogo designed the study. M Gathogo conducted all fieldwork, and M Gathogo, ER Omuse performed data curation and statistical analyses. M Gathogo wrote the manuscript. G Ong'amo, P Ndegwa, ER Omuse and E Mutitu reviewed the final version of the manuscript. All authors read and approved the final manuscript.

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