

Walk this way, fly that way: *Goniozus jacintae* attunes flight and foraging behaviour to leafroller host instar

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Abstract

Parasitoids exhibit distinct behaviours while foraging for their herbivorous hosts. Some are specialised with respect to the host stage they can utilise and even the age of individuals within a single stage. Observing the behavioural response of parasitoids to hosts of varying age may provide more practical understanding of potential biocontrol agents. A wind tunnel experiment was conducted to test for host-stage dependent foraging behaviour in *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae), a common but understudied parasitoid of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), which is a pest of grapevine, *Vitis vinifera* L. (Vitaceae), and other crops in Australia. *Goniozus jacintae* was already known to parasitise instars 3–6 of LBAM, but most readily parasitises the later instars. Later instars are larger and have a higher rate of feeding, producing more faeces and silk deposits than earlier instars, and are likely to also produce more volatile cues that may be detected by parasitoids. We found that the timing, frequency, and duration of foraging behaviours of *G. jacintae* varied significantly among host instars. Flight duration was shortest when females were exposed to leaves infested by fifth instars. The profile of behaviours exhibited was different during pre- and post-flight foraging, with the newly described *slow walking* behaviour only exhibited in close proximity to a potential host. These results contribute to the understanding of bethylid foraging, a relatively unexplored aspect of bethylid reproductive behaviour, and towards enhancing the efficacy of utilising *G. jacintae* in the control of leafroller pests.

Introduction

Parasitoids often mature in locations remote from suitable hosts (Tuda & Iwasa, 1998) and females must then disperse to forage for reproductive opportunities in complex environments. Many parasitoid species are specialised in regard to the range of species they can utilise as hosts (reviewed in Strand & Obrycki, 1996), the life-history stage of their target hosts (egg, larval stage, pupa, or adult), and even the age of individuals within a given life-history stage (e.g., early or late instars) (Mattiacci & Dicke, 1995). Therefore, finding suitable hosts is, for many parasitoids, a substantial challenge. This challenge is important to practitioners of biological pest control because it influences the

efficacy of a given parasitoid as a pest suppression agent. When designing biological control systems that deploy parasitoids, it is vital to establish which stages of the target species are susceptible to parasitism (Pandey & Singh, 1999; Canale & Loni, 2006) and to understand the abilities of parasitoids to locate such hosts at low densities (van Lenteren et al., 1976; Drost et al., 2000; Hudak et al., 2003). To understand how it locates suitable hosts, we analysed the behaviour of a bethylid parasitoid, *Goniozus jacintae* Farrugia (Hymenoptera: Bethyridae), in response to cues from one of its pest hosts, the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Danthanarayana, 1980).

The sequential phases of host searching that can lead to reproduction have been classified as host habitat location, host location, and host acceptance (Vinson et al., 1975; Vinson, 1976). In each phase, foraging behaviour is characterised by responses to environmental stimuli or cues

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(Price et al., 1980; Geervliet et al., 1994), categorised into three groups: (1) stimuli arising from the host itself, (2) stimuli arising from the host's microhabitat or food plant, and (3) stimuli indirectly associated with the presence of the host (De Moraes & Lewis, 1999). Stimuli arising from an individual host (including deposited silk and faeces) are some of the most reliable cues for a foraging parasitoid, especially when in close proximity to the host (Sternlicht, 1973). However, natural selection does not favour hosts that are easily found by natural enemies and thus, host species have evolved to minimise the emission of cues that could be utilised by foraging parasitoids (reviewed in Vet & Dicke, 1992). In response, parasitoids have evolved to use indirect cues associated with the presence or activity of the host for long-range detection (Vet & Dicke, 1992; Cardé & Bell, 1995).

Chemical information from plant volatiles related to host feeding damage is often important in mediating long-distance searching (Nordlund et al., 1988; Geervliet et al., 1994). Damage from herbivores significantly increases the emission of plant volatiles (Dicke & Sabelis, 1989), information that foraging parasitoids can readily exploit (Mattiacci & Dicke, 1995; Chiu-Alvarado et al., 2010; Chiu-Alvarado & Rojas, 2011). Some volatiles released by plants are indicators of herbivore identity (Turlings et al., 1990; Dicke et al., 1990a,b), and can even vary between different herbivore life-stages (Takabayashi et al., 1995; Gouinguéné et al., 2003), providing parasitoids and predators with more specific cues. However, not all stimuli from a host's food source are reliable: the presence of a host's food plant does not guarantee presence of a suitable host (Zanen & Cardé, 1991), and plant volatile production can be highly variable, for example, due to differences in growing conditions (Visser, 1986). This reliability versus detectability foraging challenge selects for parasitoids that can exploit both direct and indirect cues (Vet & Dicke, 1992; Cardé & Bell, 1995).

Although foraging has been studied in many parasitoid species that belong to the monophyletic group of hymenopterans known as the Parasitica (Peters et al., 2017), host finding by species in the hymenopteran family Bethyridae has had little evaluation (but see Collatz & Steidle, 2008; Chiu-Alvarado et al., 2010; Chiu-Alvarado & Rojas, 2011). Bethyrids are, unlike most other hymenopteran parasitoids, members of the Aculeata ('stinging wasps') and not of the Parasitica (Peters et al., 2017). Within the Aculeata, bethylids belong to the Chrysoidea, the sister-group of all other aculeate taxa, including bees, ants, and vespid wasps (Peters et al., 2017). Aculeates and non-aculeates diverged from their latest common ancestor some 230 million years ago (Peters et al., 2017), and thus alternative adaptations and strategies for foraging could

have evolved between members of these taxa. Bethyrids are already known to exhibit life-history characteristics that are thought to be rare (although not entirely absent) among the Parasitica, including sub-sociality (Hardy & Blackburn, 1991; Abdi et al., 2020a), quasi-sociality (Tang et al., 2014; Abdi et al., 2020b), and kin recognition (Lizé et al., 2012).

Most studies of bethylid reproduction have focussed on decisions made by females only once a host has been located (e.g., Legner & Warkentin, 1988; Hardy et al., 1992; Gao et al., 2016; Abdi et al., 2020a,b). As bethylids are encouraged and deployed as biocontrol agents against many coleopteran and lepidopteran pests of economic importance (Cock & Perera, 1987; Legner & Gordh, 1992; Batchelor et al., 2006; Jaramillo et al., 2009; Yang et al., 2014; Polaszek et al., 2019) the lack of information on their foraging behaviour is a potentially important knowledge gap.

Goniozus jacintae is a gregarious ectoparasitoid of tortricid moths. It is a commonly occurring parasitoid of LBAM (Danthanarayana, 1980), which is an invasive generalist herbivore and a pest in agro-ecosystems in Australia and elsewhere (Suckling & Brockerhoff, 2010). LBAM has particular prominence in, and economic impact on, the Australian wine grape industry (Scholefield & Morison, 2010). Despite its common occurrence as a beneficial insect, there is surprisingly little knowledge of the efficacy of *G. jacintae* as a biocontrol agent for LBAM (Danthanarayana, 1980; Paull & Austin, 2006) and no prior information on its foraging behaviours or ability to locate hosts.

Here we evaluate the foraging behaviour of female *G. jacintae* when presented remotely, in a wind tunnel, to feeding LBAM. Previous studies using wind tunnels have demonstrated that oriented flight responses of parasitoids to airborne environmental cues can usefully be studied under laboratory conditions (Drost et al., 1986; Keller, 1990; Guerrieri et al., 1993). As *G. jacintae* is known to parasitise instars 3-6 of LBAM (Danthanarayana, 1980), we investigated whether its foraging behaviour varies according to host instar, having first established that later instars generate more leaf damage and thus are likely more strongly associated with volatile cues. The ultimate aim was to use the understanding of foraging behaviour to enhance the potential of *G. jacintae* to control agricultural pests in the field. Furthermore, as this study is among the first to quantify the host location behaviour of a bethylid, the results may be applicable to further agro-ecosystems in which species of *Goniozus* and/or other bethylids have been successfully deployed as, or considered as, agents of biological pest control (Legner & Gordh, 1992; Baker, 1999; Batchelor et al., 2006; Shameer et al., 2018; Polaszek et al., 2019).

Materials and methods

Rearing *Epiphyas postvittana*

The culture of *E. postvittana* (LBAM) used in this experiment was established at the South Australian Research and Development Institute in 1994 and has since been maintained with annual additions of wild moths. LBAM was reared on an artificial diet at 22 ± 2 °C under L12:D12 photoperiod, following methods reported in Yazdani et al. (2014).

Rearing *Goniozus jacintae*

A culture of *G. jacintae* was established from individuals reared from parasitised LBAM that were collected in vineyards at McLaren Vale, South Australia, in 2017. The wasp culture was reared at 23 ± 2 °C and L14:D10 photoperiod, in cages on larval LBAM that infested plantain, *Plantago lanceolata* L. (Plantaginaceae). Adult wasps were provided with water and honey ad libitum. Wasp cocoons were isolated in glass vials (5 cm long, 18 mm diameter) containing a drop of honey and fitted with caps that had screens for ventilation. Upon emergence, females were caged serially, 2–5 at a time, with five males to allow mating, and then re-isolated and held in vials for at least 1 h before being used in experiments.

Experimental plant

Plantago lanceolata was selected as the experimental plant in this study. LBAM is a polyphagous, multivoltine leafroller that can feed on a wide diversity of plants (Suckling & Brockerhoff, 2010). *Goniozus jacintae* has been associated with parasitising LBAM on many plant species apart from grapevine, including *P. lanceolata* (Danthanarayana, 1980). *Plantago lanceolata* is commonly found in interrows and underneath the grapevine canopy in Australia, and supports LBAM populations throughout the year, including during grapevine dormancy. *Plantago lanceolata* has been used as a model species in numerous experiments

on LBAM (Tomkins et al., 1991; Yazdani et al., 2015a) as well as other life history and population studies of host plant–herbivory interactions for decades (Bowers et al., 1992; Gange & West, 1994). These observations make this plant a suitable candidate for this study.

Leaf damage by host instars

As leaf damage is a primary source of volatiles associated with the attraction of parasitoids to plant-feeding host insects (Turlings et al., 1990; Whitman & Eller, 1990), we first assayed the amount of leaf damage caused by LBAM larvae in different instars. In each replicate ($n = 20$ per instar), a fresh plantain leaf of uniform size was presented to either a single third, fourth, fifth, or sixth instar in a plastic container ($8 \times 11.5 \times 11$ cm). After 24 h, the larva was removed, and the leaf was scanned into a digital image format (jpg). This allowed for leaf area damage and subsequent perimeter of leaf damage to be measured using ImageJ for Windows (64 bit v.1.52; Schindelin et al., 2012).

Response of *Goniozus jacintae* to host instar

In order to elucidate the sequence of behaviour that leads a parasitoid to a host, we observed responses of female *G. jacintae* to leaves infested with susceptible larval stages of LBAM (instar 3–6; Danthanarayana, 1980). Instars were identified by measuring head capsule width (Yazdani et al., 2014), with measurements of 0.545 (instar 3), 0.875 (instar 4), 1.248 (instar 5), and 1.422 mm (instar 6) used accordingly. Two larvae of a selected instar were transferred to a single plantain leaf and left for 16–18 h to allow them to feed on the plant, produce frass, and deposit silk. The leaf was then hung from a bar fixed 25 cm above the floor of a wind tunnel (for details see Keller, 1990). Wind speed was set at 20 cm s^{-1} and temperature at 23 ± 2 °C. A single female wasp, which had been isolated in a glass vial (see above), was released 25 cm downwind from the infested leaf (Figure 1).

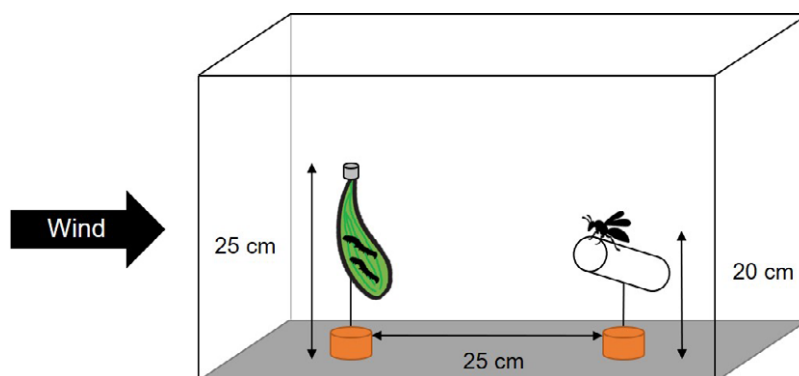


Figure 1 Diagram of the wind tunnel used in experiments.

Table 1 Behaviours exhibited by *Goniozus jacintae*

Behaviour	Description
Still	Standing still on the substrate without moving antennae
Stationary	Standing still on the substrate with moving antennae, no upwind orientation
Pointing	Standing still, facing upwind with raised head and continuously moving antennae
Grooming head	Grooming antennae and other parts of head
Grooming thorax	Grooming legs, abdomen, or wings
Walking	Walking while antennae are held forward of the head ¹
Slow walking	Walking at a slower pace, with antennae retracted and occasionally in contact with the substrate ²
Flying	Flying
Attacking	Mounted on and grappling with host while curling abdomen and attempting to insert probing stinger into host thorax ²

¹See Video S1.²Video S2.

Preliminary observations were used to distinguish and define the array of behaviours exhibited by females (Table 1; Martin et al., 1993). Observations were divided into three phases: (1) ‘pre-flight’, the time from introduction into the wind tunnel until initiation of first flight from the release vial; (2) ‘flight time’, the time from initiation of flight until first landing on the infested leaf; and (3) ‘post-flight’, the time spent on the infested leaf.

Parasitoid behaviour was recorded continuously using event-recorder software The Observer XT (Noldus Information Technology, Wageningen, The Netherlands; Zimmerman et al., 2009), with each observation lasting for either 10 min or until the wasp attacked a host larva. Observations did not continue past the host attack stage as the interactions between host and parasitoid are complex, involving multiple stinging events and ovipositional decisions, which require separate analysis (E Aspin, MA Keller & ICW Hardy, unpubl.). The mean duration and mean frequency (per min) of each behavioural category were calculated for pre- and post-flight phases within each replicate. The experiment was replicated 20× for each instar, using a different female *G. jacintae* in each replicate (n = 80).

Statistical analysis

Effects of host instar on the dimensions of leaf damage (leaf area removed and length of perimeter of leaf damage)

were analysed using two-tailed one-way ANOVAs, followed by Tukey’s honestly significant difference (HSD) post-hoc tests. Differences in the behavioural profiles exhibited by wasps exposed to different host instars were assessed using multivariate ANOVA (MANOVAs). ANOVAs were conducted when significant differences were observed, followed by Tukey’s HSD tests, to explore the effects of host instar on the occurrence of each of the behavioural categories. The significance thresholds for these ANOVAs were adjusted for multiple comparisons to control type I error rates via the false discovery rate (FDR) procedure, with the family-wide α -value set to 0.05 (Benjamini & Hochberg, 1995; McDonald, 2014). Data on the time to initiate first flights and the duration of first flights were analysed using Cox proportional hazards regression models with the ‘coxph’ function in package Survival (v.3.2-3; Therneau et al., 2020). Kaplan–Meier survival curves were constructed to illustrate how times were affected by host instar treatments. To analyse the frequencies of completed flights and host encounter occurrences, χ^2 tests of independence were used, depending on which instar group the parasitoid was presented with. All statistical analyses were conducted using R v.3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) with RStudio v.1.2.1335 (RStudio Team, Boston, MA, USA).

Results

Leaf damage by host instars

Area of consumed leaf tissue and length of the perimeter of leaf damage differed among instars (area: $F_{3,72} = 27.93$; perimeter: $F_{3,72} = 22.81$, both $P < 0.001$; Figure 2) and, for both, values were greater for instars 5 and 6 than for instars 3 and 4 (HSD test: $P < 0.001$).

Pre-flight response to host instar

There was a period of pre-flight orientation exhibited by female *G. jacintae* on the rim of the release vial which was characterised by *walking* (Video S1), *grooming*, and *pointing* behaviours. The overall behavioural profile of pre-flight behaviours exhibited differed among host instars (Table 2, MANOVAs), as did the mean duration and mean frequency of every individual behavioural category (Table 2, ANOVAs). Similarly, the proportions of time spent on each behaviour during the pre-flight phase differed among host instars (Figure 3). The mean duration of *walking* was highest when wasps were exposed to third instars but decreased when downwind of sixth instars (HSD test: $P < 0.05$; Table 2). *Pointing* was a behaviour that usually preceded flight (E Aspin, pers. obs.), and both the mean duration and mean frequency of *pointing* was significantly higher when wasps were downwind of the larger

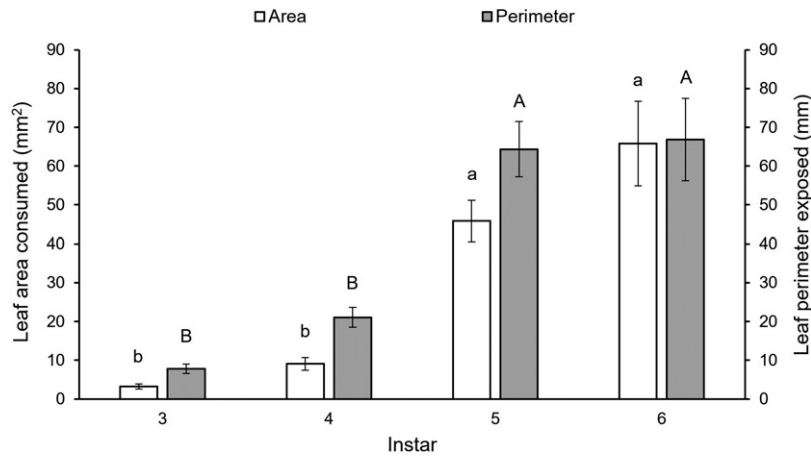


Figure 2 Mean (\pm SE) leaf area damaged (mm²) and perimeter of damaged leaf tissue (mm) caused by each of instars 3-6 of *Epiphyas postvittana* feeding on *Plantago lanceolata*. Means capped with different letters are significantly different between instars (Tukey's HSD: $P < 0.001$).

Table 2 Pre-flight mean (\pm SE) duration (s) and frequency (per min) of behaviours exhibited by female *Goniozus jacintae* according to host instar

Behaviour	Instar				ANOVAs	
	3	4	5	6	$F_{3,76}$	P
Duration						
Grooming head	10.15 \pm 1.49a	9.02 \pm 1.29a	1.68 \pm 0.64b	3.09 ¹	23.863	<0.001
Grooming thorax	6.80 \pm 0.88	7.05 \pm 0.85	8.25 \pm 0.87	10.18 \pm 1.05	2.844	0.043
Pointing	1.56 \pm 0.26b	2.17 \pm 0.23ab	2.41 \pm 0.16ab	2.85 \pm 0.27a	5.230	0.002
Stationary	10.60 \pm 1.24a	4.11 \pm 0.92b	1.87 \pm 0.50b	1.77 \pm 0.57b	23.201	<0.001
Still	25.34 \pm 3.74a	10.95 \pm 1.99b	1.38 \pm 0.79c	10.54 \pm 1.54b	18.729	<0.001
Walking	20.79 \pm 1.47a	22.86 \pm 2.65a	19.32 \pm 1.95ab	13.33 \pm 1.42b	4.465	0.006
Frequency						
Grooming head	0.92 \pm 0.10a	0.76 \pm 0.06a	0.25 \pm 0.09b	0.40 ¹	31.123	<0.001
Grooming thorax	0.42 \pm 0.05b	0.86 \pm 0.12a	0.96 \pm 0.11a	0.98 \pm 0.05a	8.031	<0.001
Pointing	0.31 \pm 0.06b	0.75 \pm 0.11b	1.48 \pm 0.15a	1.65 \pm 0.12a	30.36	<0.001
Stationary	0.89 \pm 0.08bc	1.15 \pm 0.13ab	1.52 \pm 0.12a	0.67 \pm 0.0c	12.56	<0.001
Still	0.76 \pm 0.08a	0.86 \pm 0.13a	0.12 \pm 0.07b	1.08 \pm 0.13a	11.448	<0.001
Walking	1.16 \pm 0.14c	1.91 \pm 0.24b	2.50 \pm 0.20ab	2.78 \pm 0.18a	13.664	<0.001

MANOVA, duration: Wilks' $\lambda = 0.176$, $F_{3,76} = 9.50$; frequency: Wilks' $\lambda = 0.082$, $F_{3,76} = 15.95$, both $P < 0.001$. Because six ANOVA tests were carried out, the significance criterion was adjusted via the false discovery rate (FDR) procedure: all P values remained significant following this correction.

Means within a row followed by different letters are significantly different among host instars (Tukey's HSD: $P < 0.05$).

¹Only one occurrence of *grooming head*.

instars (5 and 6) compared to the smaller instars (3 and 4; HSD test: $P < 0.05$; Table 2).

Time to initiate flight differed according to the host instar presented (Likelihood ratio test: $G = 93.14$, d.f. = 3, $P < 0.001$). Four females exposed to third instars and one female exposed to fourth instars did not take flight during the designated 10-min trial time. All other females took flight. Pre-flight times were longer when

larvae were third instars than when they were fourth ($z = 5.07$), fifth ($z = 8.84$), or sixth instars ($z = 4.27$, all $P < 0.001$). Time taken to initiate first flight when presented with fifth instar hosts was shorter than when presented with third ($z = 8.84$), fourth ($z = 6.20$), or sixth instars ($z = 6.57$, all $P < 0.001$). There was no significant difference in timing when fourth or sixth instars were presented ($z = 0.72$, $P = 0.47$; Figure 4A).

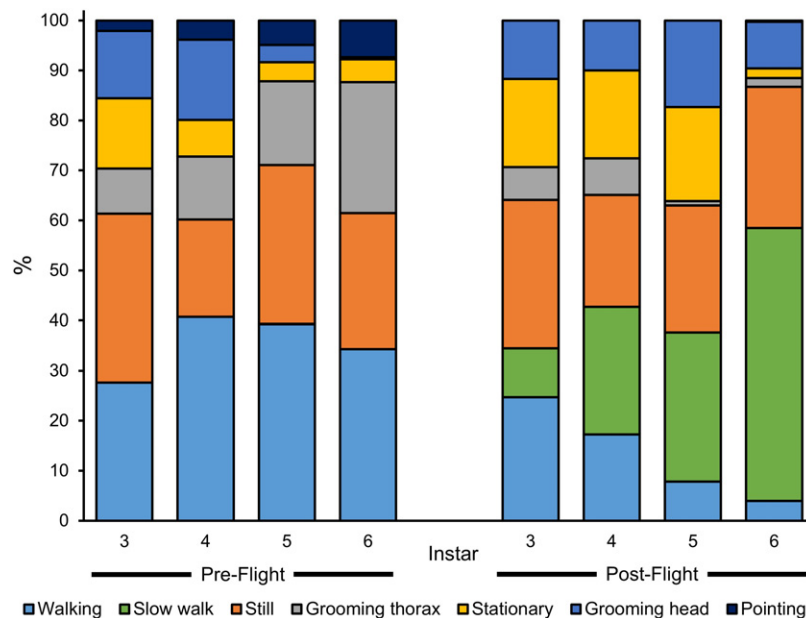


Figure 3 Percentage of total time for each of seven behaviours of female *Goniozus jacintae* in the presence of each of the four susceptible instars of *Epiphyas postvittana*. The graph is split into pre-flight and post-flight phases. Table 1 provides definitions of behaviours.

Flight response to host instar

The duration of first flight differed among host instars (Likelihood ratio test: $G = 55.46$, d.f. = 3, $P < 0.001$), with flights to the infested leaf taking longer for third instars compared to fourth ($z = 4.39$), fifth ($z = 5.61$), and sixth instars ($z = 4.91$, all $P < 0.001$). Duration of first flight towards fifth instars was shorter than fourth instars ($z = 2.52$, $P = 0.012$), but there was no significant difference between fourth and sixth instars ($z = 1.03$, $P = 0.31$; Figure 4B).

Post-flight response to host instar

The overall behavioural profile during the post-flight period differed among host instars (Table 3, MANOVAs) and mean duration and mean frequency of all behaviours differed significantly among instars (Table 3, ANOVAs). Again, the proportions of time spent on each behaviour during the post-flight phase differed among host instars (Figure 3). *Grooming thorax* behaviour was significantly lower in mean duration and mean frequency when a wasp was on a leaf infested with fifth and sixth instars compared to third and fourth instars (HSD test: $P < 0.001$; Table 3). Mean duration and mean frequency of *slow walking* differed significantly among host instars. *Slow walking* was often seen when wasps were in close proximity to a host and preceded attacking (Video S2). The mean duration and mean frequency of *slow walking* was the shortest for third instar compared to fourth, fifth, and sixth instar

(HSD test, $P < 0.05$). *Slow walking* mean frequency was longest for the sixth instar (HSD test: $P < 0.001$).

Occurrence of completed flight and host finding occurrences

As not all trials resulted in the initiation of flight or encounter of female *G. jacintae* with a larval LBAM (Table 4), a χ^2 test of independence was conducted to assess whether the instar presented influenced the frequency of parasitoid flight or LBAM encounter. The likelihood of a wasp taking flight towards an infested leaf differed among instars ($\chi^2 = 9.17$, d.f. = 3, $P < 0.05$), and the likelihood of a wasp encountering and attacking a larva was also influenced by instar ($\chi^2 = 14.01$, d.f. = 3, $P < 0.01$). The frequency of wasps that encountered and attacked a host given that they took flight differed among instars ($\chi^2 = 9.20$, d.f. = 3, $P < 0.05$). Removal of the third instar from the analysis consequently resulted in no significant difference among instars ($\chi^2 = 4.23$, d.f. = 2, $P = 0.12$), indicating that third instar hosts were attacked less frequently by *G. jacintae*.

Discussion

To our knowledge, this is the first experimental study of the foraging-flight behaviour of a bethylid wasp. We found that time for *G. jacintae* to initiate flight towards fifth instars was less than when hosts were in other instars, whereas the duration of flight towards third instar hosts

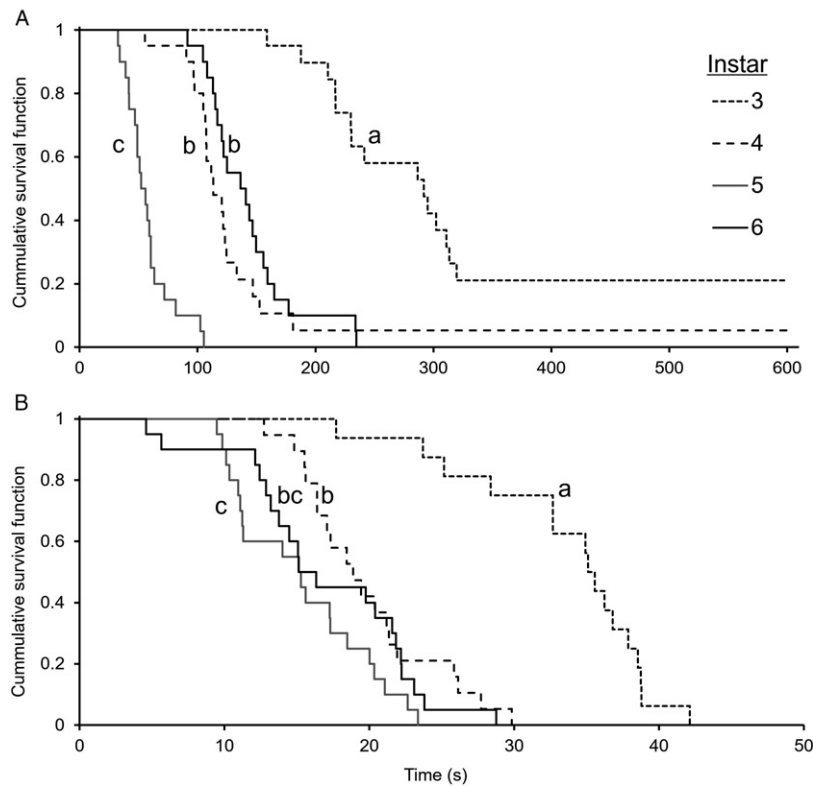


Figure 4 Kaplan–Meier survival curves for (A) time (s) from release to initiate first flight and (B) duration (s) of first flight for *Goniozus jacintae* towards each of the four susceptible instars of *Epiphyas postvittana*. Significant differences among the instars are indicated by different letters near the lines (Cox proportional hazards regression, Likelihood ratio test: $P < 0.05$).

was greater than for other instars studied. This shows that female *G. jacintae* can detect and respond to host-associated cues remotely. It suggests the females do not detect the presence of leaves infested with third instar LBAM as quickly as those infested with later instars, such as the fifth and sixth instar, and/or that they find cues emanating from third instars less attractive.

It is known from studies of other parasitoid species that herbivore-damaged plants are a source of volatiles that attract females to the locality of hosts (Nordlund et al., 1988; Turlings et al., 1991; Turlings & Wäckers, 2004), that parasitoid behaviour can be influenced by host-stage specific volatile profiles (Takabayashi et al., 1995; Turlings et al., 2000; Gouinguéné et al., 2003; McCormick et al., 2012; Yazdani et al., 2015b), and that larger lepidopteran larvae are more damaging to plants than smaller larvae (Mattiacci & Dicke, 1995; Yazdani et al., 2015b). Given that we established that larger instars of LBAM inflict more leaf damage than smaller instars, a likely explanation for the observed time-to-flight differences is that the quantity of volatile cues is higher – and thus more readily detectable by female *G. jacintae* – when the feeding larvae are larger,

although the qualitative composition of cues may also vary according to LBAM instar.

Our results indicate that *G. jacintae* has a stronger response as hosts develop through the third to sixth instar, which reflects their growth in size. This is consistent with reports of *G. jacintae* and other *Goniozus* species having greater reproductive success when attacking larger hosts (Danthanarayana, 1980; Hardy et al., 1992; Abdi et al., 2020a; E Aspin, MA Keller & ICW Hardy, unpubl.). This behaviour is also observed in parasitoid species within the Parasitica (Thompson, 1986; Godfray, 1994; Wang et al., 2016), suggesting that there has either been a retention of foraging behaviour characteristics or convergent evolution since the phylogenetic split between the Parasitica and the Chrysoidea. *Goniozus jacintae* parasitises instars 3–6 of the LBAM, whereas *Dolichogenidea tasmanica* (Cameron), another common parasitoid, parasitises instars 1–3 of the same host species and has the shortest flight duration when exposed to third instars (Yazdani et al., 2015b), indicating that flight behaviours are aligned with the range of host instars that parasitoids normally exploit. However, the observation that *G. jacintae* females would initiate

Table 3 Post-flight mean (\pm SE) duration (s) and frequency (per min) of behaviours exhibited by female *Goniozus Jacintae* according to host instar

Behaviour	Instar				ANOVAs	
	3	4	5	6	F _{3,71}	P
Duration						
Grooming head	11.35 \pm 1.21ab	8.81 \pm 0.96b	13.25 \pm 1.16a	4.51 \pm 0.60c	14.01	<0.001
Grooming thorax	6.35 \pm 1.09a	6.45 \pm 1.01a	0.68 \pm 0.37b	0.83 \pm 0.48b	16.26	<0.001
Pointing	-	-	-	2.26 [#]	N/A	N/A
Stationary	17.17 \pm 1.93a	15.50 \pm 2.12a	14.35 \pm 1.61a	0.92 \pm 0.47b	20.04	<0.001
Still	28.78 \pm 2.07a	19.70 \pm 2.08b	19.36 \pm 2.32b	13.59 \pm 2.12b	7.395	<0.001
Walking	23.99 \pm 2.44a	15.18 \pm 2.64b	5.98 \pm 1.57c	1.90 \pm 0.72c	22.61	<0.001
Slow walking	9.55 \pm 1.95b	22.43 \pm 1.52a	22.80 \pm 1.28a	26.24 \pm 3.48a	6.324	<0.001
Frequency						
Grooming head	0.75 \pm 0.08a	0.82 \pm 0.06a	0.48 \pm 0.03b	0.64 \pm 0.07a	5.115	<0.001
Grooming thorax	0.23 \pm 0.04a	0.31 \pm 0.03a	0.03 \pm 0.02b	0.05 \pm 0.03b	19.49	<0.001
Pointing	-	-	-	0.13 [#]	N/A	N/A
Stationary	0.82 \pm 0.08a	0.76 \pm 0.10a	0.67 \pm 0.08a	0.11 \pm 0.05b	15.51	<0.001
Still	0.70 \pm 0.06c	1.14 \pm 0.14ab	0.87 \pm 0.08bc	1.40 \pm 0.13a	7.398	<0.001
Walking	0.64 \pm 0.08a	0.40 \pm 0.04b	0.12 \pm 0.02c	0.22 \pm 0.09bc	12.19	<0.001
Slow walking	0.35 \pm 0.07c	0.81 \pm 0.11b	1.23 \pm 0.08b	1.96 \pm 0.21a	25.06	<0.001

MANOVA, duration: Wilks' $\lambda = 0.221$, $F_{3,71} = 11.55$; frequency: Wilks' $\lambda = 0.129$, $F_{3,71} = 11.03$, both $P < 0.001$. Because six ANOVA tests were carried out, the significance criterion was adjusted via the false discovery rate (FDR) procedure: all P values remained significant following this correction.

Means within a row followed by different letters are significantly different among host instars (Tukey's HSD: $P < 0.05$).

[#]Only one occurrence of *pointing*.

Table 4 Occurrence of flights and attacks by *Goniozus jacintae* according to host instar

	Host instar				Total
	3	4	5	6	
Frequency of flight					
Flight	16	19	20	20	75
Total	20	20	20	20	80
Proportion flying	0.80	0.95	1.00	1.00	0.94
Frequency of attack					
Attack	7	11	16	17	51
Total	20	20	20	20	80
Proportion attacking	0.35	0.55	0.80	0.85	0.64
Frequency of attack among parasitoids that flew to the host location					
Attack	7	11	16	17	51
Total	16	19	20	20	75
Proportion attacking	0.44	0.58	0.80	0.85	0.68

flight towards fifth instars significantly earlier than towards sixth instars is perplexing. Sixth instars are typically larger than fifth instars and, in this study, consumed an equal amount of leaf area, thereby exposing an equal perimeter of damaged leaf tissue as fifth instars. Volatile semiochemical cues are released from the larval damage

site (Röse et al., 1996), making the length of perimeter of leaf damage a suitable proxy for estimating degree of volatile emission per instar. This suggests that the quantities of volatiles emitted from fifth and sixth instars are similar, and thus should be detectable at a similar level. Therefore, as above, there could be qualitative differences that are influencing the response of *G. jacintae* to fifth and sixth instars.

One such difference could be the likelihood of a sixth instar being close to pupation. LBAM pass through up to six moults prior to pupation (Danthanarayana, 1983) and a host that has pupated is unsuitable for *G. jacintae* to produce offspring, as any eggs laid prior to imminent pupation would be shed along with the moulted cuticle (Danthanarayana, 1980). Larvae produce hormones that trigger morphological changes during moult or pupation (Maróy & Tarnóy, 1978; Riddiford, 1996) that in turn influence the formation of larval or pupal cuticle (Riddiford et al., 1999). Many hymenopterous parasitoids are known to inspect the surface of the host, usually as a means of discriminating between parasitised and unparasitised hosts (van Lenteren, 1981). Evidence of host discrimination mechanisms used by parasitoids include perceiving marking pheromones from other parasitoids (Vinson & Guillot, 1972) as well as physical changes in the host

surface. For example, ovipositional punctures have been suggested to function as an external marker (Boldt & Ignoffo, 1972). Considering this information, some parasitoids may be able to use surface markers or signals to identify when a potential host is close to pupation. Detecting such surface markers or pupation cues on a host at a distance seems unlikely. Although it is impossible to reduce the amount of damage that is associated with growth and development, perhaps sixth instars have evolved the ability to reduce the quantity of attractive compounds associated with their feeding. Also, if the moulting or pupation process caused notable changes to the volatile profile associated with different host stages, this could influence the behavioural response of *G. jacintae*. Hence, *G. jacintae* may have developed a mechanism to detect and avoid parasitising hosts that are close to moulting or pupation, thus limiting the costs of securing a host and laying eggs that will not develop. Further study is required to determine whether *G. jacintae* females actively avoid hosts that are close to pupation.

In addition, *G. jacintae* may respond differently to sixth instars compared to fifth instars due to differing risks involved in host attack. Later host instars are often larger and better equipped with defensive structures and behaviours, including aggressive biting behaviour, ‘corkscrewing’, and regurgitation (e.g., Video S2) that can both result in irreversible injury to, or even death of, natural enemies (Greeney et al., 2012; Abdi et al., 2020b). Sixth instar LBAM are capable of these behaviours and have been observed to use them to escape from parasitism by *G. jacintae* (E Aspin, pers. obs.). Hence, differences in flight response by *G. jacintae* between fifth and sixth instars may reflect both the risks involved in handling the largest hosts and/or host adaptation in terms of cue production.

We found that female *G. jacintae* display different behavioural profiles when exposed to different host instars and also that the exhibited behaviours vary between pre- and post-flight stages of foraging. Information gained during the pre-flight and flight phases of foraging is most likely to be transmitted via volatile chemicals. *Pointing*, for instance, involves standing still, facing upwind with raised head and continuously moving the antennae, and is very likely associated with the detection of windborne chemical cues prior to making a decision to engage in flight. Although commonly observed during pre-flight, *pointing* was only once observed post-flight. Information acquired during the post-flight stage can also include contact and potentially visual cues related to feeding damage, faeces, and silk deposits associated with hosts, and the proximity of the host itself. *Slow walking* is a distinct behaviour that was exhibited only during post-flight and showed an inverse relationship to walking. The largest distinction

between *slow walking* and *walking* is a considerably reduced pace and higher antennation of the substrate (Videos S1 and S2). Antennation is associated with orientation and searching in parasitoids (Olson et al., 2003), and *slow walking* was usually the behaviour that occurred immediately before a female attacked the host. *Slow walking* was also the most common when hosts were large, particularly in the sixth instar. It seems likely that this behaviour reflects the higher physical risk to the parasitoid that is incurred when tackling larger hosts. A slower, more cautious pace may reduce the likelihood of detection by a host and thus elicit fewer defensive behaviours, which are often detrimental to the success of a parasitoid (Waage, 1983; Gross, 1993; Greeney et al., 2012; Abdi et al., 2020b). In addition, as LBAM are leafrollers, it would be beneficial for the parasitoid to exercise caution when entering a leaf-rolled enclosure; it is not uncommon for other natural enemies, such as spiders and earwigs, to occupy these shelters, and presence of LBAM cues (frass, faeces) does not guarantee presence of a suitable host.

It is important to note that LBAM is only one of many host species that *G. jacintae* is capable of parasitising (e.g., *Merophyas divulsana* Walker; E Aspin, pers. obs.), some of which may not be leafrollers. Thus, foraging behaviour in *G. jacintae* may not be uniform across host species, as non-leafroller hosts could require a different foraging approach than that is shown towards LBAM.

Flight and subsequent host-finding success did not occur in all replicates of the wind tunnel experiment and the likelihood varied throughout each phase of foraging. The occurrence of flight towards an infested leaf and the overall frequency of attacking a larva differed among instars. Additionally, the likelihood of a wasp attacking a larva given that it had taken flight was different among instars. These results indicate a sequential process of foraging. Together with the flight time results, this shows that before flight, during flight, and after landing on the infested leaf, *G. jacintae* exhibited different responses to different susceptible instars.

This wind tunnel study of foraging behaviour of a bethylid wasp provides new understanding of the behavioural repertoires exhibited and their likely relationships with different instars of host and different classes of cues. The results suggest that hosts close to pupation may be avoided, or at least less preferred than those that are not about to undergo changes to the integument on which parasitoid eggs are deposited and further that females may approach larger hosts with caution due to the physical risks involved in host attack and suppression. Although these aspects require further investigation, we have demonstrated that (1) *G. jacintae* females can detect host-associated cues remotely and actively travel towards their

origin by a combination of flight, walking, and slow walking, and (2) behaviours are attuned to the information received regarding the developmental stage of the host. Such knowledge is useful for designing and implementing effective programmes of biocontrol of LBAM in vineyard settings, for instance, when considering how best to release mass-reared parasitoids into the field.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1. Female *Goniozus jacintae* wasp exhibiting walking behaviour across a *Plantago lanceolata* leaf. Walking behaviour characteristics: standard pace, antennae raised and not in contact with substrate.

Video S2. Female *Goniozus jacintae* wasp exhibiting slow walking behaviour before attacking and stinging a feeding light brown apple moth (LBAM) larva (*Epiphyas postvittana*) on a *Plantago lanceolata* leaf. Slow walking behaviour characteristics: considerably reduced pace, antennae making contact with substrate and host. Later LBAM instars can defensively regurgitate, which is seen in this video. They also exhibit a ‘corkscrew’ motion when attacked. In this example, however, the parasitoid stung the thorax of the larva and caused paralysis. Accessible url: <https://www.youtube.com/watch?v=T67oFiOXttI>