

Host specificity and host stage preference of *Psyllaephagus* species (Hymenoptera: Encyrtidae) towards invasive eucalypt psyllids (Hemiptera: Aphalaridae)

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Abstract

1. Invasive eucalypt psyllids pose a threat to the long-term production of eucalypts worldwide. In order to reduce their economic impact, classical biological control using *Psyllaephagus* species has been used successfully.
2. The objectives of this study were to determine the host specificity and host stage preference of *Psyllaephagus blastopsyllae*, *Psyllaephagus pilosus* and *Psyllaephagus bliteus* using related eucalypt-feeding psyllids, *Blastopsylla occidentalis*, *Ctenarytaina eucalypti*, *Glycaspis brimblecombei* and *Spondyliaspis cf. plicatuloides*.
3. To determine host specificity, three *Psyllaephagus* species were collected locally from their hosts. Five-day-old parasitoids were exposed to all five developmental stages (I–V) of host and non-host psyllids in choice and non-choice tests. Similar bioassays were used to assess parasitoid preferences for specific nymphal stages, identifying their preferred developmental stages of potential hosts.
4. *Glycaspis brimblecombei* was identified as a potential host for *P. blastopsyllae*, and *S. cf. plicatuloides* as an alternate host for *P. bliteus*, though emergence rates were three to four times lower than for their known hosts. *Psyllaephagus pilosus* showed no interest in non-host psyllids. Female parasitoids displayed stage-specific preferences for both known and newly identified hosts.
5. The current findings indicate that some *Psyllaephagus* species attack hosts within an ecological niche, whereas others are highly host specific. Additionally, the distinction between potential host range observed in laboratory conditions and ecological host range in field conditions should be considered, as laboratory tests typically exhibit a broader host range.

KEYWORDS

biological control, choice test, ecological interactions, forest pest, invasive eucalypt psyllids, no-choice test, *Psyllaephagus*

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INTRODUCTION

In many cases, parasitoids influence the population dynamics of their arthropod hosts (Godfray, 1994), and this natural insect regulation system is adopted as biological control in integrated pest management (IPM) (van Driesche et al., 2008). Concerns regarding the potential non-target effects of using biological control agents as part of an IPM strategy have prompted several studies on parasitoid ecology and evolution (Barratt et al., 2006; Clarke et al., 2019; Godfray, 1994; Hajek et al., 2016; Roitberg et al., 2001). As a result, biological control agents aimed for classical biological control are now only released into agricultural or forestry systems for the regulation of invasive insect pests following extensive host-specificity tests aimed at defining the host spectrum of potential biological control agents (Clarke et al., 2019; Hunt et al., 2008; Strong & Pemberton, 2000). Such host specificity can be influenced by various factors. For example, parasitoids are likely to select specific hosts based on the taxonomy and ecology of the hosts (Abram et al., 2021; Stireman & Singer, 2003). This is influenced by the relationship between host taxonomy and the hosts' physiological defences that may require specific adaptations by their parasitoids (Kraaijeveld et al., 1998). In addition, the host phenology, the plants that the host feed upon (Askew, 1994) and the microhabitats in which they feed (Godfray, 1994) can also influence parasitoid host specificity.

In their native range, Australia, eucalypt-feeding insects are associated with a diverse and co-evolved parasitoid fauna (Nahrung et al., 2016). Outside their native range and in the absence of their specific parasitoids, eucalyptus-feeding insects can become pests (Wingfield et al., 2008), a phenomenon commonly referred to as the enemy-release hypothesis (ERH) (Keane & Crawley, 2002). As a result, Australia is a source of natural enemies for classical biological control of alien invasive eucalypt insect pests introduced in new ranges (Hodkinson, 1999; Hurley et al., 2016; Paine et al., 2011). This includes sap-sucking psyllids, which are one of the most serious groups of invasive eucalypt pests globally, and for which natural enemies from Australia have been introduced (Makunde et al., 2020). These psyllids occupy a niche as herbivores on *Eucalyptus*, where they exploit the phloem for nutrition, often leading to reduced plant health and vigour.

Psyllids are predominantly parasitised by Chalcidoid wasps (Hymenoptera), which include representatives of the families Aphelinidae, Encyrtidae, Eulophidae and Pteromalidae (Riek, 1962). Among the encyrtids, species of the genus *Psyllaephagus* Ashmead stand out for being frequently used as biocontrol agents for invasive psyllids (Berry, 2007). The genus *Psyllaephagus* reports 246 species for Australia (Noyes, 2020), where many of the endoparasitoids are host-specific (Noyes & Hanson, 1996), such as *Psyllaephagus pilosus* Noyes in *Ctenarytaina eucalypti* (Maskell) (Hodkinson, 1999). However, some *Psyllaephagus* species have multiple hosts. *Psyllaephagus bliteus* Riek, for example, uses *Creiis costatus* Froggatt (Hemiptera: Aphalaridae), *Glycaspis* spp. and *Spondyliaspis* cf. *plicatuloides* (Froggatt) as hosts (Berry, 2007; Bush et al., 2020; Hollis, 2004).

Four Australian eucalypt psyllid species in the family Aphalaridae have been accidentally introduced into South Africa and became

established as pests in eucalypt plantations. These are the blue gum psyllid, *Ctenarytaina eucalypti* (Maskell, 1890), recorded in the 1920s (Laing, 1922); the eucalyptus psyllid, *Blastopsylla occidentalis* Taylor, recorded in 2006; the red gum lerp psyllid, *Glycaspis brimblecombei* Moore, recorded in 2012; and the shell lerp psyllid, *Spondyliaspis* cf. *plicatuloides*, recorded in 2014 (Bush et al., 2016). Nymphal instars and adults of *C. eucalypti*, *B. occidentalis*, *G. brimblecombei* and *S. cf. plicatuloides* feed on sap, causing physical damage to plant tissues. Feeding by *C. eucalypti* nymphal instars result in leaf curl, discoloration and in extreme cases may result in dieback (Hodkinson, 1999, 2007). The nymphal instars of *C. eucalypti* secrete white waxy secretions. Nymphal instars of *B. occidentalis* secrete large quantities of tiny wax-covered globules and white flocculent and, in severe infestation, results in eucalypts bud degeneration (Dzokou et al., 2020). *Glycaspis brimblecombei* nymphal instars excrete large amounts of honeydew, and plant damage is primarily the result of sooty mould on leaf surfaces (Queiroz & Burckhardt, 2007) and feeding gradually leads to leaf discoloration, leaf drop, twig dieback and in case of serious infestations, whole tree death can occur (Bella & Rapisarda, 2013; Paine et al., 2000). Infestation of susceptible eucalypts by *S. cf. plicatuloides* is normally accompanied by direct damage due to sap sucking, which results in leaf necrosis followed by defoliation, while lerps cause indirect damage by covering the leaf surfaces, reducing light interception for photosynthesis.

The co-evolved parasitoids of *B. occidentalis*, *C. eucalypti* and *G. brimblecombei*, namely *Psyllaephagus blastopsyllae* Tamesse, Soufo, Tchanatame, Dzokou, Gumovsky & Coninck, *P. pilosus* and *P. bliteus*, respectively, were accidentally introduced into South Africa together with their host (Bush et al., 2016). The specificity of these *Psyllaephagus* species, however, is currently unknown. Understanding the host specificity of these parasitoids is important for determining whether *Psyllaephagus* species play a role in regulating the populations of other psyllid species pests, including new introductions such as *S. cf. plicatuloides*. We hypothesize that parasitoids of closely related eucalypt-feeding psyllids exhibit high host specificity, influenced by phylogenetic relatedness or biochemical traits of their hosts. The objectives of this study were, therefore, to investigate the host range and specificity of *P. pilosus*, *P. blastopsyllae* and *P. bliteus* and to determine their host stage preferences.

METHODS

Source of psyllids

Adult males and females of *C. eucalypti* and *B. occidentalis* were obtained from the Forestry and Agricultural Biotechnology Institute (FABI) nursery and Biocontrol Centre at the Innovation Africa campus, University of Pretoria (25° 45.149' S, 28° 15.380' E). The identities of the two free-living psyllids, *C. eucalypti* and *B. occidentalis*, were done following keys by Maskell (1890) and Hodkinson and White (1981), as well as keys by Taylor (1985) and Burckhardt and Elgueta (2000), respectively. The two lerp forming psyllids, *G. brimblecombei* identified following keys by Moore (1964) and Halbert et al. (2001) and *S.*

cf. *plicatuloides*, were obtained from a *Eucalyptus* plantation of the National Zoological Gardens (referred to as the 'Zoo plot'), Rietondale, Pretoria, Gauteng (25° 44.139' S, 28° 14.435' E). All psyllid species were reared in potted eucalypts (0.5–1 m tall) in protected walk-in screen cages at the FABI Biocontrol Centre. This was done to ensure that the immature stages used in all the assays were free from parasitoids.

Source of *Psyllaephagus* spp.

Psyllaephagus blastopsyllae and *P. pilosus* were sourced from their respective hosts, *B. occidentalis* and *C. eucalypti*, at the FABI nursery. *Psyllaephagus bliteus* was collected from parasitised *G. brimblecombei* in the 'Zoo Plot' plantation. Eucalypt leaves and branches with psyllid mummies were collected 5 days before experimentation and placed in ventilated plastic containers. Parasitoid emergence was monitored twice daily, and the parasitoids were sexed and transferred to glass vials (30 parasitoids/vial) in a 1:1 female-to-male ratio. They were kept at 22 ± 2°C, 60%–70% relative humidity, and a LD 13:11 h cycle for 72 h to ensure maturation and mating. Parasitoids were fed honey-soaked paper towel squares. Identification of the *Psyllaephagus* species followed published keys by Tamesse et al. (2014), Riek (1962) and Noyes (1988), with identities confirmed by sequencing the mitochondrial DNA cytochrome b region (Wondrafrash et al., 2021).

No-choice host specificity testing

Behavioural assays

The host specificity of the three parasitoids, *P. blastopsyllae*, *P. pilosus* and *P. bliteus* was evaluated under a controlled environment (22 ± 2°C and 60%–70% RH) and using 5–7-day-old wasps. Each *Psyllaephagus* species was separately exposed to all four psyllids, namely *B. occidentalis*, *C. eucalypti*, *G. brimblecombei* and *S. cf. plicatuloides* with the known host of the parasitoids acting as the positive control for the different bioassays. All assays were performed following the method in Bush et al. (2020). Each parasitoid and psyllid combination had three replications, with 10 repeats per each combination per replication.

For each psyllid-parasitoid species combination, glass Petri dishes (80 × 15 mm) lined with filter paper (Whatman, 7 mm) were used. Leaf pieces (for lerp psyllids) or shoot tips (for free-living psyllids) infested with 20 individuals (four from each nymphal stage, I–V) of a single test species were affixed in the dishes. One female and one male *Psyllaephagus* were placed in each experimental unit (glass Petri dish). A small piece of honey paper strip was placed inside the lid of the glass Petri dish to feed the parasitoids during exposure time, ensuring survival and oviposition. The glass Petri dish was sealed with PARAFILM® M Laboratory film (Pechiney Plastic Packaging) to prevent the parasitoids from escaping.

In all tests, only the behaviour of the females was observed and the males remained in the petri dish for the duration of the

observations. For each parasitoid specificity test, five observed behaviours were distinguished and recorded: (1) walking, in which the female parasitoid moves on the surface of the leaves/shoot tips without contacting the psyllids; (2) antennation, in which the insect grooms its antennae or other body parts with its forelegs; (3) rest, characterized by the parasitoid remaining motionless; (4) probing, in which the parasitoid used its proboscis to probe a nymph; and (5) oviposition, in which the parasitoid holds the nymph in order to lay eggs. The behaviour (walking, antennation, rest, probing and oviposition) of the female *Psyllaephagus* was monitored for the first 30 min and then for 10 min every hour for the next 3 h. If the female did not become active within the first 30 min, she was deemed unfit for testing and was replaced. The foraging behaviours of the parasitoids (antennation, probing, and oviposition) were captured using an Olympus DP21 camera system attached to a Nikon SMZ1500 stereoscopic zoom microscope (Nikon, Tokyo, Japan).

Emergence success of *Psyllaephagus* species

Due to the inability of the nymphal instars of the lerp builders, *S. cf. plicatuloides* and *G. brimblecombei*, to survive on cut leaves, potted plants were used to investigate the reproductive output of the parasitoids. *Eucalyptus globulus*, *E. dunnii*, *E. camaldulensis* and *Corymbia ficifolia* were propagated in 5-L pots under controlled conditions. The plants were grown 2–3 months before the assays at 25 ± 1°C, with relative humidity ranging from 60% to 70%, and a LD 13:11 h photoperiod. To ensure the absence of parasitoids, the immature stages used in the assays were reared within protected walk-in screen cages. These tree species were used as they are known suitable hosts for the respective psyllid species. The trees were infested with adult psyllids: *C. eucalypti* on *E. globulus*, *B. occidentalis* on *E. dunnii*, *G. brimblecombei* on *E. camaldulensis* and *S. cf. plicatuloides* on *C. ficifolia*. Before introducing the parasitoids, psyllid samples were collected from each plant and incubated separately to ensure no parasitism was present. For each psyllid-parasitoid combination, 10 infested trees with at least 20 nymphal instars were sleeved with 45 cm × 50 cm entomological sleeves, and a pair (female and male) of the parasitoid was introduced to each sleeve. The potted eucalypt plants were watered daily. After 40 days, leaves with mummies of the four herbivorous species were collected separately and placed in separate 18 × 12 × 7 cm ventilated plastic microboxes (TP1200) that were lined with paper towel to absorb excess moisture. For 2 weeks, the paper towel was changed every second day, and emerging insects were aspirated and stored in 70% ethanol for identification. The number of *Psyllaephagus* wasps that emerged from the sleeve were recorded, and the species were morphologically confirmed using the previously mentioned keys.

Choice host specificity testing

Choice tests were performed for *P. blastopsyllae* and *P. bliteus*, as these parasitoid species had shown antennation, probing or oviposition behaviour for their known hosts and another test psyllid species

in the no-choice observational trial. The set-up of the choice tests was similar to the no-choice test set-up. In this case, leaf pieces of *G. brimblecombei*, *C. eucalypti* and *B. occidentalis* were present in each experimental unit when testing host specificity of *P. blastopsyllae*. In the same way, leaf pieces of *G. brimblecombei* and *S. cf. plicatuloides* were present in each experimental unit when testing host specificity of *P. bliteus*. In each experiment, an equal number (20 individuals where four individuals represent each nymphal stage) of all the five developmental stages (I–V) of the known host and non-host psyllid were simultaneously exposed to a 5-day-old female mated female and a male *Psyllaephagus* in a glass Petri dish for 3 h. All experiments were completed under laboratory conditions at $22 \pm 2^\circ\text{C}$ and 60%–70% RH.

Host stage preference

Host stage preference tests were performed for *P. pilosus* towards *C. eucalypti*, *P. blastopsyllae* towards *B. occidentalis* and *G. brimblecombei* and *P. bliteus* towards *G. brimblecombei* and *S. cf. plicatuloides* as these parasitoid species had shown antennation, probing or oviposition behaviour for their known hosts and another test psyllid species in the choice observational trial and parasitoid developmental trial. The host stage preferences tests were evaluated using choice and no-choice assays in a controlled environment ($22 \pm 2^\circ\text{C}$ and 60%–70% RH). Each parasitoid and psyllid nymphal stage(s) combination was replicated three times, with 10 repeats per replication.

No-choice host stage preference assays

In glass Petri dishes (80×15 mm), 20 individuals of each life stage (I–V nymphal instars) of *C. eucalypti* were separately exposed to a 5-day-old mated female *P. pilosus*. The same procedure was done for *P. blastopsyllae* towards *B. occidentalis* and *G. brimblecombei*, as well as for *P. bliteus* towards *S. cf. plicatuloides* and *G. brimblecombei*. The foraging behaviour (antennation, probing and oviposition) of the three parasitic wasps on different nymphal stages was monitored for the first 30 min and then for 10 min interval until 60 min were reached and not 3 h as we found no further activities of parasitoids after 60 min. The different nymphal instars of *C. eucalypti* were differentiated following methods by Sharma et al. (2015), whereas those of *B. occidentalis* and *G. brimblecombei* were differentiated using methods by Taylor (1985) and Ávalos et al. (2021), respectively.

Choice host stage preference assays

In a glass Petri dish, 10 individuals of each immature stage (I–V nymphal instars) of *C. eucalypti*, *B. occidentalis* or *G. brimblecombei* were separately exposed to 5-day-old mated females of *P. pilosus* (*C. eucalypti* only), *P. blastopsyllae* (*B. occidentalis* and *G. brimblecombei*

separately) and *P. bliteus* (*G. brimblecombei* and *S. cf. plicatuloides* separately). If the tested *Psyllaephagus* species showed no interest in the psyllid species after the first 10 min of observation, they were discarded. The parasitoid's behaviour was recorded as described for the no-choice host stage preference testing.

Data analysis

The host specificity of the three parasitoids on the four test psyllids was assessed by comparing the absence or presence of antennation, probing and oviposition behaviours, as well as the number of offspring per parasitoid couple, across the four-psyllid species. Additionally, the preference of parasitoids for different life stages of the psyllids was assessed by comparing the same behavioural responses across the various developmental stages of the psyllids. The presence or absence of wasps showing antennation, probing or oviposition behaviour and the number of parasitoid offspring were used as the dependant variables, and parasitoid species and psyllid host species were the independent variables. In the no-choice tests, the proportion of parasitoids exhibiting positive behaviours (antennation, probing, and oviposition) was calculated and included as part of the descriptive statistics. Further, Chi-square tests and checks for data normality and homogeneity of variances using one-way ANOVA for no-choice tests together with choice tests and host stage preference tests were conducted. For non-homogeneous variances, the Kruskal–Wallis test was used, with subsequent Dunn's test for significant pairwise differences. Alternatively, when variances were heterogeneous but data met normality assumptions, Welch's ANOVA was applied, followed by Games–Howell tests for pairwise comparisons post hoc. Statistical analyses were performed using SPSS v29.0 (IBM Inc., New York, NY, USA). Boxplots for offspring counts per female of each parasitoid on the four psyllids were generated using the ggplot2 package in R 4.4.1 (R Core Team, 2024).

RESULTS

No-choice host specificity testing

Behavioural assays

When the three potential non-target hosts, *S. cf. plicatuloides*, *G. brimblecombei* and *B. occidentalis*, were exposed to *P. pilosus* females separately for each psyllid, none of the female parasitoids showed recognition or acceptance behaviour towards the three eucalypt psyllids. However, antennation, probing and oviposition behaviours were frequently observed towards the nymphal instars of *C. eucalypti* (Table 1).

The psyllid species had a significant effect on antennation, probing and oviposition behaviours of *P. blastopsyllae* ($\chi^2 = 74,444$; $67,337$; $67,337$, $df = 3$, $p < 0.001$; $H(3) = 73,824$; $66,776$; $66,776$, $p < 0.001$) (Table 2). Dunn's post hoc pairwise comparisons with

TABLE 1 Proportion of *Psyllaephagus pilosus*, *P. blastopsyllae* and *P. bliteus* observed showing antennation, probing and oviposition to *Ctenarytaina eucalypti*, *Blastopsylla occidentalis*, *Glycaspis brimblecombei* and *Spondylaspis cf. plicatuloides* in no choice tests.

Parasitoid	Test psyllid	No. antennation attempts	No. probing attempts	No. oviposition attempts
<i>Psyllaephagus pilosus</i>	<i>Ctenarytaina eucalypti</i>	1.0 ^b	0.9 ^b	0.9 ^a
	<i>Blastopsylla occidentalis</i>	0.0 ^a	0.0 ^a	0.0 ^a
	<i>Glycaspis brimblecombei</i>	0.0 ^a	0.0 ^a	0.0 ^a
	<i>Spondylaspis cf. plicatuloides</i>	0.0 ^a	0.0 ^a	0.0 ^a
<i>Psyllaephagus blastopsyllae</i>	<i>Blastopsylla occidentalis</i>	1.0 ^c	0.9 ^c	0.9 ^c
	<i>Ctenarytaina eucalypti</i>	0.5 ^b	0.5 ^b	0.5 ^b
	<i>Glycaspis brimblecombei</i>	0.9 ^c	0.9 ^c	0.9 ^c
	<i>Spondylaspis cf. plicatuloides</i>	0.0 ^a	0.0 ^a	0.0 ^a
<i>Psyllaephagus bliteus</i>	<i>Glycaspis brimblecombei</i>	1.0 ^b	1.0 ^b	1.0 ^b
	<i>Spondylaspis cf. plicatuloides</i>	0.8 ^b	0.8 ^b	0.8 ^b
	<i>Blastopsylla occidentalis</i>	0.0 ^a	0.0 ^a	0.0 ^a
	<i>Ctenarytaina eucalypti</i>	0.0 ^a	0.0 ^a	0.0 ^a

Note: The known host psyllid of each parasitoid is indicated in bold. Each parasitoid and psyllid combination was replicated three times, with 10 repeats per replication. Different letters indicate significant differences in the presence/absence of antennation, probing and oviposition behaviours of three parasitoids among the four tested hosts ($p < 0.05$), as determined by pairwise comparisons using the Bonferroni correction. These differences apply only within each parasitoid species (Tables S1–S3).

TABLE 2 Statistical analysis of *Psyllaephagus pilosus*, *P. blastopsyllae* and *P. bliteus* in no-choice and choice tests on *Ctenarytaina eucalypti*, *Blastopsylla occidentalis*, *Glycaspis brimblecombei* and *Spondylaspis cf. plicatuloides*.

Parasitoid	Type of test	Hosts	Parameter	χ^2	df	p value	H	df	p value
<i>Psyllaephagus pilosus</i>	No-choice	<i>Ctenarytaina eucalypti</i>	Antennation	120,000	3	<0.001	119,000	3	<0.001
		<i>Blastopsylla occidentalis</i>	Probing	109,565	3	<0.001	108,652	3	<0.001
		<i>Glycaspis brimblecombei</i>	Oviposition	109,565	3	<0.001	108,652	3	<0.001
		<i>Spondylaspis cf. plicatuloides</i>							
<i>Psyllaephagus blastopsyllae</i>	No-choice	<i>Ctenarytaina eucalypti</i>	Antennation	74,444	3	<0.001	73,824	3	<0.001
		<i>Blastopsylla occidentalis</i>	Probing	67,337	3	<0.001	66,776	3	<0.001
		<i>Glycaspis brimblecombei</i>	Oviposition	67,337	3	<0.001	66,776	3	<0.001
		<i>Spondylaspis cf. plicatuloides</i>							
<i>Psyllaephagus bliteus</i>	No-choice	<i>Ctenarytaina eucalypti</i>	Antennation	103,217	3	<0.001	102,357	3	<0.001
		<i>Blastopsylla occidentalis</i>	Probing	103,217	3	<0.001	102,357	3	<0.001
		<i>Glycaspis brimblecombei</i>	Oviposition	103,217	3	<0.001	102,357	3	<0.001
		<i>Spondylaspis cf. plicatuloides</i>							
<i>Psyllaephagus blastopsyllae</i>	Choice	<i>Blastopsylla occidentalis</i>	Antennation	33,300	2	<0.001	32,930	2	<0.001
		<i>Glycaspis brimblecombei</i>	Probing	31,521	2	<0.001	31,171	2	<0.001
		<i>Ctenarytaina eucalypti</i>	Oviposition	31,521	2	<0.001	31,171	2	<0.001
<i>Psyllaephagus bliteus</i>	Choice	<i>Glycaspis brimblecombei</i>	Antennation	11,279	1	<0.001	11,091	1	<0.001
		<i>Spondylaspis cf. plicatuloides</i>	Probing	9774	1	<0.001	9611	1	=0.002
		<i>Spondylaspis cf. plicatuloides</i>	Oviposition	9774	1	<0.001	9611	1	=0.002

Note: The table presents χ^2 and H values with degrees of freedom (df) and p -values for antennation, probing, and oviposition behaviours.

Bonferroni correction revealed no significant differences in antennation, probing, or oviposition preferences of *P. blastopsyllae* between *G. brimblecombei* and *B. occidentalis* ($z = 8.000, 4.000, 4.000$; $p = 1.000, 0.602, 0.602$). In contrast, all other comparisons within and between psyllid species were statistically significant ($p < 0.001$; Tables S1 and S2). *Psyllaephagus blastopsyllae* antennated, probed and

inserted their ovipositors on *G. brimblecombei* nymphal instars found outside their lerps, as the nymphal instars have a tendency of exiting the lerps after disturbance by lerp eaters or removal of leaves with the nymphal instars from the source tree (PT Makunde, pers. obs.). Similar behaviours were observed towards *C. eucalypti* nymphal instars, as well as on its natural host, *B. occidentalis*. *Psyllaephagus*

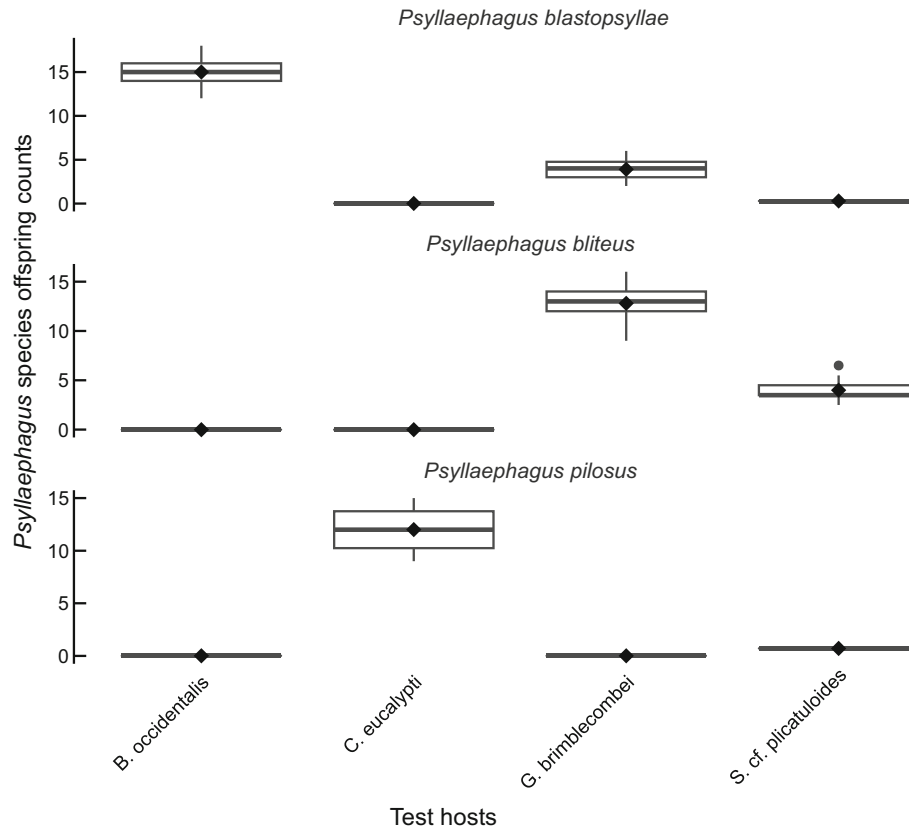


FIGURE 1 Box plots depicting the data distributions for offspring of each parasitoid on different psyllid species. The black diamonds indicate the mean number of *Psyllaephagus pilosus*, *P. blastopsyllae* and *P. bliteus* offspring that emerged from *Ctenarytaina eucalypti*, *Blastopsylla occidentalis*, *Glycaspis brimblecombei* and *Spondyliaspis cf. plicatuloides* in no-choice development trials. The Kruskal–Wallis test revealed significant differences in the offspring counts of *P. pilosus* ($H(3) = 37.975$, $p < 0.001$), *P. blastopsyllae* ($H(3) = 37.669$, $p < 0.001$) and *P. bliteus* ($H(3) = 37.681$, $p < 0.001$) across host species: *B. occidentalis*, *C. eucalypti*, *G. brimblecombei* and *S. cf. plicatuloides*. Different letters indicate significant differences in parasitoid development among the four tested hosts ($p < 0.05$), as determined by pairwise comparisons using the Bonferroni correction. These differences apply only within each parasitoid species (Tables S3 and S4).

blastopsyllae antennated towards *C. eucalypti* and only inserted their ovipositors into orange/yellow nymphal instars (I and II), avoiding nymphal instars with distinctive black spots on the head, thorax and abdomen. However, *P. blastopsyllae* females struggled to retract their ovipositors from the tiny, attacked nymphal instars. Furthermore, *P. blastopsyllae* females struggled to move on the white waxy secretions of *C. eucalypti* nymphal instars attached to the leaf tips and shoots, and continuously rubbed the secretions off their fore and hind legs. *Psyllaephagus blastopsyllae* roamed on *S. cf. plicatuloides* lerps restlessly and no typical antennation, probing or oviposition towards the psyllid was observed.

Psyllid species had a pronounced impact on the antennation, probing, and oviposition behaviours of *P. bliteus* (Table 1), with consistent χ^2 (103.217, $df = 3$, $p < 0.001$) and H values (102.357, $p < 0.001$) for all behaviours (Table 2). *Psyllaephagus bliteus* females antennated frequently to the lerp-forming psyllids, *G. brimblecombei* and *S. cf. plicatuloides*, but not the free-living psyllids, *C. eucalypti* and *B. occidentalis*. Dunn's test with Bonferroni correction of antennation, probing and oviposition behaviours identified no significant preferences between specific host pairs, *G. brimblecombei* and *S.*

plicatuloides (Table S3). The oviposition in *G. brimblecombei*, the natural host, was mostly direct through the lerps, with less incidence under the lerps, while successful oviposition in *S. cf. plicatuloides* typically occurred when the ovipositor was slipped under the edge of the lerp, rather than through the lerp.

Emergence success of *Psyllaephagus* species

The emergence of *P. pilosus* was observed exclusively on sleeved plants with *C. eucalypti*, with no emergence from other tested psyllid species (Figure 1). The progeny of *P. blastopsyllae* varied significantly among host species ($H(3) = 37.669$, $p < 0.001$). On average, 15 offspring emerged from *B. occidentalis*, four from *G. brimblecombei* and none from *S. cf. plicatuloides* or *C. eucalypti* (Figure 1). Pairwise comparisons with Bonferroni correction revealed significantly higher emergence from *B. occidentalis*, with no significant difference between *C. eucalypti* and *S. cf. plicatuloides* ($p = 1.000$; Table S4). *Psyllaephagus bliteus* emergence was significantly higher from *G. brimblecombei* than from *S. cf. plicatuloides* and other tested psyllids ($H(3) = 37.681$,

$p < 0.001$), with an average of 13 offspring emerging from *G. brimblecombei* and four from *S. cf. plicatuloides*, while no emergence occurred from *C. eucalypti* or *B. occidentalis* (Figure 1). Pairwise comparisons confirmed that *G. brimblecombei* supported significantly higher *P. bliteus* emergence compared with all other tested psyllids ($p < 0.001$ for all; Table S5). Welch's one-way ANOVA further supported that host species significantly influenced offspring production and development ($F(3, 36) = 360.00, p < 0.001$).

Choice host specificity testing

Psyllid species significantly influenced the antennation, probing and oviposition behaviours of *P. blastopsyllae* in choice tests ($\chi^2 = 33.300, 31.521, 31.521, df = 2, p < 0.001; H(2) = 32.930, 31.171, 31.171, p < 0.001$; Table 2). *Psyllaephagus blastopsyllae* exhibited higher antennation, probing and oviposition behaviours towards *B. occidentalis* compared with *G. brimblecombei* and none to *C. eucalypti* during the choice tests. Dunn's test with Bonferroni correction confirmed these differences (Tables S6 and S7). In each trial, *B. occidentalis* was consistently the first psyllid species to elicit antennation from the parasitoids. *Glycaspis brimblecombei* and *S. cf. plicatuloides* had significant effect on antennation, probing, and oviposition behaviours of *P. bliteus* in choice test ($\chi^2 = 76.767, df = 1, p < 0.001; H(1) = 76.255, p < 0.001$ (all behaviours); Table 2). In the presence of *G. brimblecombei*, host recognition and acceptance behaviours were significantly higher for *G. brimblecombei* (mean rank: 109.00) than for *S. cf. plicatuloides* (mean rank: 72.00) ($U = 2385.000, Z = -5.528, p < 0.001$; Figure S1).

Host stage preference

In choice tests, the host stage of *C. eucalypti* significantly affected the antennation, probing and oviposition behaviours of *P. pilosus* ($\chi^2 = 27.36, 22.40, 24.85, df = 4, p < 0.001; H(4) = 27.18, 22.25, 24.68, p < 0.001$; Table 3). *Psyllaephagus pilosus* attacked all five nymphal instars (I–V) of *C. eucalypti*, with post hoc comparisons showing that instars IV and V were preferred over instars I–III (Tables S8–S10). In no-choice tests, the host stage similarly affected the antennation, probing and oviposition behaviours of *P. pilosus* ($\chi^2 = 69.64, 66.38, 69.03, df = 4, p < 0.001; H(4) = 69.17, 65.94, 68.57, p < 0.001$; Table 3). Antennation, probing and oviposition occurred across all instars, with post hoc comparisons again identifying instars IV and V as the most preferred ($p < 0.05$; Tables S11–S13). In choice tests, the host stage of *G. brimblecombei* did not significantly affect the antennation, probing, and oviposition behaviours of *P. blastopsyllae* (Table 3; Tables S14–S16). However, in no-choice tests, host stage significantly influenced these behaviours ($\chi^2 = 14.19, 14.19, 23.64, df = 4, p < 0.001; H(4) = 14.09, 14.09, 23.49, p < 0.001$; Table 3). Although all five instars (I–V) were attacked in the no-choice tests, less antennation, probing and oviposition were observed towards the first instar nymphs. Pairwise

comparisons confirmed higher parasitism rates and preferences for the later instars IV and V ($p < 0.01$; Tables S17 and S18). In both choice and no-choice tests, the host stage of *C. eucalypti* significantly influenced the antennation, probing and oviposition behaviours of *P. blastopsyllae* (choice: $\chi^2 = 21.94, 24.95, 23.62, df = 4, p < 0.001; H(4) = 21.79, 24.78, 23.46, p < 0.001$; no-choice: $\chi^2 = 35.19, 33.99, 26.67, df = 4, p < 0.001; H(4) = 34.96, 33.76, 26.49, p < 0.001$; Table 3). In both experiments, *P. blastopsyllae* exclusively antennated, probed and oviposited on the first and second nymphal instars. Pairwise comparisons confirmed significant differences between instars I–II and the other stages ($p < 0.001$; Tables S19–S21). In both choice and no-choice tests, the host stage of *B. occidentalis* significantly influenced the antennation, probing, and oviposition behaviours of *P. blastopsyllae* (choice: $\chi^2 = 34.60, 29.18, 29.18, df = 4, p < 0.001; H(4) = 34.37, 28.98, 28.98, p < 0.001$; no-choice: $\chi^2 = 63.95, 63.95, 65.28, df = 4, p < 0.001; H(4) = 63.52, 63.52, 64.84, p < 0.001$; Table 3). Dunn's post hoc pairwise comparisons with Bonferroni correction confirmed a significantly higher preference for later stages IV and V ($p < 0.01$; Tables S22–S26).

In both choice and no-choice tests, the host stage of *G. brimblecombei* had a significant impact on the antennation, probing and oviposition behaviours of *P. bliteus* (choice: $\chi^2 = 27.25, 38.64, 38.64, df = 4, p < 0.001; H(4) = 27.07, 38.39, 38.39, p < 0.001$; no-choice: $\chi^2 = 33.00, 33.00, 33.00, df = 4, p < 0.001; H(4) = 32.78, 32.78, 32.78, p < 0.001$; Table 3). Dunn's post hoc pairwise comparisons with Bonferroni correction indicated a significantly stronger preference for the third and fourth instars ($p < 0.01$; Tables S27–S31). Similarly, with *S. cf. plicatuloides*, host stage significantly affected the antennation, probing and oviposition behaviours of *P. bliteus* (choice: $\chi^2 = 51.667, 40.377, 40.876, df = 4, p < 0.001; H(4) = 51.332, 40.108, 40.604, p < 0.001$; no-choice: $\chi^2 = 76.767, 76.767, 76.767, df = 4, p < 0.001; H(4) = 76.255, 76.255, 76.255, p < 0.001$; Table 3). Pairwise comparisons of the tests with Bonferroni correction revealed significant preference for the fourth and fifth instars ($p < 0.001$; Tables S32–S35).

DISCUSSION

Host specificity testing of the three parasitoids, *P. pilosus*, *P. blastopsyllae* and *P. bliteus*, towards *C. eucalypti*, *B. occidentalis*, *G. brimblecombei* and *S. cf. plicatuloides* indicated varying degrees of specificity. *Psyllaephagus pilosus* exhibited a high degree of host specificity, showing significant preference for *C. eucalypti* over the other tested psyllids. In contrast, *P. blastopsyllae* and *P. bliteus* demonstrated broader host acceptance, albeit with lower rates of development and emergence from non-host psyllids. The four psyllids are closely related and belong to the subfamily Spondylaspidinae (Burckhardt et al., 2021; Burckhardt & Ouvrard, 2012). These results confirm the findings of Berndt et al. (2009) that some parasitoids will attack hosts within a specific ecological niche, while others are highly host-specific.

TABLE 3 Statistical analysis of *Psyllaephagus pilosus*, *P. blastopsyllae* and *P. bliteus* in choice and no-choice tests across five host stages (nymphal stages).

Parasitoid	Type of test	Host and nymphal stages	Parameter	χ^2	df	p value	H	df	p value
<i>Psyllaephagus pilosus</i>	Choice	<i>Ctenarytaina eucalypti</i>	Antennation	27,360	4	<0.001	27,178	4	<0.001
			Probing	22,403	4	<0.001	22,254	4	<0.001
			Oviposition	24,845	4	<0.001	24,679	4	<0.001
	No choice	<i>Ctenarytaina eucalypti</i>	Antennation	69,638	4	<0.001	69,173	4	<0.001
			Probing	66,377	4	<0.001	65,935	4	<0.001
			Oviposition	69,033	4	<0.001	68,573	4	<0.001
<i>Psyllaephagus blastopsyllae</i>	Choice	<i>Blastopsylla occidentalis</i>	Antennation	34,598	4	<0.001	34,367	4	<0.001
			Probing	29,178	4	<0.001	28,983	4	<0.001
			Oviposition	29,178	4	<0.001	28,983	4	<0.001
	No choice	<i>Blastopsylla occidentalis</i>	Antennation	63,948	4	<0.001	63,522	4	<0.001
			Probing	63,948	4	<0.001	63,522	4	<0.001
			Oviposition	65,278	4	<0.001	64,843	4	<0.001
	Choice	<i>Ctenarytaina eucalypti</i>	Antennation	21,937	4	<0.001	21,790	4	<0.001
			Probing	24,950	4	<0.001	24,783	4	<0.001
			Oviposition	23,618	4	<0.001	23,460	4	<0.001
	No choice	<i>Ctenarytaina eucalypti</i>	Antennation	35,192	4	<0.001	34,958	4	<0.001
			Probing	33,990	4	<0.001	33,763	4	<0.001
			Oviposition	26,667	4	<0.001	26,489	4	<0.001
	Choice	<i>Glycaspis brimblecombei</i>	Antennation	4651	4	0.325	4620	4	0.329
			Probing	2450	4	0.654	2434	4	0.657
			Oviposition	2990	4	0.559	2970	4	0.563
	No choice	<i>Glycaspis brimblecombei</i>	Antennation	14,188	4	<0.001	14,094	4	=007
			Probing	14,188	4	<0.001	14,094	4	=007
			Oviposition	23,643	4	<0.001	23,486	4	<0.001
<i>Psyllaephagus bliteus</i>	Choice	<i>Glycaspis brimblecombei</i>	Antennation	27,251	4	<0.001	27,069	4	<0.001
			Probing	38,643	4	<0.001	38,386	4	<0.001
			Oviposition	38,643	4	<0.001	38,386	4	<0.001
	No choice	<i>Glycaspis brimblecombei</i>	Antennation	33,001	4	<0.001	32,781	4	<0.001
			Probing	33,001	4	<0.001	32,781	4	<0.001
			Oviposition	33,001	4	<0.001	32,781	4	<0.001
	Choice	<i>Spondylaspis cf. plicatuloides</i>	Antennation	51,667	4	<0.001	51,332	4	<0.001
			Probing	40,377	4	<0.001	40,108	4	<0.001
			Oviposition	40,876	4	<0.001	40,604	4	<0.001
	No choice	<i>Spondylaspis cf. plicatuloides</i>	Antennation	76,767	4	<0.001	76,255	4	<0.001
			Probing	76,767	4	<0.001	76,255	4	<0.001
			Oviposition	76,767	4	<0.001	76,255	4	<0.001

Note: The table presents χ^2 and H values with degrees of freedom (df) and p-values for antennation, probing and oviposition behaviours.

Female *P. pilosus* only parasitised nymphal instars of *C. eucalypti*, its known host. To the best of our knowledge, this is the first report on host specificity testing of *P. pilosus* against *Eucalyptus*-feeding psyllids. Hodkinson (1999) and Hollis (2004) reported *P. pilosus* as a host specific and monophagous parasitoid, where its natural distribution in Australia closely follows that of its host, *C. eucalypti*. Currently, there is no evidence that *P. pilosus* uses any other psyllid as its host, other than *C. eucalypti*. *Psyllaephagus pilosus* is therefore a highly host

specific parasitoid—a trait favoured for a classical biological control agent (Kenis et al., 2019). As a koinobiont endoparasitoid, the observed *P. pilosus* behaviour in our study is as expected, because the physiological relationship of koinobiont endoparasitoids and their hosts is intimate and taxonomically defined (Heimpel et al., 2021; Strand & Obrycki, 1996). This monophagous parasitoid was successfully used in the United States and Brazil to reduce the population of *C. eucalypti* to levels below the economic injury threshold (Dahlsten

et al., 1998; Kurylo et al., 2010). Narrow host ranges and high levels of specificity were reported for other psyllid-parasitoid interactions such as *Diaphorencyrtus aligarhensis* (Shafee, Alam, & Agarwal) (Hymenoptera: Encyrtidae) (Bistline-East et al., 2015) and *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae) (Hoddle & Pandey, 2014) on Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) together with *Tamarixia dryi* (Waterson) (Hymenoptera: Eulophidae) on the African citrus psyllid, *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) (Urbaneja-Bernat et al., 2019).

As far as we know, this is the first report on host specificity testing of *P. blastopsyllae* towards *Eucalyptus*-feeding psyllids. *Psyllaephagus blastopsyllae* antennated, probed and oviposited on three of the four tested psyllids, namely *B. occidentalis*, its known host, *C. eucalypti* and *G. brimblecombei*. The three psyllids are closely related and, in some instances, co-occur on one or more *Eucalyptus* species (e.g., *Eucalyptus camaldulensis* Dehnh) (PT Makunde pers. obs.). Thus, *P. blastopsyllae* appear to make use of the niche specific parasitism phenomenon. Although psyllid species may share some host plant species, their ecological niches differ significantly due to factors such as the specific plant organs affected, time of year, feeding modes and other ecological variables. This diversity in ecological niches influences the likelihood that hosts sharing similar ecological niches and host plants will also share similar parasitoids, as postulated by Kuhlmann and Mason (2002). Hall et al. (2017) demonstrated that parasitoid diversification is characterized by high host specificity and some host switching. This aligns with our findings, where *G. brimblecombei* was identified as a potential new host for *P. blastopsyllae*. Hall et al. (2017) study explored the host specificity and trophic interactions of parasitoids of psyllids, revealing a high degree of host specificity and some host switching between sympatric psyllid hosts.

Psyllaephagus blastopsyllae produced offspring on its known host and on *G. brimblecombei*, but not on *C. eucalypti*. The inability of *P. blastopsyllae* to develop on *C. eucalypti* could be attributed to death of the first and second nymphal instars it preferred for oviposition. During the no-choice observational tests, *P. blastopsyllae* females struggled to retract their ovipositors from the abdomens of the attacked nymphal instars (I and II) and thus nymphal instars could have been injured or killed during the process. Additionally, *P. blastopsyllae* struggled to move on the white waxy secretions of *C. eucalypti* nymphal instars attached to the leaf tips and shoots and continuously rubbed the secretions off their fore and hind legs. This might also have a bearing in the reduced success in parasitizing *C. eucalypti*. Similarly, Rezaei et al. (2019) reported that first and second nymphal instars of *Myzus persicae nicotianae* Blackman (Hemiptera: Aphididae) were not able to support the development of *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae). Early nymphal instars may not provide enough food for parasitoid offspring to develop successfully.

While *P. blastopsyllae* could develop on *G. brimblecombei*, *B. occidentalis* was the most preferred host and this was confirmed in choice tests. Female *P. blastopsyllae* attacked *G. brimblecombei* nymphal instars found outside the lerps and failed to antennate, probe and oviposit on *G. brimblecombei* and *S. cf. plicatuloides* nymphal

instars under white and brown lerps, respectively. It is likely that *P. blastopsyllae* only antennate, probe and oviposit on nymphal instars they observed moving freely, as the case with the nymphal instars of their natural host *B. occidentalis*, a free-living psyllid. Visual cues appear to be the primary drivers of host recognition and acceptance by *P. blastopsyllae*. As a result, visual detection of yellow nymphal instars could be a specific signal used by *P. blastopsyllae* in host finding and acceptance. Colour, for example, is used as a host cue by the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) a natural parasitoid of pea aphid, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae) (Battaglia et al., 2000; Larocca et al., 2007; Libbrecht et al., 2007) and a braconid parasitoid, *Fopius arisanus* (Hymenoptera: Braconidae) (Pérez-Otero et al., 2011). Previous studies have highlighted the importance of host specificity and trophic interactions in parasitoid communities (Hall et al., 2017).

Psyllaephagus bliteus females antennated, probed and oviposited on nymphal instars of the lerp forming psyllids, *G. brimblecombei* and *S. cf. plicatuloides*, but not the free-living psyllids, *C. eucalypti* and *B. occidentalis*. Similarly, Dahlsten et al. (2003) also reported that *P. bliteus* did not show antennation, probing and oviposition behaviour towards *C. eucalypti*. *Psyllaephagus bliteus* typically oviposited on *S. cf. plicatuloides* by slipping the ovipositor under the edge of the lerp rather than through the lerp. The stage of nymphal instars of *G. brimblecombei* determined oviposition sites. Towards *G. brimblecombei*, oviposition by *P. bliteus* in the second and third nymphal instars occurred through the lerps, while oviposition on late nymphal instars (IV–V) occurred under the edge of the lerps. These observations are consistent with the findings of Sullivan et al. (2006), who discovered that *P. bliteus* uses gaps between the leaf surface and the lerp as an avenue for oviposition. It is likely that the thick and hard lerps of *S. cf. plicatuloides* impede *P. bliteus* ovipositor penetration. However, while *P. bliteus* antennated, probed, oviposited and developed on *S. cf. plicatuloides*, *G. brimblecombei* remained the most preferred host.

In light of these observations, it seems possible that females of the parasitoids in this study employ some mechanisms to perceive and accept their known physiological hosts. Recognition and acceptance of *S. cf. plicatuloides* nymphal instars under the brown lerps, unlike the white lerps of their known host by *P. bliteus*, implied that colour and shape/structure of the lerps may not be important factors in host recognition and acceptance. Daane et al. (2005) proposed that chemical cues from the host plant expressed in the lerps initiate host recognition and acceptance by *P. bliteus*. However, the mechanism behind *P. bliteus* host selection and acceptance requires further investigation. In other studies, *P. bliteus* is reported to utilize other lerp-forming Spondylaspidinae as hosts, for example, *Creis costatus* Froggatt (Hemiptera: Aphalaridae) and *Glycaspis* spp. (Hemiptera: Aphalaridae) (Berry, 2007; Hollis, 2004). Thus, *P. bliteus* is not host specific and potentially has multiple lerp forming psyllid hosts in Australia.

A parasitoid's choice of a host among different ages is reflected by host acceptance (van Lenteren, 1981). However, because there may not always be a choice in the field, host recognition and

acceptance in no-choice tests should also be explored, especially when studying potential hosts. In the second part of our study, host stage preference of *P. pilosus*, *P. blastopsyllae* and *P. bliteus* using their respective known hosts as well as potential psyllid hosts on which they previously recognized and accepted as potential hosts in behavioural tests carried in the first part of this study were investigated. Both *P. blastopsyllae* and *P. pilosus* preferentially attacked the fourth and fifth nymphal instars of *B. occidentalis* and *C. eucalypti*, respectively. Dahlsten et al. (1998) reported similar findings for *P. pilosus* on *C. eucalypti*. Unlike *P. blastopsyllae* and *P. pilosus*, *P. bliteus* oviposited on all the five nymphal instars (I–V) of *G. brimblecombei*, although preference was slightly higher towards third and to a lesser extent fourth nymphal instars, thus agreeing with the results reported by Sime et al. (2004) and Daane et al. (2005). Sime et al. (2004) suggested that the fourth and fifth nymphal instars impede oviposition, hence were less preferred than the third nymphal instars. Understanding the parasitoid's preferred host stage is critical in biological control programs because it allows for optimal parasitoid mass rearing and timing of release (Jervis & Kidd, 1985).

Psyllaephagus blastopsyllae, a known parasitoid of *B. occidentalis*, recognized and accepted all nymphal instars of *G. brimblecombei* found outside their lerps. According to Mackauer et al. (1996), the physiological state of the parasitoid, for example, the egg load, appears to be the most important factor in host acceptance. For example, *Psyllaephagus pistaciae* Ferrière with a large egg load attacked all nymphal instars of *Agonoscena pistaciae* Burckhardt and Lauterer (Mehrnejad & Copland, 2006). As the egg load of *P. pistaciae* decreased, the wasp had greater host age preference for oviposition (Mehrnejad & Copland, 2006). Similarly, Mangel (1989) predicted that parasitoids with higher egg loads will have a broader host range by accepting lower quality hosts for oviposition. Thus, the physiological state of the *P. blastopsyllae* species could possibly have influenced the results from this study, as well as the host choice in the field.

Associative learning and previous experience have been demonstrated for parasitoids (Turlings et al., 1993; Vinson, 1998). Therefore, koinobiont parasitoids are adapted to the hosts' physiology and defence system. With experience, they detect cues from their natural host and as a result the host on which they are reared can influence their host preference. Thus, when conducting host specificity tests, it is critical to consider the potential effect of associative learning on host preference. All of the parasitoids used in this study were obtained from their known natural hosts, and it was highly anticipated that in host preference tests, they would give first preference to their natural hosts. For example, *Microctonus aethioides* Loan (Hymenoptera: Braconidae), released as a biological control agent to control an alfalfa pest *Sitona discoides* Gyllenhal (Coleoptera: Curculionidae), showed preference to *Nicaeana cervina* Broun (Coleoptera: Curculionidae) when reared on this insect, rather than its known host *S. discoides* (Louda et al., 2003). Thus, it is possible that the preference of *P. blastopsyllae* for *B. occidentalis* over *G. brimblecombei* or *C. eucalypti* could have been different if the wasp was raised from *G. brimblecombei* or *C. eucalypti*. The same could be said if *P. bliteus* was raised on *S. cf. plicatuloides*. This was difficult to test in our study because the number of parasitoids that emerged from the potential

hosts was very low, and it was necessary to determine whether the potential hosts could support successive generations. There was also a need to optimize rearing of the parasitoids on the new host in order to have enough numbers to perform bio-assays.

Overall, the findings on host stage preference revealed that first and second nymphal instars of all psyllids tested are not suitable for oviposition as they are more likely prone to death and their size may not provide adequate food for the successful development of parasitoid offspring. The current study also clearly demonstrated that host specificity among *Psyllaephagus* species differ. *Psyllaephagus pilosus* was host specific while the other *Psyllaephagus* tested were not, utilizing potential hosts either in the presence or absence of their known hosts. In conclusion, even though *P. blastopsyllae* and *P. bliteus* were not host specific, their parasitism towards *G. brimblecombei* and *S. cf. plicatuloides* was lower than their known preferred hosts. Clearly, these test species did not make good hosts and this possibly translate to lower physiological suitability of the offspring. However, further research should consider a more in-depth investigation of the impact of *P. blastopsyllae* on *G. brimblecombei* and *P. bliteus* on *S. cf. plicatuloides* in natural and/or planted eucalypt forests.

Given the host specificity of *P. pilosus*, this species holds significant promise for augmentative biological control strategies against its target psyllid hosts. Its specificity reduces the risk of non-target impacts and enhances its effectiveness in suppressing populations of specific pest psyllid species in *Eucalyptus* plantations and forests. Implementing augmentative biological control using *P. pilosus* could provide a sustainable and environmentally friendly approach to managing psyllid pests, particularly in regions where they pose significant threats to *Eucalyptus* health and productivity. Future studies should focus on optimizing rearing techniques and evaluating the field efficacy of *P. pilosus* under varying environmental conditions.

AUTHOR CONTRIBUTIONS

Privilege T. Makunde: Conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Bernard Slippers:** Conceptualization; funding acquisition; methodology; supervision; validation; writing – review and editing. **Samantha J. Bush:** Conceptualization; methodology; writing – review and editing. **Brett P. Hurley:** Conceptualization; funding acquisition; methodology; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at <https://doi.org/10.5281/zenodo.14739432>.

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REFERENCES

- Abram, P.K., Labbe, R.M. & Mason, P.G. (2021) Ranking the host range of biological control agents with quantitative metrics of taxonomic specificity. *Biological Control*, 152, 104427. Available from: <https://doi.org/10.1016/j.biocontrol.2020.104427>
- Askew, R.R. (1994) Parasitoids of leaf-mining Lepidoptera: what determines their host ranges? In: Hawkins, B.A. & Sheehan, W. (Eds.) *Parasitoid Community Ecology*. Oxford University Press, pp. 177–202.
- Ávalos, M., Betancur, C.A., Guevara, G., Bacca, T. & Gaviria-Rivera, A. (2021) Life cycle and natural enemies of *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae) in a *Eucalyptus camaldulensis* Dehnhardt forest plantation (Jericó, Colombia). *Boletín Científico Centro de Museos Museo de Historia Natural*, 25, 40–52. Available from: <https://doi.org/10.17151/bccm.2021.25.1.3>
- Barratt, B.I.P., Moeed, A. & Malone, L.A. (2006) Biosafety assessment protocols for new organisms in New Zealand: can they apply internationally to emerging technologies? *Environmental Impact Assessment Review*, 26, 339–358. Available from: <https://doi.org/10.1016/j.eiar.2005.11.008>
- Battaglia, D., Poppy, G., Powell, W., Romano, A., Tranfaglia, A. & Pennacchio, F. (2000) Physical and chemical cues influencing the oviposition behaviour of *Aphidius ervi*. *Entomologia Experimentalis et Applicata*, 94, 219–227. Available from: <https://doi.org/10.1046/j.1570-7458.2000.00623.x>
- Bella, S. & Rapisarda, C. (2013) First record from Greece of the invasive red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera Psyllidae) and its associated parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera Encyrtidae). *Redia*, 96, 33–35.
- Berndt, L.A., Withers, T.M., Mansfield, S. & Hoare, R.J.B. (2009) Nontarget species selection for host range testing of *Cotesia urabae*. *New Zealand Plant Protection*, 62, 168–173. Available from: <https://doi.org/10.30843/nzpp.2009.62.4773>
- Berry, J.A. (2007) Key to the New Zealand species of *Psyllaephagus* Ashmead (Hymenoptera: Encyrtidae) with descriptions of three new species and a new record of the psyllid hyperparasitoid *Coccidoctonus psyllae* Riek (Hymenoptera: Encyrtidae). *Australian Journal of Entomology*, 46, 99–105. Available from: <https://doi.org/10.1111/j.1440-6055.2007.00575.x>
- Bistline-East, A., Pandey, R., Kecici, M. & Hoddle, M.S. (2015) Host range testing of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) for use in classical biological control of *Diaphorina citri* (Hemiptera: Liviidae) in California. *Journal of Economic Entomology*, 108, 940–950. Available from: <https://doi.org/10.1093/jee/tov020>
- Burckhardt, D. & Elgueta, M. (2000) *Blastopsylla occidentalis* Taylor (Hemiptera: Psyllidae), a new introduced eucalypt pest in Chile. *Revista Chilena de Entomología*, 26, 57–61.
- Burckhardt, D. & Ouvrard, D. (2012) A revised classification of the jumping plant-lice (Hemiptera: Psylloidea). *Zootaxa*, 3509, 1–34. Available from: <https://doi.org/10.11646/zootaxa.3509.1.1>
- Burckhardt, D., Ouvrard, D. & Percy, D.M. (2021) An updated classification of the jumping plant-lice (Hemiptera: Psylloidea) integrating molecular and morphological evidence. *European Journal of Taxonomy*, 736, 137–182. Available from: <https://doi.org/10.5852/ejt.2021.736.1257>
- Bush, S.J., Slippers, B., Dittrich-Schröder, G. & Hurley, B.P. (2020) Host specificity tests reveals new host of a global biological control agent *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae). *African Entomology*, 28, 238–248. Available from: <https://doi.org/10.4001/003.028.0238>
- Bush, S.J., Slippers, B., Nesser, S., Harney, M., Dittrich-Schröder, G. & Hurley, B.P. (2016) Six recently recorded Australian insects associated with *Eucalyptus* in South Africa. *African Entomology*, 24, 539–544. Available from: <https://doi.org/10.4001/003.024.0539>
- Clarke, C.W., Calatayud, P.A., Sforza, R.F., Ndemah, R.N. & Nyamukondiwa, C. (2019) Parasitoids' ecology and evolution. *Frontiers in Ecology and Evolution*, 7, 485. Available from: <https://doi.org/10.3389/fevo.2019.00485>
- Daane, K.M., Sime, K.R., Dahlsten, D.L., Andrews, J.W. & Zuparko, R. (2005) The biology of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), a parasitoid of the red gum lerp psyllid (Hemiptera: Psylloidea). *Biological Control*, 32, 228–235. Available from: <https://doi.org/10.1016/j.biocontrol.2004.09.015>
- Dahlsten, D.L., Rowney, D.L., Copper, W.A., Tassan, R.L., Chaney, W.E., Robb, K.L. et al. (1998) Parasitoid wasp controls blue gum psyllid. *California Agriculture*, 52, 31–34. Available from: <https://doi.org/10.3733/ca.v052n01p31>
- Dahlsten, D.L., Rowney, D.L., Robb, K.L., Downer, J.A., Shaw, D.A. & Kabashima, J.N. (2003) Biological control of introduced psyllids on *Eucalyptus*. *Proceedings 1st International Symposium on Biological Control Arthropods*, 1, 356–361.
- Dzokou V.J., Soufo L. & Tamesse J.L. (2020) Biology of *Blastopsylla occidentalis* (Hemiptera: Psylloidea: Aphalaridae), a pest of *Eucalyptus globulus* (Myrtaceae) in Yaoundé, Cameroon. *Journal of Applied and Natural Science*, 12, 30–35. Available from: <https://doi.org/10.31018/jans.v12i1.2220>
- Godfray, H.C.J. (1994) *Parasitoids: Behavioural and evolutionary ecology*, Vol. 67. Princeton, NJ: Princeton University Press.
- Hajek, A.E., Hurley, B.P., Kenis, M., Garnas, J., Bush, S.J., Wingfield, M.J. et al. (2016) Exotic biological control agents: a solution or contribution to arthropod invasions? *Biological Invasions*, 18, 953–969. Available from: <https://doi.org/10.1007/s10530-016-1075-8>
- Halbert, S.E., Gill, R. & Nisson, J.N. (2001) Two *Eucalyptus* psyllids new to Florida (Homoptera: Psyllidae). *Entomology Circular-Florida Department of Agriculture and Consumer Services-Division of Plant Industry*, 407, 1–2.
- Hall, A.A., Steinbauer, M.J., Taylor, G.S., Johnson, S.N., Cook, J.M. & Riegler, M. (2017) Unravelling mummies: cryptic diversity, host specificity, trophic and coevolutionary interactions in psyllid-parasitoid food webs. *BMC Evolutionary Biology*, 17, 1–15. Available from: <https://doi.org/10.1186/s12862-017-0959-2>
- Heimpel, G.E., Abram, P.K. & Brodeur, J. (2021) A phylogenetic perspective on parasitoid host ranges with implications for biological control. *Current Opinion in Insect Science*, 44, 95–100. Available from: <https://doi.org/10.1016/j.cois.2021.04.003>
- Hoddle, M.S. & Pandey, R. (2014) Host range testing of *Tamarixia radiata* (Hymenoptera: Eulophidae) sourced from the Punjab of Pakistan for classical biological control of *Diaphorina citri* (Hemiptera: Liviidae: Euphyllurinae: Diaphorini) in California. *Journal of Economic Entomology*, 107, 125–136. Available from: <https://doi.org/10.1603/EC13318>
- Hodkinson, I.D. (1999) Biocontrol of *Eucalyptus* psyllid *Ctenarytaina eucalypti* by the Australian parasitoid *Psyllaephagus pilosus*: a review of current programmes and their success. *Biocontrol News and Information*, 20, 129–134. Available from: <https://doi.org/10.1079/cabireviews/20063049830>
- Hodkinson I.D. (2007) A new introduced species of *Ctenarytaina* (Hemiptera, Psylloidea) damaging cultivated *Eucalyptus parvula* (= *parvifolia*) in Europe. *Deutsche Entomologische Zeitschrift*, 54, 27–33. Available from: <https://doi.org/10.1002/mmnd.200700002>
- Hodkinson, I.D. & White, I.M. (1981) The neotropical Psylloidea (Homoptera: Insecta): an annotated check list. *Journal of Natural*

- History, 15, 491–523. Available from: <https://doi.org/10.1080/00222938100770361>
- Hollis, D. (2004) *Australian Psylloidea: jumping plant lice and lerp insects*. Canberra, Australia: Australian Biological Resources Study.
- Hunt, E.J., Kuhlmann, U., Sheppard, A., Qin, T.K., Barratt, B.I.P., Harrison, L. et al. (2008) Review of invertebrate biological control agent regulation in Australia, New Zealand, Canada and the USA: recommendations for a harmonised European system. *Journal of Applied Entomology*, 132, 89–123. Available from: <https://doi.org/10.1111/j.1439-0418.2007.01232.x>
- Hurley, B.P., Garnas, J., Wingfield, M.J., Branco, M., Richardson, D.M. & Slippers, B. (2016) Increasing numbers and intercontinental spread of invasive insects on eucalypts. *Biological Invasions*, 18, 921–933.
- Jervis, M.A. & Kidd, N.A. (1985) Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews*, 61, 395–434. Available from: <https://doi.org/10.1111/j.1469-185X.1986.tb00660.x>
- Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17, 164–170.
- Kenis, M., Hurley, B.P., Colombari, F., Lawson, S., Sun, J., Wilcken, C. et al. (2019) *Guide to the classical biological control of insect pests in planted and natural forests*, FAO forestry paper No. 182. Rome: FAO.
- Kraaijeveld, A.R., Van Alphen, J.J. & Godfray, H.C. (1998) The coevolution of host resistance and parasitoid virulence. *Parasitology*, 116, 29–45.
- Kuhlmann, U. & Mason, P.G. (2002) Use of field host range surveys for selecting candidate non-target species for physiological host specificity testing of entomophagous biological control agents. In: Van Driesche, R.G. (Ed.) *Proceedings of the first international symposium on biological control of arthropods*. Morgantown, WV: United States Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, pp. 370–377.
- Kurylo, C.L., Garcia, M.S., Costa, V., Tibola, C., Ramiro, G. & Finkenauer, E. (2010) Occurrence of *Ctenarytaina eucalypti* (Maskell) (Hemiptera: Psyllidae) and its natural enemy *Psyllaephagus pilosus* Noyes (Hymenoptera: Encyrtidae) in *Eucalyptus globulus* in the state of Rio Grande do Sul, Brazil. *Neotropical Entomology*, 39, 671–673. Available from: <https://doi.org/10.1590/S1519-566X2010000400031>
- Laing, F. (1922) *Rhinocola eucalypti* Maskell in England. *Entomologist's Monthly Magazine*, 58, 141.
- Larocca, A., Fanti, P., Romano, V.A., Cardioverter, E.M., Isidoro, N., Romani, R. et al. (2007) Functional bases of host-acceptance behaviour in the aphid parasitoid *Aphidius ervi*. *Physiological Entomology*, 32, 305–312. Available from: <https://doi.org/10.1111/j.1365-3032.2007.00578.x>
- Libbrecht, R., Gwynn, D.M. & Fellowes, M.D.E. (2007) *Aphidius ervi* preferentially attacks the green morph of the pea aphid, *Acyrtosiphon pisum*. *Journal of Insect Behavior*, 20, 25–32. Available from: <https://doi.org/10.1007/s10905-006-9055-y>
- Louda, S.M., Pemberton, R.W., Johnson, M.T. & Follett, P. (2003) Nontarget effects—the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology*, 48, 365–396. Available from: <https://doi.org/10.1146/annurev.ento.48.060402.102800>
- Mackauer, M., Michaud, J.P. & Volkl, W. (1996) Host choice by aphid parasitoids (Hymenoptera, Aphididae), host recognition, host quality, and host value. *The Canadian Entomologist*, 128, 959–980.
- Makunde, P.T., Slippers, B., Burkhardt, D., de Queiroz, D.L., Lawson, S.A. & Hurley, B.P. (2020) Current and potential threat of psyllids (Hemiptera: Psylloidea) on eucalypts. *Southern Forests*, 82, 233–242. Available from: <https://doi.org/10.2989/20702620.2020.1813650>
- Mangel, M. (1989) Evolution of host selection in parasitoids, does the state of the parasitoid matter. *The American Naturalist*, 133, 688–705. Available from: <https://doi.org/10.1086/284945>
- Maskell, W.M. (1890) On some species of Psyllidae in New Zealand. *Transactions and Proceedings of the New Zealand Institute*, 22, 157–170.
- Mehrnejad, M.R. & Copland, M.J. (2006) Host-stage selection and oviposition behaviour of *Psyllaephagus pistaciae*, parasitoid of the common pistachio psylla *Agonoscena pistaciae*. *Biological Control*, 36, 139–146. Available from: <https://doi.org/10.1016/j.biocontrol.2005.07.005>
- Moore, K.M. (1964) Observations on some Australian forest insects. 18. Four new species of *Glycaspis* (Homoptera: Psyllidae) from Queensland. *Proceedings of the Linnean Society of New South Wales*, 89, 163–166.
- Nahrung, H.F., Loch, A.D. & Matsuki, M. (2016) Invasive insects in Mediterranean forest systems: Australia. In: Paine, T. & Lieutier, F. (Eds.) *Insects and diseases of Mediterranean forest systems*. Cham: Springer, pp. 475–498.
- Noyes, J.S. (1988) Encyrtidae (Insecta: Hymenoptera). *Fauna of New Zealand*, 13, 1–188.
- Noyes, J.S. (2020) *Universal Chalcidoidea Database*. World Wide Web electronic Publication. Available from: <http://www.nhm.ac.uk/chalcidooids> [Accessed 02 December 2024].
- Noyes, J.S. & Hanson, P. (1996) Encyrtidae (Hymenoptera: Chalcidoidea) of Costa Rica: the genera and species associated with jumping plant-lice (Homoptera: Psylloidea). *Bulletin of the British Museum (Natural History) Entomology*, 65, 105–164.
- Paine, T.D., Dahlsten, D.L., Millar, J.G., Hoddle, M.S. & Hanks, L.M. (2000) UC scientists apply IPM techniques to new *Eucalyptus* pests. *California Agriculture*, 54, 8–13. Available from: <https://doi.org/10.3733/ca.v054n06p8>
- Paine, T.D., Steinbauer, M.J. & Lawson, S.A. (2011) Native and exotic pests of *Eucalyptus*: a worldwide perspective. *Annual Review of Entomology*, 56, 181–201. Available from: <https://doi.org/10.1146/annurev-ento-120709-144817>
- Pérez-Otero, R., Borrajo, P., Mansilla, J.P. & Ruiz, F. (2011) Primera cita en España de *Psyllaephagus bliteus* Riek (Hymenoptera, Encyrtidae), parasitoid de *Glycaspis brimblecombei* (Hemiptera, Psyllidae). *Boletín de Sanidad Vegetal Plagas*, 37, 37–44.
- Queiroz D.L. & Burckhardt D. (2007) Introduced *Eucalyptus* psyllids in Brazil. *Journal of Forest Research*, 12, 337–344. Available from: <https://doi.org/10.1007/s10310-007-0035-7>
- R Core Team. (2024) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available from: <http://www.R-project.org/>
- Rezaei, M., Talebi, A.A., Fathipour, Y., Karimzadeh, J. & Mehrbadi, M. (2019) Foraging behavior of *Aphidius matricariae* (Hymenoptera: Braconidae) on tobacco aphid, *Myzus persicae nicotianae* (Hemiptera: Aphididae). *Bulletin of Entomological Research*, 109, 840–848.
- Riek, E.F. (1962) The Australian species of *Psyllaephagus* (Hymenoptera, Encyrtidae), parasites of psyllids (Homoptera). *Australia Journal of Zoology*, 10, 684–757. Available from: <https://doi.org/10.1071/ZO9620684>
- Roitberg, B.D., Boivin, G. & Vet, L.E.M. (2001) Fitness, parasitoids, and biological control: an opinion. *Canadian Entomologist*, 133, 429–438. Available from: <https://doi.org/10.4039/Ent133429-3>
- Sharma, A., Raman, A., Taylor, G.S., Fletcher, M.J. & Nicol, H. (2015) Development, feeding and oviposition behaviour of *Ctenarytaina eucalypti* (Maskell) (Hemiptera: Psylloidea: Aphalaridae) on *Eucalyptus globulus* (Myrtaceae) in the central tablelands of New South Wales. *Austral Entomology*, 54, 117–136.
- Sime, K.R., Daane, K.M., Dahlsten, D.L., Andrews, J.W. & Rowney, D.L. (2004) Constraints on the effectiveness of *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae), a biological control agent for the red-gum lerp psyllid (Hemiptera: Psylloidea) in California. In: *Proceedings of the fourth California conference on biological control*. Berkeley, CA: University of California, pp. 141–144.
- Stireman, J.O. & Singer, M.S. (2003) What determines host range in parasitoids? An analysis of a tachinid parasitoid community. *Oecologia*, 135, 629–638. Available from: <https://doi.org/10.1007/s00442-003-1235-2>

- Strand, M.R. & Obyrcki, J.J. (1996) Host specificity of insect parasitoids and predators. *Bioscience*, 46, 422–429.
- Strong, D.R. & Pemberton, R.W. (2000) Biological control of invading species—risk and reform. *Science*, 288, 1969–1970. Available from: <https://doi.org/10.1126/science.288.5473.1969>
- Sullivan, D.J., Daane, K.M., Sime, K.R. & Andrews, J.W., Jr. (2006) Protective mechanisms for pupae of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), a parasitoid of the red-gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psylloidea). *Australian Journal of Entomology*, 45, 101–105. Available from: <https://doi.org/10.1111/j.1440-6055.2006.00496.x>
- Tamesse, J.L., Soufo, L., Tchanatame, E.C., Dzokou, V.J., Gumovsky, A. & De Coninck, E. (2014) Description of *Psyllaephagus blastopsyllae* sp. n. (Encyrtidae), new species, endoparasitoid of *Blastopsylla occidentalis* Taylor (Psyllidae, Spondyliaspidinae) in Cameroon. *Journal of Biodiversity and Environmental Sciences*, 5, 228–236.
- Taylor, K.L. (1985) Australian psyllids: a new genus of Ctenarytainini (Homoptera: Psylloidea) on *Eucalyptus*, with nine new species. *Journal of the Australian Entomological Society*, 24, 17–30. Available from: <https://doi.org/10.1111/j.1440-6055.1985.tb00179.x>
- Turlings, T.C., Wäckers, F.L., Vet, L.E., Lewis, W.J. & Tumlinson, J.H. (1993) Learning of host-finding cues by hymenopterous parasitoids. *Insect Learning: Ecological and Evolutionary Perspectives*, 3, 51–78. Available from: https://doi.org/10.1007/978-1-4615-2814-2_3
- Urbaneja-Bernat, P., Pérez-Rodríguez, J., Krüger, K., Catalán, J., Rizza, R., Hernández-Suárez, E. et al. (2019) Host range testing of *Tamarixia dryi* (Hymenoptera: Eulophidae) sourced from South Africa for classical biological control of *Trioza erytrae* (Hemiptera: Psyllidae) in Europe. *Biological Control*, 135, 110–116. Available from: <https://doi.org/10.1016/j.biocontrol.2019.04.018>
- van Driesche, R., Hoodle, M. & Center, T. (2008) *Control of pests and weeds by natural enemies: an introduction to biological control*. Oxford, UK: Blackwell Publishing, p. 473.
- van Lenteren, J.C. (1981) Host discrimination by insect parasitoids. In: Nordlund, D.A., Jones, R.L. & Lewis, W.J. (Eds.) *Semiochemical, their role in Pest control*. New York: Wiley, pp. 153–179.
- Vinson, S.B. (1998) The general host selection behaviour of parasitoid hymenoptera and a comparison of initial strategies utilized by larviphagous and oophagous species. *Biological Control*, 11, 79–96. Available from: <https://doi.org/10.1006/bcon.1997.0601>
- Wingfield, M.J., Slippers, B., Hurley, B.P., Coutinho, T.A., Wingfield, B.D. & Roux, J. (2008) Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests*, 70, 139–144.
- Wondafraash, M., Slippers, B., Asfaw, B.A., Makowe, I.A., Jenya, H., Bush, S. et al. (2021) Tracing the distribution of natural enemies of non-native invasive eucalypt insect pests in sub-Saharan Africa. *Southern Forests*, 83, 205–214. Available from: <https://doi.org/10.2989/20702620.2021.1960218>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting information.

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