

Short- and long-range cues used by ground-dwelling parasitoids to find their host

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Abstract Parasitoids of phytophagous insects face a detectability–reliability dilemma when foraging for hosts. Plant-related cues are easily detectable, but do not guarantee the presence of the host. Host-related cues are very reliable, but much harder to detect from a distance. Little is known in particular about the way coleopteran parasitoid females use these cues when foraging for a suitable place to lay their eggs. The question is of interest because, unlike hymenopteran larvae, coleopteran parasitoid larvae are highly mobile and able to forage for hosts on their own. We assessed whether females of the parasitoid rove beetle *Aleochara bipustulata* (L.) (Coleoptera: Staphylinidae) are attracted to plant (Swede roots, *Brassica napus*) and host-related cues [pupae of the cabbage root fly *Delia radicum* (L.) (Diptera: Anthomyiidae)]. In the field, *A. bipustulata* adult females were captured in selective pitfall traps containing pieces of roots damaged by *D. radicum* larvae, but not in traps containing pieces of healthy roots or *D. radicum* pupae. However, in the laboratory, the odour of *D. radicum* pupae attracted *A. bipustulata* females to mini-pitfalls. Video

monitoring in the laboratory showed that foraging *A. bipustulata* females preferred a zone containing *D. radicum* pupae and larval tracks rather than one containing an extract of *D. radicum*-infested roots. Our results suggest a behavioural sequence where *A. bipustulata* females use plant-related cues at a distance, but then switch their preference to host-related cues at a close range. This would be the first observation of this behaviour in coleopteran parasitoids.

Keywords Optimal foraging · *Aleochara* · Cabbage root fly · Coleopteran parasitoids · Behaviour

Introduction

Parasitoid insects bear a particularly strong selective pressure to find their hosts efficiently because hosts directly yield offspring once parasitized (Godfray 1994). The parasitoid's foraging behaviour generally starts with locating the habitat of their hosts, then a host patch and finally selecting a suitable host (Vet and Dicke 1992; Rutledge 1996; Wajnberg et al. 2008). Each step is mainly driven by the perception and use of infochemicals emitted by the attacked plant (either due to the depletion of tissues or the induction of a specific production of infochemicals), host kairomones, the hosts themselves and all their metabolic waste (Dicke and Sabelis 1988; Godfray 1994; Rutledge 1996; Hilker and McNeil 2008). Parasitoids generally locate host habitat using infochemicals which are easily detectable at long range, such as plant volatiles. However, finding the host itself remains a more difficult task: since hosts have a low biomass, they release much smaller amounts of volatiles than plants, making them harder to detect at a distance (Vet et al. 1991; Turlings and Loughrin 1995). The difficulty is further enhanced when the host is sessile (egg, pupal stage) or buried (in plant tissue or underground).

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The reliability–detectability dilemma faced by parasitoids (Vet et al. 1991) has been well documented in the Hymenoptera (see Vet and Dicke 1992; Godfray 1994; Wajnberg et al. 2008 for reviews). In contrast, other taxa such as Coleoptera have received little attention in spite of their original behaviour: in these species, females lay their eggs at a distance from hosts, and it is the mobile larvae that search, evaluate and parasitize hosts (Eggleton and Belshaw 1993). Although females lay their eggs without direct contact with hosts, the site selected for oviposition remains essential to ensure their reproductive success because first instars have limited dispersion capabilities and energy reserves, and they cannot feed before finding a host (Royer et al. 1998; Brodeur and Boivin 2004).

This particularity represents a major distinction with hymenopteran parasitoids because coleopteran females must not find the host itself, only its whereabouts. This host selection strategy of coleopteran parasitoids shows similarities with that of dipteran parasitoids with mobile searching larvae, such as some species of tachinids (Diptera: Tachinidae) with “indirect oviposition strategies” (Stireman et al. 2006) as well as bee flies (Diptera: Bombyliidae) (Boesi et al. 2009) or robber flies (Diptera: Asilidae) (Crespo and Castelo 2010). In these examples, the parasitoid females lay their eggs at a distance from host, and active mobile larvae are involved in host research. However, in the latter parasitoids, females are usually not egg limited and display a low investment in direct host searching (especially in Bombyliidae and Asilidae). Parasitization success often relies on a large number of eggs being laid in environments favourable to chance encounters with hosts (Yeates and Greathead 1997; Stireman et al. 2006; Boesi et al. 2009).

Compared to the abundant knowledge accumulated about hymenopteran parasitoids or even the more scattered information available on dipteran parasitoids, the pre-oviposition behaviour of coleopteran parasitoids is poorly known. This is especially true regarding the nature and the relative value of the cues used by females to select an egg laying site. The present study aims at identifying the foraging cues used by these atypical parasitoids. Specifically, we have measured if plant volatiles attract a female to the vicinity of a host patch, and we have determined to what extent hosts themselves provide females with relevant cues to select an oviposition site.

Rove beetles of the genus *Aleochara* (Coleoptera: Staphylinidae) are ground-dwelling parasitoids of Diptera including the cabbage root fly *Delia radicum* (L.) (Diptera: Anthomyiidae); adults also predate *D. radicum* eggs and larvae (Fuldner 1960). *D. radicum* infests brassicaceous plants, which therefore provide both food and hosts to *Aleochara* sp. (Fournet et al. 2001).

Root feeding by *D. radicum* larvae induces plant damage culminating when third instar maggots leave the root to

pupate in the nearby soil. The only study of oviposition behaviour in *Aleochara* showed that the presence of such damaged roots resulted in larger clutch sizes of *Aleochara bilineata* Gyllenhaal females compared to healthy roots (Fournet et al. 2001). In a field study, Ferry et al. (2007) further showed that the odour of roots damaged by *D. radicum* attracted individuals of both sexes in *A. bilineata* and the close species *Aleochara bipustulata* (L.). This odour could then be a suitable cue for an *Aleochara* female in search of a site to lay its eggs, even if root damage by *D. radicum* larvae does not guarantee the current presence of pupae (which are the only host stage suitable for *Aleochara* larvae). Moreover, because pupae themselves probably do not emit large amounts of volatiles or other cues (they are underground, immobile and exchanges between the nymph and the environment are restricted by the thick puparium), we hypothesize that the blend emitted by a damaged plant would be the first cue used by females to locate a host patch at a distance. However, the location of the damaged root itself does not match exactly that of the host, since *D. radicum* can pupate in the soil up to 20 cm from the plant (Fuldner 1960). Therefore, once reaching a damaged plant, a female would probably enhance the parasitisation success of its larvae by perceiving host-derived cues which would allow her to lay its eggs closer from the pupae. Royer and Boivin (1999) found that starved *A. bilineata* adults respond positively to maggot frass odour in an olfactometer. We thus expect that, once they have found a damaged plant, *Aleochara* sp. females would move closer from pupae by using as a guide the odour of larval tracks (including frass) left behind by the maggot when it leaves the root to pupate into the soil. Because responses to environmental cues depend on physiological state (Browne 1993), only fed and mated females should be used to assess the value of cues on the oviposition behaviour of *Aleochara* sp. parasitoids, which are also predators (Fuldner 1960). Indeed, starved or unmated females would rather use cues to search for food (i.e., eggs or young instars that are still feeding on plant tissue) than an egg laying site.

In the present work, we aimed at finding cues used by *Aleochara* sp. females to select their egg laying site. More specifically, we wanted to know if they would use (a) the volatile blend from a plant damaged by *D. radicum* and (b) larval tracks or other host-derived cues. To do so, we used our designed methods adapted to these atypical ground-dwelling parasitoids, mixing complementary approaches both in field and controlled conditions. We worked with *A. bipustulata* (L.), which is the most common species of the *Aleochara* genus in Brittany, but is less studied than its close competitor *A. bilineata*. We first tested in the field the attractiveness of volatile cues from plant (indirect cues) and host (direct cues) on individuals. Then, we compared this response in controlled conditions using mated females

fed ad libitum, which were thus ready to lay their eggs, but probably not looking for food. Finally, to better understand the relative importance of attractive cues, we evaluated the behaviour of females toward putative cues in choice experiments using video tracking in experimental arenas, which is a convenient method for studying these roving parasitoids.

Material and methods

Host and parasitoid strains

Insects were reared in controlled conditions (20 ± 1 °C, 60 ± 10 % RH and a 16-L:8-D photoperiod).

Host

The *D. radicum* population was established in 2009 from pupae collected in broccoli fields at Le Rheu, Brittany, France ($48^{\circ}06' N$, $01^{\circ}47' W$). The strain was maintained on Swede roots (*Brassica napus*) following a method derived from Keymeulen et al. (1981).

Parasitoid

The *A. bipustulata* population was established in 2009 from parasitized *D. radicum* pupae collected in broccoli fields at Kerbabu, Brittany, France ($48^{\circ}40' N$, $3^{\circ}50' W$). Adults were kept in plastic boxes ($16 \times 9.5 \times 8$ cm) filled up with moistened vermiculite containing *D. radicum* pupae and minced beef ad libitum as a food source. Once a week, parasitized pupae were collected from the rearing box and stored in another one with moistened vermiculite until parasitoid emergence. Emerging adults were either used for maintaining the rearing or for the experiments.

Plant and host cues tested

The following cues were tested in field and laboratory experiments: (a) healthy root: 1 g of uninfested rutabaga root, (b) damaged root: 1 g of a rutabaga root infested by *D. radicum* larvae during 3–4 weeks at 20 °C (corresponding to the average time needed by *D. radicum* larvae to complete their development and leave the root to pupate; Keymeulen et al. 1981), remaining maggots and pupae were removed just before the experiment, (c) host pupae: 5 (laboratory) or 10 (field) *D. radicum* pupae cleaned with water, then gently dried on absorbent paper to remove residual plant material and (d) control: empty pitfall. Infested plant extracts used during video tracking experiments were obtained as follows: an infested rutabaga was sampled from the *D. radicum* rearing after the larvae had left the root to pupate; then,

the rutabaga was wrapped in a textile bag and pressed to filter-out a crude extract.

Response of *A. bipustulata* females toward plant and host cues in the field

The attractiveness of plant and host odour sources in the field was recorded by size-selective odorous pitfalls derived from Ferry et al. (2007). Pitfalls were made of a 35-mL plastic cup (33 mm high, 40 mm upper diameter, P100, Solo Cup Co., Urbana, IL, USA) containing the odour source and a piece of moistened textile keeping captured individuals alive throughout the experiment. Pitfalls were closed with a piece of Parafilm TM perforated in its centre ($\varnothing = 1\text{--}2$ mm). Three odour sources were tested: healthy root, damaged root and host pupae. Cups containing only the moistened textile were used as controls. The trial took place in April and July 2011 in two experimental 50×50 m broccoli fields (*Brassica oleracea* cultivar Italica) located 2 km apart at Le Rheu, Brittany, France ($48^{\circ}06' N$, $1^{\circ}47' W$).

Sampling plots (48–49 per site) were placed regularly all around the edges of the fields. At each plot, four pitfalls were randomly arranged at the corners of an 80×80 -cm square. They were left for 48 h in the fields, after which trapped individuals were brought to the laboratory to be identified, counted and sexed. Only female individuals were taken into account for this study.

Response of *A. bipustulata* females toward plant and host cues in the laboratory

Short-range orientation

The *A. bipustulata* females used—mated and fed ad libitum—had never been in contact with host-infested rutabaga roots or hosts before the experiments and were less than 1 month old. A change in egg production has not been reported through the life time of regularly mated females and 1 month appears to be relatively young for this species where females easily live more than 100 days (Fournet et al. 2000; Josso, personal communication). Short-range attractiveness of volatile compounds emitted by the damaged plant and the host was evaluated using mini-pitfalls in dual tests. These mini-pitfalls consisted of two 1.5-mL Eppendorf TM tubes in a plastic cup filled up with sand ($\varnothing = 8$ cm, $h = 7.5$ cm). The two tubes were positioned diametrically opposed to each other in the cup and were buried vertically in the sand (up to the rim) at 0.5 cm from the inner surface of the cup, allowing a parasitoid to move along the inner perimeter of the arena without encountering a pitfall entrance. Finally, to prevent insect escape by flight, the device was covered with the top of a transparent plastic box ($\varnothing = 9$ cm, $h = 2$ cm) where a hole ($\varnothing = 3$ cm) was covered by a

layer of tulle allowing air circulation. Tubes were either empty (control) or contained an odour source. They were closed by a piece of Parafilm TM bearing a small hole ($\varnothing=1\text{--}2\text{ mm}$) allowing odour emission and selective entrance of the insects. Thus, the females could not accidentally fall into the trap: they had to sneak their way through the hole. One female was released at the centre of the arena and the experiment was monitored for up to 2 h, by which time all females had entered one of the traps. Three dual-choice tests were made: (a) damaged root vs control; (b) host pupae vs control; and (c) damaged root vs host pupae. Each dual choice was replicated 40–60 times and the position of pitfalls was randomized among replicates to avoid asymmetry bias in the device. The proportion of females entering each kind of mini-pitfall was recorded.

Short-range behaviour observation by video tracking

The behaviour of females—mated and fed ad libitum before the experiments—was recorded by video tracking in an arena containing two odorous test zones (the odour being plant or host related) separated by a central buffer zone. The arena consisted of a transparent plastic box ($16\times 9.5\times 8\text{ cm}$) filled up with 1 cm of moistened sand. Each zone was $5.33\times 9.5\text{ cm}$ as seen from above. In a first series of test, one test zone was sprayed with 10 mL of a solution extracted from an infested rutabaga root (see above). In the second test zone, the clean sand was removed and replaced by 10 *D. radicum* pupae and the moistened sand where they had previously pupated. In a second series of test, the first test zone contained the moistened sand in which 10 *D. radicum* larvae—previously rinsed in water to remove superficial residues of damage plant tissues—had pupated (but not the pupae themselves) and the other zone contained 10 *D. radicum* pupae but not the sand in which they had pupated (i.e., the pupae—cleaned with water—were manually buried in clean moistened sand). In particular, sand in which larvae pupated will contain larval tracks and possibly faeces.

At the beginning of the experiment, one female was placed at the centre of the arena on the buffer zone. The cumulated time spent on each zone was then recorded continuously for 2 h using a video camera connected to a computer running the video tracking software Ethovision 8.0 XT (Noldus Information Technology, Wageningen, Netherlands).

Statistical analysis

Statistical analyses were performed using R 2.14.0 software (R Development Core Team 2011). Unless otherwise stated, all the following statistical tests were conducted at a critical level of $\alpha=0.05$.

The average number of female trapped in field pitfalls was compared between treatments using a generalized linear mixed model (GLMM) with Poisson distribution (counts), including the odour source as fixed effect and the experimental field as random effect (Fig. 1). Multiple comparison tests were performed between treatments with a contrast analysis, using the function “esticon” of the package “doBy” (Højsgaard 2012). The proportion of females trapped in laboratory mini-pitfalls in dual tests was analysed using exact binomial tests ($H_0: P=0.5$).

The mean cumulated residence times in each zone of the arena during video tracking experiments were compared every 10 min (females did not display an orientated behaviour until 10 min; Goubert, personal observation) by using a general linear model with Gaussian distribution of errors. To take into account that for each repetition one female generates the time of the three zones, the time spent in each zone was linked to the total time spent by the female in the arena with the following log function:

$$\ln(T_{zi}) = \ln(z_i) + \ln(T_{tot})$$

where T_{zi} is time in zone i (test zones 1 and 2 or buffer) and z_i is a contribution of zone i to T_{tot} , the total time spent in the three zones. Multiple comparisons were

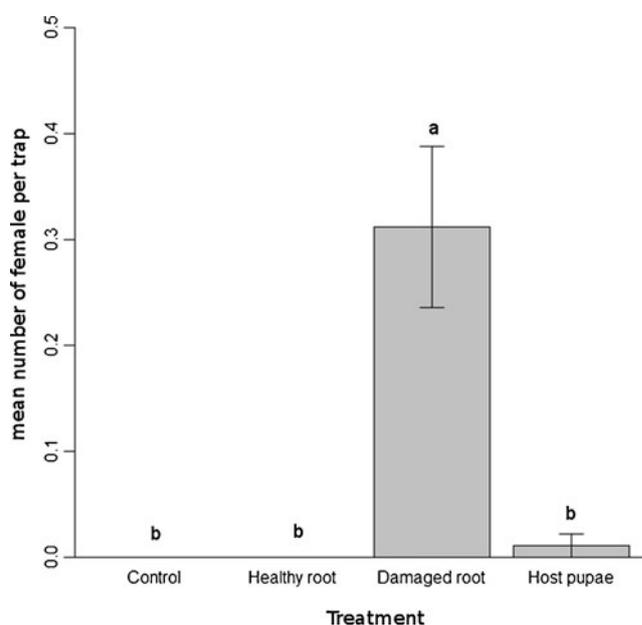
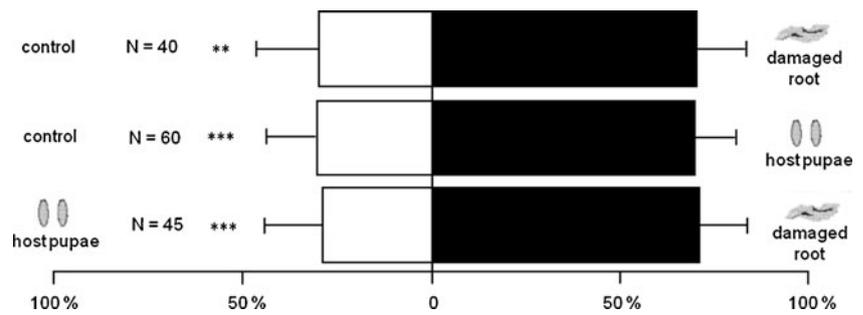


Fig. 1 Mean number (\pm SE) of *A. bipustulata* females trapped per pitfall in a field experiment. Mean of two sampling sites 2 km apart, 48–49 pitfalls per site and per treatment (total $N=30$ females). Different letters indicate a significant difference between means (GLMM Poisson followed by analysis of contrast with FDR correction $q\text{-val}<0.05$)

Fig. 2 Proportion ($\pm 95\%$ confidence interval) of *A. bipustulata* females trapped in mini-pitfalls during dual test experiments in the laboratory. *N*: number of females tested per dual test. Exact binomial test with $H_0: P=0.5$; ** $P<0.01$; *** $P<0.001$



carried out with an analysis of contrast as described previously.

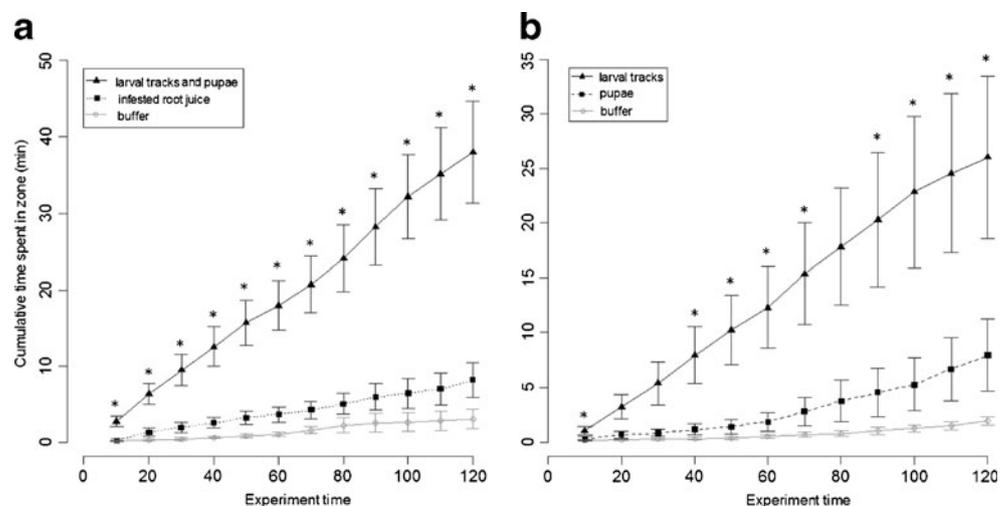
In multiple comparison tests, *P* values were adjusted with a false discovery rate (FDR) method (Benjamini and Hochberg 1995), using the “esticon.adjust” function of the package “RVAideMemoire” (Hervé 2012).

Results

Attraction of *A. bipustulata* females toward cues in the field

The pitfalls at the two sites captured a total of 30 *A. bipustulata* females, 29 of which were found in pitfalls containing pieces of rutabaga roots damaged by *D. radicum* larvae (Fig. 2). Only one female was found in a trap containing *D. radicum* pupae, and none was found in control traps or in traps containing healthy roots, demonstrating the selectivity of the device. There was a significant treatment effect (GLMM: $df=3$, $\chi^2=53.95$, $P<0.001$); traps containing damaged roots being more attractive than all other treatments (analysis of contrast, all pairwise comparisons: $P<0.001$). The other treatments did not differ significantly (all comparisons: $P>0.75$).

Fig. 3 Cumulative time (mean \pm SE) spent in each zone of the arena during video tracking of *A. bipustulata* females by step of 10 min during the 2 h of experiment (central buffer zone separating two odorous zones). Asterisks: significant difference between test zones (black vs black-dotted lines) by analysis of contrast with FDR correction, $P<0.05$. **a** Infested root extract vs naturally buried pupae. **b** Larval tracks vs artificially buried pupae



Attraction of *A. bipustulata* females toward cues in the laboratory

Short-range attraction

Significantly more *A. bipustulata* females were found in mini-pitfalls containing either a piece of damaged root or five *D. radicum* pupae than in the empty control pitfalls (Fig. 2, exact binomial test, $P<0.01$). When both odour sources were compared, significantly more females entered the pitfalls containing damaged root (exact binomial test, $P<0.05$), indicating either that this odour was more attractive or that it was more easily perceived.

Short-range behaviour observation by video tracking

For each dual test, females spent significantly more time in a zone containing one odorous cue compared to the other, and both zones were more attractive than the central buffer zone (Fig. 3, GLM, damaged root extract vs larval tracks with pupae: $P<0.001$; larval tracks vs pupae: $P<0.05$).

Specifically, females spent significantly more time in the zone where *D. radicum* larvae had naturally pupated than in the zone where infested rutabaga solution was sprayed (GLM, analysis of contrast with FDR control, $P<0.001$).

Moreover, the time spent in the zone containing larval tracks but no pupae was for most of the experiment significantly superior to the time spent in the zone containing artificially buried pupae but no larval tracks (GLM, analysis of contrast with FDR control, $P < 0.05$).

Discussion

The aim of our study was to assess to what extent the plant and the host would provide cues leading a coleopteran parasitoid to select an oviposition site. We found that the odour of roots damaged by the host was the most attractive cue at a distance, but that host larval tracks were the preferred cue at close range. We also found that females were able to detect immobile pupae and found them attractive at close range. Our results suggest that *A. bipustulata* females could use most of the evidence available to lay their eggs as close as possible from their host.

Our field experiment demonstrated that a piece of a healthy Swede root is no more attractive to *A. bipustulata* females than an odourless control (not a single female was found in those traps). This shows that neither females looking for food nor those looking for an egg laying site will rely on the odour of a healthy plant, even if it has been damaged recently (here, the root tissue had been cut off using a blade). We also found a strong attraction for infested root, all females but one being captured in traps containing pieces of roots damaged by *D. radicum* (but from which the larvae had been removed prior to the experiment). Such a material contains not only rotten root, but also larval faeces and gives off a very noticeable odour. Ferry et al. (2007) have already shown that the volatiles released by decaying plant material are attractive to *Aleochara* sp. in the field. These authors further found that this attraction was at least in part due to the release of dimethyl disulfide, a natural by-product of sulphur-rich tissue degradation (Ferry et al. 2007). The fact that the odour of a rotten root would attract *Aleochara* sp. is not surprising since such a root might signal the presence of food on the plant (*D. radicum* eggs and larvae) and possibly hosts (pupae) in the surrounding soil.

We did not capture more than a single female in field traps containing washed pupae of *D. radicum*. Since at least some females from those two fields were searching for laying sites, this result shows that in field conditions at least, the odour of a few pupae is not sufficient to attract females at a distance, even though host cocoon odours are attractive in some parasitoid species (Afsheen et al. 2008, Filella et al. 2011). As we used washed pupae to avoid a confounding effect of remaining plant material, their attractiveness might have been reduced. However, water did not remove hydrophobic cuticular hydrocarbons, which are known to provide relevant signals to insects (Howard 1993).

Royer et al. (1998) demonstrated that starved and unmated males or females of the close relative *A. bilineata* orientated toward odour of infested plant, host larvae and their faeces to find their food. Although testing starved and unmated individuals in *A. bipustulata* would also be informative, the data we obtained in controlled conditions had the extra interest that females were all mated and fed ad libitum. Thus, such females were expected to focus on finding a suitable egg laying site rather than food (since both *A. bipustulata* and *A. bilineata* species feed on *D. radicum* eggs and larvae; Fuldner 1960). Our results confirmed the strong attraction of host-damaged roots even in those females, suggesting that this cue is indeed used by females searching for a host patch and not only looking for food. Moreover, we found in dual tests that the odour of pupae was not more attractive than a control when offered as an alternative to host-damaged roots. Yet, when the odour of pupae was tested in a dual test against the control, a significant majority of females were trapped in the pitfall containing pupae. This demonstrates an important point: the odour of pupae is detected by *A. bipustulata* at close range—even when pupae have been washed in water—which indicates that adult females are theoretically able to search and detect pupae hidden in the soil. It also suggests that, although coleopteran parasitoid larvae are very mobile and able to forage for themselves, coleopteran parasitoid females do not necessarily lay their eggs at random near infested plants, relying completely on their larvae to scan the zone and find the pupae.

Our tracking experiment further revealed that females spent significantly more time foraging in a zone containing pupae and the sand in which they pupated (i.e., larval tracks + pupae + possibly larval faeces) than they spent time in a zone sprayed with an odorous *D. radicum*-damaged root extract. This might be due to the fact that on one side of the experiment, the odour of the host was not swamped by that of the plant and therefore easier to find. An alternative explanation (developed later) is that once the female has found the infested plant, it would switch its preference and search specifically for the host itself.

Since larval tracks and the pupae themselves are two different kinds of cues, we used a final experiment offering a dual choice between a zone containing pupae but no larval tracks and another containing larval tracks but no pupae. Here, perhaps surprisingly, the females spent significantly more time foraging in the zone with larval tracks, although no pupae were present, than in the zone where pupae were readily available (although covered by approximately 0.5 cm of moistened sand). This demonstrates that larval tracks left by *D. radicum* last instars when tunnelling in the substrate to pupate are prime cues for *A. bipustulata* females foraging for hosts. Tracks could represent a kind of “infochemical detour” (Vet et al. 1991), particularly relevant for parasitoids attacking buried pupae (Filella et al. 2011) and more generally buried hosts (Rogers and Potter 2002; Inoue

and Endo 2008). Since larvae were briefly rinsed in water to remove superficial residues of damaged plant tissues before the experiments, the only cues composing tracks should be faeces, mostly made of consumed root tissues and known to be attractive for starved *Aleochara* sp. (Royer and Boivin 1999), and maybe some cuticular hydrocarbons.

This switch from cues from the patch to ones from the hosts fits the classical pattern followed by hymenopteran parasitoids looking for a host (Wajnberg et al. 2008, Louâpre et al. 2011). Egg parasitoids, in which hosts are also sessile, use cues from host faeces, which are both volatile and contact cues, to locate their target at short range (Fatouros et al. 2008). Other hymenopteran parasitoids foraging for pupal or concealed host (such as wood borers) also use larval tracks, including faeces, to orientate in host galleries after being first attracted at long range by plant volatiles (Sullivan et al. 2000; Afsheen et al. 2008; Xiaoyi and Zhongqi 2008; Granchietti et al. 2012). Our results also suggest that *Aleochara* sp. behaviour could be close to oviposition strategies used by parasitoid flies of the Tachinidae family, where females are able to use a wide range of cues, including host-associated odours, to locate their host or an egg laying site. This is particularly true for tachinids laying directly on their host or those laying “microtype” eggs at a distance, which are then ingested by the host (Mondor and Roland 1997; Stireman et al. 2006; Ichiki et al. 2012). Unfortunately, the cues used by adult Tachinidae with mobile searching larvae (probably the dipteran parasitoids most comparable to *Aleochara* sp.) have not been investigated to our knowledge (Stireman et al. 2006). Finally, the behaviour of the coleopteran parasitoids studied here appears to be distinct from other dipteran parasitoids with mobile searching larvae. For instance, *Mallophora ruficauda* (Diptera: Asilidae) parasitizes coleopteran larvae buried in the soil, but females lay their eggs on tall grass, their larvae being later dispersed by the wind (Crespo and Castelo 2010). In the Bombyliidae family, fly larvae parasitize wild bee larvae in their underground nests: depending on the species; females can lay their eggs at random in the substrate or at the entrance of a nest, but do not venture closer from the host (Yeates and Greathead 1997; Boesi et al. 2009).

Our results allow us to propose a putative scenario of the behavioural sequence by which *A. bipustulata* females might lay their eggs in the optimal place for the survival of their larvae. As a first step, females would be attracted from a distance by odours from *D. radicum*-infested roots. This plant-related cue is much easier to detect than pupae (high detectability), but it does not guarantee the presence of pupae (low reliability) because more than 2 weeks elapsed (at 20 °C) between the initial damage by *D. radicum* larvae and the first pupae produced (average time observed from laboratory breeding, unpublished data). Upon reaching the infested plant, the female would then switch to another cue,

i.e., larval tracks in the soil. Indeed, only late *D. radicum* third instars are bound to leave tracks in the soil when they leave the root to pupate, and each of those tracks leads to a pupa. Following those tracks at least partly (the elongated shape of roving beetle is very fit for this purpose), *A. bipustulata* females would be able to lay their eggs as close from the host as possible, considerably increasing the chances that their own larvae find a suitable host upon hatching. The fact that we observed several tunnels dug by *A. bilineata* females in the sand is also consistent with the idea that females tried to reach the pupae. Interestingly, investment in host research by the female turns out to be more important than for reported cases of dipteran parasitoids having mobile searching larvae. This may come from the large difference in the daily fecundity between parasitoids studied in these orders, from hundreds or thousands eggs a day in Diptera (Yeates and Greathead 1997; Boesi et al. 2009) to around 10 eggs a day in Coleoptera of the genus *Aleochara* (Fournet et al. 2000), forcing these females to lay as close as possible to their hosts to ensure their reproductive success.

In conclusion, we have shown that host-damaged plant odours are used by *A. bilineata* females foraging for an egg laying site, but that at closer range, females may switch their preference for odours directly linked to the hosts, i.e., tracks left by third instar *D. radicum* larvae. We have also shown that at close range, *A. bipustulata* females can be attracted to the odour of *D. radicum* pupae themselves. Together, our results suggest a behavioural sequence akin to that found in “classical” parasitoid wasps, where females use successively a detectable but unreliable cue linked to the host plant (long-range detection), then a less detectable but highly reliable cue linked to the host (short-range detection) (Vet et al. 1991). Despite of their mobile larvae, some parasitoid beetles might go all the way to the host when they can.

In our next experiments, we will try to find out if they do, by establishing where exactly *A. bipustulata* females lay their eggs with respect to underground pupae. Our results also suggest the need to investigate in details the composition of larval tracks, which seems to take an important part in host finding, as well as the relative importance of potential herbivore-induced plant volatiles, released this time by brassicaceous leaves, during *D. radicum* attack. Such volatiles attract *Trybliographa rapae*, a hymenopteran parasitoid of *D. radicum* (Pierre et al. 2011), but their effect has not been investigated yet on *Aleochara* sp.

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These experiments comply with the current laws of France.