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(RESEARCH ARTICLE)

Establishment of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) as a bio-control agent for controlling eucalyptus pest *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae) in Uganda

Violet Mirembe Namuyanja *, Ronald Kisekka, Sarah Ssempala, Rose Kakayi and Peter Kiwuso

National Agricultural Research organization (NARO), P. O. Box 295, Entebbe, Uganda.

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Abstract

The global demand for Eucalyptus trees has increased due to their utility in producing timber and energy. However, the trees face the threat of invasive pests like the Red gum lerp psyllid (*Glycaspis brimblecombei*). To address this issue, *Psyllaephagus bliteus* was introduced in Uganda in 2017 to control the Red gum lerp psyllid. A recent study evaluated the parasitoid's establishment in the country by collecting samples from 10 release sites across Uganda from August 2018 to December 2021. Infested leaf samples were collected from various parts of the Eucalyptus tree crown and the number of *G. brimblecombei* nymphs were counted. The study found that mummified nymphs of *G. brimblecombei* were observed in all 10 release sites and even as far as 179 km away from the nearest release site. Parasitized nymphs of *G. brimblecombei* were found on both plantations and standalone trees, with no significant parasitism differences on different leaf surfaces or along the tree crown. These findings suggest that *P. bliteus* has been successfully established in Uganda and has the potential to substantially control *G. brimblecombei*. However, it is crucial to further study the social and economic impacts of the parasitoids.

Keywords: *Glycaspis brimblecombei; Psyllaephagus bliteus;* Uganda; Eucalyptus; Parasitism; dispersal

1. Introduction

Globally, there is an increasing demand for energy and timber, which has exacerbated human dependence on natural resources. Uganda like other Sub-Saharan countries depends largely on wood biomass and the demand is projected to grow exponentially in the future which could lead to forest degradation (Sassen et al., 2015). To cope with this situation, people often opt to plant fast-growing, highly utilizable, exotic tree species like Eucalyptus (Zerga 2015). Eucalyptus gives superior and versatile benefits compared to other tree species thus preferred in plantation forestry, particularly by smallholder farmers in tropical and subtropical regions (Hailemicael 2012). In Uganda Eucalyptus is estimated to cover 70 % of the total hardwood plantations (11,000 ha) (FAO, 2009).

Among the many Eucalyptus genotypes, two are common in Uganda; *Eucalyptus grandis* suited to cooler and wetter areas and *Eucalyptus camaldulensis* which grows well in hotter and drier climates (Kilimo Trust, 2011). Hybrid trees combine the desirable properties of different species such as fast and uniform growth, high productivity, resistance to pests and diseases and straight stems (Kilimo Trust, 2011). In 2002, National Agriculture Research Organization (NARO) / National Forestry Resources Research Institute (NaFORRI) introduced six clones of Eucalyptus, including *E. grandis x E. camaldulensis* (GCs) and *E. grandis x E. urophylla* (GUs). Specific clones were matched to the most

* Corresponding author: Violet Mirembe Namuyanja; Email: viomire12@yahoo.com

appropriate sites in 12 AEZs of Uganda. GCs that are drought-resistant in dry parts of Uganda and GUs planted on moister sites (Epila-Otara & Ndhokero, 2009).

Eucalyptus is being threatened by invasive pests like *Glycaspis. brimblecombei* Moore (Hemiptera: Aphalaridae) with growth in international trade and an evident increase in climate change such pests are set to increase (Lamichhane *et al*., 2015). *G. brimblecombei* is a highly invasive pest and has been included in the European and Mediterranean Plant Protection Organization (EPPO) list of quarantine species since 2002 (CABI, 2021). *G. brimblecombei* has been reported in California (the USA), Portugal, Spain, Italy, France, Morocco, Algeria, Montenegro, Greece, Tunisia, Zimbabwe (Brennan et al., 1999; Valente & Hodkinson, 2009; Reguia & Peris-Felipo, 2013; Bella & Rapisarda, 2013 ; Attia & Rapisarda, 2014; Ndlela, 2016).

Glycaspis brimblecombei can cause 20% to 30% defoliation, crown thinning, and tree mortality (Queiroz et al., 2013). Valente et al., (2018) estimated that Red gum lerp psyllid infestation of 46% can cause losses of 648 M Euros within 20 years. *G. brimblecombei* was detected in Uganda for the first time in May 2016 at Patiko prison Eucalyptus plantation in Gulu district. Studies carried out by National Agricultural Research Organization (NARO) estimated 80% of Eucalypts in Uganda to be infested with Red gum lerp psyllid (NARO , 2016, unpublished data).

Following successful control of *Glycaspis brimblecombei* using parasitic wasps *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) which is host specific to *G. brimblecombei* in countries like the USA, Brazil, Portugal, Italy, Spain, and Algeria (Dahlsten et al., 2005; Ferreira Filho et al., 2008; Dhahri et al 2014; Caleca et al., 2011). Uganda imported mummified *G. brimblecombei* nymphs from the Forestry Agricultural and Biotechnology Institute (FABI) University of Pretoria (South Africa) in February 2017. The wasps were reared and multiplied at the National Crops Resources Research Institute (NaCORRI) of NARO insectary. In the same month, NaFORRI released the parasitoid in three Agro-ecological Zone (AEZs) i.e. Northern Moist Farmlands, Lake Victoria crescent, and Lake Albert crescent (figure 1, table 1). Previous surveys had shown a high infestation of *Glycaspis brimblecombei* in the above regions and hot spots for release had been established (NARO, 2017 unpublished data). Over the years, NaFORRI has introduced the parasitoid in most parts of the country with Red gum lerp psyllid infestations and set up various points to evaluate the establishment of parasitoids in the field.

Knowing the dispersal of a biological control agent can help optimize distances needed between release sites and the appropriate number of agents released in order to avoid negative impacts from Allee effects. Propensity and potential ability to disperse (i.e. speed, distance, and activity) without assistance differ among species (Gaudon et al., 2018), Besides, The level of parasitism exerted on the target pest population by a parasitoid, is in part driven by its dispersal capacity (Gaudon et al., 2018). Therefore, determining the dispersal capacity of a biological control agent is an important consideration when developing any biological control program.

Thus, the aim of this study was to update on the establishment of *Psyllaephagus bliteus* as a biological control agent for *Glycaspis brimblecombei* in release sites and beyond in Uganda following its initial release in 2017.

2. Material and methods

2.1. Recovery of *Psyllaephagus bliteus* from release sites

Surveys were carried out at different time in all the sites above were *Psyllaephagus bliteus* was released starting from August 2018 to December 2021. Each Eucalyptus stand was divided into two or three blocks of about equal size. Within each block, plots of 10 × 10 tree lines (2 m × 2 m escapement) were marked out from which 10 trees were sampled (Nyeko et al., 2010). Branch tips measuring 30 – 40 cm were cut from psyllid- infested trees (Daane et al., 2012).

The Eucalyptus tree crown was divided into three approximately equally sections (low, mid, top) where the infested branch samples were collected. For short young trees, the branches were directly cut otherwise a pruning knife was used to collect the samples (Jere et al., 2019). For each crown section ten (10) infested leaves were picked per branch (Erbilgin et al., 2004). The leaves were placed in clearly marked zip-lock bags and were transported in a cooler box to the laboratory for analysis.

2.2. Survey for dispersal of *Psyllaephagus bliteus*

Monitoring for dispersal of *Psyllaephagus bliteus* was carried out co-currently with the recovery of *P. bliteus* from release sites survey. Samples were picked from plantations or stand – alone Eucalyptus trees were *P. bliteus* was never released. The sampling involved cutting branches as described above from four randomly selected trees per Eucalyptus plantation (Satta & Floris, 2018). GPS recordings were got to aid in calculation of distance from release sites. The GPS was got outside the plantation if the canopy was closed or inside the plantation for shorter trees.

2.2.1. Level of parasitism

The total number of *Glycaspis brimblecombei* nymphs on the leaf upper and lower surfaces was counted separately, then the nymphs were examined for parasitized ones (mummified, grayish nymphs without mobility, consumed, or presence of a small round emergence orifice on the nymph). The health/intact nymphs are orange with darker points and mobile depending on the nymph stage (figure 2). Only 3 – 5th install nymphs were counted. The parasitism was calculated using the formula: PN/ (PN+HN) * 100, with (PN) being the number of parasitized nymphs and (HN) the number of healthy nymphs. (Erbilgin et al, 2004).

Figure 1 A-Team setting up cages for release of *P. bliteus* to control Eucalypts pest *G. brimblecombei (*Soroti Municipality/Ramathan (Kichinjagyi) release site); B-Tying the sleeves of the Muslim cloth to enclose *P. bliteus* in cage (release site in Asuret Sub County, Soroti District); C-A cage properly marked and bio agents already released for parasitism by *P. bliteus* (Photo Credit: Ronald Kisekka, NaFORRI)

Figure 2 Nymphs of *Glycaspis brimblecombei* (red gum lerp psyllid) A: live nymph B. consumed nymph with wasp exit hole (Photo Credit: Jack Kelly Clark)

2.3. Data analysis

Descriptive statistics were used to compare means of different release sites at a given time. A Generalized Linear Mixed model (GLM) was used to compare mean parasitism of the different crown sections and the different leaf surfaces. Site was used as the random variable and mean parasitism was the response variable. The explanatory variables were crown section and the leaf surface. Data was analyzed using GLMM owing to nested structure of different host crown sections and leaf surfaces under the different sites (Bates et al., 2010). The significance level was set at $p < .05$. Levene's test was used to test for homogeneity of Variance. The test was statistically significant ($F(3, 56) = 3.97$, $p < .05$), indicating a departure from homoscedasticity. Data was Arcsine transformed to conform to normality required by parametric test.

2.4. Calculation of dispersal distance

The dispersal distance of *Psyllaephagus bliteus* was estimated by measuring the direct distance between surveyed Eucalyptus plantations and the nearest parasitoid release site. We used the formula posted by (BlueMM 2007) in Microsoft Excel;

Distance=ACOS(COS(RADIANS(90-Lat1)) *COS(RADIANS(90-Lat2)) + SIN(RADIANS(90- Lat1))*SIN(RADIANS(90-Lat2))*COS(RADIANS(Long1-Long2))) *R

Where:
Lat1 = Latitude of the release point;
Lat2 = Latitude of the sampling point;
Long1 = Longitude of the release point;
Long2 = Longitude of the sampling point;
R = Earth's radius (mean radius = 6,371 km).

3. Results

3.1. Recovery of *Psyllaephagus bliteus* from release sites

Mummified nymphs of *Glycaspis brimblecombei* and nymphs with orifice were recovered in all the ten release sites in table 1 (100%). The overall mean percentage parasitism was 53% for the sampling duration, highest parasitism was 80% recorded in Kichinjaji Soroti district with coppices of *Eucalyptus camadulensis* in December 2020 and the lowest was 15% in Kabarole district with GC clones in January 2020. The highest mean parasitism in a year recorded was in 2020 (60%) while the lowest was 2021 (50.6%) (Figure3). More mummified nymphs were collected from the mid and low crown sections compared to the top crown section (Table 2), however, the difference was not statistically significant (p=.33) and so was parasitism on the different the leaf surfaces (p = .86).

Table 1 Established hot spots of *G. brimblecombei* where *P. bliteus* was released in Uganda

District /Site	Aver temp (°C)	Age of trees at time of release (years)	Eucalyptus species	Total area of plantation (acre)
Mbale/ Namanyonyi	32	2	GC clones	1
Namutumba/ Magadda	32	2	GC clones	0.5
Soroti/ Asuret	32	1	GC clones	2.5
Soroti / Kichinjaji	32	4	*E. camaldulensis*	5
Kabale / Kitumba	17	1	*E. grandis*	2
Kabale/ Kahama	17	4	*E. grandis*	2
Kyakwanzi /Kikonda	31	2	GC clones	20
Kabarole	20	3	*E. grandis*	2
Jinja/ Agricultural show grounds	22	3	clones	Exhibition trees
Mukono/NaFORRI	21	seedlings	*E. camaldulensis*	Rearing

3.2. Survey for dispersal of *Psyllaephagus bliteus*

Mummified nymphs of *Glycaspis brimblecombei* were found in all of the surveyed Eucalyptus plantations (100%) (Figure 4, Table 3). Elevation of the plantations ranged from 622 M to 1885 M and average annual temperature varied from 17 °C to 35 °C. Mummified nymphs were also found on all Eucalyptus species and clones common in Uganda (*E. grandis*, *E. camaldulensis*, *E. tereticornis* and clones). The furthest distance recorded was 295 km in Arua district (table 3).

Table 2 Mean parasitism by *P. bliteus* on *G. brimblecombei* for the different tree crown sections and leaf sides

Crown section	Leaf side	Mean parasitism (%)
low	Abaxial	53.1
	Adaxial	53.2
Section total		**53.1**
Mid	Abaxial	54.0
	Adaxial	52.9
Section total		**53.5**
Top	Abaxial	53.0
	Adaxial	52.6
Section total		**52.8**
Grand Total		**53.2**

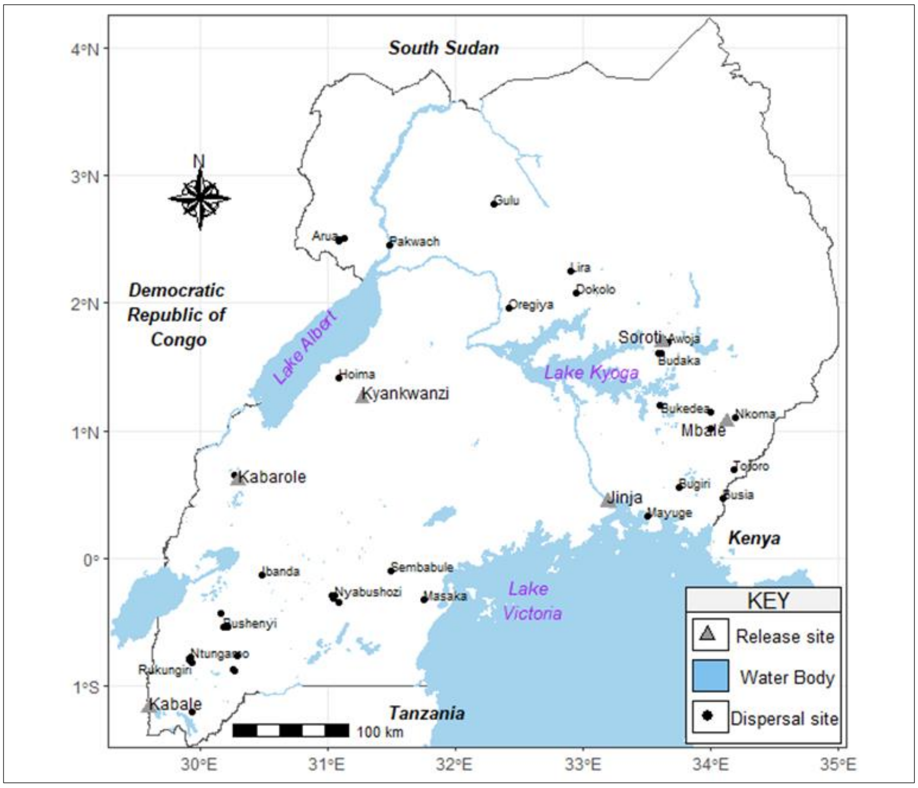

Figure 3 Map of Uganda showing surveyed districts where mummified nymphs of *G. brimblecombei* were recovered

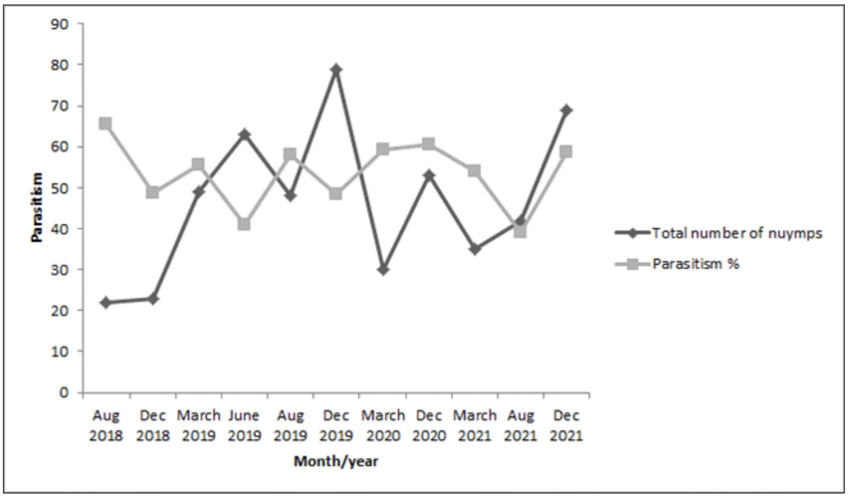

Figure 4 A graphical representation of the level of parasitism by *P. bliteus* a biological control agent of a Eucalyptus pest *G. brimblecombei* in Uganda after four years of monitoring

Table 3 Collection of mummified *G. brimblecombei* nymphs in Eucalyptus plantations across Uganda from August 2018 to March 2022. Dispersal distance = distance between nearest release site and plantation surveyed. Latitude and longitude co-ordinates are the World Geodetic System 1984

District	Latitude	Longitude	*Eucalyptus* species	Dispersal distance
Nyabushozi	-0.2886126	31.028745	*E.grandis*	188
Nyabushozi	-0.293666	31.0545839	*E.camadulensis*	190
Nyabushozi	-0.3529481	31.0889862	*E.grandis*	190
Nyabushozi	-0.3071659	31.0440679	*GC*	188
Ibanda	-0.1339462	30.4913529	*E.grandis*	153
Bushenyi	-0.5420352	30.1920686	*E.camadulensis*	97
Bushenyi	-0.540735	30.1839077	*E.grandis*	97
Bushenyi	-0.5437829	30.2176864	*E.camadulensis*	99
Bushenyi	-0.4341701	30.1566488	*E.grandis*	104
Bushenyi	-0.8671572	30.2614454	*E.camadulensis*	82
Ntungamo	-0.7842884	29.9251729	*E.camadulensis*	57
Ntungamo	-0.7885323	29.9242139	*E.grandis*	57
Rukungiri	-0.799788	29.9232928	*E.grandis*	56
Rukungiri	-0.8204721	29.9446689	*E.grandis*	56
Kabale	-1.2480608	29.9841506	*E.grandis*	43
Kabale	-1.2530497	29.9910071	*E.grandis*	44
Kabale	-1.250595	29.9920721	*E.camadulensis*	44
Kabale	-1.2134084	29.938444	*E.grandis*	38
Kabale	-1.2724998	30.0296665	*E.grandis*	49
Kabale	-1.244425	30.0539571	*E.grandis*	51

Kabale	-0.7696517	30.287699	*E.grandis*	89
Fortportal	0.651847	30.273483	*GC*	217
Pakwach	2.4641456	31.480073	*E. tereticornis*	253
Pakwach	2.4512568	31.481777	*E.camadulensis*	252
Nebbi	2.5139905	31.1339741	*E. tereticornis*	291
Arua	2.494943	31.090955	*E.camadulensis*	295
Arua	2.4960319	31.0918839	*E.camadulensis*	295
Dokolo	2.0753568	32.9464077	*E.camadulensis*	86
Dokolo	2.0753568	32.9464077	*GC*	86
Soroti	1.7239321	33.6240465	*E.camadulensis*	4
Awoja	1.6866298	33.6725593	*GC*	6
Awoja	1.686698	33.6725593	*GC*	6
Otatai	1.6112991	33.609708	*GC*	9
Otatai	1.6112970	33.609708	*GU*	9
Oregiya	1.6084163	33.5899809	*E.camadulensis*	10
Nkoma	1.1018247	34.1936916	*E.camadulensis*	91

4. Discussion

The aim of this study was to determine the establishment of *Psyllaephagus bliteus* a larvae parasitoid of Eucalyptus pest *Glycaspis brimblecombei* by surveying release sites for recovery of parasitized nymphs of *G. brimblecombei* and evaluating the dispersal of *P. bliteus* beyond areas where the parasitoid wasp was released.

The overall mean parasitism of 53% depicted in figure 3 is in range with results noted in other countries where the parasitoid has been introduced. For example Brazil recorded values ranging between 30.32 and 79.34% (Peris- felipo et al., 2011), Portugal 48% (Boavida et al., 2016) and Chile 80 % (Huerta et al., 2011). However parasitism by *Psyllaephagus bliteus* was low in countries like Tunisia, 6.8% (Dhahri et al., 2014) and 15% in the interior valleys of California (The USA) (Daane et al., 2012). The contrast in parasitism levels of *P. bliteus* in different countries is probably due to temperature differences but not latitude (Daane et al., 2012; Ferreira - Filho et al., 2018).

A study on environmental factors impacting parasitism by *Psyllaephagus bliteus* noted daily temperatures in the atmosphere as the most limiting factor (Caleca et al., 2018), in support Daane et al., (2005) suggested that development time and longevity of *P. bliteus* is influenced by temperature. *P. bliteus* development rate is in the range of 22 and 30 °C (Daane et al., 2012). Similar results have been noted in other parasitoid species for example 75% parasitism by *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) is explained by temperature (Gullan & Cranston, 2010). Temperature in Uganda is within the optimal range 30 °C of the parasitoid threshold this partly explains the results of this study. Hence the relatively high mean parasitism recorded in this study.

Research also attributes the establishment of parasitoids in new territories to parasitoid traits. Specialist parasitoids are more likely to establish compared to generalist (Rossinelli & Bacher, 2014; Kimberling, 2004; Stiling, 1990). This is because specialist parasitoids have coevolved with plant species (including imported crops) such that when a particular plant species is attacked by a herbivore, it releases herbivore-induced plant volatiles which attract the parasitoid to pest infested plants (Abdala et al., 2019). Another parasitoid trait that could have favored results from this study is koinobiont parasitoids which are believed to be more specialized and are more likely to establish compared to idiobiont parasitoids that are generalised (Jarrett &Szucs, 2022; Quicke, 1997). *Psyllaephagus bliteus* is a koinobiont parasitoid specialist to *Glycaspis brimblecombei* (Daane et al., 2005).

Features of host biology also appear to influence the establishment rates of parasitoids. Parasitoids to monophagous hosts are more likely to establish compared to parasitoids of polyphagous. Monophagous parasitoids may find it easier to locate their hosts if the hosts are confined to one plant species rather than spread over many. The nature of the host habitat also has an effect on parasitoid establishment, parasitoid hosts in forests are more likely to establish since forests offer stable environments compared to annual crops (Stiling, 1990). *Glycaspis brimblecombei* is known to be monophagous onto Eucalyptus trees that grow as forest trees hence the favorable results from this study (CABI, 2021).

Parasitism by *Psyllaephagus bliteus* along Eucalyptus tree crown was also evaluated. There were no significant difference (p = .33) even if mean parasitism indicated more parasitized nymphs from the mid and low sections compared to parasitism in the top section of the crown (table 2). Similar results were noted in other parasitoid species *Trissolcus japonicas* (Hymenoptera: Scelionidae) an egg parasitoid of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) emerged more from eggs collected from mid-canopy compared to other canopy sections (Quinn, 2019), Leite et al*.,* (2020) recorded more parasitism levels in the mid and low section of *Eurytoma sp* (Hymenoptera: Eurytomidae) on *Caryocar brasiliense* camb. (Malpighiales: Caryocaraceae) tree.

On evaluation of parasitism on the different leaf sides (abaxial and adaxial), parasitism on the abaxial was not different from that on adaxial side. This indicates that parasitism of *Glycaspis brimblecombei* by *Psyllaephagus bliteus* is not affected by leaf surface. Even if *G. brimblecombei* prefers to oviposit on the abaxial surface of the leaf, to avoid competition nymphs of *G. brimblecombei* moves to the adaxial yet *P. bliteus* prefers third to fourth instar nymphs that are seemingly more agile thus can move widely (Daane et al., 2005). Probably this is why mummified nymphs of *G. brimblecombei* where collected from both leaf sides.

Psyllaephagus bliteus was able to dispersal all over the region of Sicily (Italy) a short time after release (Caleca et al., 2018), in California in the USA, *P. bliteus* was recovered on sticky traps as early as eight (8) weeks following initial release (Dahlsten et al., 2005). No wonder, the parasitoid was able to dispersal in Uganda. Similar, results were attainted for other parasitoid species for example *Closterocerus chamaeleon* Girault (Hymenoptera: Entedoninae), rapidly spread through short and long distance dispersal after 18 months (Caleca et al., 2011).

Taylor (1963) established that poikilothermic including insects' flight behavior is affected by ambient temperature. For example, *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) maximum flight propensity occurred between 25 and 30 °C (Forsse et al., 1992), *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) wasps flew significantly farther as the ambient temperature increased (Gaudon et al., 2018) thus its speculated that warm tropical temperatures in Uganda facilitated the dispersal of *Psyllaephagus bliteus.*

5. Conclusion

Recovery of mummified nymphs of *Glycaspis brimblecombei* in all release sites four years after the initial release of *Psyllaephagus bliteus* is an indication of establishment of *P. bliteus* in Uganda. Research suggests that establishment and control are correlated. Control is only likely after a parasitoid has established. Literature categories degree of control into four groups: none, partial, substantial, or complete. Thus, since *P. bliteus* has established in Uganda we can assume substantial control of *G. brimblecombei*. The dispersal of *P. bliteus* for a maximum distance of 295 km indicates that it transverses approximately 74 km per year. This shows that the use of bio-agents will sustainably manage invasive forestry pests in Uganda. However, there is a need to determine the social-economic impacts of classical biological control of pests along the Eucalyptus value-chain in Uganda.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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