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Accumulation of Glucosinolates by the Cabbage Aphid *Brevicoryne brassicae* as a Defense Against Two Coccinellid Species

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Abstract *Brassica nigra* plants, characterized by high levels of sinigrin, and artificial aphid diets to which sinigrin was selectively added were used to rear the crucifer specialist, *Brevicoryne brassicae*. Aphids were provided as a food source to two species of polyphagous ladybird, *Adalia bipunctata* and *Coccinella septempunctata*. First instar *A. bipunctata* were unable to survive when fed with *B. brassicae* reared on *B. nigra* or diets containing 0.2% sinigrin, but when fed with aphids reared on diets containing 0% sinigrin, survival rates were high. By contrast, first instar *C. septempunctata* were able to survive when fed with aphids reared on *B. nigra* or artificial diets containing up to 1% sinigrin. However, the presence of sinigrin in the aphid diet decreased larval growth and increased the time necessary for larvae to reach second instar for this species of ladybird. These results indicate that the presence of sinigrin in the diet of *B. brassicae* makes this aphid unsuitable as a food source for *A. bipunctata* but not for *C. septempunctata*, although for this ladybird species, there appear to be costs associated with feeding on aphids that contain this secondary metabolite.

Keywords *Adalia bipunctata* · *Coccinella septempunctata* · Glucosinolate · Chemical defense · Tritrophic interactions

Introduction

Secondary metabolites are important for plant survival in the environment, forming a chemical defense against pests and diseases (Wink 1988; Jander et al. 2001; Kliebenstein et al. 2005). Glucosinolates (anionic thioglucosides) are the main secondary metabolites accumulated by cruciferous plants (Brassicaceae). The plants also possess a myrosinase (β-thioglucoside glucohydrolase, EC 3.2.3.1; Bones and Rossiter 1996, 2006; Halkier and Gershenzon 2006). These two components are spatially segregated (Kelly et al. 1998; Koroleva et al. 2000) but are brought together upon attack by a pest or pathogen; glucosinolates are then hydrolyzed to biologically active products including nitriles, epithionitriles, thiocyanates, and isothiocyantes (Bones and Rossiter 1996, 2006; Halkier and Gershenzon 2006).

Despite this potent defense, crucifer specialists have evolved in several insect orders with counter adaptive biochemical mechanisms that allow feeding on plants that contain glucosinolates (Ratzka et al. 2002; Wittstock et al. 2004). The cabbage aphid *Brevicoryne brassicae* (L.) and the turnip aphid *Lipaphis pseudobrassicae* (=*erysimi*) (Kaltenbach) are not only able to feed on crucifers but have also developed a chemical defense system that exploits and mimics that of their host plants (Bridges et al. 2002; Kazana et al. 2007). These two aphid species are able to accumulate glucosinolates from their host plants and produce their own myrosinase that is compartmentalized into crystalline microbodies, thus avoiding internal glucosinolate hydrolysis under normal conditions. However, tissue damage as a result of attack by a predator results in production of hydrolysis products such as isothiocyanates (Francis et al. 2001; Kazana et al. 2007). Therefore, the aphid mimics the chemical defense system of its host plants and probably derives benefit in terms of protection from natural enemies. In addition, isothiocyanates also have been shown to synergize the response of *L. erysimi* to the aphid alarm pheromone, *E*-β-farnesene (Dawson et al. 1987) and may play a role in dispersing aphid colonies after attack.

Aphidophagous coccinellids have been used extensively in biological control programs for the control of aphid pests

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(Obrycki and Kring 1998). Indeed, the seven-spot ladybird, *Coccinella septempunctata* (L.), is commonly found predating on *B. brassicae* (Acheampong and Stark 2004). By contrast, larval survival rates of the two-spot ladybird, *Adalia bipunctata* (L.), fed with *B. brassicae* reared on a range of crucifer host-plants were low (Francis et al. 2001). However, *A. bipunctata* larvae were able to develop when fed with peach-potato aphids, *Myzus persicae* (Sulzer), reared on the same species of crucifer. This result may reflect the fact that whereas *B. brassicae* accumulates glucosinolates from its host, *M. persicae* appears to excrete these compounds in the honeydew (Weber et al. 1986; Merritt 1996). In addition, *M. persicae* lacks myrosinase activity and, therefore, does not produce toxic hydrolysis products when attacked (Francis et al. 2001).

The use of a range of crucifer host plants on which *B. brassicae* were reared and fed to *A. bipunctata* larvae indicates that the myrosinase–glucosinolate system may be central to the aphids' defense against this natural enemy (Francis et al. 2001). In this study, an artificial aphid diet system was used to manipulate the levels of glucosinolate ingested by *B. brassicae.* The aphids were fed to *A. bipunctata* larvae to investigate the effects of glucosinolates on the next trophic level. This approach was extended to include *C. septempunctata* to determine what, if any, defense glucosinolate accumulation affords *B. brassicae* against this natural enemy.

Methods and Materials

Artificial Diets Parafilm© "M" (American Can Company, Greenwich, CT, USA) was stretched over circular curtain rings of 25 mm diameter. Three hundred microliters of an artificial aphid diet (see Diet B in Dadd 1967), to which 0.0, 0.2, 0.4, 0.6, 0.8, or 1.0% sinigrin had been added, was applied to the surface of the Parafilm. A second sheet of Parafilm was stretched over the ring to create a 'sachet' containing the diet. Excess Parafilm was removed, and diet rings were stored in a freezer until required.

Insects B. brassicae and *M. persicae* cultures were each reared on *Brassica nigra* (L.) Koch. Each plant was enclosed within a perforated bread bag and maintained at 18°C with a 16L/8D photoperiod.

B. brassicae were reared on artificial diet sachets by first transferring 5–10 adult wingless aphids to the underside of a diet ring. Diet rings were then placed into Petri dishes, which were covered with semi-transparent green discs to mimic leaf spectral reflectance. The adults were left for 72 hr before being removed. Aphid nymphs produced during this 72-hr period were transferred to fresh diet rings. Aphids used in experiments were undifferentiated nymphs (wing buds not visible), aged between 3–6 d. Aphids were reared on diet rings at 18°C with a 16L/8D photoperiod.

A. bipunctata and *C. septempunctata* cultures were each maintained at 20°C with a 16L/8D photoperiod. Adult ladybirds were kept, in groups of approximately 20, within ventilated sandwich boxes and were fed daily with an excess of pea aphids, *Acyrthosiphon pisum* (Harris), which were reared on tic bean (*Vicia faba* var. *minor* L.) seedlings. Ladybird eggs were collected by transferring three to four *C. septempunctata* or five to six *A. bipunctata* to ventilated 90 mm diameter Petri dishes and were provided with an excess of *A. pisum*. Adult ladybirds were removed after 24 hr, and any eggs laid were checked daily until they had hatched.

Effect of Sinigrin on Growth of B. brassicae Nymphs Adult wingless *B. brassicae* were transferred onto diet rings containing 0% sinigrin, as previously described, except that they were removed after 24 hr. Ten nymphs were selected at random and weighed before being returned to a fresh diet, also containing 0% sinigrin for 5 d. Similarly, 10 *B. brassicae* nymphs were selected, weighed, and then reared for 5 d on diet rings containing 1% sinigrin. All aphids were kept at 18°C with a 16L/8D photoperiod. After 5 d, the aphids reared on the 0 and 1% sinigrin diets were reweighed.

Growth and Survival of A. bipunctata and C. septempunctata Larvae Newly hatched *A. bipunctata* larvae were selected at random from various egg clutches and weighed (Mettler Toledo MX5, Switzerland) and isolated to separate glass tubes (internal diameter 18 mm, height 51 mm). Each larva was assigned a treatment and supplied with an excess of aphids (approximately 7 for *A. bipunctata* larvae and 10 for *C. septempunctata*). Ladybird larval weight and survival was recorded every 24 hr until the first molt was reached or until the larva died. After weighing, larvae were transferred to a clean tube, and fresh aphids were provided. Ten replicates of the experiment were carried out for each treatment. Experiments were conducted at 21°C, 16 hr light-phase/18°C, 8 hr dark-phase regime.

The following three experiments were completed with each ladybird species:

I. Larvae were fed mixed aged *B. brassicae* or *M. persicae* nymphs (aphids had been reared on *B. nigra*).
II. Larvae were fed 3- to 6-d-old *B. brassicae* nymphs reared on artificial diets containing either 0 or 1% sinigrin. An additional 'starved' treatment was included in this experiment to compare the effects of sinigrin with complete absence of food.

III. Larvae were fed 3- to 6-d-old *B. brassicae* nymphs reared on artificial diets containing 0, 0.2, 0.4, 0.6, 0.8, or 1% sinigrin.

Analysis Data were analyzed by using Student's *t* test and chi-square tests with Yates correction with the exception of the experiments investigating the effect of a range of sinigrin concentrations in the artificial aphid diet on ladybird growth, where an analysis of variance was completed using GenStat 8th Edition. Ladybird growth was analyzed by comparing larval weight after 24 hr. There was some variation in the weights of newly hatched ladybird larvae among different experiments, presumably because some batches may have hatched earlier than others, with more opportunity for egg-case consumption and sibling egg cannibalism (Omkar et al. 2007). However, initial larval weight did not differ significantly among treatments within each experiment (data not shown).

Results

Effect of Sinigrin on Growth of *B. brassicae*

Twenty *B. brassicae* nymphs were reared for 5 d on two artificial aphid diets that were identical with the exception that sinigrin was present at one of two levels, 0 and 1%, respectively (Table 1). Initial mean weights of *B. brassicae* nymphs (<24 hr old) born on either artificial diet did not differ significantly. Similarly, after 5 days continued feeding on these two artificial diets, mean nymph weights were not different.

Growth and Survival of *A. bipunctata* and *C. septempunctata* Larvae

Experiment I Weight gain and survival data of newly hatched *A. bipunctata* and *C. septempunctata* larvae fed with either *B. brassicae* or *M. persicae* nymphs reared on *B. nigra* were recorded (Table 2). The weight of *A. bipunctata* larvae fed with *M. persicae* nymphs was significantly higher (t=3.48, P=0.003) than that for larvae fed with *B. brassicae* nymphs after the first 24 hr of the experiment. Survival of *A. bipunctata* larvae was also affected by the aphid species provided as a food source, with 90% of *M. persicae*-fed ladybird larvae surviving to second instar, compared with 0% of larvae fed with *B. brassicae* nymphs. For *C. septempunctata*, the weight of larvae fed *M. persicae* nymphs was also higher (t=3.28, P=0.005) than for larvae fed *B. brassicae* nymphs. By contrast with survival data for *A. bipunctata*, an equal number, 90%, of *C. septempunctata* larvae survived to second instar when fed either *M. persicae* or *B. brassicae*. However, larvae fed *B. brassicae* nymphs took longer (t=3.16, P=0.006) than larvae fed *M. persicae* to reach this stage.

Table 1 Weight gain of *Brevicoryne brassicae* nymphs reared on artificial diets containing 0% or 1% sinigrin

	Sinigrin Content in Aphid Artificial Diet		Significance
	0%	1%	
Mean weight in mg (0 hr)	0.035± 0.002	0.034± 0.002	t=0.32, P=0.753
Mean weight in mg (120 hr)	0.122± 0.006	0.107± 0.006	t=1.74, P=0.100

Mean weights±SE, N=10

Experiment II Newly hatched *A. bipunctata* and *C. septempunctata* larvae were starved or fed with *B. brassicae* nymphs reared on artificial diets containing 0 or 1% sinigrin (Table 3). During the first 24 hr, whether *A. bipunctata* were starved or fed with *B. brassicae* nymphs reared on artificial diets containing 0 or 1% sinigrin significantly affected larval weights (F=16.59, P<0.001). Individual contrasts between means, using least significant difference (LSD) indicated that the mean weight of larvae (after 24 hr) fed *B. brassicae* reared on diet containing 0% sinigrin was significantly higher than for larvae that were starved or fed *B. brassicae* reared on the 1% sinigrin diet. Larvae that were starved or fed *B. brassicae* nymphs reared on diet containing 1% sinigrin had mean weights that did not differ significantly. Of the *A. bipunctata* larvae fed with *B. brassicae* nymphs reared on diet containing 0% sinigrin, 100% survived to second instar. By contrast, 0% of larvae that were starved or fed with *B. brassicae* reared on the 1% diet reached their first molt. Growth of *C. septempunctata* larvae during the first 24 hr of the experiment was also affected (F=36.08, P<0.001) by the food source provided. Analysis of differences between means, using least significant difference (LSD), indicates that, as for *A. bipunctata* larvae, weights after 24 hr for *C. septempunctata* larvae fed with *B. brassicae* reared on artificial diet containing 0% sinigrin were significantly higher than for larvae that were starved or fed *B. brassicae* reared on the 1% sinigrin diet. Weights of larvae fed *B. brassicae* nymphs reared on the 1% sinigrin diet were not significantly different from starved larvae. Although a greater number of *C. septempunctata* larvae fed with *B. brassicae* reared on the 0% sinigrin diet reached second instar compared with larvae fed with *B. brassicae* nymphs reared on the 1% sinigrin diet, this difference was not significant. In addition, *C. septempunctata* larvae took less time to reach second instar when fed with *B. brassicae* reared on the 0% sinigrin diet

Table 2 Survival, mean weight after 24 hr and time to second instar data for *Adalia bipunctata* or *Coccinella septempunctata* first instar larvae fed *Brevicoryne brassicae* or *Myzus persicae* reared on *Brassica nigra*

	Aphid Species		Significance
	Myzus persicae	*Brevicoryne brassicae*	
Adalia bipunctata			
% survival to second instar	90	0	χ^2=11.99, P<0.001
Mean weight after 24 hr (mg)	0.216±0.021	0.134±0.007	t=3.48, P=0.003
No. days to second instar	2.9±0.1	-	N/A
Coccinella septempunctata			
% survival to second instar	90	90	χ^2=0, P=NS
Mean weight after 24 hr (mg)	0.332±0.014	0.276±0.010	t=3.28, P=0.005
No. days to second instar	3.0±0.0	3.6±0.2	t=3.16, P=0.006

Mean weights±SE, N=10; *N/A* not applicable

compared to larvae fed *B. brassicae* reared on diet containing 1% sinigrin (t=6.52, P<0.001). Again, no larvae that were starved reached second instar.

Experiment III Weight gain and survival of *A. bipunctata* and *C. septempunctata* larvae fed with *B. brassicae* nymphs reared on artificial diets containing a range of sinigrin concentrations were recorded (Table 4). After 24 hr of feeding, growth of *A. bipunctata* larvae fed *B. brassicae* reared on artificial diets containing 0, 0.2, 0.4, 0.6, 0.8, or 1% sinigrin differed significantly (F=31.59, P<0.001). Individual contrasts between means, using LSD, indicates that the 24-hr weights of larvae fed *B. brassicae* nymphs reared on the 0% sinigrin diet were higher than for larvae fed *B. brassicae* reared on all other diets. Mean weight of larvae fed *B. brassicae* reared on diet containing 0.2% sinigrin was also higher than for larvae fed aphids reared on diets containing 0.4% sinigrin. However, larval weights did not differ significantly among the other treatments. Only larvae fed with *B. brassicae* reared on artificial diet containing 0% sinigrin reached second instar (90%). Sinigrin content of artificial diets used to rear *B. brassicae* nymphs, which were fed to *C. septempunctata* larvae, did not affect larval weights (F=1.05, P>0.05). This lack of overall significance suggests that individual contrasts between means are not appropriate. However, time to second instar was affected (F=5.20, P<0.001). Individual contrasts between means, using LSD, indicates that *C. septempunctata* larvae fed aphids reared on the 0% sinigrin diet reached second instar significantly faster than larvae fed aphids reared on other diets. However, survival was not affected, with similar numbers of larvae reaching second instar, regardless of the sinigrin content of the diet used to rear the aphids provided as a food source.

Discussion

Survival of *A. bipunctata* larvae fed with *B. brassicae* is known to be affected by the species of cruciferous plant on which the aphids were reared (Francis et al. 2001). This effect has been correlated with glucosinolate content of the host plant. In this study, we demonstrated through the use

Table 3 Survival, mean weight after 24 hr and time to second instar data for *Adalia bipunctata* or *Coccinella septempunctata* either starved or fed *Brevicoryne brassicae* reared on artificial diets containing 0 or 1% sinigrin

	Starved	Sinigrin Content in Aphid Artificial Diet		Significance
		0%	1%	
Adalia bipunctata				
% survival to second instar	0	100	0	χ^2=16.20, P<0.001
Mean weight after 24 hr (mg)	0.092±0.005	0.172±0.014	0.114±0.004	F=16.59, P<0.001
No. days to second instar	-	2.8±0.1	-	N/A
Coccinella septempunctata				
% survival to second instar	0	100	80	χ^2=0.56, P<0.456
Mean weight after 24 hr (mg)	0.150±0.002	0.415±0.036	0.209±0.014	F=36.08, P<0.001
No. days to second instar	-	2.7±0.2	5.4±0.4	t=6.52, P<0.001

Mean weights±SE, N=10; *N/A* not applicable.

Table 4 Survival, mean weight after 24 hr and time to second instar data for *Adalia bipunctata* or *Coccinella septempunctata* fed *Brevicoryne brassicae* reared on artificial diets containing a range of concentrations of sinigrin

	Sinigrin Content in Aphid Artificial Diet						Significance
	0%	0.2%	0.4%	0.6%	0.8%	1.0%	
Adalia bipunctata							
% survival to second instar	90	0	0	0	0	0	N/A
Mean weight after 24 hr (mg)	0.220±0.013	0.130±0.006	0.101±0.008	0.116±0.004	0.108±0.007	0.108±0.005	F=31.59, P<0.001
No. days to second instar	2.8±0.1	–	–	–	–	–	N/A
Coccinella septempunctata							
% survival to second instar	100	90	90	80	70	80	χ^2=1.57, P=0.905
Mean weight after 24 hr (mg)	0.324±0.039	0.289±0.029	0.263±0.019	0.264±0.020	0.256±0.022	0.256±0.022	F=1.05, P=NS
No. days to second instar	3.7±0.2	4.4±0.3	4.7±0.2	5.0±0.2	4.9±0.1	5.0±0.3	F=5.20, P<0.001

Mean weights±SE, N=9 for *A. bipunctata* and 10 for *C. septempunctata*; *N/A* not applicable

of artificial aphid diets how the presence of a single glucosinolate, sinigrin, affects survival of first instar *A. bipunctata*. By contrast, survival of larvae of a second polyphagous species of ladybird, *C. septempunctata*, was not compromised by the presence of sinigrin in the diet of *B. brassicae*. However, more subtle costs are apparent with extended development times in larvae fed *B. brassicae* reared on diets containing sinigrin compared with larvae fed with aphids reared on diets without sinigrin.

When aphids were reared on *B. nigra*, survival rates of first instar *A. bipunctata* larvae were 90% when fed with *M. persicae*, but 0% when fed with *B. brassicae*, confirming the earlier findings of Francis et al. (2001). The apparent suitability of the generalist *M. persicae* as a food source for *A. bipunctata* may reflect the fact that this species of aphid, although able to colonize crucifers, does not accumulate glucosinolates (Weber et al. 1986). Indeed, when *M. persicae* were fed to *A. bipunctata*, the species of cruciferous plant on which the aphids were reared did not significantly affect larval mortality (Francis et al. 2001). By contrast, the crucifer specialist, *B. brassicae*, not only accumulates glucosinolates in the hemolymph (Kazana et al. 2007) but also possesses, like its host plants, the ability to hydrolyze these secondary metabolites to biologically active products including isothiocyanates (MacGibbon and Allison 1968; Bridges et al. 2002). As these hydrolysis products are known to be toxic to both insects and fungi, it has been suggested that they may provide a direct defense against generalist natural enemies (Bridges et al. 2002; Bones and Rossiter 1996, 2006) as appears to be the case in this study for *A. bipunctata*. Air entrainments show that aphids reared on a glucosinolate-containing diet certainly release isothiocyanate when attacked by foraging ladybirds (Kazana et al. 2007).

Bridges et al. (2002) also suggested that as with specialist crucifer-feeding insects, natural enemies of these herbivores are probably adapted to toxic glucosinolate hydrolysis products. The polyphagous ladybird *C. septempunctata* is known to successfully predate upon *B. brassicae* (Blackman 1967), and results presented in this paper confirm that first instar *C. septempunctata* are able to develop successfully on both *M. persicae* and *B. brassicae*. However, *C. septempunctata* larvae performed better, in terms of weight after the first 24 hr of the experiment and time to second instar, when fed *M. persicae* as opposed to *B. brassicae*.

Results from Experiment I and the earlier work by Francis et al. (2001) suggest a possible direct defensive role for glucosinolates accumulated and hydrolyzed by *B. brassicae*. However, by providing *M. persicae* and *B. brassicae* as a food source, it is not possible to discriminate between aphid morphology, behavior, or chemical composition as possible explanations for the observed differences in ladybird performance (Omkar 2005). Therefore, subsequent experiments were completed where *A. bipunctata* and *C. septempunctata* larvae were fed with *B. brassicae* reared on artificial aphid diets to which a glucosinolate, sinigrin, was selectively added.

Results from these experiments confirm that the presence of sinigrin in the diet of *B. brassicae* results in this aphid becoming unsuitable as prey for *A. bipunctata* larvae. Indeed, whereas 100% of larvae tested were able to reach second instar when fed *B. brassicae* nymphs reared on a diet containing 0% sinigrin, no larvae were able to develop when fed nymphs reared on a 1% sinigrin diet. The presence of sinigrin in aphid diets had such a strong inhibitory effect on *A. bipunctata* that larval growth and survival were similar to those insects that were assigned to the starved treatment and completely deprived of aphid food. To confirm the suitability of *B. brassicae* reared on a diet containing 0% sinigrin for *A. bipunctata*, a small number of larvae were allowed to continue feeding on this group of aphids, and the predators then successfully completed their development (unpublished observations).

C. septempunctata larvae were able to predate upon *B. brassicae*, when fed aphids reared on diets containing either 0 or 1% sinigrin. However, the presence of the glucosinolate in the aphid diet appears to have consequences for the performance of this ladybird species. Larval weights were greater and time required to reach second instar shorter for *C. septempunctata* larvae supplied with *B. brassicae* reared on a 0% sinigrin diet compared with larvae fed aphids reared on a 1% sinigrin diet. However, there was no difference in survival of first instars fed with *B. brassicae* reared on diets containing either 0 or 1% sinigrin. By contrast with *A. bipunctata*, first instar *C. septempunctata* appear to have a mechanism that at least partially negates the effects of sinigrin or the toxic hydrolysis products produced by *B. brassicae* after tissue damage. It is, however, unclear from these data whether the mechanism involved is based on tolerance or detoxification. Interestingly, glutathione transferase levels in *A. bipunctata* increase after exposure to isothiocyanates (Francis et al. 1999).

A. bipunctata larvae did not reach second instar when fed *B. brassicae* nymphs reared on artificial diets containing 0.2, 0.4, 0.6, 0.8, or 1% sinigrin. Again, only when fed aphids reared on the 0% sinigrin diet were *A. bipunctata* larvae able to develop. The higher weight of larvae fed *B. brassicae* reared on a diet containing 0.2% sinigrin compared with larvae fed aphids reared on diet containing 0.4% sinigrin indicates that *A. bipunctata* can perhaps tolerate low levels of allyl isothiocyanate to some extent. However, given an estimated phloem sinigrin content of >0.4% in *B. nigra* (Merritt 1996), it is perhaps not surprising that *A. bipunctata* larvae were unable to survive when fed with *B. brassicae* reared on this host-plant.

Growth (after 24 hr) and survival of *C. septempunctata* larvae fed *B. brassicae* was not significantly affected by sinigrin content of the diet on which the aphids were reared. However, costs were apparent (in terms of extended development time) when larvae were fed *B. brassicae* reared on any of the diets containing sinigrin. There is some evidence that this cost increased with increasing concentration of sinigrin added to the artificial diet. Indeed, when diets containing 0, 0.2, and 0.4% sinigrin are considered, times to second instar were 3.7, 4.4, and 4.7 days, respectively. Interestingly, these trends appear to level out when larvae were fed *B. brassicae* reared on diets containing higher concentrations of sinigrin. Thus, the level of defense afforded to 3- to 6-d-old *B. brassica* nymphs through the accumulation of sinigrin appears to be a function, over a limited range, of the concentration of sinigrin present in the aphid's diet. The precise relationship between dietary sinigrin concentration and the level of this glucosinolate in aphid body tissues has not been investigated. It has been shown that wingless *B. brassicae* contain approximately 3.5 times higher levels of sinigrin when reared on 1% than on 0.1% (Kazana et al. 2007). Over the range of sinigrin concentrations tested, it is qualitative aspects of host-plant chemistry that determine the interaction between *B. brassicae* and *A. bipunctata*, whereas quantitative factors may be important in determining the interaction between this species of aphid and *C. septempunctata*.

By contrast with the results for the two species of ladybird, rearing *B. brassicae* on artificial diets that contain either 0 or 1% sinigrin had no effect on the weight gain of nymphs. Studies have previously demonstrated that sinigrin acts as a strong phagostimulant to *B. brassicae* (Wensler 1962; Nault and Styer 1972). However, the fact that aphids did not perform better when feeding on diet containing 1% sinigrin vs 0% sinigrin diet may reflect the artificial conditions encountered by aphids probing through Parafilm. Indeed, when probing plants, *B. brassicae* may be able to recognize host plants when the stylets make contact with mesophyll tissue, before the phloem is reached (Gabrys and Tjallingii 2002).

Results from this study indicate that accumulation of sinigrin and production of allyl isothiocyanate by *B. brassicae* appears to affect negatively the performance of *C. septempunctata* by slowing development. Use of crops with lower levels of glucosinolates may, therefore, enhance the performance of *C. septempunctata*, making this species perhaps more effective as a biological control agent for *B. brassicae*. However, crops with lower levels of glucosinolates may bring an increased risk from generalist herbivores (Raybould and Moyes 2001).

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