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Plants under multiple herbivory: consequences for parasitoid search behaviour and foraging efficiency

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In the field, plants are attacked by several herbivore species both simultaneously and in isolation. Spatial variation in damage to plants by different herbivores may affect the search behaviour of parasitoid wasps, but the consequences of this variation for host–parasitoid interactions are still little understood. We examined the effects of multiple herbivory on the search behaviour of the parasitoid *Cotesia glomerata*. Reduced parasitism was found in a field tent experiment, when both the host small cabbage white, *Pieris rapae*, and the nonhost cabbage moth, *Mamestra brassicae*, were present on *Brassica oleracea* plants. When hosts and nonhosts occurred on the same or neighbouring plants, this reduction in parasitism was similar, but the underlying behavioural mechanisms were different. In wind tunnel bioassays, parasitoids were equally attracted towards plants infested by the two herbivore species but were more attracted to plants with both herbivore species than to plants with only one. Differences in arrival tendencies therefore could not explain the reduced parasitism in the tent experiment with mixed infestations. Experiments showed that parasitoids readily left nonhost patches, whereas leaving tendencies from mixed patches of hosts and nonhosts were the same as from pure host patches. Therefore, reduced leaving tendencies and reduced host encounters explained the lower parasitism rate in mixed infestations in the tent experiment. Our results show that the spatial context in which hosts and nonhosts attack plants determines the foraging efficiency of parasitoids, with consequences for host–parasitoid interactions.

Trait-mediated indirect interactions (TMII) occur when one species (e.g. a plant) affects populations of another species (e.g. a herbivore) through changes in the behaviour of a third species (e.g. a predator or parasitoid; Müller & Godfray 1999; Werner & Peacor 2003). The use of herbivore-induced plant volatiles (HIPVs) by parasitic wasps represents a case of TMII, in which parasitoid foraging success is influenced by the variation in information use from different trophic levels, that is, from the herbivore and its food plant. HIPVs may provide cues that are conspicuous for the parasitoid and may increase parasitoid search efficiency (Vet et al. 1991; Kessler & Halitschke 2007). However, HIPVs do not always reliably predict host identity and suitability, which may negatively affect foraging efficiency of parasitoids (Vet & Dicke 1992; Vos et al. 2001; Halitschke et al. 2008). For example, different herbivore species may induce qualitatively similar volatile blends, which can reduce the ability of parasitoids to discriminate between herbivore species at a distance from the plant (Agelopoulos & Keller 1994). To evaluate the role of HIPVs under increasingly realistic conditions, experimental approaches are needed that link behavioural variation to parameters (e.g. parasitism) that are related to population-level processes (Werner & Peacor 2003; Ohgushi 2005).

Recent studies have observed variable effects of the presence of nonhosts on the success of parasitoids to locate and parasitize hosts (e.g. Vos et al. 2001; Shiojiri et al. 2002; Van Veen et al. 2005; Erb et al. 2010). The study of Shiojiri et al. (2002) demonstrated that the presence of nonhosts might affect parasitism rates through infochemically mediated indirect interactions. These effects on parasitism may be either negative or positive, depending on how infochemicals, induced by multiple herbivore species, were used by herbivores and parasitoids to make foraging decisions (Shiojiri et al. 2002). Other studies have related changes in parasitoid foraging

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success either to the interference or attraction caused by the presence of nonhosts in association with hosts. Van Veen et al. (2005) showed that the foraging efficiency of the parasitoid *Aphidius ervi* was reduced when the host aphid, *Acyrthosiphon pisum*, was associated with the nonhost aphid *Megoura viciae*. Rodriguez-Saona et al. (2005) found a positive effect of nonhosts on parasitoid attraction, where plants infested by both hosts (*Spodoptera exigua*) and nonhosts (*Macrosiphum euphorbiae*) were more attractive to the parasitoid *Cotesia marginiventris* than plants infested by a monoculture of either herbivore species. Subsequent studies showed that simultaneous damage to plants by different herbivores might result in decreased or increased attack rates of the herbivore host by parasitoids under field or semifield conditions (e.g. White & Andow 2006; Soler et al. 2007a). Such effects on parasitoids may be mediated by different mechanisms, such as changes in the emission of similar or novel compounds by plants. In turn, qualitative and quantitative changes in the infochemical profile of plants may represent different infochemical contexts for the parasitoid (Soler et al. 2007a, b; Rasmann & Turlings 2007; Schröder & Hilker 2008). Alternatively, when different herbivores attack a plant, the resulting changes in the kairomonal context within the herbivore patch can also affect foraging decisions of parasitoids.

Most studies, however, have focused on the consequences of multiple herbivory by correlating parasitoid attraction to plant–herbivore complexes with differences in parasitism rates. Thus, the effects of multiple herbivory on foraging success within herbivore patches have been overlooked. Rewarding experiences such as host encounters will determine residence times, but host-related cues (i.e. kairomones and plant cues) also play an important role in determining leaving tendencies from patches (Waage 1979; Vos et al. 1998; Hilker & McNeil 2008). Thus, the presence of nonhosts in the direct vicinity of or within patches may affect not only arrival tendencies to host patches, but also the processes of patch exploitation and patch-leaving decisions. Moreover, herbivore distributions in the field show variation both within and between plants. Since HIPV production by plants after attack by a single herbivore may be different from volatile production after multiple herbivory (Dicke et al. 2009), the consequences for parasitoid search behaviour may depend on how hosts and nonhosts are distributed relative to each other. As far as we know, no studies have related changes in the host selection process to parasitism, when plants in the local habitat patch experience different spatial distributions in herbivory.

The secondary chemistry of plants plays an important role in shaping herbivore–parasitoid interactions. *Brassicas*, for example, exhibit large inter- and intraspecific variation in herbivory-induced infochemicals (Renwick 2002; Halkier & Gershenzon 2006; Gols & Harvey 2009). Glucosinolates, the characteristic secondary chemicals of the Brassicaceae, and their breakdown products (e.g. isothiocyanates and nitriles) play an important role in host plant location and acceptance by specialized herbivores. Both this specific group of compounds and general green leaf volatiles are important for mediating attraction of parasitoids to herbivore-damaged *Brassicas* (Gols & Harvey 2009; Poelman et al. 2009). *Cotesia glomerata* is a gregarious endoparasitoid attacking larvae of the large cabbage white, *Pieris brassicae*, and small cabbage white, *Pieris rapae*, butterflies. Headspace volatiles of cabbage will differ depending on the herbivore species that damages it and *C. glomerata* exhibits a limited ability to discriminate between host- and nonhost-induced changes in HIPVs (Geervliet et al. 1996; Vos et al. 2001).

In this study, we analysed the foraging behaviour of the parasitoid *C. glomerata* in relation to variation in the reliability of infochemical signals, and related our findings to parasitism rates. Cabbage plants were induced by pure patches of the host *P. rapae*, the nonhost cabbage moth, *Mamestra brassicae*, or a mixture of both hosts and nonhosts. A field mesocosm experiment was devised to evaluate the effects of the presence of nonhosts in the vicinity or in mixtures with host patches on parasitism rates. In wind tunnel bioassays, we compared the attraction of parasitoids towards herbivore-induced plants that were attacked by the host only, by the nonhost only or by a mixture of both herbivores. Using greenhouse bioassays, we studied the behaviour and patch exploitation by parasitoids when the infochemical cues and herbivore species composition within a patch were manipulated. We hypothesized that the presence of the nonhost would affect parasitism rates, where this effect would depend on how herbivore- and plant-related cues interact with the different phases of the host selection process (i.e. attraction to patches and patch residence times).

METHODS

Plants

Brassica oleracea cv capitata Christmas Drumhead was used in all experiments. Plants were reared in the greenhouse (60% relative humidity; 22 ± 2 °C; 16:8 h L:D) in pots (0.7 litre) filled with standard potting soil (Lentse potgrond). All plants were 7–8 weeks old with six or seven leaves, when used in experiments.

Insects

Insect cultures were maintained at 20 ± 2 °C, 60 ± 10% relative humidity and 16:8 h photoperiod at the Laboratory of Entomology of Wageningen University. Herbivores were reared on Brussels sprout plants, *B. oleracea* var gemmifera 'Cyrus', and *C. glomerata* was reared on *P. brassicae*. Parasitoid cocoons were collected weekly from culture cages and were placed in cages without the host or plant material present. Emerging wasps were provided with water and honey ad libitum and were allowed to mate. Mated females of 5–7 days of age with no oviposition experience were used in experiments. In all experiments, we used early second-instar larvae of *P. rapae*, and late second instar *M. brassicae* for infesting plants. This was done to compensate for the different amounts of foliar damage caused by the two herbivore species.

Experimental Set-ups

Field tent experiment

Field experiments were carried out in 12 tents (3 × 4 m and 2 m high) made of fine (diameter 0.6 mm) insect screen. Each tent received 16 cabbage plants arranged in a 4 × 4 grid, planted 3 weeks before the experiment. Four tents belonged to each of the three treatments (Fig. 1). In the control (hosts only), 10 *P. rapae* caterpillars were introduced on the first fully grown leaf of every second plant. In the treatments in which nonhosts were also present in the tent, *M. brassicae* (nonhosts) larvae were added in two different spatial arrangements to the tents, while the distribution of *P. rapae* caterpillars remained the same as in the control. In one treatment (i.e. separate infestation), 10 *M. brassicae* caterpillars per plant and 10 *P. rapae* were placed on neighbouring plants (Fig. 1). In the other treatment with nonhosts (i.e. mixed infestation), five *M. brassicae* caterpillars were present on all plants within a tent, and on plants containing the 10 *P. rapae*, the hosts and nonhosts were placed on the same leaf. This way, the total number of herbivores and the distribution of the host *P. rapae* were standardized (Fig. 1). Sixteen hours after infestation, four female and four male *C. glomerata* were introduced into each tent by releasing them from a vial placed on the ground in the middle of the plant patch. Although parasitoids were well fed when released, a petri dish with drops of honey was placed in each tent. Caterpillars were collected 36 h after releasing the