Host specificity tests reveals new host of a global biological control agent *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae)

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Glycaspis brimblecombei (Hemiptera: Aphalaridae) is an invasive, sap-sucking eucalypt pest in various parts of the world. *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) was first released as a biological control agent against this pest in California in 2000. Since then, *P. bliteus* has been found with its insect pest host, with no intentional introduction, in various South American, European and North African countries, and recently South Africa. Here we report on host specificity studies of *P. bliteus* in South Africa in order to determine potential risks to non-target hosts. Non-target test insects included the only native lerp-forming psyllid in South Africa, *Retroacizzia mopani* (Hemiptera: Psyllidae), as well as two free-living and one lerp-forming psyllid that are not native but also feed on *Eucalyptus* species. *Psyllaephagus bliteus* was monitored during no-choice tests for antennation, probing and oviposition behaviours towards the test insects. In addition, *P. bliteus* was enclosed on live plants infested with the test insects which were then monitored for offspring of *P. bliteus*. *Retroacizzia mopani* and the non-native free-living psyllids were not attacked, but *P. bliteus* did attack and develop on the non-native lerp psyllid, *Spondyliaspis* cf. *plicatuloides* (Hemiptera: Aphalaridae). Choice tests were then undertaken with *G. brimblecombei* and *S.* cf. *plicatuloides*, and *G. brimblecombei* was found to be the preferred host. The results indicate that the risk of *P. bliteus* to native insects is low, but that the host range of *P. bliteus* is not restricted to *Glycaspis* spp. and *Creiis costatus* (Hemiptera: Aphalaridae) as previously thought. The host range of *P. bliteus* may thus include other lerp-forming insects on eucalypts, especially those within the Spondyliaspidinae.

Key words: *Spondyliaspis*, *G. brimblecombei*, *P. bliteus*, no-choice test, choice test, parasitoid, non-target, risk, psyllid.

INTRODUCTION

The control of a pest by using natural enemies from the target organism's native range is known as classical biological control (Eilenberg *et al*. 2001; Heimpel *et al*. 2004). If successful, classical biological control is usually permanent and irreversible (Howarth 1983; Duan & Messing 2000; Brodeur 2012), which is considered a major benefit of this management approach, as further introductions of natural enemies are not needed to control the pest. However, the irreversibility of this approach has also become an area of concern as potential negative effects of classical biological control were realised (van Lenteren *et al*. 2006; Brodeur 2012). Until the 1980s the release of arthropod natural enemies or parasitoids was considered to be almost risk-free, but perceptions among biological control practitioners and regulators have since changed (Kuhlmann & Mason 2003; Sands & van Driesche 2003). It is now recognised that introducing natural enemies could cause non-target impacts, including changes to the abundance and distribution of non-target organisms, potentially resulting in global or local extinctions, hybridisation with closely related native insects or vectoring harmful organisms (van Lenteren *et al*. 2006; Brodeur 2012).

Host specificity testing to understand the potential attack and subsequent impact of a natural enemy on non-target organisms became self-regulated among researchers since the 1980s (Hajek *et al*. 2016). Many countries now have legislation requiring host specificity tests before allowing the release of a potential biological control agent (Hajek *et al*. 2016). This involves

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Received 20 February 2020. Accepted 9 April 2020

ISSN 1021-3589 [Print]; 2224-8854 [Online]
DOI: https://doi.org/10.4001/003.028.0238

African Entomology **28**(2): 238–248 (2020)

selecting and testing potential non-target species of the biological control agent. Selection of non-target insects should include not only closely related species, but also those that share ecological similarities, species of conservation concern, those that are attacked by congeners, and species within other biological control programmes that could be affected (van Driesche & Hoddle 1997; van Lenteren *et al*. 2006; Hogendoorn *et al*. 2013).

Glycaspis brimblecombei Moore (Hemiptera: Aphalaridae) is one pest species against which classical biological control programmes have been initiated (Daane *et al*. 2005). *Glycaspis brimblecombei* (Fig. 1A, B) is a pest on *Eucalyptus* outside of its native range in Australia (Brennan *et al*. 1999). It feeds on the leaves of *Eucalyptus* trees and the nymphs form characteristic cone like structures, called lerps, from carbohydrates and proteins that they exude while feeding (Fig. 1C). These lerps promote the growth of sooty mould which can reduce the ability of the leaves to photosynthesise. The feeding on the leaves as well as the impact of sooty mould can cause leaf wilt, leaf death, branch dieback and death of the tree (Brennan *et al*. 1999; Paine *et al*. 2000; Dahlsten *et al*. 2002; Huerta *et al*. 2011). These negative impacts resulted in an investigation for a suitable biological control agent, resulting in the release of the parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) (Daane *et al*. 2005; Plascencia-González *et al*. 2005; Ide *et al*. 2006).

Psyllaephagus bliteus was first used as a biological control agent against *G. brimblecombei* when it was released in California in 2000, after testing determined it was host specific (Daane *et al*. 2005). It was subsequently released in Mexico in 2002 (Plascencia-González *et al*. 2005) and Chile in 2003 (Ide *et al*. 2006). In addition to these deliberate introductions, *P. bliteus* has been found with its pest host in various countries around the world, including Brazil (Berti-Filho *et al*. 2003), Spain (Pérez-Otero *et al*. 2011), Greece (Bella & Rapisarda 2013), Portugal (Dhahri *et al*. 2014), Tunisia (Dhahri *et al*. 2014), Turkey (Karaca *et al*. 2015), Algeria (Reguia & Peris-Filipo 2013), and recently in South Africa (Bush *et al*. 2016).

Prior to the discovery of *P. bliteus* in South Africa, investigation into the host specificity of *P. bliteus* had started, with the intention to consider its release in the field if suitably host specific. Testing was continued after the discovery to determine what impact *P. bliteus* may have on non-target hosts, and this is reported here. No-choice and choice host specificity tests were undertaken using one native lerp-forming psyllid and three eucalypt-feeding psyllids native to Australia, one of which is lerp-forming.

MATERIAL AND METHODS

Selection of non-target test insects

There are no species of conservation concern or biological control agents that would be affected by the release of *P. bliteus*, or insect species native to South Africa that are closely related to *G. brimblecombei* (D. Burckhardt, pers. comm.; I. Millar, pers. comm.). Non-target hosts selected for testing included two free-living psyllids, *Blastopsylla occidentalis* Taylor (Hemiptera: Aphalaridae) and *Ctenarytaina eucalypti* Maskell (Hemiptera: Aphalaridae), as well as one lerp psyllid, *Spondyliaspis* cf. *plicatuloides* Froggatt (Hemiptera: Aphalaridae). These psyllids are not native to South Africa but feed on the same host as *G. brimblecombei*, namely *Eucalyptus* species, and belong to the same subfamily as *G. brimblecombei*; the Spondyliaspidinae (Burckhardt & Ouvrard 2012). A fourth test psyllid, *Retroacizzia mopani* Pettey (Hemiptera: Psyllidae) (mopane psyllid) (Fig. 1L) is not closely related to *G. brimblecombei* as it is placed in the Macrocorsinae, but is the only native lerp-forming psyllid in South Africa. Host plant can play a role in parasitoid specificity (Meiners *et al*. 2000), the host plant of *R. mopani*, *Colophospermum mopane*, is part of the Fabaceae, while *Eucalyptus*, the host of the remaining psyllids is part of the Myrtaceae. All four of these insects are attacked by congeners of *P. bliteus*. *Glycaspis brimblecombei* was included as a positive control.

Source of test psyllids

Nymphs of *G. brimblecombei* (Fig. 1A), *B. occidentalis* (Fig. 1D), *C. eucalypti* (Fig. 1G) and *S.* cf. *plicatuloides* (Fig. 1M) were obtained from either the nursery at the Forestry and Agricultural Biotechnology Institute (FABI) on the Experimental Farm of the University of Pretoria (25°45.149′S 28° 15.380′E) or the 'Zoo Plot' of the National Zoological Gardens (25°44.139′S 28°14.435′E). *Retroacizzia mopani* (Fig. 1J) was collected from Zwigodini, Limpopo (22°29.952′S 30°38.082′E) and brought back to the FABI Biocontrol Centre for the observation trials.

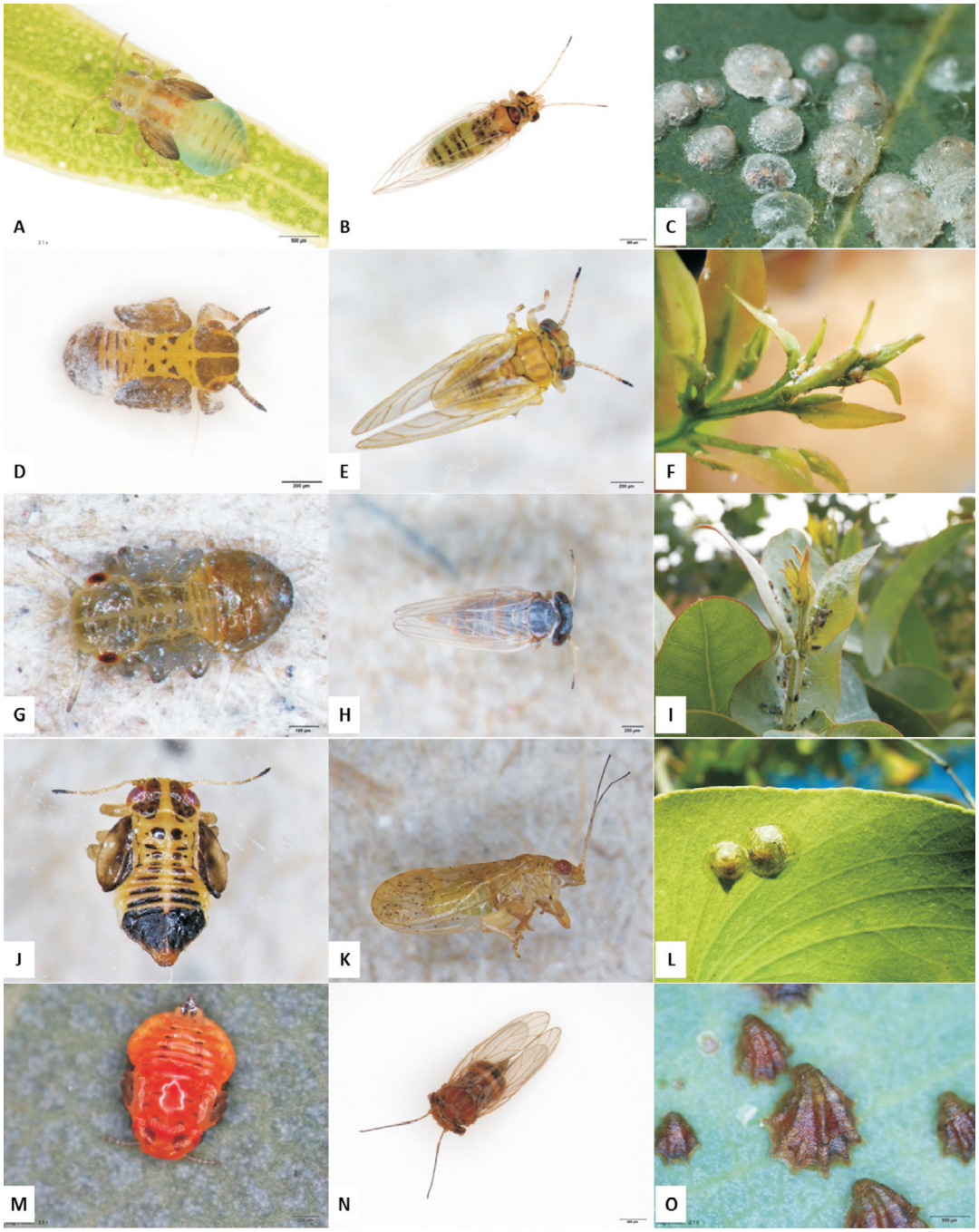

Fig. 1. *Glycaspis brimblecombei*: **A**, nymph; **B**, adult; **C**, lerps (L. Eksteen). *Blastopsylla occidentalis*: **D**, nymph; **E**, adult; **F**, infested leaf. *Ctenarytaina eucalypti*: **G**, nymph; **H**, adult; **I**, infested leaf (J. Roux). *Retroaccizia mopani*: **J**, nymph; **K**, adult; **L**, lerp. *Spondyliaspis* cf. *plicatuloides*: **M**, nymph; **N**, adult; **O**, lerps.

Source of P. bliteus

A culture of *P. bliteus* was imported from Brazil in 2014 to use for host specificity tests. However, in 2015, *P. bliteus* was detected in South Africa, likely introduced unintentionally with its host, *G. brimblecombei* (Bush *et al*. 2016). The cytochrome *b* region of the mitochondrial DNA (mtDNA) of the two sources of *P. bliteus*, namely from Brazil and field-collected in South Africa, were compared with insect specimens from Australia to confirm that they were the same species (Bush *et al*. 2016). The sequence results indicated that the 'Brazilian' and 'South African' *P. bliteus* collections were the same species, but genetically distinct populations, differing by two nucleotide base pairs. The Brazilian culture of *P. bliteus* was culled and subsequent tests used *P. bliteus* collected from the 'Zoo Plot' of the National Zoological Gardens in Pretoria, South Africa. Females and males were collected and stored between 6 and 10 °C for no longer than 10 days before use.

No-choice observation trial

For each repetition the bottom of an 8 cm diameter glass Petri dish was lined with a sheet of Whatmann Watt No. 8 filter paper. Small squares of leaf pieces or shoot tips infested with nymphs of a single test species were affixed to the filter paper, with at least 20 nymphs per Petri dish. *Psyllaephagus bliteus* will oviposit into all instars of *G. brimblecombei* (Daane *et al*. 2005), thus a range of non-target instars were also provided. *Psyllaephagus bliteus* was observed to oviposit throughout the day, as such tests were not limited to a particular time, as they would have been for a diurnal species. One female and one male *P. bliteus* were placed in the Petri dish and the dish was then sealed with Parafilm to prevent escape. A small block of honey paper was placed inside the top of the Petri dish for *P. bliteus* to feed on during testing. A minimum of 10 repetitions were performed per test species. More repetitions were performed if test psyllids were available. For lerp-forming host species, the number of nymphs could only be confirmed at the end of the repetition. In these cases, repetitions where less than 20 psyllids emerged were excluded and additional repetitions were set to meet the minimum number of repetitions per species.

After *P. bliteus* was placed in the Petri dish, the female was observed for antennation, probing and oviposition for the initial 30 min and then for 10 min every hour for 3 h. If the female was not active within the first 30 min she was considered unsuitable for testing and replaced with a fresh female. Once the observation period was complete, the *P. bliteus* were moved to a Petri dish with *G. brimblecombei* nymphs and lerps on leaf pieces. If the *P. bliteus* female did not show interest in the *G. brimblecombei* lerps within half an hour she was considered unsuitable and the repetition was discarded. This was to ensure that the *P. bliteus* used in the tests would show positive behaviour if exposed to a suitable host. Each *P. bliteus* was only used once.

No-choice development trial

A constraint of the no-choice observation trial was that the nymphs used would not survive to complete development as the host material did not last under these conditions and the nymphs did not survive being moved to fresh material; as a result, whether or not *P. bliteus* could develop in the non-target species could not be known. A second set of tests was therefore conducted to check for the emergence of the parasitoid after exposure to the test species. Host plants infested with at least 20 nymphs of a single test psyllid species of *G. brimblecombei*, *B. occidentalis* or *C. eucalypti* were brought into a quarantine glasshouse, enclosed in a sleeve and exposed to one female and one male *P. bliteus*. Sleeves were kept on the plants for 40 days and then opened. The identity and number of species that emerged during this period was recorded. A repetition where no *P. bliteus* emerged was considered successful if at least 20 adults of the test psyllid emerged (Fig. 1B, E, H). This was to ensure that there were sufficient viable nymphs available in that repetition. A minimum of 10 repetitions was performed per test species.

The host plant of *R. mopani*, *Colophospermum mopane*, could not be commercially sourced and are also slow growing, thus *R. mopani* could not be reared at the FABI Biocontrol Centre. When *P. bliteus* was found in South Africa the opportunity was taken to conduct the no-choice development trial in the field, at Zwigodini, Limpopo (22°29.952'S 30°38.082'E), where there is a natural infestation of *R. mopani* on *C. mopane* trees. Branches infested with *R. mopani*, and free of other psyllids, including *G. brimblecombei*, were enclosed in sleeves and a single female and male *P. bliteus* were added. These sleeves were removed and

branches taken back to the FABI Biocontrol Centre one month later. The branches were placed in unventilated plastic containers (9.5 l) that were lined with paper towel to absorb excess moisture. The paper towel was changed every second day, for two weeks, and at the same time, emerging insects were aspirated and stored in 100 % ethanol for identification. Only repetitions where 20 adults of the host insect *R. mopani* emerged (Fig. 1K) were included in the results, to ensure that there had been sufficient viable nymphs available for parasitism.

During the course of the study, mummies of *Spondyliaspis* cf. *plicatuloides* were collected from the 'Zoo Plot' of the National Zoological Gardens. The parasitoid species that emerged from the mummies was sequenced and confirmed as *P. bliteus* (G. Dittrich-Schröder, unpubl.). As the development of *P. bliteus* on *S.* cf. *plicatuloides* was thus confirmed in the field, there was no need to conduct development trials for this non-target insect.

Choice tests

Choice tests were performed for the test psyllids where antennation, probing or oviposition were observed for *P. bliteus* as in the no-choice observation trial. Choice test arenas were constructed the same way as the no-choice test, but leaf pieces with the preferred host, *G. brimblecombei*, and the non-target test species were present in each repetition. Within each Petri dish, the leaf pieces with *G. brimblecombei* and the leaf pieces with the non-target test species were interspersed in an alternating pattern. For each test species there was a minimum of 10 repetitions, with a range of instars present in each repetition. To ensure that there were sufficient viable nymphs available to elicit behavioural responses, a minimum of 10 *G. brimblecombei* and 10 of the test species were needed for a repetition to be considered successful. Repetitions with less than this number were excluded and additional repetitions were set. All *P. bliteus* used for the choice tests were from the South African culture.

RESULTS

No-choice observation trial

In total, 10 no-choice repetitions were performed for *G. brimblecombei*, *C. eucalypti* and *S.* cf. *plicatuloides*, 12 for *B. occidentalis* and 19 for *R. mopani* (Table 1).

Psyllaephagus bliteus did not show any host recognition or acceptance behaviours towards *B. occidentalis*, *C. eucalypti* or *R. mopani* (Table 1). *Psyllaephagus bliteus* did, however, show these behaviours towards the target *G. brimblecombei* and non-target *S.* cf. *plicatuloides* (Table 1). Of the three repetitions performed for *S.* cf. *plicatuloides* using the Brazil-sourced culture of *P. bliteus*, the only behaviour shown was one instance of antennation. For the *S.* cf. *plicatuloides* that were exposed to the South African culture of *P. bliteus*, four out of seven repetitions showed antennation and probing. For each of these four repetitions, this host recognition behaviour resulted in a single oviposition attempt (Table 1).

All five of the repetitions using the Brazilian culture of *P. bliteus* showed multiple instances of antennation, probing and oviposition towards *G. brimblecombei*. One female of the South African culture did not antennate, probe or oviposit in the *G. brimblecombei* lerps; however, this female did

Table 1. Number of repetitions performed for no-choice observation trials for each test species and culture and number of antennation, probing and oviposition attempts. Values in brackets are the number of repetitions where the behaviour was displayed.

Test insect	No. repetitions per culture (*, ^)	No. antennation attempts (*, ^)	No. probing attempts (*, ^)	No. oviposition attempts (*, ^)
Glycaspis brimblecombei	5, 5	51(5), 45(4)	44(5), 36(4)	26(5), 17(4)
Blastopsylla occidentalis	2, 10	0, 0	0, 0	0, 0
Ctenarytaina eucalypti	0, 10	–, 0	–, 0	–, 0
Retroaccizia mopani	10, 9	0, 0	0, 0	0, 0
Spondyliaspis cf. *plicatuloides*	3, 7	1(1), 12(4)	0, 9(4)	0, 4(4)

* = Repetitions using the culture of *P. bliteus* from Brazil.

^ = Repetitions using the field collected or 'South African' *P. bliteus*.

– = No repetitions set for that culture.

attempt to oviposit during the viability check at the end of the repetition. A second *P. bliteus* female showed a single attempt of each of the behaviours towards *G. brimblecombei* and the remaining three females each attempted to antennate, probe and oviposit multiple times (Table 1).

No-choice development trial

Ten repetitions each were performed for *B. occidentalis* and *C. eucalypti*, 20 repetitions for *G. brimblecombei* and 23 repetitions for *R. mopani* (Table 2). Offspring of the Brazilian culture of *P. bliteus* emerged from each of the 10 *G. brimblecombei* no-choice development repetitions, while eight of the 10 repetitions set using the South African culture of *P. bliteus* with *G. brimblecombei* resulted in *P. bliteus* offspring. *Psyllaephagus bliteus* offspring did not emerge from the tested non-target insects, *i.e. B. occidentalis*, *C. eucalypti* and *R. mopani* (Table 2). As mentioned above, the development of *P. bliteus* on *S.* cf. *plicatuloides* was confirmed from field observations.

Choice trial

Two out of 10 *P. bliteus* antennated *S.* cf. *plicatuloides* in the presence of *G. brimblecombei*, with a single subsequent probing attempt and no attempt to oviposit. In contrast, for each of the repetitions, *P. bliteus* antennated the *G. brimblecombei* lerps, and three females probed and subsequently oviposited. The number of host recognition and acceptance behaviours was greater towards *G. brimblecombei* than *S.* cf. *plicatuloides* (Table 3), and for each repetition *G. brimblecombei* was the first test insect antennated.

DISCUSSION

Host specificity tests for *P. bliteus* on the only native lerp-forming psyllid in South Africa, as well as on three introduced non-native psyllids occurring on eucalypts, indicated that *P. bliteus* is not completely host specific. *Psyllaephagus bliteus* did not parasitise the native lerp-forming psyllid, *R. mopani* or the non-native free-living psyllids tested. While the parasitoid could develop on the introduced lerp psyllid *S.* cf. *plicatuloides*, *G. brimblecombei* was the preferred host. Nine out of the 10 *P. bliteus* repetitions for the no-choice observation test showed host recognition and acceptance behaviours towards *G. brimblecombei* and 18 out of 20 repetitions for the development trial produced offspring. These results indicate that the methods used were appropriate and increases confidence in the results of the host specificity tests.

Table 2. Number of repetitions performed for no-choice development trials for each test species and the culture and number of *Psyllaephagus bliteus* that emerged.

Test insect	No. repetitions per culture (*, ^)	Mean no. of *P. bliteus* that emerged (*, ^)
Glycaspis brimblecombei	10, 10	23.6, 11.1
Blastopsylla occidentalis	9, 1	0, 0
Ctenarytaina eucalypti	0, 10	–, 0
Retroaccizia mopani	9, 14	0, 0
Spondyliaspis cf. *plicatuloides*	No repetitions performed	–

* = Repetitions using the culture of *P. bliteus* from Brazil.

^ = Repetitions using the field collected *P. bliteus*.

– = No repetitions performed for that culture.

Table 3. Antennation, probing and oviposition attempts by *Psyllaephagus bliteus* when exposed to both *Glycaspis brimblecombei* and *Spondyliaspis* cf. *plicatuloides* in the choice trial. Values in brackets are the number of females displaying the particular behaviour.

Test insect	Antennation	Probing	Oviposition
Glycaspis brimblecombei	37 (10)	11 (3)	6 (3)
Spondyliaspis cf. *plicatuloides*	2 (2)	1 (1)	0

Glycaspis brimblecombei, *B. occidentalis*, *C. eucalypti* and *S.* cf. *plicatuliodes* are closely related as they fall within the same subfamily (Burckhardt & Ouvrard 2012), namely the Spondyliaspidinae, and share the same habitat as they all occur on leaves of eucalypts (Hollis 2004). *Psyllaephagus bliteus*, however, showed no interest towards *B. occidentalis* and *C. eucalypti*. The phylogenetic approach to selecting non-target species, which is used in weed biological control, is not always suitable for arthropod biological control (Berndt *et al*. 2009). This is in part because the taxonomic relationships of insects are often not as well studied, and the numbers of insects that can be tested is usually much higher than for plants (Messing 2001). Factors such as life history, plant hosts or the habitats of the target species may be equally or more important than phylogenetic relatedness (Kuhlmann & Mason 2003). Although some parasitoids will attack hosts within an ecological niche, other parasitoids are strongly host specific (Messing 2001; Berndt *et al*. 2009). According to Sands & van Driesche (2004), kionobionts, such as *P. bliteus*, are more likely to be host specific as they need to overcome a host's physiological defences.

Both *R. mopani* and *S.* cf. *plicatuloides* are lerp-forming psyllids; however, the lerps of *R. mopani* are similar in shape and colour (whitish) to *G. brimblecombei* while those of *S.* cf. *plicatuloides* are brown and scallop-shaped (Fig. 1C, L, O). As *P. bliteus* showed no interest in *R. mopani*, but showed interest towards *S.* cf. *plicatuloides* it is possible that the colour and shape of the lerp structure is not a cue for the parasitoid. This is different to some other parasitoids where the visual detection of a host has been shown to provoke an attack response. For example, the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) uses colour as a host cue (Larocca *et al*. 2007), and opiine braconids, used to control fruit flies in Hawaii, use fruit shape, size and colour as cues (Duan *et al*. 1997). These results support the hypothesis of Daane *et al*. (2005) that oviposition is initiated by chemical cues on the lerp. Chemical cues linked to physical structures that stimulate oviposition have been found in other parasitoids, for example *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) responds to the chemical cues on waxy filaments secreted by *Eupulvinaria hydrangea* Steinweiden (Hemiptera: Coccidae) (Merlin *et al*. 1996). Lopez & Kairo (2003) proposed that the attack of *Nephaspis bicolor* Gordon (Coleoptera: Coccinellidae) on non-target aleyrodid hosts was influenced by the texture, chemistry and constitution of the flocculent wax they produced, or did not produce. Lopez & Kairo (2003) further proposed that the effect of the flocculent wax might act at a species level.

Plant volatiles are an important primary mechanism for parasitoid host location and may be required to induce parasitic behaviour (Whitman & Eller 1990; Fatouros *et al*. 2005). By including host plant material in the study, the possible role of the host plant in the attraction of *P. bliteus* to *G. brimblecombei* and *S.* cf. *plicatuloides*, on Myrtaceae, and lack of interest in *R. mopani*, on Fabaceae, cannot be eliminated. However, the lack of interest in *B. occidentalis* and *C. eucalypti*, which also occur on Myrtaceae, indicates that if host plant volatiles do play a role, it is more likely as part of a combination of cues.

Spondyliaspis cf. *plicatuloides* is a new host record for *P. bliteus*. Known hosts of *P. bliteus* include *Creiis costatus* Froggatt (Hemiptera: Aphalaridae) and *Glycaspis* spp. (Berry 2007; Hollis 2004). Like *S.* cf. *plicatuloides*, *C. costatus* is a lerp-former recorded on *Eucalyptus* (Hollis 2004) and part of the Spondyliaspidinae (Burckhardt & Ouvrard 2012). There are many lerp-forming Spondyliaspidinae infesting eucalypts in Australia, for example *Austrapsylla revoluta* Frogatt, *Blepharocosta marmorata* Froggatt and *Cardiaspina alba* Froggatt (Hollis 2004) and these might be as yet unrecorded hosts of *P. bliteus*. In addition, there are Spondyliaspidinae that occupy deserted lerps, such as *Anoeconeossa aquilonia* Taylor, *Blastopsylla adnatarinae* Taylor and *Cryptoneossa minuta* Taylor (Hollis 2004) and depending on the cues *P. bliteus* uses for host choice, these might also be unrecorded hosts.

When the number of attacks by *P. bliteus* towards *G. brimblecombei* and *S.* cf. *plicatuloides* in the no-choice test are compared, it appears that *Spondyliaspis* cf. *plicatuloides* is a less preferred host. This preference was confirmed during the choice tests as *P. bliteus* attacked *G. brimblecombei* first in every repetition and the number of attacks towards *G. brimblecombei* was greater. No-choice tests maximise the probability of acceptance of the test species by the natural enemy and indicate the physiological host range of the natural enemy, which may be an overestimate of the actual host range (Barratt *et al*. 2010). Once species are known to be attacked by a natural enemy from the

no-choice test, choice tests are used to provide a more realistic impression of the ecological host range (Barratt *et al*. 2010).

For host specificity tests, it is important to consider the influence of the host material on host choice in subsequent generations. The preference of *P. bliteus* towards *G. brimblecombei* over *S.* cf. *plicatuloides* may possibly have changed if the *P. bliteus* was reared from *S.* cf. *plicatuloides*. An example of the relevance of this consideration is *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) that was released in New Zealand to control an alfalfa pest *Sitona discoides* Gyllenhal (Coleoptera: Curculionidae). The offspring of this parasitoid that developed from the non-target *Nicaeana cervina* Broun (Coleoptera: Curculionidae) preferred to attack this host instead of the target host (Louda *et al*. 2003). This effect was not investigated in this study as the *P. bliteus* used in the choice-test were all reared from *G. brimblecombei*.

When determining the host range of a natural enemy, published host records and host specificity tests of other countries or regions should be used with caution. Host specificity tests in other regions will give an indication of the degree of host specificity, but are limited by the test insects used. Researchers in California, for example, conducted host specificity tests on *P. bliteus* and found it to be host specific for the insects they tested and safe to release in their environment (Daane *et al*. 2005). Lerp-forming Spondyliaspidinae were not available as test insects and thus the decision to release *P. bliteus* was valid for their situation. The available literature should also be used with caution as the host records may be incomplete (Sands & van Driesche 2003; van Driesche *et al*. 2008). This would be especially true for natural enemies that were previously of no interest.

Kuhlmann *et al*. (2005) provided a framework for the selection of non-target insects and by their estimates, there would be between 10 and 22 non-target species in the test list for a proposed parasitoid. This study falls below this estimate with four test insects and *G. brimblecombei* as a positive control. More psyllids species could have been tested, however they would have been included simply to increase the number of test species, not because they were expected to be potential hosts. With the acceptance of *S.* cf. *plicatuloides*, a related lerp-forming eucalypt feeder as a host and rejection of the related, free-living eucalypt feeders *B. occidentalis* and *C. eucalypti*, as well as the native lerp-forming *R. mopani*, we are confident that the type of insects chosen provide an accurate indication of the type of insects *P. bliteus* will attack. No other insects currently in South Africa fit this attack profile.

The population of *P. bliteus* found with *G. brimblecombei* in South Africa differed genetically from both the culture sourced from Brazil, as well as a population in Australia. Genetic variation of parasitoids has been thought to improve the chance of success of biological control agents (Louzier *et al*. 2008). This is because in their native range, different populations of a parasitoid may be locally adapted to environmental conditions, or the organisms with which they interact (Louzier *et al*. 2008). For example, when different populations of the parasitoid *Microctonus hyperodae* (Hymenoptera: Braconidae) were introduced to New Zealand to control a weevil, *Listronotus bonariensis* (Coleoptera: Curculionidae), the population of *M. hyperodae* that was sourced closer to the origin of *L. bonariensis* outcompeted those sourced from other locations (Lozier *et al*. 2008; Phillips *et al*. 2008). Based on this reasoning it is possible that the three sources of *P. bliteus* are differently adapted and may provide better control in different environments. However, caution should be used when introducing multiple populations of a biological control agent. Barratt *et al*. (2010) provided the example of *Microtonus aethiopoides*, a parasitoid of the clover root weevil *S. lepidus* where the hybridisation of two biotypes of *M. aethiopoides* would have resulted in offspring that were inferior biological control agents.

The potential risk of releasing a biological control agent *versus* the risk posed by the target organism is a common theme in biological control (De Clercq *et al*. 2011). The risk to non-target species must be weighed against the risk the target species poses to the environment, both ecologically and economically. This means that a degree of acceptable host specificity may be warranted (Gilbert & Morrison 1997; Brodeur 2012). For example, Valente *et al*. (2017) conducted host specificity tests on an egg parasitoid *Anaphes inexpectatus* (Hymenoptera: Mymaridae). Their study found that *A. inexepectatus* developed on two test species, however the parasitism of these hosts was lower than the preferred host. This indicated that the test species were suboptimal hosts and Valente *et al*. (2017) recommended the release of

A. inexpectatus. If *P. bliteus* had not already been found in the field in South Africa, the recommendation made to the relevant legislating body would be that it is safe to release. Like *A. inexpectatus*, the number of attacks on *S.* cf. *plicatuloides* was lower than the preferred host *G. brimblecombei*, indicating that it is a suboptimal host. However, unlike *A. inexpectatus*, the suboptimal host is non-native and a pest itself, which would have strengthened the argument for releasing *P. bliteus*.

ACKNOWLEDGEMENTS

We thank C. Wilcken from São Paulo State University who provided the Brazilian culture of *P. bliteus*, E. Kunjeku (University of Venda, South Africa) who assisted in the collections of *R. mopani*, as well as various staff and students at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, who assisted in collecting parasitoids and test insects; including D. Arriagada Cares, C. Gevers, L. Eksteen, J. Khadile, O. Gayiya and S. Jali. We thank S. Neser for his assistance throughout the project. L. Eksteen provided Fig. 1C and J. Roux Fig. 1I. Members of the Tree Protection Co-operative Programme (TPCP) and the University of Pretoria (UP) provided financial support.

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