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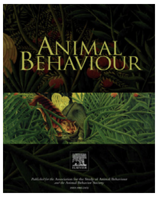

Flexible parasitoid behaviour overcomes constraint resulting from position of host and nonhost herbivores

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Parasitoids face several hurdles and distractions while foraging for their hosts, one of which is the presence of nonhost herbivores. Nonhost herbivores may interfere with plant volatile-mediated location of host-infested plants and reduce encounter rates with hosts on the plant. This results in a lower foraging efficiency. In this study, we tested whether the feeding position of a host and nonhost herbivore on the same plant influences foraging decisions and parasitism efficiency of parasitoids. We confined host and nonhost herbivores to either higher positions, i.e. younger leaves (preferred by the host) or lower positions on the plant, i.e. older leaves (preferred by the nonhost). Host and nonhost herbivores fed either on separate leaves or on the same leaf. Results from laboratory experiments show that during the first phase of foraging when plant volatiles are used to locate a host-infested plant, parasitoids were misled when host and nonhost were positioned in an unnatural way on the individual plant (host on the older leaves). The positions of host and nonhost partly influenced parasitoids during the second phase of foraging, when the host is located on the plant by using host cues. Total host-finding efficiency, as tested in a semifield set-up, was not affected by herbivore position. We conclude that parasitoid foraging behaviour has enough flexibility to overcome constraints resulting from an unexpected distribution of herbivores over a plant.

Foraging is essential for all animals and, therefore, the behaviour when foraging for food or other resources has been well studied in many groups of organisms (Nonacs, 2001; Stephens, 2008). Of these studied groups, the behaviour of parasitic wasps (parasitoids) when foraging for hosts is especially interesting. Foraging for hosts is expected to be subject to strong selection pressure, because the success of foraging for hosts is strongly linked to parasitoid fitness (Thiel & Hoffmeister, 2009). Parasitoids lay their eggs in or on the host (usually other arthropods) and after emergence the larvae are dependent on this host as their food source (Godfray, 1994; Thiel & Hoffmeister, 2009). To be able to find their herbivorous hosts, parasitoids have evolved foraging strategies that can be divided into two phases (De Rijk, Dicke, & Poelman, 2013; Van Alphen, Bernstein, & Driessen, 2003). During the first phase, plant volatiles are used as cues to locate the plant on which the herbivore host is feeding (Hare, 2011; Van Alphen et al., 2003; Vet & Dicke, 1992). These so-called herbivore-induced plant volatiles (HIPVs) are known to be produced by plants in response to herbivore attack (Arimura, Ozawa, & Maffei, 2011; Stam et al., 2014; Vet & Dicke, 1992). During the second phase of foraging, host cues (e.g. from frass or feeding damage) are used to locate the host on the plant after which the host is recognized and examined for its quality before being accepted (Colazza, Cusumano, Lo Giudice, & Peri, 2014; De Rijk et al., 2013; Van Alphen et al., 2003).

Hosts are ‘hidden’ in an environment full of possible hurdles and distractions for the parasitoid. For example, landscape structure (Nelson & Forbes, 2014; Roitberg & Gillespie, 2014), vegetation composition (Bezemer et al., 2010; Gols et al., 2005) and, on a smaller scale, host patch size (Bezemer et al., 2010) or the presence

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of competitors (Almohamad & Hance, 2014; Couchoux & van Nouhuys, 2014) could affect the ability of parasitoids to find their host. Furthermore, the presence of herbivores that are not suitable as a host could impair host location (De Rijk et al., 2013; Desurmont et al., 2014; Ponzio, Gols, Weldegergis, & Dicke, 2014). Finding these nonhosts alongside hosts is common, as in both natural and agricultural ecosystems plants are commonly attacked by more than one herbivore species simultaneously as well as sequentially (Stam et al., 2014; Vos, Berrocal, Karamaouna, Hemerik, & Vet, 2001). Like hosts, nonhosts also induce the plant to produce volatiles. Nonhost feeding could modify the induction of volatiles by the host herbivore (Dicke, Van Loon, & Soler, 2009; Soler et al., 2012; Zhang et al., 2013; Zhang et al., 2009) and could, therefore, interfere with the detectability of host-induced cues by the parasitoid. In addition, nonhosts that feed on the same plant also produce cues, such as frass and feeding damage, that could lead the parasitoid away from the infochemicals produced by the host (Takabayashi & Takahashi, 1990). Here, we use the term ‘misleading’ to describe when nonhost-induced plant volatiles and nonhost infochemicals lead the parasitoid away from cues induced and released by hosts.

Previously, it was found that the foraging efficiency of parasitoids could be differently affected by nonhost herbivores belonging to different feeding guilds (De Rijk, Yang, Engel, Dicke, & Poelman, in press; Moujahed et al., 2014). It is, however, not known whether the positions of the host and these nonhosts on the leaves of the same plant would influence the decisions and foraging efficiency of parasitoids (Bukovinszky et al., 2012). It is known that parasitism rates can be affected by plant structure (Obermaier, Heisswolf, Poethke, Randlkofer, & Meiners, 2008; Romeis, Babendreier, Wäckers, & Shanower, 2005), position of the infested leaf (El-Wakeil, 2011) and feeding on either leaves or flowers by host herbivores (Lucas-Barbosa et al., 2014). Therefore, adding a nonhost herbivore on the same plant but at a separate leaf could possibly also affect parasitoid foraging behaviour and efficiency. When host and nonhost herbivores are feeding on different leaves of the same plant, both will induce the plant to defend itself. Plant defence is often expressed not only locally but also systemically (Gutbrodt, Mody, Wittwer, & Dorn, 2011; Heil & Ton, 2008; Marti et al., 2013; Shah, 2009) and systemic signal distribution can be affected by plant architecture (Jones, Hopper, Coleman, & Krischik, 1993; Mousavi, Chauvin, Pascaud, Kellenberger, & Farmer, 2013; Orians, 2005; Utsumi, Ando, & Miki, 2010). It is not clear whether two herbivores feeding on different parts of the plant will induce one cue emitted from the entire plant or whether different plant parts provide distinct cues that can be used during the first phase of foraging to determine the position of the host herbivore (Utsumi et al., 2010). In either case, when herbivores are feeding on separate leaves, parasitoids would have to choose on which leaf to land. During the second foraging phase the influence of the separated positions of host and nonhost on parasitoid behaviour is expected to depend on the leaf selected. When the parasitoid lands on a nonhost-infested leaf, it is likely to encounter nonhosts and their products. This may result in either one short visit to the plant without the parasitoid parasitizing any hosts (Bukovinszky et al., 2012; Sato, Takabayashi, Yano, & Ohsaki, 1999; Shiojiri, Takabayashi, Yano, & Takafuji, 2000; Vos et al., 2001) or prolonged visits during which the parasitoid wastes time by encountering nonhosts, but eventually finds the host-infested leaf. In contrast, when it lands on a host-infested leaf, no constraints are present on this smaller scale, so the parasitoid could be as efficient as when no nonhosts are around.

Here, we present the results of three experiments that studied the effect of herbivore position on the foraging behaviour of the parasitoid *Cotesia glomerata*, which specializes on *Pieris* caterpillars. For this study, we selected its most common host, the gregariously feeding *Pieris brassicae*, and a nonhost, *Mamestra brassicae*, which lays eggs in batches and the caterpillars collectively feed for the first few days on the leaf on which they hatched. These caterpillars feed on brassicaceous plants, including cultivated *Brassica oleracea*. In general, the specialist *P. brassicae* caterpillars feed on the younger, and so higher up, leaves (Gutbrodt, Dorn, Unsicker, & Mody, 2012; E. H. Poelman, personal observations), while generalist caterpillars such as *M. brassicae* feed on the older, less defended and lower leaves of *B. oleracea* (Gutbrodt et al., 2012). First, we tested in a wind tunnel whether host and nonhost positions on the plant influenced parasitoid decisions to land on infested leaves. Second, we observed in a flight chamber set-up how the behaviour of the parasitoid after landing on the plant was affected by herbivore position. Third, we tested in a semifield set-up whether the total host-finding efficiency of the parasitoid was affected by herbivore position.

METHODS

Plants and Insects

Five-week-old *B. oleracea* var gemmifera Cyrus plants were used in all three experiments. The plants were grown by Unifarm of Wageningen UR in a greenhouse (20 ± 2 °C, 60 ± 10% relative humidity, 16:8 h light:dark photoperiod, SON-T light 500 μ mol/m^2 per s) in pots of 0.7 litre filled with potting soil (Lentse potgrond, no. 4; Lent, The Netherlands) and fertilized two to three times per week using a liquid fertilizer (EC 2.1 mS/cm, pH 5.8). No pest control measures were taken.

Pieris brassicae and *M. brassicae* cultures were maintained on *B. oleracea* plants at 20 ± 2 °C, 60 ± 10% relative humidity, 16:8 h light:dark photoperiod for over 10 generations. Both caterpillar species were originally collected from agricultural fields in The Netherlands. In the experiments first-instar caterpillars were used. A *C. glomerata* culture was maintained on *P. brassicae* larvae at 20 ± 2 °C, 60 ± 10% relative humidity, 16:8 h light:dark photoperiod for over 10 generations and the culture was renewed with field-collected parasitoids yearly. The parasitoids were originally collected from an agricultural field in Wageningen, The Netherlands. *Cotesia glomerata* cocoons were collected from rearing cages and emerged adults were kept in cages with water and honey ad libitum at 21 °C and 16:8 h light:dark photoperiod. The parasitoids were allowed to mate and 1–10-day-old parasitoids inexperienced with herbivores and plants were collected from the cages for the experiments.

Wind Tunnel Experiment

To study whether host and nonhost positions on the plant influenced the decision of *C. glomerata* to land on a leaf, a wind tunnel as described by Geervliet, Vet, and Dicke (1994) was used in July and August 2013. The parasitoid was given the choice of all leaves of a single plant and the one chosen was recorded. *Brassica oleracea* plants were infested with herbivores according to four treatments: (1) 10 first-instar *P. brassicae* larvae on the higher leaf,