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Host finding and host discrimination ability in *Anaphes nitens* Girault, an egg parasitoid of the *Eucalyptus* snout-beetle *Gonipterus scutellatus* Gyllenhal

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Abstract

We investigated host finding and host discrimination ability in *Anaphes nitens*, a solitary egg parasitoid that attacks a gregarious host, the egg capsules of the *Eucalyptus* snout-beetle, *Gonipterus scutellatus*. In a first experiment, females were assigned to three treatments: no experience, one experience on an unparasitized egg capsule or one experience on a parasitized egg capsule. We combined this variable with three types of hosts: unparasitized, previously parasitized by the experimental female, or parasitized by conspecifics. Females were observed in a no-choice test, and results indicate that: (1) naive females can discriminate but do not refrain from superparasitism, (2) previous experience and the type of egg capsule affect host acceptance and visit duration, and (3) there is no evidence of self-discrimination. The acceptance of parasitized hosts decreased from 90% for the inexperienced females at their first encounter with the host, to 45% for the experienced females, and visit duration from 17.2 to 9.2 min. In a second experiment, a choice test was performed to assess *A. nitens* preference towards hosts of different age. Females oviposited preferentially in very young hosts. A field experiment tested the ability to find new hosts within the window of vulnerability of the egg capsules, by manipulation of the time that hosts were exposed to parasitism. Results showed that parasitoids need one day to discover the hosts and that parasitism does not increase after three days. We conclude that *A. nitens* biocontrol success is due to its ability to find fresh hosts, and to its discrimination ability, even if the female is inexperienced.

Keywords: Mymaridae; Biological control; Oviposition behaviour; Experience; Learning; Host quality; Host age

1. Introduction

Parasitoids used as biocontrol agents are expected to be highly efficient in finding hosts and to be able to discriminate among hosts of different quality (DeBach and Rosen, 1991). While the first ability avoids host escape from parasitism due to their development stage, the second optimizes parasitoid reproductive effort. We designate the "window of vulnerability" for parasitism (Craig et al., 1990) as the time period between the moment the host is first available for parasitism and the time it is too old to be successfully attacked. An efficient parasitoid must be able to find most of the hosts during this temporal window.

Once the host has been located, final acceptance (i.e., egg laying) depends on host species and quality, expressed as age, size, or previous parasitism (Vinson, 1976), and on factors related to parasitoid history, such as age, egg load, innate responses, experience, learning, ability to detect chemical marks, encounters with other conspecifics (Godfray, 1994; Vinson, 1998). In contrast to typical predators, parasitoids do not remove attacked hosts, which can be re-encountered by the female herself or by other females, creating opportunities for superparasitism. In gregarious parasitoids many larvae can develop in the same host, while in solitary parasitoids, the simultaneous presence of additional larvae (superparasitism) results in strong competition (Boivin and van Baaren, 2000; van Baaren et al., 1999) that leads to physical or physiological suppression of supernumerary individuals (Vinson, 1980). Under the best

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circumstances a smaller parasitoid will emerge from a superparasitized host, but under the worst circumstances, nothing will eclose (Anderson and Paschke, 1969; Harvey et al., 1993; Potting et al., 1997). Nevertheless, when there is the risk that a second female can encounter a host, or if hosts are scarce or show defensive mechanisms, then superparasitism could be a good strategy for both solitary and gregarious species (van Alphen and Visser, 1990).

Host discrimination is the ability of a female to distinguish parasitized from unparasitized hosts, probably detecting external and/or internal host-marking pheromones using the antennae and the ovipositor (Nufio and Papaj, 2001; Salt, 1961; van Lenteren, 1981; Vinson, 1976). Such behaviour is called self-discrimination when the female recognizes hosts already parasitized by herself, intra- or conspecific discrimination when the hosts have been parasitized by other conspecific females, and interspecific discrimination when the female recognizes hosts parasitized by heterospecific females (van Baaren et al., 1994).

There is some evidence that the use of parasitoids for biological control of pests might fail because of a high rate of superparasitism that causes elevated parasitoid larval mortality and therefore a substantial decrease in their population abundance (DeBach and Rosen, 1991; Iyatomi, 1943). The ability to avoid superparasitism or to use it as a means to compete with other foragers is an important aspect of parasitoid reproductive behaviour, that influences without any doubt parasitoid population stability (van Alphen and Visser, 1990).

Anaphes nitens (Hymenoptera: Mymaridae), native to Australia, is a solitary egg parasitoid of the *Eucalyptus* snout beetle *Gonipterus scutellatus* (Col.: Curculionidae). The weevil is a generalist herbivore of *Eucalyptus* spp., whose adults and larvae consume young and tender leaves, buds and shoots, causing a reduction in tree growth, and contorting and eventually killing branches (Tooke, 1955). Adults are strong fliers and may live six to twelve months in the field, but up to two years in laboratory (Mansilla, 2001). Females deposit hard brown egg capsules, mainly composed of faeces and containing a mean of eight eggs, on shoots and the youngest leaves. Snout beetle larvae have four instars: neonates emerge after 7–10 days and feed on leaf surface, while later instars consume the entire leaf blade.

The weevil and its parasitoid have been intensively studied since the beginning of the 20th century by entomologists from South Africa (Tooke, 1955), North America (Hanks et al., 2000; Kidd and Jervis, 1997), South America (Marelli, 1928), and Europe (Arzone and Vidano, 1978; Cordero Rivera et al., 1999; Mansilla, 1992) because of the important damage caused by the snout beetle to eucalypt stands and the use of the parasitoid for biological control. Despite its economic importance, details of *A. nitens* foraging behaviour are almost unknown, therefore we consider that this study will contribute substantially to this gap in our knowledge.

In Spain, *Eucalyptus globulus* Labill. (325,000 ha) and *E. camaldulensis* (175,000 ha) are the most commonly planted species, cultivated for the paper industry. In Galicia (NW Spain), monocultures of *E. globulus* represent 40% of the Spanish production and constitute a basic economic resource in areas where agriculture is not profitable (Montoya, 1995). *G. scutellatus* was accidentally introduced to Galicia in 1991 (Mansilla, 1992). At the end of 1993 *A. nitens* was released as a biocontrol agent, obtaining excellent results very soon after its release (Mansilla and Pérez Otero, 1996).

More than 10 different species of the genus *Anaphes* have been studied for their importance as biocontrol agents (Aeschlimann, 1977; Aeschlimann et al., 1989; Blossey and Ehlers, 1991; Boivin, 1986; Boivin et al., 1990; Carrillo et al., 1994; Charlet and Balsbaugh, 1984; Cormier et al., 1996; Hogg and Kingsley, 1983; Jackson, 1987). In most cases, *Anaphes* hosts are eggs laid alone or in clusters, but always without any cover, although sometimes embedded in plant tissue (Conti et al., 1996). In the case of *A. nitens* the situation is different: hosts are hidden inside hard egg capsules. This circumstance makes *A. nitens* a solitary parasitoid that develops in a quasi-gregarious fashion, because even if each larva develops alone within an individual host egg, the adults emerge together from the capsule, mating immediately (Tooke, 1955). The study of the oviposition behaviour of this species of *Anaphes* is therefore challenging because it is impossible to know which of the eggs hidden inside the capsule were encountered by individual female parasitoids. This may pose problems for the parasitoid too, because a female might detect if other individuals have encountered an egg capsule, but may not be able to detect whether all the eggs inside the capsule are parasitized until she checks them with the ovipositor.

Previous field studies showed that at a small spatial scale *A. nitens* shows a tendency for positive spatial density dependence when parasitism is low and negative spatial density dependence when parasitism is high. This suggests that parasitoids remain at patches with a high density of hosts only when parasitoid density is low (Cordero Rivera et al., 1999). When there are too many parasitoids, the risk of superparasitism is so high that females gain more by dispersing and searching for patches with a lower density of egg capsules (and also competitors). This led us to predict that there should be a high selection pressure on female *A. nitens* to avoid parasitized eggs. Nevertheless, there is evidence that female *A. nitens* sometimes superparasitize in the field, with up to 17 larvae found in a single host egg (Tooke, 1955).

Our first aim was therefore to test if females of *A. nitens* are able to assess host quality, discriminating between parasitized (by herself or conspecifics) and unparasitized hosts, and whether previous experience (particularly on unparasitized hosts) is necessary to achieve such host discrimination ability. In a second experiment, we investigated if females can differentiate among hosts of different age, using age as a measure of host quality. In a last experiment, by estimating parasitism of egg capsules exposed for different periods of time in the field, we examined the parasitoid's ability to find hosts within the probable window of vulnerability of the egg capsules.

2. Methods

All the adult parasitoids employed in the experiments were reared in laboratory from *G. scutellatus* egg capsules collected in the field in Galicia (NW Spain). Fresh egg capsules for experiments were obtained from a colony of adults collected in the field and maintained in laboratory, feeding on fresh leaves of *E. globulus*.

1. *Experiment 1: host discrimination*. Egg capsules collected in the field were introduced individually into sterile plastic tubes (6 × 1 cm) and stored at room temperature (18–20 °C), to await emergence of adult parasitoids. We selected only virgin females that emerged from single-sex broods. Each female was gently introduced with a soft-hair brush into a 6 × 1 cm plastic tube and mated individually with a male immediately before the beginning of the experiment. Courtship and mating behaviour lasted approximately 60 s, so we could verify mating status under a binocular microscope. For the experiment we used 142 one-day old females of *A. nitens*.

   The experimental arena was a petri dish (10 cm diameter, 2 cm high) containing only one fresh egg capsule (no-choice test), 24 h old, on a disk of wet filter paper (ALBET, 73 g/m^2). We tried to offer egg capsules of similar size to all females. The female was introduced with a soft-hair brush into the petri dish and continuously observed under a binocular microscope. Earlier observations permitted oviposition behaviour of female *A. nitens* to be classified as follows:
2. *Host acceptance or rejection*. When a female encounters an egg capsule, she climbs onto the capsule for inspection, and walks on the hard cover gently drumming the surface with her antennae while unfolding her ovipositor. The inspection includes insertion of the ovipositor presumably to detect internal pheromones, as described for *A. victus* and *A. listronoti* (van Baaren et al., 1995). The short duration of the puncture and the absence of oviposition movements led us to interpret this behaviour as a sting inspection and not an oviposition. The dissection of 20 egg capsules confirmed this hypothesis. A female can reject the host after this kind of inspection or, in some cases, rejection can be the result of a simple antennal inspection, as also described for *A*. n. sp. and *A. sordidatus* (van Baaren et al., 1994).
3. *Oviposition*. During oviposition the antennae remain still and parallel with the surface of the egg capsule. Normally the ovipositor is correctly positioned on the first trial. A female must grasp an egg capsule very firmly to be able to drill through the cover. The ovipositor is almost completely inserted with a slow vertical movement and the abdomen is squeezed for 4–5 s. After the squeezing a female remains still for a few seconds and then displays first slowly and then rapid vertical abdominal vibrations that precede ovipositor extraction and withdrawal. We assume that, as described for *A. victus* and *A. listronoti*, the abdominal contractions indicate egg deposition (van Baaren et al., 1995). However, with *A. nitens* we were unable to detect the passage of eggs along the ovipositor. The total duration of oviposition lasts from 20 s to more than 1 min.

   On every encounter, we recorded if a female accepted or rejected an egg capsule and the total visit duration. We considered that a female accepted a host if she made at least one oviposition (showing the typical movements), while the absence of oviposition and the abandoning of the host for longer than 2 min was considered as host rejection. To calculate the total visit duration, we considered the interval between the moment of contact with the egg capsule and as the end when the female left it for more than 2 min.

   The experimental protocol is summarised in Fig. 1. We used three types of egg capsules: unparasitized egg capsules (UE), egg capsules parasitized 30 min before by the female herself during the first trial and offered again in the second trial (SPE, self-parasitized eggs), and egg capsules parasitized by a group of three conspecific females 30 min before the test, in this case to simulate a situation of competition (CPE, conspecific-parasitized eggs). To obtain conspecific parasitized hosts (CPE treatment) we used groups of three fresh egg capsules introduced into a petri dish (10 cm diameter, 2 cm high) on a disk of wet filter paper, and groups of three mated females (not included in the tests). *A. nitens* females attack a gregarious, concealed host, so that it is possible that during a single encounter females do not parasitize all the eggs inside the capsule. Considering that the objective of the experiment was to test host discrimination ability by calculating visit duration and rejection/acceptance rate, we observed females' behaviour during one hour under the binocular microscope, to ensure that every egg capsule was effectively parasitized by two to three females.

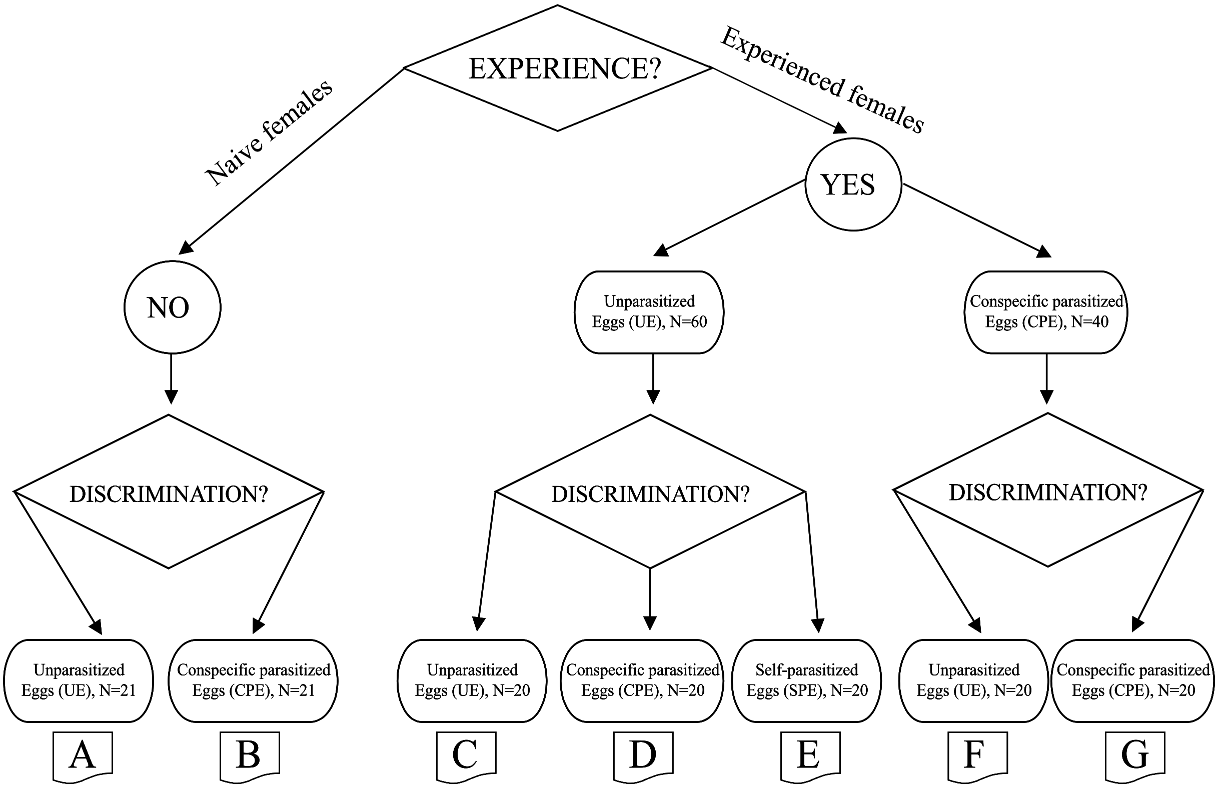

Fig. 1. A summary of the treatments used in Experiment 1.

The females used in the experiment were given one of three types of preliminary experience: absence of experience (N, naive females), one experience on an unparasitized egg capsule (UE females), or one experience on an egg capsule parasitized by conspecific females (CPE females). Females could feed during the test on droplets of honey. For UE and CPE females, the interval between the first and the second visit was less than 10 min. We removed with forceps the accepted/rejected egg capsules, while the female remained in the petri dish. This short interval was selected assuming that the learning effect has a short persistence (Miura et al., 1994). On the other hand, given that host discrimination could be time dependent, due to the time taken for a marking cue or host physiological changes to be detectable (Chow and Mackauer, 1986; Nufio and Papaj, 2001; Outreman et al., 2001), we decided that CPE capsules should have an interval of 30 min before being offered to the females of groups B, D, F, and G. Consequently, to make the treatments uniform for experienced females SPE egg capsules were also held for 30 min.

Means are presented with standard errors. Visit duration was square-root transformed before parametric tests and P values were adjusted for multiple comparisons using the Bonferroni method (Sokal and Rohlf, 1995). We then analysed host acceptance and visit duration to answer the following questions:

1. *Are naive females able to discriminate?* To this aim we compared groups A and B of Fig. 1 by means of ANOVA (visit duration) and Fisher's exact tests (acceptance ratio).
2. *Are host acceptance and visit duration affected by preliminary experience and type of host?* To this aim, we compared groups A–B, C–D, and F–G of Fig. 1. Host acceptance (accepted = 1; rejected = 0) was analysed with a logistic regression model. Visit duration was analysed with an ANOVA with type of experience and type of host as treatments, including an interaction term. We planned a priori to compare groups A–B with C–D + F–G (effect of presence or absence of previous experience), and C–D with F–G (effect of the type of first egg capsule encountered on second visit).
3. *Are females able to discriminate between egg capsules parasitized by themselves and those parasitized by other females?* This question was addressed by comparing groups D and E of Fig. 1, by means of ANOVA and Fisher's exact tests.

2.1. Experiment 2: host age preference

We used 24 h old, inexperienced females ($n = 10$) to assess host age preference. Each female was introduced into a petri dish (14 cm diameter, 2 cm high) that contained four egg capsules of different ages on a disk of wet filter paper (Albet, 73 g/m^2). Females were left in the dishes for 36 h to give them time to use most of their egg load (45.6 ± 3.73 eggs ($n = 27$)), (Santolamazza-Carbone and Cordero Rivera, 2003) and manifest preferences. The number of egg capsules was selected to ensure that there were approximately the same number of hosts available as the typical egg load of a single female. Each egg capsule had a different age: 0 (just laid), 1, 2, and 3 days. The experiment was performed at room temperature (18–20 °C). We considered parasitism rate as a measure of host age preference. To calculate parasitism rate (parasitoids/(parasitoids + host larvae)), at the end of each trial egg capsules were introduced individually in a plastic tube (6 × 1 cm) and checked daily for one month, recording the number of adult parasitoids and/or beetle larvae emerged.

Results were analysed with an ANOVA, where the response variable was parasitism rate (asin square root transformed), the fixed factor was age of host, and female identity was a random factor.

2.2. Experiment 3: host finding and window of vulnerability

This experiment was carried out during March–April 2001 in a field plot of *E. globulus* heavily attacked by *G. scutellatus* in NW Spain. At the start of the experiment we randomly selected 8 small trees (1–2 m high) and removed all the egg capsules. The trees were marked and inspected daily, and each new egg capsule found was marked by writing a number on the leaf with a permanent marker. This allowed us to track the date of oviposition of each egg capsule. During the first week, we collected egg capsules each day to obtain hosts that were exposed to parasitism for 1–7 days. The experiment required two additional weeks to increase sample size, because many egg capsules were lost due to leaf abscission. Further groups were obtained by collecting egg capsules found and marked in the morning and collected the same afternoon, after 0.2 days of exposure to parasitoids, and egg capsules that remained 8–9 days in the field. Control egg capsules (that remained an unknown time exposed to parasitism) were collected at the start of the experiment (all the removed egg capsules) and on two further occasions on nearby trees. A total of 439 egg capsules were collected. In order to calculate percentage parasitism, these were maintained in laboratory at room temperature in individual tubes (6 × 1 cm), and checked daily for one month, recording the number of parasitoids and beetle larvae that emerged. There is some evidence that superparasitism can increase mortality during the early stages of parasitoid development (Hanks et al., 2000; Santolamazza-Carbone, 2002). Therefore, all the egg capsules were dissected to count the number of parasitoids dying inside the host and the number of undeveloped host eggs. We calculated a conservative estimate of parasitism as the number of parasitoids (including those found dead inside egg capsules) divided by the total number of eggs inside the egg capsules. Nevertheless, undeveloped eggs could be parasitized eggs that died due to competition between parasitoid larvae (Hanks et al., 2000). We therefore also calculated a maximum rate of parasitism by assuming that undeveloped eggs were also parasitized.

The response variable was the number of eggs successfully parasitized. The effect of the treatments (days of exposure to parasitoids) was analysed with a Generalized Linear Model with binomial errors and logit link, using the number of eggs in each capsule as the binomial denominator (Crawley, 1993). To account for overdispersion, we used procedure Extrabinomial in GenStat 4.2 software (GenStat, 2000). The effect of the week could not be analysed because sample size was too small, but parasitism remained high during the period of study (41–65%). All analyses were performed with SPSS 10.0 and GenStat 5th edition for Windows.

3. Results

3.1. Experiment 1: host discrimination

Percent host acceptance and visit duration are presented in Table 1 for all experimental groups.

3.1.1. Are naive females able to discriminate?

There were no significant differences in the percentage of acceptance of parasitized (90%) or unparasitized (100%) egg capsules by naive females (Table 1; Fisher's exact test, two-tailed, $P = 0.49$). Nevertheless, visit duration was clearly briefer (17 versus 42 min; Table 1) when females encountered parasitized egg capsules (ANOVA, $F = 27.65$, $P < 0.001$).

3.1.2. Are host acceptance and visit duration affected by preliminary experience and type of host?

Experienced females always accepted unparasitized egg capsules (UE), either offered as first or second host, and visit duration was rather constant (39–41 min; Table 1). As occurred with the inexperienced females (N), parasitized eggs (CPE) were also accepted in 84–90% of cases when presented as first hosts, but only in 45–60% of cases when presented as second hosts (Table 1). Host acceptance was significantly affected by preliminary experience (logistic regression coefficient, $b = -1.667$, $P = 0.04$) and by type of egg capsule ($b = -1.092$, $P = 0.005$).

Visit duration was also influenced by preliminary experience and by type of egg capsule, without interaction between both variables (ANOVA, type of experience, $F = 6.53$, $P = 0.002$; type of host, $F = 176.71$, $P < 0.001$; interaction, $F = 1.76$, $P = 0.29$). Planned

Table 1
Percentage acceptance of hosts and encounter duration (min, means ± SE) for female *A. nitens* with different preliminary experience (Group), and treatment

Group: preliminary experience	Preliminary encounter acceptance (%)	Preliminary encounter duration	Treatment	Second encounter acceptance (%)	Second encounter duration
A: none	—	—	UE	100	42.3 ± 4.17
B: none	—	—	CPE	90	17.2 ± 2.32
C: UE	100	40.0 ± 3.16	UE	100	35.9 ± 3.21
D: UE	100	41.4 ± 3.49	CPE	45	9.2 ± 1.55
E: UE	100	38.6 ± 3.40	SPE	60	12.7 ± 2.53
F: CPE	84	15.1 ± 1.60	UE	100	36.7 ± 3.78
G: CPE	90	19.8 ± 2.69	CPE	60	8.3 ± 1.36

Sample size is 21 females for the treatments without preliminary experience and 20 for the rest (see also Fig. 1).
UE, unparasitized eggs; CPE, eggs parasitized by conspecifics; and SPE, eggs previously parasitized by the experimental female.

contrasts between means indicate that, in terms of second visit duration, there are no differences between the experience on a parasitized host (CPE) or on an unparasitized one (UE, $P = 1.00$) but both are significantly different from the absence of experience (N, $P = 0.045$).

To assess if the short visit duration to the second parasitized egg capsule in group D (Table 1) was due to a depleted egg load or because the female was tired, 10 min after the end of the second visit we offered a third new fresh unparasitized egg capsule to six females. All hosts were accepted and the duration of this third visit was on average 40.8 ± 1.7 min.

Dissection of a random sample of 40 egg capsules parasitized by naive females showed that on average they contained 7.7 ± 2.3 eggs, and 7.2 ± 2.8 eggs were parasitized.

3.1.3. Are females able to discriminate between egg capsules parasitized by themselves and those parasitized by other females?

CPE and SPE hosts were accepted in similar proportion (45–60%; Table 1, Fisher's exact test, two-tailed, $P = 0.53$). Visit duration was not significantly different between groups D and E of Fig. 1 (Table 1, ANOVA, $F = 1.308$, $P = 0.26$).

3.1.4. Host rejection

Females rejected hosts mostly after a sting inspection (25 cases), rather than after antennal inspection (3 cases).

3.2. Experiment 2: host age preference

Females parasitized 62% of freshly laid eggs (0 days), but only 14–24% of older eggs (Fig. 2; ANOVA, fixed effects, $P = 0.04$).

3.3. Experiment 3: host finding and window of vulnerability

Both estimates of percentage parasitism (Fig. 3) show that parasitism increased with time of exposure in the field (GLM, $P < 0.001$ in both cases). Using the conservative estimate of parasitism and the control egg capsules as a reference group, there were significantly lower rates of parasitism on day 1 of exposure ($P = 0.003$), and day 2 ($P = 0.04$). Using the maximal estimate of parasitism, there were significantly lower rates of parasitism on day 0.2 ($P = 0.005$) and day 1 ($P = 0.007$), but days 2–9 of exposure did not differ from control eggs ($P > 0.38$ in all cases).

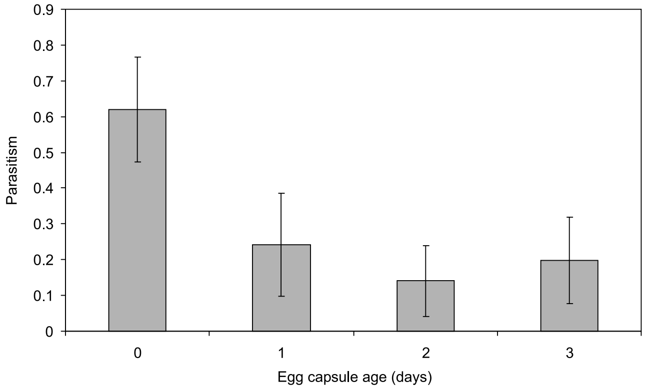

Fig. 2. The relationship between host age and parasitism (means ± SE) in a choice test in the laboratory ($n = 10$ females). There are significant differences among treatments (ANOVA, $P = 0.04$).

4. Discussion

We found clear evidence for host discrimination in *A. nitens*, an ability previously found in *A. iole* (Conti et al., 1997), *A. victus* (van Baaren and Boivin, 1998), *A*. n. sp., and *A. sordidatus* (van Baaren et al., 1994). The first question we addressed was if females have to learn to discriminate hosts (van Lenteren and Bakker, 1975) or if it is an innate ability (van Alphen et. al., 1987). In spite of a general acceptance of parasitized and unparasitized hosts, naive females of *A. nitens* changed their behaviour when encountering parasitized hosts, shortening visit duration and probably also reducing clutch size,

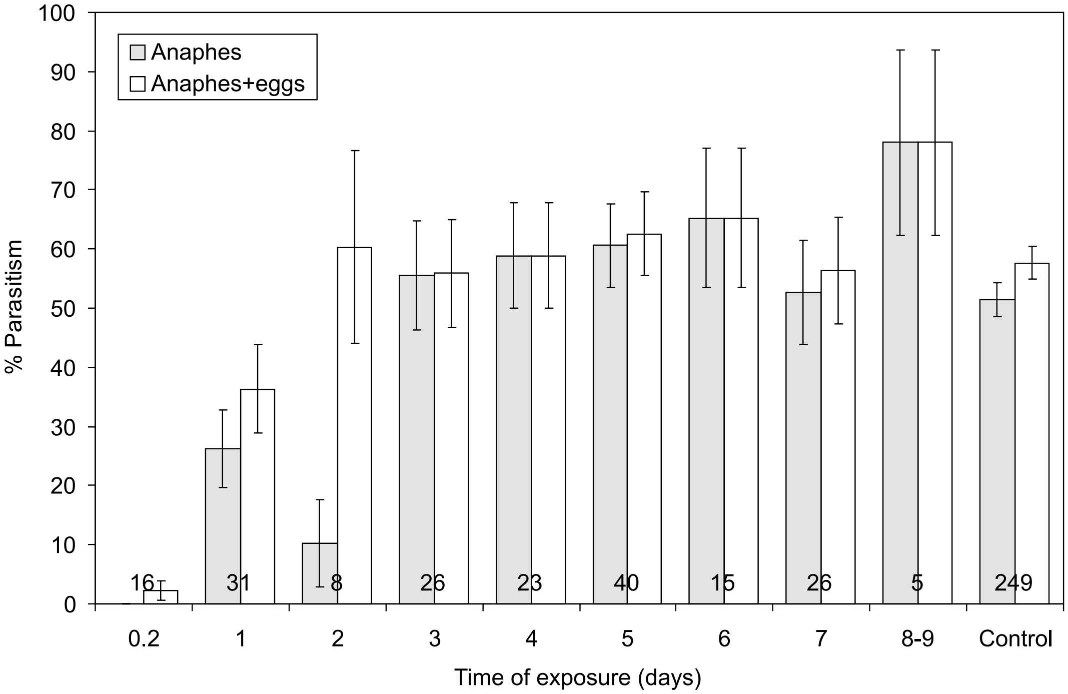

Fig. 3. Estimation of the temporal window for parasitism of *G. scutellatus* eggs by *A. nitens* under field conditions. Parasitism was estimated conservatively as the number of parasitoids divided by the number of eggs per capsule, or maximally as the number of parasitoids and undeveloped eggs divided by the total. Numbers inside bars indicate sample size (egg capsules analysed).

avoiding wastage of time and eggs. Moreover, in agreement with Henneman et al. (1995) results using *Leptopilina heterotoma*, we found that host discrimination ability improves by having just one previous experience with any kind of host (Table 1). We did not find a clear qualitative difference between having a preliminary experience on a parasitized or on an unparasitized host, because both seem equally to improve the performance on the second parasitized host offered (groups D, E, and G).

Previous studies on two species of *Anaphes* using a choice experiment indicated that females never accept self-parasitized eggs (van Baaren and Boivin, 1998; van Baaren et al., 1994). In particular, van Baaren et al. (1994) pointed out that when *A.* n. sp. females had to choose between eggs parasitized by a conspecific and by themselves, they preferred to oviposit in those attacked by conspecifics. We expected a similar response in *A. nitens*, but our results suggest that females do not discriminate between CPE and SPE hosts. It is possible that females did not make a distinction between hosts attacked by themselves or by other conspecifics because the chemical marks left over or in the host were very similar (Godfray, 1994). This might happen if parasitoids are genetically very close or even identical due to the small number of founders of the artificially introduced population (in this case only nine adults; Mansilla, 2001). A choice experiment is needed to assess if in another context females would show this ability.

Egg load and previous experience might influence clutch size decisions (Rosenheim and Rosen, 1991), but also host acceptance (Minkenberg et al., 1992). Nevertheless, egg depletion has no effect on discrimination behaviour in *Anaphes victus* (van Baaren and Boivin, 1998). In our experiment, if visit duration is a measure of clutch size, then the similar duration of first and second visit on unparasitized hosts in group C females, demonstrates that there is no influence of egg limitation. Moreover, some group D females, that visited a third unparasitized host (see Section 3), showed a visit duration of 40.8 min on a third host. This suggests that only host quality influences visit duration.

The dissection of a sample of unparasitized egg capsules offered to naive females showed that they have a great efficiency, parasitizing almost all of the available eggs. This means that the second female visiting a parasitized egg capsule must choose between rejection and superparasitism, and both outcomes were observed (Table 1). The shorter visit duration of second females to parasitized egg capsules (self- and conspecific), is thus in agreement with theory that predicts that a superparasitizing female should lay less eggs (Waage, 1986; Werren, 1980). In this case, the non avoidance of superparasitism does not indicate the absence of host discrimination ability (van Lenteren et al., 1978). Host discrimination can also be measured in terms of number of eggs laid, and if visit duration reflects clutch size, then the tested females behaved in agreement with this prediction. As concluded by van Alphen et. al., 1987 for *L. heterotoma* and *Trichogramma evanescens*, we consider that naive *A. nitens* females are able to discriminate, but decide to superparasitize because they lack any information about patch density or quality and about competing female density. Under these circumstances, to accept a certain percentage of low quality hosts could be more adaptive than to reject them all.

Among hymenopteran parasitoids learning seems to be extremely diffuse (Prokopy and Lewis, 1993), and host discrimination has often been related to learning (van Baaren and Boivin, 1998). When a female of *A. victus* encounters a parasitized host, she learns to associate the presence of an external pheromone marker with the presence of the internal one. Antennal rejection is much faster than sting rejection, so this behaviour could be adaptive; consequently they have learned to reject parasitized hosts after antennal drumming (van Baaren and Boivin, 1998). However, females showed such a change in behaviour only after at least six encounters. In our experiment, each female encountered only two egg capsules and they seem to prefer sting rejection. This is probably not because they need many more trials to learn, but rather that hosts of *A. nitens* are hidden beneath a cover that might impede effective detection of pheromone markers without piercing the cover. This characteristic probably explains the occurrence and greater frequency of sting rejection by *A. nitens* during host inspection.

In a developmental and temporal sense, eggs represent a variable and transitory host stage. Holometabolous embryogenesis can be very rapid in some species of Coleoptera, so that for an egg parasitoid the most suitable host age should be quantified in terms of a few days (Strand, 1986). Successful parasitism of eggs declines with host age (Godfray, 1994; Vinson, 1998): as host embryogenesis proceeds, there are changes in the amount of yolk and in the internal physiological condition, that affect host suitability (Vinson, 1980). Moreover, older host eggs have a hardened chorion and thus handling time should increase (Vinson, 1998). Parasitoid ability to assess host age is then crucial, especially for solitary species, because host nutritional insufficiency may lead to pre-emergence mortality or may affect adult fitness in terms of reduced size (Vinson, 1980).

Under laboratory conditions, *A. nitens* will never parasitize six day old egg capsules, while four day old eggs are accepted at a low rate (Williams et al., 1951). Considering that *G. scutellatus* larvae need only a week to emerge at 20–25 °C (Tooke, 1955), it is not surprising that under laboratory conditions *A. nitens* showed preference for very young hosts. The design of our experiment, where females that have an average egg load of 46 eggs were offered 32 hosts during 36 h, might have encouraged them to use the less preferred egg capsules or to superparasitize the most preferred. Therefore, even stronger preferences might be shown under field conditions. The mechanism by which *A. nitens* detects host age is actually unknown, but it is probably related to antennal drumming and sting probing (Godfray, 1994; Vinson, 1976).

Field results have shown that female parasitoids need only one day to find fresh hosts and 2–3 days to fully parasitize them. After 3 days of exposure to parasitoids, parasitism rate does not increase significantly. Therefore, it is reasonable to consider a range of 0–3 days as the window of vulnerability of *G. scutellatus* eggs. Parasitism of eggs or pupae is especially challenging because these kinds of hosts are sessile and inactive and probably release very few host location cues (Vinson, 1998). Under laboratory conditions, *A. nitens* females accept and successfully parasitize eggs deprived of the capsule, showing that the cover is not necessarily involved in host location and acceptance (Santolamazza-Carbone, 2002). These factors lead us to predict that host location cues should be strongly related to feeding and mating activity of *G. scutellatus* adults. Further studies on the physiological mechanism that allows host location (e.g., response to volatiles, vibrational, or visual cues) would be illuminating.

In conclusion, our results clearly show that female *A. nitens* have the ability to locate fresh hosts quickly and to discriminate among hosts of different quality. These factors might contribute to the great success of this species as a biocontrol agent, representing one of the few cases of successful biological control developed by an egg parasitoid acting alone (DeBach and Rosen, 1991; Greathead, 1986).

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