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On the role of sinigrin (mustard oil) in a tritrophic context: plant–aphid–aphidophagous hoverfly

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Abstract. 1. Plant secondary metabolites can govern prey–predator interactions by altering the diet breadth of predators and sometimes provide an ecological refuge to prey. Brassicaceae plants and their specialist pests can be used as a model system for understanding the role of chemically mediated effects restricting the diet breadth of natural enemies, and consequently the occurrence of enemy-free space for the specialist pest.

2. The objective of the present study was to test the performance of the generalist predator *Episyrphus balteatus* De Geer (Diptera: Syrphidae) fed on the specialist herbivore *Brevicoryne brassicae* L.(Homoptera: Aphididae), reared on two different brassica species: black mustard (*Brassica nigra*), a wild species with high levels of sinigrin; and canola (*Brassica napus*), a cultivated species without sinigrin.

3. The preference and performance of the predator and the performance of the prey were measured. Sinigrin was quantified by high-performance liquid chromatography in both leaf samples and aphids reared on the two host plants.

4. The cabbage aphid performed better on canola than on black mustard. The performance of the predator on this aphid when reared on canola was clearly better than when reared on black mustard. Females had a higher overall preference for cabbage aphids reared on canola than on black mustard.

5. The ability of aphids reared on plants with high glucosinolate content to reduce the performance of their generalist predators indicates that the presence of *B. nigra* may provide enemy-free space for the cabbage aphid from its predator, a concept that has useful application in the context of biological control for agricultural systems.

Key words. Black mustard, *Brevicoryne brassicae*, enemy-free space., *Episyrphus balteatus*, sinigrin.

Introduction

Several factors govern prey–predator interactions, such as habitat (Bell, 1990), host plants (Almohamad *et al.*, 2007; Kos *et al.*, 2011), prey species (Hodek & Honek, 1996; Sadeghi *et al.*, 2014), prey availability (Sutherland *et al.*, 2001; Almohamad *et al.*, 2006; Amiri-Jami & Sadeghi-Namaghi, 2014), semiochemicals (Francis *et al.*, 2004; Harmel *et al.*, 2007; Verheggen *et al.*, 2008; Almohamad *et al.*, 2010), competition (Hindayana *et al.*, 2001; Almohamad *et al.*, 2010) and, in some cases, third parties such as ants associated with some aphids (Stadler *et al.*, 2003).

The study of the aphid prey–natural enemy relationship is generally focused on the top-down pressures of predators on their prey, but in fact they have evolved in a multitrophic context (Gilbert, 2005). Plants have an important role in mediating the interaction (Malcolm, 1992; Chaplin-Kramer *et al.*, 2011), and bottom-up factors such as plant secondary metabolites can alter the diet breadth of predators (Dyer, 1995) and sometimes provide an ecological refuge to prey (Jeffries & Lawton, 1984;

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Stamp, 2001; Chaplin-Kramer *et al.*, 2011). Many wild plants contain higher levels of defence compounds than their domesticated congeners (Cole, 1997; Gols & Harvey, 2009), and specialised herbivores can often utilise those toxins to compile their own chemical arsenal to escape enemies (Nishida, 2002; Hopkins *et al.*, 2009). If this chemical refuge allows pests to escape their enemies more effectively than when feeding on crops, the occurrence of such plants around farmland could support populations of pests that are then unregulated by their enemies. The occurrence of such enemy-free space reveals important subtleties in the relationship between these trophic groups (Chaplin-Kramer *et al.*, 2011), a concept that has useful application in the context of biological control for agricultural systems. In agricultural systems, sometimes the efficiency of biocontrol agents is low, and the cause can be interpreted by considering the concept of bottom-up along with top-down factors (Vanhaelen *et al.*, 2002; Kos *et al.*, 2011).

Glucosinolates are a well-studied class of plant secondary metabolites, mainly because they occur in important crops such as cabbage, broccoli and oilseed rape (Hopkins *et al.*, 2009). More than 120 different glucosinolates are known of which four common glucosinolates are sinigrin, sinalbin, glucobrassicanapin, and glucobrassicin (Hopkins *et al.*, 2009). Generally crop plants contain lower levels of secondary metabolites in comparison with wild species (Cole, 1997; Gols & Harvey, 2009). The term ‘mustard oil bomb’ has been applied in the description of chemically mediated effects in this plant–herbivore interaction (Ratzka *et al.*, 2002). The defence system is activated when damage occurs (such as pest damage), releasing glucosinolates from vacuoles that come into contact with myrosinase enzymes in the cytoplasm, resulting in the formation of toxic hydrolysis products such as isothiocyanates, nitriles, and thiocyanates (Heaney & Fenwick, 1995; Hopkins *et al.*, 2009). Generalist herbivores are affected negatively by these hydrolysis products, e.g. by deterring feeding or decreasing survival (Halkier & Gershenzon, 2006; Hopkins *et al.*, 2009). In contrast, specialist herbivores of brassicaceous plants have evolved special adaptations to detoxify glucosinolates (Ratzka *et al.*, 2002; Wittstock *et al.*, 2004), and use these compounds as feeding or oviposition stimulants (van Loon *et al.*, 1992; Miles *et al.*, 2005). The influence of glucosinolates of brassica plants is not limited to herbivores. Several specialist natural enemies of herbivores are attracted by the volatile breakdown products of glucosinolates (Bradburne & Mithen, 2000; Blande *et al.*, 2007; Mumm *et al.*, 2008). Parasitoids can use hosts containing glucosinolates as enemy-free space (Ode, 2006), but in contrast, generalist predators feeding on glucosinolate-containing prey can be negatively affected (Francis *et al.*, 2001; Vanhaelen *et al.*, 2002; Sznajder & Harvey, 2003; Kazana *et al.*, 2007; Pratt, 2008; Kos *et al.*, 2011). The extent of this negative effect depends on the prey being generalist or specialist (Kos *et al.*, 2011). The oviposition behaviour of generalist predators also plays an important role in relation to the fate of the developing larvae, especially for predators with sedentary larvae, such as aphidophagous syrphids (Sadeghi & Gilbert, 1999, 2000a). Overall, it is expected that females avoid ovipositing in host/prey combinations with high potential risk, and consequently specialist herbivores could escape from their natural enemies via appropriate choices of host plant (Aliabadi *et al.*, 2002; Chaplin-Kramer *et al.*, 2011).

Brassicaceae plants and their specialist pests can be used as a model system for understanding the role of chemically mediated effects restricting the diet breadth of natural enemies, and consequently the occurrence of enemy-free space for the specialist pest.

A few studies have evaluated the effects of sequestration of glucosinolates by the cabbage aphid *Brevicoryne brassicae* on a few aphid predators (Francis *et al.*, 2001; Kazana *et al.*, 2007; Pratt, 2008; Kos *et al.*, 2011). In most cases, these studies have assessed the performance of predators in relation to the total concentrations of glucosinolates or glucosinolate profiles among aphids and linked it to the glucosinolate concentrations in foliar materials of the host plant. Mostly the performance of a generalist predator feeding on a generalist herbivore has been compared with its performance feeding on a specialist herbivore.

The objectives of the present study were: (i) to investigate the performance of the generalist predator *Episyrphus balteatus* De Geer (Diptera: Syrphidae) fed on *Brevicoryne brassicae* L. (Homoptera: Aphididae) reared on two different brassica species: canola (*Brassica napus*), a cultivated brassica species without any sinigrin, and black mustard (*Brassica nigra*), a wild brassica species with high levels of sinigrin; and (ii) to investigate the precise relationship between the host plant sinigrin concentration and the level of this glucosinolate in aphid body tissues, which has not been investigated thoroughly (Pratt, 2008). We hypothesised that, based on the difference in concentrations of sinigrin and its breakdown products, the performance of *E. balteatus* will be lower when fed *B. brassicae* reared on black mustard than when fed this aphid reared on canola. Furthermore, we expect that variation in glucosinolate composition among the host plant species would affect the glucosinolate composition of the aphids feeding on these species, as well as the possible creation of enemy-free space, and thereby the performance of the generalist predator feeding on these host plant–aphid combinations, a concept that has useful application in the context of biological control for agricultural systems.

Material and methods

Canola *Brassica napus* var. Hyola 308 (from the Torogh Agricultural Research Center, Razavi Khorasan Province, Iran) and black mustard *Brassica nigra* (from the Pakan-Bazr Co., Isfahan Province, Iran) were grown in a greenhouse at 25 ± 2 °C, 60 ± 10% RH and under an LD 16:8 h photoperiod. The seeds were sown in April 2014 in plastic pots (20 cm diameter, 30 cm height, containing a 2 : 2 : 1 mixture of soil : sand : leaf compost). Plants at the stage of six to seven fully expanded leaves were selected for aphid rearing and were also sampled for high-performance liquid chromatography (HPLC) analysis. For mass rearing of the cabbage aphid, the selected plants were infected with apterous females collected from a single colony of *Brevicoryne brassicae* in an oilseed field to obtain a suitably homogenous population.

A stock culture of *E. balteatus* was established using gravid females captured at the campus of Ferdowsi University of

Mashhad, north-eastern Iran, in May 2014. The stock culture was maintained in a constant environment of 22–23 °C under an LD 15:9 h photoperiod. To obtain a group of larvae of the same age, females were induced to lay eggs on cut sections of broad bean plants (*Vicia faba* L.) infested with black bean aphids. For experimental purposes, eggs laid over a period of 12 h were selected and placed in a large Petri dish in an incubator (20 ± 1 °C, 60–70% RH, LD 16:8 h photoperiod) to hatch.

Chemical analysis

A total of 50 apterous aphids in their penultimate instar were selected from colonies reared on each plant–aphid combination for at least six generations (from at least five different plants in each treatment) and preserved in 5 ml methanol 90% for HPLC analysis. Approximately 1 g of fresh leaf matter from fully expanded leaves of six different plants in each treatment was collected and immediately frozen in liquid nitrogen and preserved at −80 °C until HPLC analysis.

The extraction method of sinigrin from plant and aphid samples was made according to the EEC Regulation N1864/90. Duplicated samples (400 mg leaf powder and 50 aphids related to each treatment) were extracted with 5 ml hot methanol 70% for 10 min at 80 °C, and centrifuged at 6708 ***g*** for 20 min. Aliquots of 2 ml extract were loaded twice onto a mini-column filled with 0.6 ml DEAE Q-Sepharose anion-exchange resin (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), conditioned with 25 mM acetate sodium buffer, pH 5.6. After washing with 3 ml buffer, volumes of 250 μl commercial sulphatase (catalogue no. S9626-5KU; Sigma Ltd, Oakville, Canada) were loaded onto each mini-column, left overnight at room temperature, and the desulfo-sinigrin was eluted with 3 ml of ultrapure water and then analysed by HPLC.

The chromatographic analyses were carried out on an HPLC system (Knauer Corporation, Scientific Instruments, Berlin, Germany) equipped with a manager 5000, pump 1000 and UV detector 2600. Sinigrin and desulfo-sinigrin were separated on a Eurospher (C 18) 100 A column (Knauer Corporation) (250 × 4.6 mm, 5 μm) at 30 °C. The data were processed using the software EZ CHROM ELITE (Shimadzu Corporation, Kyoto, Japan). The flow rate was 0.5 ml min^{-1} and the injection volume was 20 μl. The mobile phases consisted of water (eluent A) and acetonitrile (eluent B), using a gradient programme as follows: 3 min 1% B; 9 min linear gradient up to 10% B; 3 min linear gradient down to 1% B. Elution of sinigrin was monitored at 229 nm. The sinigrin in both plants and aphids was identified and quantified in relation to a previously purified standard (sinigrin hydrate; catalogue no. 85440, Sigma).

Performance of Brevicoryne brassicae

Fertility components of life table parameters were calculated for individual cabbage aphids in each aphid–host plant combination. A total of 30 apterous adults of *B. brassicae* were placed individually in clip-cages, resulting in five clip-cages per plant on each of six plants per treatment (the total sample size was 60). Each clip-cage was placed on the upper side of a fully expanded leaf. The location of the clip-cage was changed every 3–4 days to avoid leaf damage and pseudoreplication effect. Once the first newborn nymph was observed, the adult aphids were removed from the clip-cage, and the nymphs were observed daily until they became adults and then until they died. The number of nymphs produced day^{-1} was noted and removed after counting. The daily rate of reproduction was recorded until the adults died, allowing for a calculation of total fecundity and longevity. All replicates in which nymphs died within the first 24 h of being born, or were lost during the experiment, were omitted from analyses (six and 13 replicates for canola and black mustard, respectively). Daily observations provided the primary data of l_x (the probability of being alive at age x) and m_x (mean number of female offspring produced day^{-1} by a female aged x), which were used to construct age-specific life tables for aphids in the different treatments. All offspring produced were female.

Lifetime fecundity was calculated using data obtained from the experiment described earlier. The intrinsic rate of increase (r_{m}), as a composite index of survival, developmental time, and fecundity of the whole population, was estimated using the Euler–Lotka equation (Birch, 1948): $\Sigma\, L_x \mathrm{m}_x \mathrm{e}^{-r_{\mathrm{m}} x^{*}=1}$, where x^* is the midpoint of each age group $= (x_i + x_{i+1})/2$. The values of L_x were taken at the midpoint of each age group as $(l_{xi} + l_{xi+1})/2$, and m_x is number of female offspring per female at the midpoint of each age group. The value of r_{m} was estimated by substituting trial values in the above equation. After r_{m} was calculated for the original data (r_{all}), differences in r_{m} values were tested for significance by estimating the variances using the jackknife method (Meyer *et al.*, 1986).

Other parameters in the fecundity life table were derived as follows: net reproductive rate ($R_0 = \Sigma L_x\ m_x$), mean generation time ($T = \ln R_0/r_{\mathrm{m}}$), population doubling time ($\mathrm{DT} = \ln 2/r_{\mathrm{m}}$), and finite rate of increase ($\lambda = \mathrm{e}_{r\mathrm{m}}$). Variances of all these parameters were estimated using the jackknife method.

Performance of Episyrphus balteatus

Because the newly emerged larvae are very delicate and difficult to handle, they were left in groups for the first 2 days and allowed to feed on cabbage aphids supplied from colonies reared for at least two generations on each host plant separately. After that, larvae were isolated individually in experimental Petri dishes (9 × 1.5 cm) and fed every other day *ad libitum* with the selected combination of aphid-host plant. Each day the experimental Petri dishes were observed and survival recorded, continuing through the pupation period to emergence. Developmental time was then calculated. The resulting adult females were reared for 24 h after emergence and their potential fecundity was then measured by dissecting and counting the number of ovarioles (both ovaries).

Individual fitness (r) was calculated as a performance measure (McGraw & Caswell, 1996; Sadeghi & Gilbert, 2000b) by integrating development time (D), survival [m, either 1 (survived) or 0 (died)] and potential fecundity (V) via the equation: $r = [\ln(mV)]/D$. Fitnesses were calculated for surviving females only and for all females including non-survivors. Because female larvae/pupae cannot be differentiated from males, we assumed half the mortality to be female, rounding up when an odd number had died.

Oviposition preferences were determined in the laboratory using females of known age. One culture of males and females of *E. balteatus* obtained from a cohort of larvae of the same age (supplied from laboratory stock culture) was established initially. After 3–4 days, mating occurred and the ovaries began to enlarge about 7–10 days after emergence. Usually at 12 days after emergence, the majority of females are ready to lay eggs, but to be certain that all females were ready, oviposition preference tests began 15 days after eclosion. Females were initially naive, having had no previous exposure to the test aphids. Test aphids were then introduced to singly-caged females on a potted host plant (plastic pots, 10 cm diameter, 15 cm height, filled with the same mixture of soil used for rearing plants) and great care was taken to provide equal aphid densities, by making sure that all potted plants were approximately the same age and size (15–20 cm tall with four to five fully expanded leaves) and infested with 40 aphids (of various instars).

Singer (1986) discussed different techniques for measuring preference, concluding that many insects cannot perceive more than one host at a time, encountering them sequentially even when they are presented simultaneously. Courtney *et al.* (1989) and Sadeghi and Gilbert (2000a, 2000b, 2000c) followed this idea and proposed either a no-choice or a sequential-choice test as the best design for testing the prediction of whether an insect is more or less likely to accept a particular food. They pointed out that if a multiple choice test is used, then indeed relative preferences are assessed instead of acceptability of the given host/prey. Thus each day, aphids were presented in a randomised sequence to each female (i.e. a no-choice situation, with only one aphid–plant combination available at any time). Each presentation of an aphid–plant combination lasted for 45 min. The number of eggs laid on each aphid–plant combination was counted and it was then replaced by the other treatment. Each day, both aphid–plant combinations were offered to all test females; presentations continued for 10 successive days (the period during which most oviposition occurs).

Statistical analysis

Life table parameters of the cabbage aphid for two treatments were compared using a *t*-test in the statistical software SAS version 9.2 (SAS Institute, Cary, NC, USA). Data were transformed where appropriate to satisfy assumptions of normality and homogeneity of variance for ANOVA. A Mann–Whitney test was used for the analysis of syrphid performance measures: because this assumes equal dispersion of data within the groups, and the data were uniformly zero for one group, we tested whether the other group was different from zero in a one-sample Mann–Whitney test (equal to a one-sample Wilcoxon rank sum test) using R software (R Core Team, 2011).

To analyse the oviposition preference of female syrphids, because each egg was laid separately, we treated each egg as an independent event and hence used a binomial GLM using R to test for differences among individual females, and for an effect of time: these factors are known to affect selectivity of females (Sadeghi & Gilbert, 1999, 2000c). The mean selectivity of females for cabbage aphids on canola at the start of the oviposition testing was obtained from the 'intercept' via back-transforming the logistic transformation. Thereafter, to test whether any significant choice (preference) existed between two host/prey treatments, a paired *t*-test was carried out using SAS, with the data being the overall proportion of eggs laid on one host/prey minus the proportion laid on the other, for each female.

Results

Sinigrin was detected in high concentrations in *B. nigra* in both leaf samples (mean ± SE $10.83 \pm 0.337\ \mu\text{mol g}^{-1}$) and aphids reared on this host plant (145.7 ± 15.6 nmol per aphid), but no sinigrin was detected in *B. napus* in either the leaf samples or the aphids reared on this host.

The intrinsic rate of increase (r_m) of *B. brassicae* was significantly higher for aphids reared on *B. napus* than for those reared on *B. nigra* (Fig. 1a; $t = 3.17$, d.f. = 39, $P = 0.003$), because of the faster development, higher daily progeny production (Fig. 2a), and higher survivorship (Fig. 2b). Aphids in the *B. napus* treatment had a significantly higher net reproductive rate (R_0) than those in the *B. nigra* treatment (Fig. 1b; $t = 2.78$, d.f. = 39, $P = 0.008$), and a longer mean generation time (Fig. 1c; $t = 2.31$, d.f. = 39, $P = 0.03$) and finite rate of increase (Fig. 1d; $t = 2.66$, d.f. = 39, $P = 0.01$). Doubling time was significantly longer when aphids were reared on *B. nigra* than when they were reared on *B. napus* (Fig. 1e; $t = 2.18$, d.f. = 39, $P = 0.04$).

The values of the performance measures of *E. balteatus* fed on *B. brassicae* reared on black mustard and canola are shown in Table 1. There was a highly significant difference in performance measures between the two host plant groups. As a consequence of the absence of harmful secondary metabolites, all hoverfly larvae fed on canola-grown aphids achieved the pupal stage and most emerged as adults, whereas nearly all of those fed on aphids on black mustard died before pupation and those that did pupate did not emerge as adults (Fig. 2c).

The oviposition preference of females did not vary significantly among individuals, but females became less choosy with time (Fig. 3a). In relation to the overall proportion of eggs laid per female, there was a significant preference for cabbage aphids on canola (Fig. 3b; $t = 11.86$, d.f. = 11, $P < 0.0001$).

Discussion

The performance of a natural enemy should be positively correlated with the performance of its host or prey (Bernays & Graham, 1998; Sznajder & Harvey, 2003), and this is exactly what we found. The relative performance of the aphidophagus hoverfly *E. balteatus* when fed cabbage aphids reared on two host plants differing greatly in sinigrin content reflected the relative performance of the aphid on these host plants. The cabbage aphid performed better on canola, and probably as a consequence of the absence of harmful secondary metabolites, especially sinigrin, the performance of the predator on this aphid reared on canola was clearly better too.

The syrphid could not complete development on *B. brassicae* reared on black mustard, while its survival rate when fed this

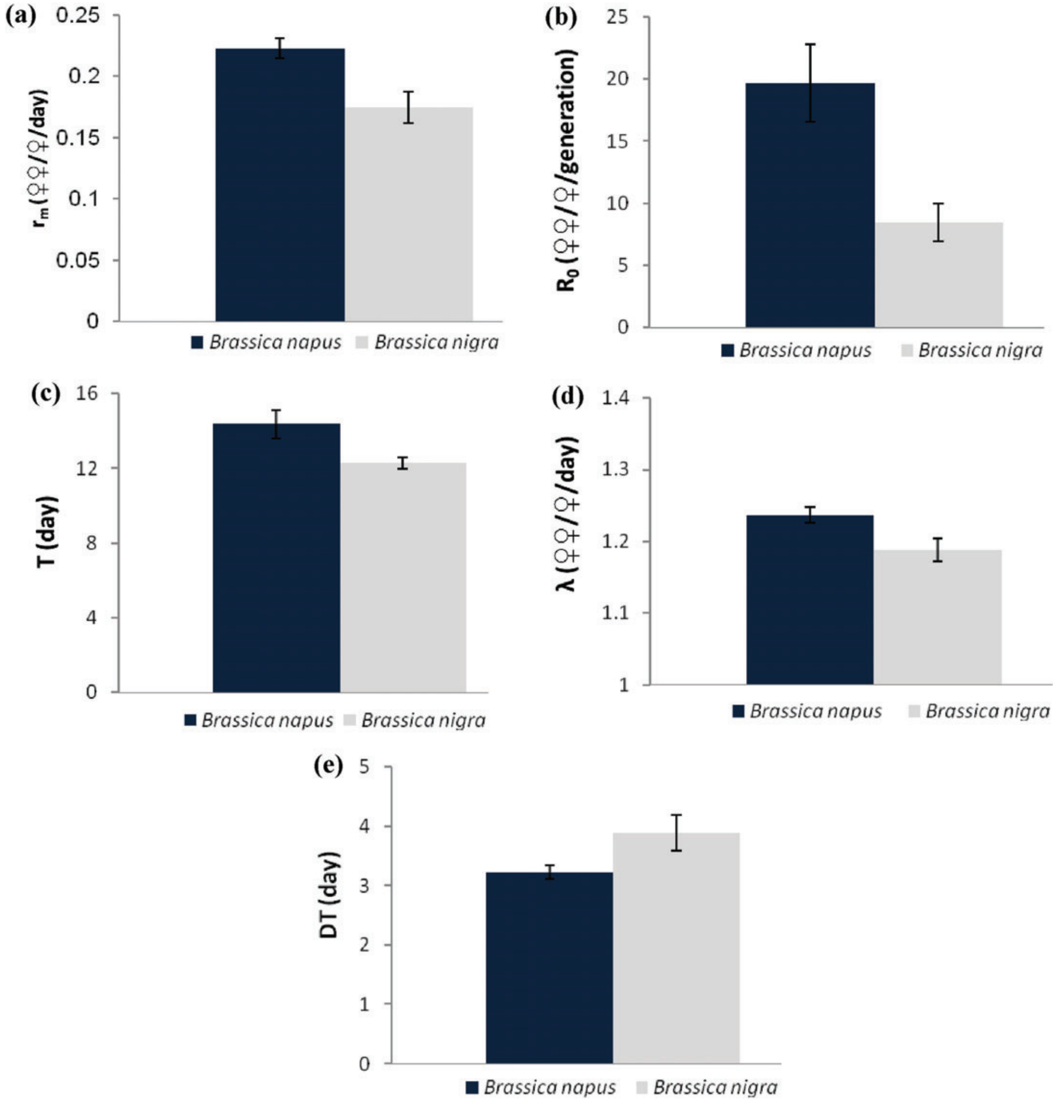

Fig. 1. Effect of host plant (*Brassica napus* vs. *Brassica nigra*) on fertility life table parameters of *Brevicoryne brassicae* (means ± SE). (a) r_{m}, intrinsic rate of increase; (b) R_0, net reproductive rate; (c) T, mean generation time; (d) λ, finite rate of increase; (e) DT, doubling time.

aphid reared on canola was about 85%. Although the two *Brassica* species differ in many ways, we suspect that the observed difference in predator performance can be attributed, to an important extent, to the dominant presence and concentration of the glucosinolate sinigrin and its hydrolytic products in *B. nigra*. Cabbage aphid on black mustard sequesters sinigrin in high concentrations (e.g. Francis *et al.*, 2001; Chaplin-Kramer *et al.*, 2011; Kos *et al.*, 2011) and, as it contains its own aphid-specific myrosinase (Hopkins *et al.*, 2009), it is able to hydrolyse the sinigrin in its body upon damage by predators. When the aphid is reared on a host plant without sinigrin, it can no longer harm its predator. Thus sinigrin can contribute to the antagonistic relationship between some plants and insect predators.

These findings are supported by other studies. For example, predatory stinkbugs given caterpillars reared on some of the allelochemicals found in tomato leaves took longer to develop and were smaller in size (Stamp *et al.*, 1991; Traugott & Stamp, 1996). Prey fed milkweed seeds (containing cardenolides) reduced the consumption and growth rate of their praying mantid predators (Paradise & Stamp, 1993). Francis *et al.* (2001) showed that toxicity of *B. brassicae* for *Adalia bipunctata* larvae increases with the increasing glucosinolate content of the host plant. The fitness of the hoverfly *E. balteatus* was significantly affected when fed *B. brassicae* reared on the crucifer *Sinapis alba*, which has high levels of glucosinolates (Vanhaelen *et al.*, 2002). The performance of predators fed the brassica specialist aphid *B. brassicae* reared on different white cabbage cultivars was lower than when fed a non-sequestering generalist aphid (*Myzus persicae*) reared on the same plants: in this experiment, differences in predator performance matched differences in glucosinolate profiles among the aphids (Kos *et al.*,

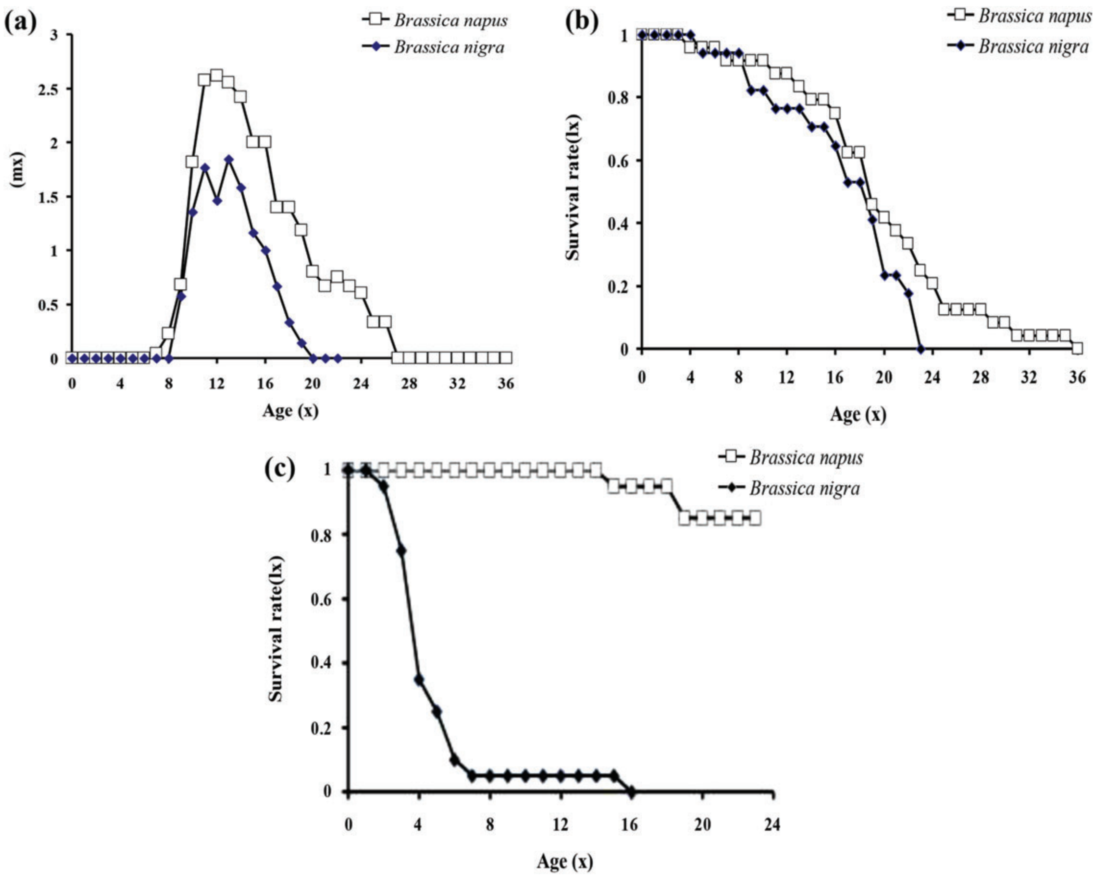

Fig. 2. (a, b) Mean daily fecundity (a) and survival rate (b) of *Brevicoryne brassicae* on two host plants. (c) Survival rate of *Episyrphus balteatus* from egg hatching until adult emergence.

Table 1. The performance measures of *Episyrphus balteatus* fed on *Brevicoryne brassicae* reared on black mustard *Brassica nigra* and canola *Brassica napus*.

Measure	Black mustard	Canola	Test statistic
Larval survival (%)	0.05 ± 0.05 (20)	100 ± 0.00 (20)	$V = 210$***
Survival to emergence (%)	0 (20)	85 ± 0.08 (20)	$V = 153$***
Fecundity (ovariole number)	0 (20)	50.4 ± 1.59 (10)	$V = 55$**
Fitness, r of survivors (day^{-1})	0 (20)	0.198 ± 0.003 (10)	$V = 55$***
Fitness, r, all individuals (day^{-1})	0 (20)	0.165 ± 0.018 (12)	$V = 55$**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Each value in the table gives the mean ± SE, with the sample size in brackets. All means between two treatments are compared by Mann–Whitney test.

2011). However, other studies show that under some conditions allelochemical-fed prey have little or no effect on some invertebrate predators (Malcolm, 1992; Osier *et al.*, 1996; Stamp *et al.*, 1996). Whether allelochemical-fed prey have a negative impact on invertebrate predators or not depends on various factors, e.g. the detoxification ability of the predator (Yu, 1987), the concentration of the allelochemical in the diet of the prey (Traugott & Stamp, 1996), prey species (Drummond *et al.*, 1984; Kos *et al.*, 2011), plant cultivar (Kos *et al.*, 2011) and the age of the insect (Stamp *et al.*, 1996).

If a generalist predator suffers a reduction in fitness when it has a diet of prey containing detrimental plant chemicals, as was observed in the present study, it would be expected that adult females avoid selecting those kinds of prey. In this experiment, a small percentage of eggs of *E. balteatus* were laid on black mustard aphid containing sinigrin. This unexpected response might simply be a result of errors made by females, but there are several factors that could also affect oviposition behaviour. For example, Sadeghi and Gilbert (2000c) observed that age and physiological condition of females (such as egg load) affect oviposition behaviour of aphidophagous hoverflies. Moreover, as has been suggested by Bridges *et al.* (2002), natural enemies of specialist crucifer-feeding insects are probably adapted to toxic glucosinolate hydrolysis products of their hosts. This suggestion has been supported by other studies: for example, Pratt (2008) reported that the polyphagous ladybird *Coccinella septempunctata* is able to develop successfully on *B. brassicae* as well as on *M. persicae* (which does not sequester glucosinolates); although lacking

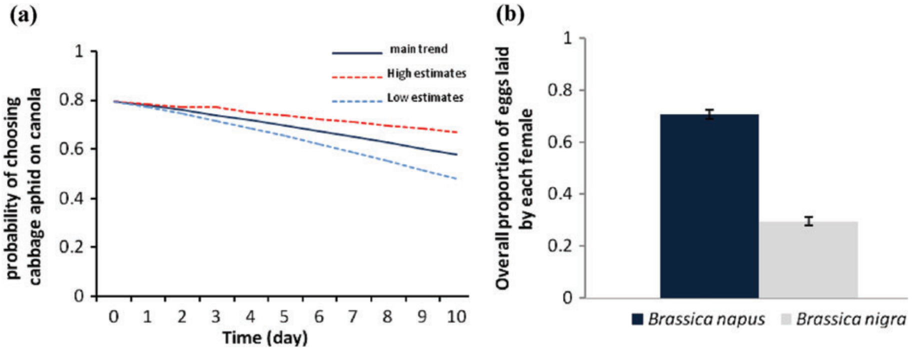

Fig. 3. (a) Relationship between selectivity of females for *Brevicoryne brassicae* on *Brassica napus* and time; (b) mean ± SE of proportion of eggs laid on each host/prey by each female during 10 successive days.

myrosinases that could break down the glucosinolates in the gut into toxic breakdown products, instead it excretes them in the honeydew (Francis *et al.*, 2001).

Differences in performance of predatory insects are influenced by diet breadth. In the study by Pratt (2008), the presence of sinigrin in the diet of *B. brassicae* made this aphid unsuitable as a food source for *A. bipunctata* but not for *C. septempunctata*. As we have shown (Fig. 3b), females had a higher overall preference (about 70% vs. 30%) for cabbage aphids reared on canola compared with those reared on black mustard. Although naive females had a high preference (about 80%), with time (increasing age) this preference declined (Fig. 3a). In a field experiment, Chaplin-Kramer *et al.* (2011) observed that aphidophagous syrphids were found at far lower densities in *B. nigra* patches than in nearby crop plants (*Brassica oleracea*). In contrast, Newton *et al.* (2009) could not find any difference in syrphid larval abundance on wild *B. oleracea* phenotypes differing in the presence and absence of the glucosinolate sinigrin. As the sinigrin content was not quantified in the latter study, the observed result could have arisen because the plants lacked an adequate concentration of sinigrin to escape the natural enemy.

Our performance measures showed significantly lower values of demographic parameters when *B. brassicae* was reared on *B. nigra* rather than on *B. napus*. Ulusoy and Olmez-Bayhan (2006) also observed that, when reared on a wild brassica species (*Sinapis arvensis* L.), *B. brassicae* had lower reproductive rates than when reared on a cultivated brassica species (*B. oleracea*). In contrast, Chaplin-Kramer *et al.* (2011) found that *B. brassicae* performed better when reared on *B. nigra* than on *B. oleracea*. To explain this discrepancy, it has been suggested that to understand the mechanism governing these biological effects, it would be better to consider the specific glucosinolate compounds rather than the overall total content (Chaplin-Kramer *et al.*, 2011). Cole (1997) demonstrated that the intrinsic rate of increase of cabbage aphid depended on the type of glucosinolate compound present in the host plant, and that amino acid concentrations also influenced aphid performance on brassica plants. Extensive studies on the performance of aphids, mainly using chemically defined diets, have revealed the central roles of sucrose concentration, amino acid concentration and composition, and the sucrose:amino acid ratio in shaping aphid performance (Auclair, 1963; Dadd, 1985; Douglas, 1993; Kazemi & van Emden, 1992; Sandström & Pettersson, 1994; Karley *et al.*, 2002). However, a study by Weibull and Melin (1990) showed that the suitability of *Brassica juncea*, *B. napus* and *Brassica campestris* as hosts for the mustard aphid, *Lipaphis erysimi*, was found to be unrelated to the composition or concentrations of amino acids. This suggests that differences in aphid performance might be due to factors other than nutritional status of the phloem sap. Information on the plant nutritional factors that influence aphid performance might lead to the development of crop management practices that aid aphid pest management.

In conclusion, our data on the performance of *B.brassicae* reared on a wild and on a cultivated brassicaceous plant, and on the preference and performance of their generalist predator *E. balteatus*, is consistent with the enemy-free space hypothesis, which states that insect herbivores escape their enemies by being scarce in space and time, and/or chemically defended via sequestering plant allelochemicals. However, an offset effect could exist in the studied system because of observed decrease in the performance of the test aphid when it was reared on wild brassica species (black mustard) compared with cultivated brassica species (canola). Several factors are involved in creating enemy-free space, e.g. adaptations in morphology and size, position, interspecific interaction, visibility and chemistry (Berdegue *et al.*, 1996; Chaplin-Kramer *et al.*, 2011). Thus host plant chemistry via their effects on enemies is just one of several potential factors that could confer enemy-free space on invertebrate herbivores.

The ability of aphids reared on plants with high glucosinolate content to reduce the performance of their generalist predators indicates that weedy *B. nigra* may provide enemy-free space for the cabbage aphid from one of its main predators, *E. balteatus*. An important consideration for pest control may be the potential for non-crop plants occurring in and around crop systems to provide a reservoir for aphid pests where natural enemies cannot suppress them, as well as to reduce populations of biocontrol agents through the provision of an ecological trap.

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