Bulletin of Entomological Research (2005) **95**, 429–436 DOI: 10.1079/BER2005374

Role of host feeding niches and host refuges in habitat-related behaviour of *Hyssopus pallidus* (Hymenoptera: Eulophidae), a larval parasitoid of the codling moth

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Abstract

Parasitoid fitness depends largely on the capability to locate a host in an ecosystem. A parasitoid of a polyphagous host might not be able to find or to access the host in all its feeding niches. This study evaluated the niche selection of *Hyssopus pallidus* (Askew), a larval parasitoid of *Cydia pomonella* (Linnaeus), at the plant level with the goal of assessing its potential for biological control on different fruit crops throughout the plant cycle. Parasitoid behaviour during host location and reproduction rate were investigated on host caterpillars actively feeding on apple, pear, apricot or plum, and on caterpillars diapausing under the bark. Under laboratory conditions, the host searching behaviour of *H. pallidus* varied depending on the fruit species offered and the infestation of the fruits. Parasitoid females searched longer on apples than on other fruit species, and they searched longer on infested than on uninfested apples. Female wasps were able to locate and parasitize host caterpillars under the tree bark, and their behaviour did not vary with host accessibility. The numbers of caterpillars attacked by *H. pallidus* depended on the fruit species. The highest numbers of caterpillars were parasitized in apples and apricots. Their accessibility (i.e. position) within the fruit or on the branch did not influence parasitism success. Although hosts were parasitized throughout the season, the best results were achieved with early and late releases. Therefore, the host niche selection behaviour of *H. pallidus* most likely co-evolved with the host *C. pomonella* on apples, which renders *H. pallidus* a valuable biocontrol agent for successful release at different times of the season into apple orchards.

Keywords: *Hyssopus pallidus*, *Cydia pomonella*, Hymenoptera, Lepidoptera, Tortricidae, Eulophidae, apple, behaviour, host location, niche selection

Introduction

The fitness of parasitoids depends largely on their capability to locate the host in an ecosystem. The challenge of finding a host is complicated by the fact that the parasitoids may emerge in a location where hosts are not present or at a time when hosts are not at the stage suitable for attack. A parasitoid has to make foraging decisions, as for example where to search for hosts and for how long (Vet *et al.*, 1995). The first important decision involves selecting a host niche that guarantees optimal development of the progeny. A parasitoid of a polyphagous host might not be able to find the host in all its feeding niches, such as in different plant species or parts of a plant, or throughout the season, and the foraging strategy will be decisive for its survival and

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reproductive success. Thus, searching for a host in a non-suitable niche might not be adaptive for a parasitoid (Godfray, 1994).

As a defensive strategy to escape attack from natural enemies, some herbivores have adapted a concealed lifestyle. It has been hypothesized that endophytic feeding provides a physical refuge for herbivores. Feeding inside plant tissue makes it more difficult for enemies to locate and attack (Hawkins, 1994). However, some parasitoids have developed behavioural and morphological characteristics allowing them to utilize concealed hosts. Given the difficulty of reaching a host hidden in plant tissue, it is likely that such parasitoids adapt more sophisticated strategies than do parasitoids of exophytic hosts. For instance, visual and acoustic cues (Sugimoto *et al*., 1988), vibrational cues (Meyhöfer *et al*., 1994; Fischer *et al*., 2004), and host-derived kairomones (Dutton *et al*., 2000) can play a role in the location of endophytic hosts by parasitoids.

In the current study, the caterpillar hidden in plant substrate which serves as a host to a parasitoid, is the codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), a major pest on apples worldwide (Dorn *et al*., 1999). This species also attacks pear, apricot, plum, quince, almond, peach and walnut (van der Geest & Evenhuis, 1991). After hatching, young larvae bore into the fruit and tunnel to the core, where they feed on developing seeds (Ohlendorf, 1991). Fifth instar larvae leave the fruit and pupate or overwinter in cocoons, mainly under the bark and in crevices in the host tree (Wearing & Charles, 1989). Amongst the many egg, larval and pupal parasitoids of *C. pomonella* that have been identified (van der Geest & Evenhuis, 1991), *Hyssopus pallidus* (Askew) (Hymenoptera: Eulophidae) is a candidate species for biological control. It has a short generation time, is easy to rear as compared to other parasitoids, and females deposit eggs throughout their lifespan which may last 60 days when a food source is available (Tschudi-Rein & Dorn, 2001). Furthermore, this species has a highly female-biased sex-ratio of about 96% females to 4% males (Zaviezo & Mills, 1999). The reduction of the *C. pomonella* population at its larval stage is a promising component of a sustainable management system. In fact, a considerable proportion of a *C. pomonella* population is relatively sedentary and, when left uncontrolled, will endanger a fruit orchard in the subsequent generations (Dorn *et al*., 1999). In addition, removal of *C. pomonella* infested fruits from orchards as a sanitation measure has been reported as a useful component of a control system based on mating disruption (Judd *et al*., 1997). Such elimination of infestation sources by means of a biological control agent might be more economic than by manual labour. A number of laboratory bioassays was carried out as a basis for future field releases. Female *H. pallidus* are able to find the host by entering the fruit through the fruit calyx or the host larval tunnel (Mattiacci *et al*., 1999). The small female wasp stings the host larva, paralyses it, and deposits 10 to 30 eggs on or beside the host larva. Neonates crawl to the moribund host, penetrate its integument with their mouth parts (Tschudi-Rein & Dorn, 2001), and ingest body fluids. Fully developed parasitoid larvae then crawl a short distance (<1 cm), void their guts (meconia), and pupate (Brown, 1996). This gregarious ectoparasitoid of late instar *C. pomonella* larvae occasionally also attacks *Cydia molesta* (Busck) (Lepidoptera: Tortricidae), a key pest of stone fruits (Boucek & Askew, 1968; Tschudi-Rein *et al*., 2004).

However, very little is known about the degree of niche specialization of *H. pallidus*. The objective of our study was to elucidate *H. pallidus* niche preference through an investigation of the searching behaviour on infested and uninfested fruits of different species and on infested branches of apple trees. The parasitism rate in the different ecological niches of *C. pomonella* was also assessed, in order to evaluate whether this parasitoid could be used for biological control in different fruit crops.

Materials and methods

Rearing procedures

The insect rearing conditions and methods used in this study were the same as described previously (Mattiacci *et al*., 1999). In brief, *H. pallidus* was collected in France, then maintained in the laboratory by T. Unruh (USDA-ARS Research Laboratory, Wapato Washington, USA), and since 1997 by Applied Entomology, ETH Zurich. As *H. pallidus* is an ectoparasitoid, fifth instar *C. pomonella* larvae (L5) were used to obtain the largest number of progeny. Parasitoids were reared on two different *C. pomonella* strains, as previously described (Gandolfi *et al*., 2003b). To obtain a strain reared on artificial diet, *C. pomonella* larvae were transferred upon eclosion onto an artificial medium (medium 'B', Huber *et al*., 1972) which did not contain any fruit material. To obtain a strain reared on apple, newly hatched first instar *C. pomonella* larvae were placed on the surface of an apple. They burrowed into the fruit within 24 h and reached the fifth instar in 16–21 days. To facilitate recovery of fifth instar larvae before pupation, apples were covered with plastic cylinders on the 14th day from infestation and were carefully dissected after 18 to 21 days. Larvae of the two strains, those reared on artificial diet and those reared on apple, were kept at 25°C, 60 ± 10% rh and 18:6 LD and were offered to female *H. pallidus* at a 1:1 ratio of parasitoid:host (see Mattiacci *et al*., 1999 for more details). The *C. pomonella* larvae came from a laboratory colony which originated from diapausing larvae collected in cardboard strips around high-stem apple trees in north-eastern Switzerland. The colony had been maintained for 20 generations in culture.

All bioassays were conducted with four to seven days old, mated *H. pallidus* females of the 35th–38th generation after split rearing. The females had no oviposition experience before the bioassay. As the rearing environment might influence parasitoid searching behaviour (Gandolfi *et al*., 2003b) females from the strain reared on artificial diet were used for observations of parasitoids searching on different fruit species. On the other hand, for observations on tree branches females from the apple strain were used as experiments were carried out only on apple plants.

Searching behaviour in different host niches

The objective of this bioassay was to determine whether the different host niches (fruits and bark) and the presence of the host caterpillar within the niche influence the searching behaviour of *H. pallidus* females.

Plant treatments

Parasitoid foraging behaviour was observed on the most common fruit hosts of *C. pomonella*: apples (Rosaceae, *Malus*

Table 1. Average size, firmness and origin of fruits used in the behavioural bioassay ($n=30$).

Fruit	Diameter (cm) average ± SD	Length (cm) average ± SD	Firmness[1] (N) average ± SD	Origin (in northern Switzerland)
Apple	2.41 ± 0.24	2.58 ± 0.19	11.43 ± 2.48	Steinmaur
Pear	6.18 ± 0.17	8.46 ± 0.80	3.15 ± 1.10	Waltenschwil
Apricot	2.76 ± 0.25	3.08 ± 0.27	1.58 ± 0.18	Wädenswil
Plum	2.91 ± 0.40	2.42 ± 0.42	4.07 ± 1.40	Villmergen

[1] Force required to penetrate the fruit skin.

domestica Borkh. cv. Bohnäpfel), apricots (Rosaceae, *Prunus armeniaca* L. cv. Breda), pears (Rosaceae, *Pyrus communis* L. cv. Kaiser Alexander), and plums (Rosaceae, *Prunus domestica* L. cv. Fellenberg). Diameter and length of all fruits used were measured with a ruler and the firmness of the fruits was measured with a hand-penetrometer (table 1). In addition, as diapausing larvae overwinter under the bark of infested trees, the foraging behaviour was observed on branches of an apple tree.

Infestation with all caterpillar instars induced the emission of volatiles from apple fruit which might contribute to the parasitoid's host location (Hern & Dorn, 2001). As previous studies suggested (Mattiacci *et al.*, 1999), however, female parasitoids are likely to enter a fruit for parasitism either through the fruit calyx or through the relatively large tunnel made by fifth instar larvae. Therefore, fruits that contained fifth instar larvae were used for the bioassay. To obtain infested fruits, a third instar larva was placed into a small plastic dish (diameter 17 mm, height 11 mm). Each detached fruit was placed on top of the dish so that the larva could only enter through the calyx (apple and pear) or through the bottom part of the fruit (apricot and plum, which have no calyx). Fruits were then kept at the same environmental conditions as in the rearing, in order to achieve similar conditions of larval infestation. For the bioassay, fruits were used 7–11 days after infestation, the time needed by *C. pomonella* larvae to reach fifth instar. Uninfested fruits were stored under the same environmental conditions.

To obtain caterpillar-bearing bark, rough branches with a diameter of 1.5 to 3 cm were sawed into 6–7 cm long pieces. These were suspended in a plastic box (9 × 9 × 8 cm) by a nail through the cut ends, so that the branch did not touch the bottom of the box and could be turned to facilitate observations of the searching parasitoids. *Hyssopus pallidus* females are small (less than 2 mm body length) and of a dark colour, similar to that of the bark, making them difficult to detect. A diapausing *C. pomonella* larva was placed on the branch and kept for 1–3 days until it wove a cocoon under the same environmental conditions as the fruit bioassays. Three different locations of the cocooned larva were defined: 'exposed', if the larva was visible on the bark; 'semi-exposed', if the larva was under the bark but still visible; and 'hidden', if the larva was completely under the bark or in the wood, and therefore not visible. The thickness of the bark was measured as 1.87 ± 0.97 mm, on average.

Behavioural bioassays

A 25 × 25 × 25 cm Plexiglas cage was used as the bioassay arena to observe parasitoids searching on fruits. On the day of the bioassay, one fruit was placed on a 4 × 5.5 cm metal grating hanging 10 cm from the ceiling of the cage. This swing-like structure gave the parasitoids access practically to the whole fruit.

Immediately before the bioassay, individual females of the strain reared on artificial diet were placed in 5 ml glass vials. They were then released 1 cm away from the calyx (apples and pears) or the entrance hole of the host (plums and apricots) or on the corresponding area of an uninfested fruit.

To observe parasitoids searching on infested branches in the box used as the bioassay arena, individual females from the apple strain were released on the bark immediately before the bioassay. The branches were sprayed with water at least one hour before the bioassay to enhance emission and/or perception of chemical cues, since in preliminary observations, reduced responsiveness to dry bark was observed. Parasitoid activity was continuously recorded with a hand-held computer (Psion organizer) using the software package 'The Observer 3.0'; a computer software package for event recording and data analysis used in behavioural research (Noldus, 1991).

Activities were recorded as follows: 'searching', defined as an intensive drumming with the antennae while either walking or standing on fruits or bark, 'inside', defined as the behaviour inside the calyx of a fruit, or inside the tunnel bored by the caterpillar in a fruit or under the bark; 'standing', defined as either standing motionless or preening; 'away' defined as leaving the fruit or branch by flying elsewhere in the cage or walking away. Females were used only once for an observation, with a total of 15 females per treatment.

For each observation, a new branch or a new fruit was used. Observations took place from 0900 h to 1630 h and were carried out under natural daylight conditions (540–1566 lux), 23 ± 2°C, 55 ± 15% rh. One set of bioassays was performed on at least three different days, to compensate for a possible effect of daily variation in response. Treatments were randomized on different days. An observation was discontinued if a parasitoid remained motionless for 15 min at the same location, if it did not leave the tunnel or calyx within 15 min of entering, if it left the fruit for 5 min, or if the total searching time exceeded 45 min. Preliminary observations showed that searching on the apple was seldom resumed after 15 min of 'standing' and that a parasitoid did not return to the apple after having left it for more than 5 min. As the average searching time of naïve *H. pallidus* on ripe infested apples is 15 min (Mattiacci *et al.*, 1999), the observation was discontinued after a total searching time of 45 min.

Infested fruits and the bark were dissected on the same day as the bioassay to check for presence and condition of the host larvae. Fruits from which larvae could not be recovered were excluded from the analysis.

Data analysis

The data obtained from observations on the duration of behavioural activities on fruits and those from the behavioural bioassay of searching on branches were subjected to the analysis of variance (ANOVA), followed by a Fisher's PLSD post hoc test. Infestation treatment (infested vs. uninfested) and plant species were chosen as fixed factors. Differences within one plant species between searching times on infested and uninfested fruits were determined by t-test.

The causal relationships of the number of parasitoids entering a fruit or bark (dependent variable: '1' for females which entered fruits or bark and '0' for females which did not enter), with infestation treatment and plant species, chosen as independent variables, were analysed with a logistic regression and the respective effect of each independent variable was examined using Likelihood Ratio test (SPSS Inc. 1997).

Reproductive success in different host niches

The reproductive success in different niches was examined by offering infested fruits of different species to the parasitoid and recording successful parasitism and reproduction. In order to avoid problems due to laboratory storage of the soft fruits (plums), infestation took place in the field. The exposure of infested fruits to *H. pallidus* females was carried out in the laboratory.

Infestation of fruits

In the field, fruits from trees without any pesticide treatment were each infested with two first instar larvae of *C. pomonella*. The infested fruits were covered with gauze to prevent dispersal of the larvae and to protect them from predators. The following fruits were infested: apple (*M. domestica* cv. Kidds orange, $n = 59$), apricots (*P. armeniaca* cv. Breda, $n = 101$), plums (*P. domestica* cv. Fellenberg, $n = 80$), and pears (*P. communis* cv. Kaiser Alexander, $n = 87$).

In a separate experiment, the effect of different levels of maturation of apples on parasitoid reproductive success was studied. Apples were infested 19 weeks (cv. Bohnäpfel, early, $n = 81$), 14 weeks (cv. Bohnäpfel, intermediate, $n = 113$) and 9 weeks (cv. Bohnäpfel, late, $n = 68$) before harvest.

Two weeks after infestation, all infested fruits were picked and kept in the laboratory at 25°C for maximum of five days before the bioassay.

For the behavioural observations, a fruit was placed on a 4 × 5.5 cm metal grating, so that the single female parasitoid placed on it had access from all sides. The parasitoid was provided with a droplet of honey as food source. A Plexiglas cylinder, 8.2 cm in diameter and 5.1 cm in length covered with gauze, was positioned over the fruit. The behavioural observations were directly related to the fruit as the unit, rather than to a certain surface area, as there was no significant correlation between searching time and surface area within the total of the data gained, and within each level of maturation and each species of fruit (linear regression analysis).

Infestation of branches

Infestation of branches was carried out as described for the behavioural bioassays. Three location treatments, i.e. 'exposed', 'semi-exposed' and 'hidden', were defined as explained above. A total of 20 to 21 observations was carried out for each location. The thickness of the bark was 1.5 ± 0.5 mm.

Data recording

After seven days, the parasitoids were removed and four days later the fruits or branches were dissected. Host condition, position of the host and the number of progeny were recorded. For the parameter 'host condition', the host larva was defined as 'parasitized' if parasitoid larvae or pupae could be observed on the host's body, 'paralysed' if no parasitoid progeny was present but host larvae were characteristically immobile, turgid and of a pink colour, 'alive' if still moving or it had pupated. For the parameter 'host position', the location of the larva upon dissection was recorded, as in the 'calyx', 'core', 'stem', 'side' or, 'stone' (only for apricots and plums). If no larva was found in a fruit or the larva found was dead, that fruit was discarded from the analysis. This resulted in the following number of replicates: apple (*M. domestica* cv. Kidds orange), $n = 12$, apricots (*P. armeniaca* cv. Breda) $n = 22$, plums (*P. domestica* cv. Fellenberg) $n = 21$, pears (*P. communis* cv. Kaiser Alexander) $n = 7$, early apple (cv. Bohnäpfel) $n = 22$, intermediate apple (cv. Bohnäpfel) $n = 25$ and late apple (cv. Bohnäpfel) $n = 3$. As the number of replicates was very low for late apple, the categories 'intermediate' and 'late' apple were combined to a category 'intermediate-late' for the data analyses.

Data analysis

The Chi-square analysis was applied to examine the respective effect of fruit species or maturation, and of the location of the larva on the branch on the total number of *C. pomonella* larvae attacked by *H. pallidus*. To meet the requirements of the Chi-square test, the categories 'parasitized' and 'paralysed' were combined for data analyses to a category 'attacked' and compared with the category 'alive'. The effects of host position and fruit species on the level of attack (using the number of larvae alive, paralysed or parasitized as dependent variables) were analysed with a logistic regression, based on Likelihood Ratio test (SPSS Inc. 1997). The Kruskal-Wallis test was conducted to analyse possible differences in the number of progeny on a parasitized *C. pomonella* larva in the different fruit species and from a differently exposed larva in bark.

Results

Searching behaviour on different host niches

Fruits

Fruit species significantly influenced the searching time of *H. pallidus* (ANOVA, $F = 7.37$, $df = 3$, $P < 0.001$). *Hyssopus pallidus* searched longer on apple than on pear, apricot, and plum (fig. 1). The infestation treatment of the fruit, i.e. whether the fruit was infested or not also significantly influenced the searching duration (ANOVA, $F = 12.87$, $df = 1$, $P < 0.001$). Female wasps searched longer on infested apples (t-test, $t = -3.02$, $P = 0.007$) than on uninfested apples, but they did not discriminate between infested and uninfested fruits of apricots ($t = -0.40$, $P = 0.695$), pears ($t = -1.68$,

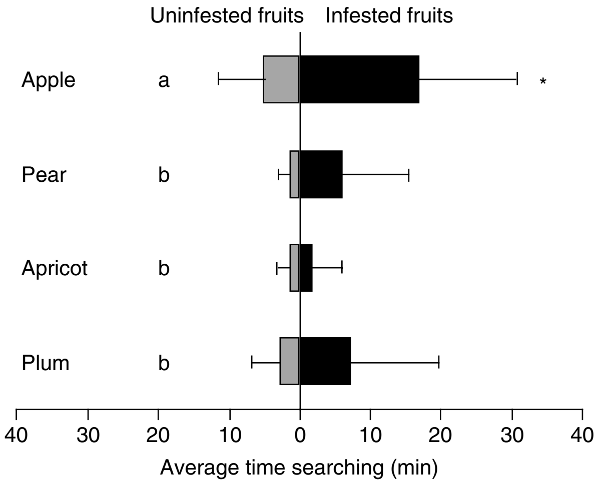

Fig. 1. The average total time spent 'searching' by *Hyssopus pallidus* on infested and uninfested fruits of different plant species (average ± SD, $n = 15$). 'Searching' was defined as an intensive drumming with the antennae while either walking or standing on fruits. Letters next to bars indicate significant differences between fruit species (ANOVA, $P < 0.05$). The asterisk indicates the significant preference for infested over uninfested fruits (t-test, $P < 0.05$).

$P = 0.114$) or plums ($t = -1.33$, $P = 0.201$). The time spent 'standing' on the fruit was significantly influenced by the fruit species (ANOVA, $F = 3.30$, $df = 3$, $P = 0.023$), but not by infestation treatment ($F = 0.81$, $df = 1$, $P = 0.372$). Parasitoids spent the longest time standing on infested apples (table 2). The time parasitoids spent 'away' from the fruit was influenced by the fruit species (ANOVA, $F = 5.79$, $df = 3$, $P = 0.001$) as well as by the infestation treatment ($F = 8.31$, $df = 1$, $P = 0.005$). The longest average time spent 'away' occurred when the parasitoids encountered uninfested pears (table 2).

The number of females entering the fruits depended on the infestation treatment (Likelihood Ratio test, $\chi^2 = 15.53$, $df = 1$, $P < 0.001$) and plant species ($\chi^2 = 19.43$, $df = 3$, $P < 0.001$) (table 3). The highest total numbers of *H. pallidus* females were observed entering infested apples and plums (table 3). Uninfested fruits were not entered by females except in the case of apple where they entered through the calyx.

Branches

The accessibility of the host in the bark did not affect a parasitoid's time allocation to the different activities defined: searching time (ANOVA, $F = 0.91$, $df = 2$, $P = 0.409$; time spent on hidden host, average ± SD: 3.81 ± 5.95 min, semi-exposed host 9.27 ± 17.7 min, and exposed host 9.04 ± 11.1 min), time spent 'standing' ($F = 1.59$, $df = 2$, $P = 0.216$; hidden, average ± SD: 5.21 ± 9.20 min, semi-exposed 8.25 ± 11.5 min, exposed 12.9 ± 14.3 min) and time spent 'away' ($F = 2.82$, $df = 2$, $P = 0.071$; hidden, average ± SD: 5.97 ± 0.84 min, semi-exposed 4.26 ± 2.67 min, exposed 4.20 ± 2.92 min).

Table 2. Average duration of total time spent 'standing' and 'away' by *Hyssopus pallidus* on infested and uninfested fruits of different species ($n = 15$).

Fruit		Duration (min, average ± SD)	
		standing	away
Apple	uninfested	10.6 ± 11.8	4.18 ± 2.97
	infested	12.9 ± 13.8	1.01 ± 2.07
Apricot	uninfested	5.09 ± 8.18	5.24 ± 3.29
	infested	4.04 ± 7.28	5.44 ± 3.43
Pear	uninfested	4.26 ± 6.67	5.65 ± 3.06
	infested	8.16 ± 10.71	3.47 ± 2.75
Plum	uninfested	4.98 ± 8.57	5.09 ± 1.73
	infested	6.25 ± 8.52	4.50 ± 1.94

Table 3. Total number of female *Hyssopus pallidus* entering different infested and uninfested fruit species during the behavioural bioassay ($n = 15$).

	Apple	Pear	Apricot	Plum
Infested	8	4	0	11
Uninfested	1	0	0	0

Accessibility and reproductive success

Fruits

The numbers of *C. pomonella* larvae attacked (parasitized or only paralysed) by *H. pallidus* during the seven days of exposure depended on the fruit species (Chi-square, $\chi^2 = 12.17$, $df = 3$, $P = 0.007$). The highest percentages of parasitized larvae were found in apple and apricot (fig. 2).

The level of maturation of apple fruits significantly affected parasitism success (Chi-square, $\chi^2 = 8.53$, $df = 1$, $P = 0.003$). Most parasitized larvae were found on young apples (fig. 3).

Parasitism success was significantly affected by the fruit species (Likelihood Ratio test, $\chi^2 = 28.09$, $df = 6$, $P < 0.001$) but not by the position of the larva (i.e. 'calyx', 'core', 'stem', 'side' and 'stone') ($\chi^2 = 13.52$, $df = 8$, $P = 0.095$). There were no significant interactions between fruit species and position of the larva ($\chi^2 = 4.83$, $df = 8$, $P = 0.776$).

No significant differences were found in the brood size of the parasitoid obtained from different infested fruit species (Kruskal-Wallis test, $H = 2.75$, $df = 2$, $P = 0.253$). As no progeny were obtained from pears due to difficulties in storing the fruit in the laboratory, data relating to this plant were excluded from the analysis. There was no significant difference in the number of *H. pallidus* progeny from apples at different stages of maturation (Kruskal-Wallis test, $H = 3.91$, $df = 2$, $P = 0.142$).

Branches

Under the bark, 40–58% of the host larvae were parasitized regardless of their position (exposed, semi-exposed, hidden; Chi-square, $\chi^2 = 0.22$, $df = 2$, $P = 0.896$) (fig. 4). The location of the *C. pomonella* larvae on the branches significantly affected the number of parasitoid progeny (Kruskal-Wallis test, $H = 6.41$, $df = 2$, $P = 0.041$). On average, 20.8 ± 5.4 parasitoid progeny were found when the parasitized

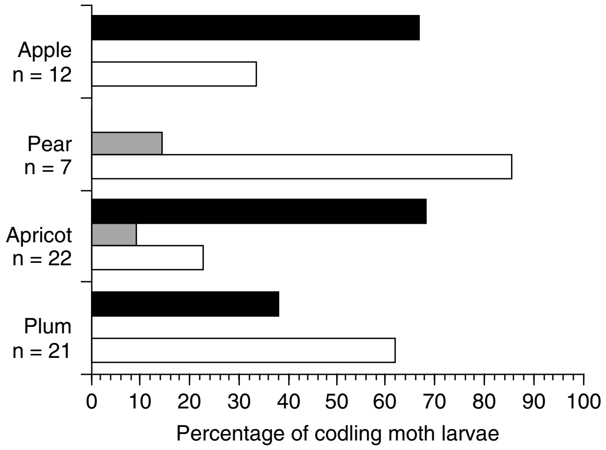

Fig. 2. The state of *Cydia pomonella* larvae (■, parasitized; ▨, paralysed; □, alive) in different fruit species after a seven-day exposure to the parasitoid *Hyssopus pallidus*.

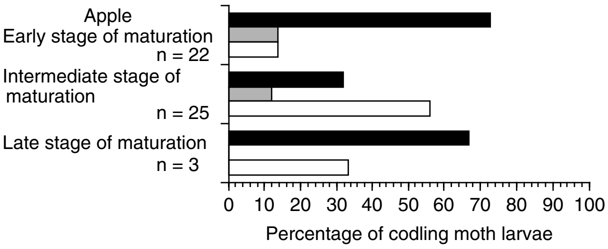

Fig. 3. The state of *Cydia pomonella* larvae (■, parasitized; ▨, paralysed; □, alive) in apples of an early, intermediate and late stage of fruit maturation after a seven-day exposure to the parasitoid *Hyssopus pallidus*.

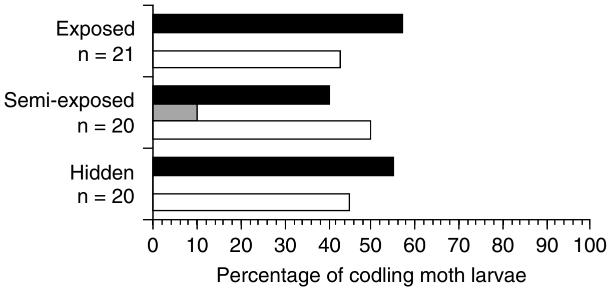

Fig. 4. The state of *Cydia pomonella* larvae (■, parasitized; ▨, paralysed; □, alive) in different locations on the branch after a seven-day exposure to the parasitoid *Hyssopus pallidus.*

C. pomonella larvae were exposed, 20.3 ± 4.6 when the larvae were semi-exposed, and 16.3 ± 3.2 when they were hidden.

Discussion

Studies on the niche selection of parasitoids attacking fruit-feeding herbivores are scarce (Leyva *et al.*, 1991; Morgan & Hare, 1998). Earlier work carried out for biological control purposes focused mainly on the reproduction rate of parasitoids attacking hosts feeding on different fruit species (Leyva *et al.*, 1991; Liquido, 1991). In the present study, not only was the reproductive success of *H. pallidus* on various fruit species examined but also the searching behaviour. Furthermore, the behaviour of this larval parasitoid of *C. pomonella* on bark, one of the host's preferred overwintering sites (van der Geest & Evenhuis, 1991), was elucidated.

The searching behaviour of *H. pallidus* on mature apples observed in a previous study revealed some of the stimuli that determine host location in this system. Female wasps showed more complex and intensive searching on infested apples than on mechanically damaged or uninfested apples (Mattiacci *et al.*, 1999). Remarkably, the parasitoid retained a relatively long searching time in the presence of stimuli originating from the natural host habitat (apple), even after 15–20 generations of rearing on hosts fed on fruit-devoid artificial diet (Mattiacci *et al.*, 2000).

Theory predicts that during assessment of a host patch (or niche), foraging parasitoids will search longer on good patches and spend a shorter time on poor patches (Godfray, 1994). Indeed for *H. pallidus*, the time spent searching reflects the suitability of a habitat for the adult female moth (Gandolfi *et al*., 2003b). Therefore, in the present study it was hypothesized that the parasitoid might benefit from niche-related cues for oviposition in the most suitable niches, where chances for host encounter are highest. This criterion is met when the host presence is highly correlated with a specific niche, as described for *C. pomonella* and apples (van der Geest & Evenhuis, 1991). The present study clearly documents that *H. pallidus* females searched longer on infested apples than on any other fruit species or uninfested fruits. The level of response to apple cues found in this study appears to be innate, as parasitoids were reared on hosts feeding on a diet devoid of fruit, and had therefore not been able to learn apple associated cues (Gandolfi *et al*., 2003a,b). Apple cues have a pivotal function for *H. pallidus* as they are used in host habitat location (present data), for host location (Mattiacci *et al.*, 1999) and they trigger and enhance parasitoid host handling and reproduction (Mattiacci *et al.*, 2000).

In contrast to the clear behavioural preference for apple, parasitoid reproduction rate was not affected by the host's feeding niche. Under the conditions of the bioassay, *H. pallidus* females attacked as many larvae in apples as in apricots (apples 66.7%, apricots 68.2%). In addition, the brood size of *H. pallidus* was not affected by the fruit species. However, wasps were kept for several days in a confined environment to increase the chance of actually observing parasitism, because in *H. pallidus* host searching and host handling are remarkably slow, compared to parasitoids of exophytic herbivores (Mattiacci *et al.*, 1999, 2000). It is possible that in this type of single-choice situation females will attack every host available, while in the field they would have discontinued searching.

Interestingly, more host larvae were attacked in apples at an early and a late stage of maturation. Although these results should be interpreted with caution as the number of replicates was low, the observed seasonality is in good agreement with quantitative changes in release of chemical cues by apples with progressing season. In fact, a clear pattern of volatile emission from apple fruits at different sampling times during fruit development was recently observed in the field (Hern & Dorn, 2003). Generally, volatiles were detected in greater amounts early in the season, after which a sharp decrease was found with hardly detectable emissions. Later in the season, another maximum of volatile emission was reached. The temporal dynamics in volatile emission makes the fruit more or less attractive for searching *H. pallidus* (Rott *et al*., 2005) and therefore might influence host location success. Similarly, this variation in emission influenced the attraction of *C. pomonella* females to healthy and infested fruit throughout the season (Hern & Dorn, 2002, 2004; Vallat & Dorn, 2005).

Hyssopus pallidus is able to attack not only actively feeding *C. pomonella* larvae in a fruit but also diapausing larvae under the tree bark. The parasitism rate of diapausing larvae is relatively high at 40–58%, probably because diapausing larvae cannot defend themselves. Actively feeding larvae can prevent parasitism by wriggling intensively (C. Hausmann, personal observation).

Surprisingly, the accessibility, i.e. the position of the larva within the fruit or on the branch, did not influence the level of attack by *H. pallidus*. This indicates that *H. pallidus* females have an excellent capability of reaching a host concealed in the plant. This is a considerable advantage, as inundative releases of this parasitoid are expected to significantly reduce codling moth populations in infested fruits and also under the tree bark. Success of parasitism might be optimized by an accurate choice of the release period. Such biological control should be integrated into a more comprehensive programme using, for example, the sterile insect release technique or mating disruption as the key method of control. These key methods require a low population level of *C. pomonella* which could be achieved by releasing *H. pallidus*. The recent first record of *H. pallidus* in Switzerland (Tschudi-Rein *et al*., 2004) will allow for field experiments with this parasitoid under the regulations of the country.

The data presented further indicate that this parasitoid species is highly adapted to apple. The niche selection of *H. pallidus* underlines the significance of plant-related trophic interactions in this ectoparasitoid (Mattiacci *et al*., 2000; Gandolfi *et al*., 2003a,b), as this selection is most likely a reflection of a co-evolution with *C. pomonella* on apple as the most important and principal host plant species.

Acknowledgements

Thanks are due to Hainan Gu for advice with statistical analysis and for valuable comments on previous versions of the paper. The authors thank Anja Rott, Kathrin Tschudi-Rein and two anonymous referees for useful comments on the manuscript.

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(Accepted 3 May 2005)