Reproductive characteristics of invasive hyperparasitoid *Baeoanusia albifunicle* have implications for the biological control of eucalypt pest *Paropsis charybdis*

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Abstract

Hyperparasitoids can impede the establishment of primary parasitoid biological control agents or limit their control capacity. Although modern quarantine practices generally prevent hyperparasitoids being introduced with biological control agents, introductions can occur via natural pathways or accidentally with incoming passengers and cargo. In New Zealand, *Baeoanusia albifunicle* Girault is a self-introduced hyperparasitoid of *Enoggera nassaui* Girault, an intentionally introduced control agent of the eucalypt pest *Paropsis charybdis* Stål. A self-introduced primary parasitoid, *Neopolycystus insectifurax* (Girault), also parasitises *P. charybdis* in New Zealand. We assessed *B. albifunicle* biology to better understand its potential to disrupt *P. charybdis* control. It was determined that *B. albifunicle* is an obligate solitary hyperparasitoid with a longer lifespan, lower fecundity and longer generation time than its host. The hyperparasitoid reduced effective parasitism by *E. nassaui* to <10% in the lab, indicating it may limit control of the first *P. charybdis* generation by slowing spring population growth. It was confirmed that *N. insectifurax* is not hyperparasitised by *B. albifunicle* and therefore has some potential to substitute for any hyperparasitoid-driven decline in *E. nassaui*.

Keywords: Hyperparasitoid interactions, Encyrtidae, Pteromalidae, *Enoggera*, *Neopolycystus*, New Zealand

1 Introduction

Internationally, records of deliberately or accidentally introduced hyperparasitoids are limited (e.g. Charles 1993; Day 2002; Gaines and Kok 1999; Peck et al. 2008; Wang and Messing 2004). However, considering the numerous examples of accidentally introduced pest insects, and a small but relevant number of primary parasitoids (e.g., Bjørnson 2008; Calcaterra et al. 2007; Charles 1993; Johnson et al. 2001; Peck et al. 2008), it is likely that hyperparasitoid incursions occur at higher frequencies than reported. While herbivore incursions and their damage may be conspicuous, the arrival of minute parasitoids is likely to go unnoticed unless they occur in intensively managed systems, yet their presence could have important consequences.

Obligate hyperparasitoids are generally considered integral in the regulation of primary parasitoids in their native range and detrimental to their use as biological control agents (BCAs) elsewhere (Rosenheim 1998). Hyperparasitoids can jeopardise BCA establishment by limiting population growth before (Bain and Kay 1989) or after (Gaines and Kok 1999) release, or prevent established agents from attaining densities sufficient to suppress their hosts (Höller et al. 1993; May and Hassell 1981; Rosenheim 1998; Sullivan and Völkl 1999). Consequently, screening to exclude hyperparasitoids from introduction with primary parasitoid BCAs has become a standard component of classical biological control in New Zealand and other countries with 'risk adverse' biosecurity policies. When selecting BCAs it is now also common to consider hyperparasitoid associations in the agent's native range and potential susceptibility to hyperparasitoids already present in the receiving country (Berry and Mansfield 2006).

Basic invasion theory predicts ample opportunity for natural and accidental introductions of Australian insects to New Zealand as a result of geography, wind patterns, high volume trade and travel (Close et al. 1978; Fox 1973; Ridley et al. 2000). Incursions of specialist eucalypt herbivores occur with particularly high frequency and several Australian parasitoids have been imported to New Zealand to control these pests in exotic eucalypt plantations (Withers 2001). In addition to intentional introductions, at least five primary parasitoids of introduced eucalypt psyllids and one of paropsine beetles have been detected since the 1860s. Two hyperparasitoids also appear to have established without intentional human assistance (Berry 2006): *Coccidoctonus psyllae* Riek (Hymenoptera: Encyrtidae) attacking the psyllid parasitoid *Psyllaephagus* sp. (Encyrtidae), and *Baeoanusia albifunicle* Girault (Encyrtidae) attacking *Enoggera nassaui* Girault (Hymenoptera: Pteromalidae), the primary parasitoid

BCA of *Paropsis charybdis* Stål (see below). As parasitoid BCAs introduced from Australia to New Zealand may associate with several hosts or share host species with other parasitoid species in Australia, the high rate of trans-Tasman dispersal could result in BCAs encountering new hosts, competitors, or natural enemies from their native range that were absent when they were initially introduced.

In New Zealand, four species of Australian paropsine beetles (Chrysomelidae: Paropsini) are established and others have been intercepted at the border or eradicated (Withers 2001; Bain 2013). In Australia these defoliators are regulated by a range of dipteran and hymenopteran primary parasitoids, themselves regulated by a suite of hyperparasitoids (de Little 1982; Greaves 1966; Tribe 2000). In New Zealand, *P. charybdis* initially constrained the establishment of a commercial eucalypt industry and remains the most serious eucalypt plantation defoliator today (New Zealand Forest Service 1976; Withers 2001). Early attempts at biological control failed, often because imported agents arrived heavily hyperparasitised by their Australian natural enemies (Bain and Kay 1989). Control was eventually achieved following the introduction of the solitary egg parasitoid, *E. nassaui*, from Western Australia in 1987 (Kay 1990). However, recent self-introductions of *Neopolycystus insectifurax* (Girault) (Pteromalidae) (Berry 2003), a solitary primary egg parasitoid that competes directly with *E. nassaui* for hosts, and the solitary hyperparasitoid *B. albifunicle* (Jones and Withers 2003) are thought to have altered these control dynamics.

The biology and behaviour of *E. nassaui* and *N. insectifurax* are compared elsewhere (Mansfield et al. 2011; Murray et al. 2009). Interactions between them have not been assessed in the absence of *B. albifunicle* in Australia and the biology of *B. albifunicle* itself has received little attention. Tribe (2000) noted large head and mandibles of larvae, a female-biased sex ratio, and concluded *B. albifunicle* was an obligate hyperparasitoid, but experiments were limited in replication and host eggs were presented to both primary and secondary parasitoids simultaneously. Field monitoring in New Zealand has shown *E. nassaui* is heavily hyperparasitised by *B. albifunicle*, leading to speculation that *P. charybdis* control has been disrupted (Berry and Mansfield 2006; Jones and Withers 2003; Murray et al. 2008). As there is no evidence that *N. insectifurax* is also hyperparasitised, it could potentially complement or substitute for control provided by *E. nassaui*. Here, we investigate the biological characteristics of *B. albifunicle* to assess its potential to disrupt the previously well-established biological control of *P. charybdis*.

2 Materials and methods

2.1 Insect cultures

All insect colonies were maintained at 22 ± 2°C, 65% r.h., and L14:D10. *Paropsis charybdis* were reared in ventilated perspex cages (1.0 m x 0.7 m x 0.7 m). Fresh cut field-grown *Eucalyptus nitens* (Deane et Maiden) Maiden flush foliage was provided as food. Egg batches laid on the foliage were collected every 2-3 days and stored at 4°C for up to five days. Individual *E. nassaui* and *N. insectifurax* females ≥3-days-old were presented with these egg batches for 24 h in 90 mm Petri dishes in a separate room. Parasitised batches were maintained in Petri dishes in groups of five until emergence. Progeny were supplied undiluted honey on 20 mm^2 paper-towel, and left to mate in the presence of the natal host eggshell.

Baeoanusia albifunicle were maintained in 65 mm Petri dishes in a controlled climate cabinet (Custom made, Scion). *Paropsis charybdis* egg batches, parasitised by *E. nassaui* over the preceding 24 h, were presented to groups of five 3-5 day-old *B. albifunicle* females (sexed using antennae morphology: female flagellum clubbed, male plumose) for 48 h. *Enoggera nassaui* that escaped hyperparasitism were removed at emergence nine days later. Hyperparasitoids emerged after 14 days and were provided undiluted honey as above.

Experiments were conducted in growth cabinets (22°C, 65-70% r.h., L14:D10) in which all egg batches exposed to parasitoids were subsequently held for up to 21 days to record their fate. As neither *E. nassaui* nor *N. insectifurax* are sexually dimorphic it was not possible to expose the desired number of hosts to female parasitoids in every experiment. Generally, behavioural observation at the beginning of each experiment verified the wasps used were female. All parasitoids were dissected after experiments to confirm their sex and hosts exposed to males were discarded as replicates.

2.2 Obligate or facultative hyperparasitism

Tribe (2000) reported *B. albifunicle* to be an obligate hyperparasitoid following exposure of paropsine eggs to *B. albifunicle* either without or simultaneously with a primary parasitoid. Murphy (2002) described *B. albifunicle* as ovipositing into unparasitised *P. charybdis* eggs and developing only if the host was subsequently parasitised by a primary parasitoid. If *B. albifunicle* is an obligate hyperparasitoid this strategy could provide opportunity for it to evolve facultative hyperparasitism. The following trials were conducted to confirm if *B. albifunicle* is an obligate hyperparasitoid and when it must oviposit relative to its primary parasitoid host. In trial one, *P. charybdis* egg batches were placed in separate Petri dishes, provisioned with honey, and exposed to individual 3-day-old females of either *B. albifunicle*

or *E. nassaui* for 24 h (n = 20 per species). Half (n = 10) the batches exposed to each species were then immediately presented to the other species for a further 24 h. In trial two, egg batches were exposed to *E. nassaui* (2 h) followed by *B. albifunicle* for a further 2 h after an interval of 2, 4, 6, 12 or 24 h since the start of the first exposure period (n = 10 per time interval). For trial one and two the number of *P. charybdis*, *E. nassaui* and *B. albifunicle* that later emerged was recorded. Trial three followed the procedures of trial two but with exposure to *B. albifunicle* after intervals of 30, 1, 24, 12, 18, 15, 17 and 16 h (n = 20 per interval). As appropriately aged insects were limited, one interval was tested per day in the order indicated above until a minimum interval between primary and secondary parasitism was determined. On each occasion five egg batches were also exposed to *E. nassaui* alone (2 h) as a control. Parasitised and control batches were subsequently submerged in sodium hypochlorite 5% v/v to partially dissolve the chorion. The softened eggs were pressed onto a glass slide with a coverslip and viewed at 100-200 x magnification to record the presence of primary and secondary parasitoid eggs. Percent hyperparasitism was compared between intervals by non-parametric Wilcoxon ranked-sums tests (SAS Version 9.1) and P-values adjusted for multiple tests using the sequential Bonferonni procedure (R Development Core Team 2009).

2.3 Hyperparasitism of *N. insectifurax*

As two primary parasitoids of *P. charybdis* exist in New Zealand the impact of *B. albifunicle* on *P. charybdis* control will depend on its ability to exploit each of them. *Neopolycystus insectifurax* is not thought to be a host for *B. albifunicle*, which if true may limit the hyperparasitoid's reduction of *P. charybdis* control. To confirm this, individual *P. charybdis* egg batches were exposed to either individual 3-day old female *E. nassaui* (1 h, n = 20), *N. insectifurax* (24 h as this species is much slower to initiate parasitism (Mansfield et al. 2011), n = 17) or no primary parasitoid (n = 20). All batches from the three treatments were then individually exposed to solitary 3-day-old hyperparasitoid females for 2 h, beginning 24 h after primary parasitoids had been first introduced. Slides were prepared as above (section 2.2) and viewed at 200 x mag. to record the number of eggs of each wasp species per *P. charybdis* egg batch.

In a second experiment, egg batches were exposed for 24 h to a solitary 2-day-old *E. nassaui*, or 6-day-old *N. insectifurax* female (n = 20 each), followed immediately by a single *B. albifunicle* female for 24 h. Eggs were allowed to develop and the number of each species that emerged per batch was recorded.

2.4 Fecundity, sex ratio and percent hyperparasitism

Comparing fecundity, progeny sex ratios and parasitism success between *B. albifunicle* and *E. nassaui* may indicate the hyperparasitoid's ability to suppress *E. nassaui*. Upon emergence, ten *B. albifunicle* females were placed into individual 65 mm Petri dishes. Each was presented with honey and a batch of *P. charybdis* eggs that had been exposed to *E. nassaui* for the preceding 24 h. This was repeated daily for the lifetime of each female. The number of *P. charybdis*, *E. nassaui* and *B. albifunicle* that emerged per batch was recorded and percent hyperparasitism, total number of offspring and offspring sex ratio was determined for each parent.

3 Results

3.1 Obligate or facultative hyperparasitism

In trial one, only *P. charybdis* larvae emerged from eggs exposed to *B. albifunicle* alone. *Enoggera nassaui* emerged from hosts exposed to *B. albifunicle* followed by *E. nassaui*, or to *E. nassaui* alone (Fig 1). Of the eggs presented to *B. albifunicle* after *E. nassaui*, 91.8% were hyperparasitised and *E. nassaui* emerged from a further 2.7%. Only one hyperparasitoid emerged per host egg. The highest host-egg mortality (8.4%) occurred when eggs were exposed to *B. albifunicle* only (Fig 1 'collapsed').

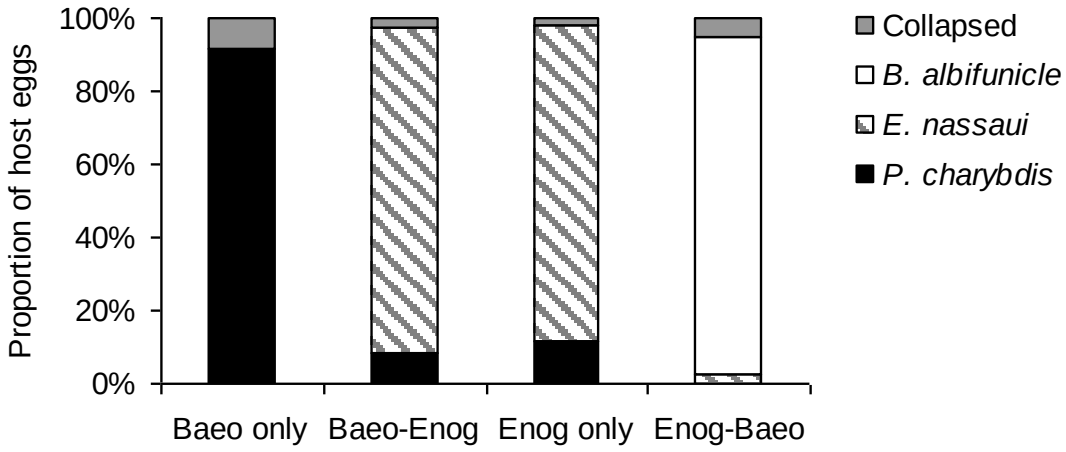

Fig 1 Proportion of *P. charybdis* eggs from which *P. charybdis*, *E. nassaui*, *B. albifunicle* or nothing (collapsed) emerged following exposure in the laboratory to; *B. albifunicle* (= Baeo only), *B. albifunicle* followed by *E. nassaui* (= Baeo-Enog), *E. nassaui* (= Enog only) or *E. nassaui* followed by *B. albifunicle* (= Enog-Baeo). Hosts were exposed to the first parasitoid for 24 h followed immediately by the second parasitoid for the succeeding 24 h (22^{o}C, 70% r.h.)

In trial two no hyperparasitoids emerged from *P. charybdis* eggs exposed to *B. albifunicle* 2 h (n = 68), 4 h (n = 62) or 6 h (n = 73) after primary parasitism. When exposed to hyperparasitoids 12 h and 24 h after primary parasitism, *B. albifunicle* emerged from 1/81 and17/62 *P. charybdis* eggs respectively. For the 24 h interval this represents successful hyperparasitism of 11.6% of *P. charybdis* eggs and 27.4% of individual *E. nassaui*.

In trial three, *E. nassaui* eggs were detected in 97.5% of control egg batches by dissection. No hyperparasitoid eggs were found in hosts exposed < 16 h after primary parasitism (Table 1). From 16-24 h, hyperparasitism of *E. nassaui* increased steadily from 1% to 62% then declined to 39% at 30 h. The proportion of primary parasitoids super-hyperparasitised (i.e. > 1 *B. albifunicle* egg present, see Fig 2c) followed a similar pattern. Super-hyperparasitism was highest (25%, Table 1) after the 24 h interval, and almost half the hyperparasitoid eggs were located externally adjacent to *E. nassaui*. When only one or two hyperparasitoid eggs were present per host, all were located inside an *E. nassaui* egg or larva (Fig 2).

Table 1 Number of *E. nassaui* (1^o) and *B. albifunicle* (2^o) dissected from *P. charybdis* eggs that had been exposed to *B. albifunicle* 1-30 h after *E. nassaui* (22 ^{o}C, 70% r.h., L14:D10). The proportion of 1^o parasitoids hyperparasitised (% of 1^o), and super-hyperparasitised (% Super.) and the proportion of 2^o parasitoid eggs found within or external to 1^o parasitoid eggs and larvae are indicated.

	P. charybdis	**Parasitoid eggs**		**Hyperparasitism**			
Interval	**eggs**	**1^o**	**2^o**	**% of 1^o**	**% Super.**	**% Inside**	**% Outside**
1 h	157	121	0	0.0	-	-	-
12 h	106	110	0	0.0	-	-	-
15 h	79	56	0	0.0	-	-	-
16 h	66	56	1	1.8	0.0	100.0	0.0
17 h	77	72	24	26.4	6.9	100.0	0.0
18 h	81	66	32	33.3	9.0	87.5	12.5
24 h	78	71	128	62.0	25.4	50.8	49.2
30 h	101	109	58	39.4	10.1	93.1	6.9

3.2 Hyperparasitism of *N. insectifurax*

Hyperparasitoid eggs were dissected from 90% of batches parasitised by *E. nassaui* but none of the unparasitised batches nor those parasitised by *N. insectifurax*. In the second trial, successful parasitism by *E. nassaui* and *N. insectifurax* was 48% and 58% respectively. Hyperparasitoids emerged from 95% of eggs parasitised by *E. nassaui* (n = 304 eggs) and 0% parasitised by *N. insectifurax* (n = 314 eggs).

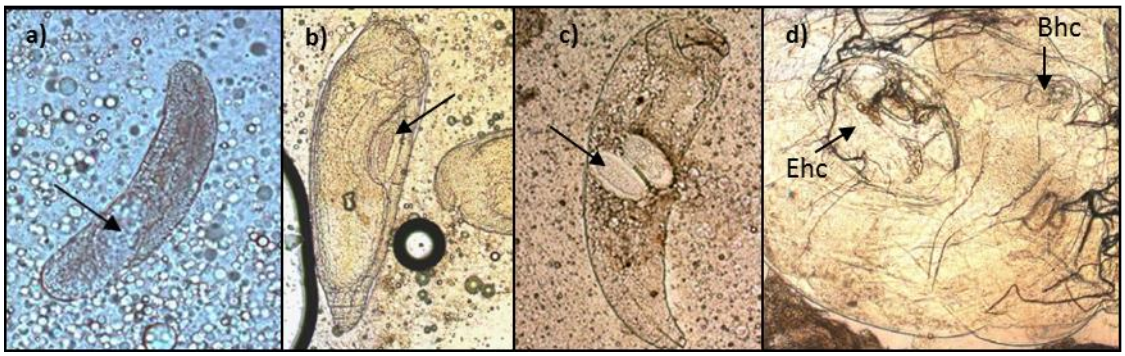

Fig 2 *Baeoanusia albifunicle* eggs within a) 16 h old *E. nassaui* egg (maintained at 22°C), b) early-instar *E. nassaui* larva, c), mid-instar *E. nassaui* larva. d) *B. albifunicle* larva developing within late instar *E. nassaui* (Ehc = *E. nassaui* head capsule, Bhc = *B. albifunicle* head capsule)

3.3 Fecundity, sex ratio and percent hyperparasitism

On average, female *B. albifunicle* oviposited for 19 days after emergence and survived five more days once oviposition ceased (Table 2). Mean lifetime fecundity was 127.2 progeny (max = 182) and 69.9% were female. Progeny sex ratio per parent ranged from 1.2 to 3.9 females ($\bar{x}$ = 2.6 ± 0.3) to 1 male. Males ($\bar{x}$ = 2.4 per egg batch) emerged first and three parents produced only males for their last 5-7 reproductive days. *Baeoanusia albifunicle* successfully hyperparasitised 41.8% of *P. charybdis* eggs and *E. nassaui* emerged from an additional 7.9% (Table 2). Assuming obligate hyperparasitism and no *E. nassaui* mortality without hyperparasitism, this indicates 84.1% of *E. nassaui* were hyperparasitised and 15.9% escaped.

Table 2 Lifespan and fecundity of *B. albifunicle* (n = 10) when provided daily with fresh hosts (22°C, 70% r.h., L14:D10). 'Reproductive' = number of days that females continued to oviposit. Mean number of host eggs exposed per female from which emerged *P. charybdis* (P.c.), *E. nassaui* (E.n.) or *B. albifunicle* (B.a.) are shown with the total proportion of host eggs from which each emerged (remaining 47.3% host eggs collapsed without hatching). The sex of *B. albifunicle* offspring is also shown.

	# Days reproductive	Total lifespan	# Host eggs presented	Species emerged			*B. albifunicle* sex	
				P.c.	E.n.	B.a.	Female	Male
Min.	13.0	14.0	223.0	0.0	3.0	87.0	62.0	19.0
Max.	27.0	32.0	421.0	21.0	67.0	182.0	124.0	74.0
Mean	18.8	24.2	304.7	9.2	24.1	127.2	88.9	38.3
Total	-	-	3047	3.0%	7.9%	41.8%	69.9%	30.1%

4 Discussion

4.1 Biology of *Baeoanusia albifunicle*

Biological characteristics determine the ability of introduced parasitoid BCAs to establish, disperse and regulate their pest host (Pschorn-Walcher 1977). For example, in New Zealand *E. nassaui* has characteristics that allow it to regulate *P. charybdis* more effectively than does *N. insectifurax* (Mansfield et al. 2011). The same is true with regard to an invasive hyperparasitoid's capacity to reduce parasitoid density below the level necessary to control the herbivore pest. Schooler (2011) hypothesised that hyperparasitoids with a higher intrinsic rate of increase (*r*) than their host pose the greatest risk to biological control. Generation time, fecundity, sex ratio and parasitism level are all important determinants of *r*, hence the impact of *B. albifunicle* on *P. charybdis* in New Zealand depends on how these parameters compare between *B. albifunicle* and *E. nassaui* and, possibly, *N. insectifurax*. All of these parameters are expected to be lower for secondary than primary parasitoids (Sullivan and Völkl 1999). At 22^{o}C, *B. albifunicle* has a longer generation time (14 days) than *E. nassaui* (9 days) and *N. insectifurax* (11 days) (Murray 2010) but females have similar longevity when fed undiluted honey in the absence of host; *B. albifunicle* max = 102.5 days, $\bar{x}$ = 56.7 ± 6.5 (Murray, 2010), *E. nassaui* max = 109, $\bar{x}$ = 42.5 ± 6.1, *N. insectifurax* max = 107, $\bar{x}$ = 37.6 ± 7.6 (Mansfield et al. 2011). The sex ratio of *B. albifunicle* (69.9% female) is comparable to *E. nassaui* in Western Australia (68.5%; Tribe 2000) but lower than reported in New Zealand (88.3%; Murray 2010). As *P. charybdis* egg size, and therefore quality, is highly uniform within egg-batches, *B. albifunicle* likely exhibits a set pattern of sex allocation, producing only enough males to fertilise their sisters. Hyperparasitoid fecundity was only slightly lower (103 progeny in 14 days) than previously recorded for *E. nassaui* (123 progeny in 14 days, Mansfield unpub. data) and total levels of hyperparasitism recorded across the experiments here ranged from 84.1% to 95%. These combined data suggest *B. albifunicle* has the potential to reduce effective parasitism of *P. charybdis* by *E. nassaui* to <16%. A reduction of this scale was observed in some North Island field sites between 2003 and 2005 (Jones and Withers 2003; Mansfield et al. 2011).

Mackauer and Volkl (1993) argued that even when local rates of hyperparasitism are high, population impacts on the host are limited by longer generation times, lower lifetime fecundity (due to lower egg storage capacity) and higher temperature requirements delaying spring appearance. The relative biology of *B. albifunicle* to its host generally supports these assertions. It is established that *E. nassaui* exert limited spring control of *P. charybdis* because higher temperature requirements delay population growth relative to the pest. The

delay between spring appearance of *E. nassaui* and *B. albifunicle* has not been fully assessed but field observations (Jones and Withers 2003) suggest that even if *B. albifunicle* does appear later, it may reach densities that reduce the *E. nassaui* population by the time *E. nassaui* has itself built up sufficiently to control *P. charybdis*.

4.2 Host range and host suitability

Baeoanusia albifunicle is not host specific at the herbivore level. Low levels of hyperparasitism have been recorded from field-collected *Chrysophtharta decolorata* (Chapuis), *Chrysophtharta amoena* (Clark), *Paropsis geographica* Baly and *Paropsis atomaria* Olivier in Australia (Tribe 2000), and *Dicranosterna semipunctata* (Chapuis) in the laboratory (Murray et al. 2010). The arrangement, placement and chorion texture of these beetle's eggs vary; suggesting *B. albifunicle* searches a variety of surfaces for parasitoid cues. It is likely, however, that *B. albifunicle* is specific to the genus *Enoggera,* having only been confirmed from eggs hosting *E. nassaui* and *E. reticulata* Naumann (Cumpston 1939; Tribe 2000). In south-east Queensland *B. albifunicle* reportedly hyperparasitise *Neopolycystus* sp. at low levels but this has not been definitively confirmed. Rather, *Neopolycystus* sp. was the only primary parasitoid to emerge from extensive field collections of *P. atomaria* eggs, which was taken to indicate that no other species was available to have hosted the hyperparasitoid (Nahrung and Duffy 2008). In New Zealand, we are confident that *B. albifunicle* is restricted to *E. nassaui* because parasitised *P. charybdis* eggs subsequently develop distinct colour patterns. All field-collected host eggs from which hyperparasitoids have emerged (Jones and Withers 2003) have shown the colour pattern consistent with *E. nassaui* not *N. insectifurax*. The present study has confirmed the restriction to *E. nassaui* experimentally. The high levels (47.3%) of *P. charybdis* egg collapse observed after sequential exposure to *E. nassaui* and *B. albifunicle* suggest however, that *E. nassaui* is not an ideal host, especially as only 15.9% of eggs collapse when exposed to *E. nassaui* alone (Mansfield unpub. data). Contrariwise, is also possible that the lengthy test period induced repeated probing by *B. albifunicle* after successful oviposition, inducing the host-egg collapse and an underestimate of hyperparasitism (see Tribe 2000).

Host dissections indicated that *B. albifunicle* oviposits directly into *E. nassaui*, which only becomes susceptible to hyperparasitism 12-16 h after oviposition. It may be that *E. nassaui* eggs are too small to detect, reach, or accommodate the hyperparasitoid before this (e.g. Strand and Vinson 1984). Hyperparasitism decreased when the delay exceeded 24 h, possibly

due to the larval integument toughening, insufficient time for hyperparasitoid development, or physical and immunological host defences. We conclude *E. nassaui* is susceptible to hyperparasitism for only a short period and *B. albifunicle* should require high host-finding efficiency and seasonal synchrony with *E. nassaui* to cause a significant reduction in the *E. nassaui* population.

4.3 Implications for biological control

High rates of hyperparasitism alone do not reliably indicate significant impact on the efficacy of a primary parasitoid (Rosenheim 1998). Some models predict hyperparasitism will disrupt biological control (May and Hassell 1981) while others predict a stabilising effect that improves control if there are fluctuations in the host-primary-parasitoid system (Beddington and Hammond 1977). Experimental studies show some BCAs perform poorly in the presence of hyperparasitoids (e.g. Wang and Messing 2004; Gains and Kok 1999) while others remain economically successful (e.g. Day 2002; Hammond and Neuenschwander 1990). The high levels of parasitism achieved in the absence of hyperparasitism, as evidenced by the success of *E. nassaui* controlling *P. charybdis* in warmer regions of New Zealand (Kay 1990; Murphy and Kay 2000) and of *E. reticulata* controlling *T. tincticollis* in South Africa (Tribe 2000), are indicative that hyperparasitoids can exert considerable regulatory pressure on hosts in their native range (de Little 1982; Tribe 2000). It is therefore appropriate to assume that accidental or self-introductions of BCAs native hyperparasitoids have the potential to significantly weaken classical biological.

The arrival of *B. albifunicle* to New Zealand was expected to devastate *P. charybdis* control. As an obligate hyperparasitoid with only one available host, *B. albifunicle* presumably exhibits strong synchrony with *E. nassaui* as it has successfully established, dispersed and proliferated (Jones and Withers 2003; Murray et al. 2008). We have confirmed that *B. albifunicle* has the biological capacity to reduce effective parasitism by *E. nassaui* to just 5-16%, similar to field-survey estimates made shortly after *B. albifunicle's* detection (Jones and Withers, 2003). The confirmation that *N. insectifurax* is not also exploited supports the notion that it may substitute for control lost to hyperparasitoid-driven reductions in *E. nassaui*. Up to 2008, *B. albifunicle* was recorded from four New Zealand regions where *N. insectifurax* was not, but these distributions are expected to eventually overlap given the climatic ranges each species already exploits (Murray et al. 2008). Currently, *E. nassaui* remains the primary BCA active against the spring (most damaging) generation of *P. charybdis* as *N. insectifurax* has a

slower developmental rate, longer pre-oviposition period, and is less well synchronised with *P. charybdis* oviposition peaks (Mansfield et al. 2011). Although obligate hyperparasitism does not reduce intra-generational impact on herbivore density (Jones and Withers 2003), heavy late-summer hyperparasitism is likely to reduce the *E. nassaui* population going into winter. As winter mortality is already high (Murphy and Kay 2000), *E. nassaui* may become even more scarce in early spring, increasing the time taken to attain levels sufficient to suppress *P. charybdis*. This capacity of *B. albifunicle* to further reduce *P. charybdis* control has yet to be quantified in the field. Additional assessment will determine the geographical and temporal scales on which control is affected and whether *N. insectifurax*, now well established, is substituting for *E. nassaui*.

Despite BCA import regulations and strict border biosecurity in New Zealand accidental introductions will continue, especially from Australia. Most will fail to establish, but those that do have the potential to become pests or place established BCAs at risk. Selecting BCAs with a low native hyperparasitoid load may maximise their likelihood of remaining hyperparasitoid-free to provide long term pest suppression. Continued monitoring of biological control programs may assist early detection of organisms disrupting control; facilitating better management and identification of the pathways by which parasitoid/hyperparasitoid incursions occur.

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