Article (refereed) - postprint

2012 Predator mortality depends on whether its prey feeds on organic or conventionally fertilised plants. Biological Control, 63 (1). 56-61.
10.1016/j.biocontrol.2012.05.008

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Predator mortality depends on whether its prey feeds on organic or conventionally fertilised plants

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Keywords: Adalia bipunctata; Brevicoryne brassicae; glucosinolate; Myzus persicae; pest control; sustainable agriculture;

Running head: Fertiliser alters predator mortality
Abstract

Natural enemy abundance and diversity can be increased under sustainable farming systems, but this has not been shown to consistently increase predation and parasitism rates or decrease herbivore abundance. ‘Top-down’ regulation of herbivore populations may depend on ‘bottom-up’ factors such as plant quality, and not solely on predator diversity or abundance. Specialised herbivore species can sequester secondary chemicals from plants to use in a defensive system against predators which mimics that of their host plants, but this herbivore defence may vary with the concentration of plant defences. We investigated whether fertiliser type and concentration alter the mortality of coccinellids feeding on two aphid species from Brassica plants growing in fertilisers typical of organic and conventional farming systems, due to differences in concentrations of defensive glucosinolate compounds cascading up the food chain. Coccinellid larval mortality was 10% higher when feeding on aphids from synthetically fertilised plants compared with those in organic fertilisers, regardless of the aphid species. Concentrations of both constitutive foliar glucosinolates, and those induced by aphids, varied with fertiliser type but this did not affect the glucosinolate concentrations sequestered by the aphids. The efficacy of predators as biological control agents may thus differ between conventional and sustainable farming systems.
1. Introduction

Interest in sustainable farming systems has led to comparisons of herbivore, predator and parasitoid invertebrate communities under organic and conventional agriculture in the context of both increased biodiversity and enhanced pest control (Letourneau and Bothwell, 2008; Macfadyen et al., 2009). Although species richness and abundance of predators and parasitoids can be increased under organic farming systems, this has not been shown to translate into a consistent reduction in populations of herbivores (Garratt et al., 2011; Letourneau and Bothwell, 2008), or an increase in rates of predation and parasitism (Garratt et al., 2010a; Macfadyen et al., 2009). The use of biological control programmes or measures to enhance natural enemy diversity assume that herbivore populations are regulated from the ‘top-down’ by their natural enemies (Hairston et al., 1960). A growing body of evidence shows that bottom-up factors such as plant quality can interact to affect the efficacy of natural enemies (Chaplin-Kramer et al., 2011; Price et al., 1980), but this has not been investigated in the context of organic and conventional farming systems.

Insect herbivore species differ in their response to the types of fertiliser used in organic and conventional agriculture, with some showing increased abundance on plants grown in synthetic fertilisers (Alyokhin et al., 2005; Garratt et al., 2010b; Ponti et al., 2007), while others are more abundant on plants in organic fertilisers (Culliney and Pimentel, 1986) or show no effect (Bengtsson et al., 2005; Costello and Altieri, 1995; Letourneau et al., 1996). This may be due to differences in herbivore species responses to plant structure or nutritional quality. For example, a specialist aphid feeding on Brassicas had increased abundance on plants grown in organic animal manure which had three times the concentrations of secondary metabolites (glucosinolates) found in synthetically fertilised plants, while
populations of a generalist aphid were reduced (Staley et al., 2010). These changes to plant
defensive chemistry in response to fertiliser type have been shown to alter competition
between two herbivore species (Staley et al., 2011) and also have the potential to alter
interactions between natural enemies and their prey.

Predators and parasitoids use volatile organic compounds (VOCs) emitted by the plant to
locate their prey. These VOCs are breakdown products of secondary metabolites emitted by
plants in response to herbivore feeding damage (Hopkins et al., 2009; Vet and Dicke, 1992).
Some herbivore species have the ability to sequester these defensive chemicals, to use in their
own defence against predators (Ratzka et al., 2002; Winde and Wittstock, 2011). The
specialist Brassica aphid, *Brevicoryne brassicae*, has a defence system which mimics that of
its host plants. *Brevicoryne brassicae* produces an enzyme (myrosinase), which catalyses the
hydrolysis of glucosinolates to potentially toxic isothiocyanates (Kazana et al., 2007). The
aphid accumulates sinigrin and other glucosinolate compounds, which are present in higher
concentrations in the aphid than its host plant. If *B. brassicae* is attacked by a predator the
myrosinase comes into contact with glucosinolates and volatile isothiocyanates are released
(Francis et al., 2001b) resulting in high mortality of the first instar of the coccinellid
*Adalia bipunctata* (Kazana et al., 2007; Pratt et al., 2008). By contrast, a more generalist
aphid species (*Myzus persicae*) does not accumulate high concentrations of glucosinolates,
and mortality of *A. bipunctata* larvae was lower when fed *M. persicae* compared with
*B. brassicae* (Francis et al., 2001b). Some predators of *B. brassicae* also have reduced
performance if feeding on hosts that have developed on plant species or cultivars with high
concentrations of glucosinolates (Chaplin-Kramer et al., 2011; Kos et al., 2011), suggesting a
direct link between plant glucosinolate concentration and herbivore defence against their
natural enemies.
The concentration and type of fertiliser supplied to a plant can alter the concentration of foliar glucosinolates. For example, four of five glucosinolate compounds had higher concentrations in *Brassica oleracea* grown in organic fertilisers compared to synthetic fertilisers, in a field experiment (Staley et al., 2010). This may be because organic fertilisers provide a wide range of nutrients for plants, while the conventional mineral fertiliser only supplied nitrogen. For example, sulphur is an important prerequisite for the production of glucosinolates in Brassicas (Hopkins et al., 2009). Fertiliser type and herbivore feeding damage may also interact to affect foliar glucosinolate concentrations (Staley et al., 2011). We tested whether fertiliser type and concentration can affect the mortality of coccinellid larvae feeding on two aphid species from Brassica plants growing in fertilisers typical of organic and conventional farming systems. Glucosinolate concentrations were analysed in *B. oleracea* foliage and *B. brassicae* from the various fertiliser treatments. We hypothesised that sinigrin and other aliphatic glucosinolates would be present in higher concentrations in plants grown in organic fertilisers, and this would result in higher coccinellid larval mortality for those feeding on the specialist aphid *B. brassicae*. No effect of fertiliser was expected on coccinellid mortality when feeding on the more generalist *M. persicae*.
2. Materials and methods

2.1 Fertiliser treatments

Soil was collected in May 2009 from a field experiment conducted at the University of Reading, UK (51°24’ N, 0°57’ W). Four fertiliser treatments had been added to 6 x 6 m plots in a fully factorial design for two years: 1) a conventional high concentration treatment consisting of ammonium nitrate (Nitram, 34.5% N) at 200 kg nitrogen per ha; 2) a conventional low concentration fertiliser treatment (ammonium nitrate (Nitram, 34.5% N) at 100 kg nitrogen per ha); 3) an organic high concentration fertiliser treatment (a green manure crop (Trifolium repens var. Milvus, approximately 2.6 % N) plus chicken manure pellets (4.5% N; Greenvale, Yorkshire, UK) at 200 kg nitrogen per ha in total); 4) an organic low concentration fertiliser treatment (green manure only (T. repens var. Milvus, approximately 2.6 % N) at approximately 100 kg nitrogen per ha). The green manure was a crop of Trifolium repens var Milvus sown at a rate of 108 g of seeds per plot the preceding September. These treatments standardised on the amount of total nitrogen added at either a ‘high’ or a ‘low’ rate, with half the quantity of nitrogen added to the ‘low’ treatments compared with the ‘high’ treatments. The percentages of N, P, K and S in each fertilizer were as follows: chicken manure, 4.5% N, 2.5% P, 2.5% K and 0.2% S; Nitram, 34.5% N, 0.0% P, K and S (Pope et al., 2012). For further details of the field experiment design see Staley et al. (2010).

For the current study twelve litres of soil were removed from each of sixteen plots, comprising four plots for each of the four fertiliser treatments. The soil was sieved through a 1cm² grid to remove large stones and invertebrates. Soils from the four plots corresponding
to the same fertiliser treatment were thoroughly mixed after sieving. The soil from each field experiment treatment had an identical fertiliser treatment added in May 2009, to mimic the field experiment treatments in the two previous years. All fertilisers were crushed with a pestle and mortar and mixed thoroughly with the experimental soil.

2.2 Plant cultivation

*Brassica oleracea* var *capitata* cv. Derby Day seeds (Tozer seeds, Sussex, UK) were sown into peat plugs (diameter 22 mm, length 50 mm, LBS Horticulture, UK) and kept in a controlled environment (CE) room (20°C, 70% RH, 16 L : 8 D). Following germination seedlings were transferred to a greenhouse (20°C min, 16 L : 8 D), where overhead lighting was supplied by mercury halide and sodium bulbs to ensure a light intensity minimum of 300 watts/m². When seedlings had developed two true leaves, usually two weeks after germination, each plug was transplanted into a 15 cm pot containing one litre of experimental soil. Twenty-five plants were potted per fertiliser treatment. The potted plants were grown outside in netted cages for five weeks and then moved to a CE room (as above).

2.3 Aphid treatment

*Myzus persicae* and *B. brassicae* were obtained from mixed clone long-term laboratory cultures at Rothamsted Research (Harpenden, UK). The insects were cultured on *B. oleracea* var *capitata* cv. Derby Day grown in John Innes no. 2 compost (Monro Horticulture, Kent, UK). Each potted plant was enclosed in an individual perforated bread bag in a CE room (as above). Twenty five apterous adult aphids were placed onto the third and fourth oldest leaves on the plants: ten plants per treatment were inoculated with *B. brassicae*, ten with *M.*
*persicae*, and five left uninfested. Seven days later adult aphids were removed leaving only deposited nymphs, and after a further 7 days aphids from the colonies were used for predator bioassays or glucosinolate analyses.

2.4 Predator performance

Two-spot coccinellid beetles (*Adalia bipunctata*) were reared on a diet of *Acyrthosiphon pisum* aphids (cultured on *Vicia faba* var. Aquadulce Claudia), and maintained in a CE room (as above). Larvae hatching within 24 h of each other were collected from multiple egg clusters, weighed on a microbalance (Sartorius MP3, Sartorius AG, Germany) and placed individually into a Petri dish (11cm diameter). For each fertiliser-treated aphid-infested plant, three coccinellid larvae were fed an excess of adult apterous aphids collected from that plant. Each experimental replicate thus consisted of the response of three coccinelid larvae to feeding on aphids from a colony reared for a generation on a single plant under one of four fertiliser treatments. Coccinellid larvae were maintained in a CE room (conditions as above) and survival was recorded daily throughout the first instar. The weight of larvae surviving and moulting into the second instar was recorded. First instar *A. bipunctata* larvae are more susceptible to sequestered defences in their prey than later instars (Francis *et al.*, 2001a), and ongoing development to adulthood is dependent on survival through this initial developmental stage.

2.5 Foliar glucosinolate concentration

Five *B. brassicae*-infested plants were selected at random from each of the four fertiliser treatments. The fourth oldest leaf was excised from each of these plants and from five
uninfested plants from each fertiliser treatment. Each leaf was wrapped in aluminium foil and frozen immediately in liquid nitrogen. The samples were transferred into an -80°C freezer, prior to being placed in a freeze-drier (ThermoSavant, Micro Modulyo) for 48 h before glucosinolate analysis. The dried leaves were crushed and 20 mg from each placed into an Eppendorf tube for glucosinolate extraction and analysis.

Following the addition of 1ml methanol, 20 µl of 2 mg/ml benzyglucosinolate as a standard, and a methanol-conditioned steel bead to the Eppendorf tubes, the samples were run through a tissue-lyser for 2 min and then vortexed. Desulphoglucosinolates were then extracted as detailed by (Kazana et al., 2007). Samples were analysed by high-performance liquid chromatography (HPLC; Agilent 1200 series with a Synergy (150 x 2 mm) column, in a 229 nm variable wavelength detector), where desulphoglucosinolates were separated on a water-acetonitrile gradient. Glucosinolate concentrations were then calculated.

2.6 Aphid glucosinolate concentration and weight

Ten apterous adult *B. brassicae* aphids were removed and weighed (Sartorius MP3, Sartorius AG, Germany) from the five selected infested plants for each fertiliser treatment. They were frozen in liquid nitrogen within one hour of removal from the plant, and then transferred to an -80°C freezer. The samples were then freeze-dried for 48 hours, before being re-weighed to establish dry weight and transferred to an Eppendorf tube for glucosinolate extraction and analysis. Sample preparation and analysis process was the same as used for foliar glucosinolates (described above).

2.7 Statistical analyses
Glucosinolate concentrations were grouped into indole, aliphatic and total glucosinolates, and
log transformed prior to analysis. The response of individual glucosinolates is available in
the electronic supplementary material (Tables 1 & 2). The effects of fertiliser type and
concentration on aphid glucosinolate concentrations, aphid weight and coccinellid relative
growth rates were tested using ANOVA. The effects of fertiliser type, fertiliser concentration
and whether the plant was infested with aphids on foliar glucosinolate concentrations were
tested using ANOVA. Following a significant result for a factor or interaction in an
ANOVA, posthoc Tukey’s Honestly Significant Difference (HSD) tests were used to
determine which factor levels differed (Crawley, 2007). The effect of fertilisers on
coccinellid mortality was analysed using a generalised linear model with a binomial
distribution (Crawley, 2007). All analyses were conducted in R version 2.12.1 using package
nlme (Pinheiro et al., 2011; R Core Development Team, 2010).

3. Results

3.1 Predator performance

Mortality of *A. bipunctata* was reduced when feeding on *M. persicae* compared with
*B. brassicae* ($Z_{1,75} = 9.26, P < 0.001$; Figure 1). *Adalia bipunctata* feeding on aphids from
synthetically fertilised plants had higher mortality compared with those eating aphids from
organically fertilised plants ($Z_{1,75} = -2.51, P = 0.012$). Fertiliser concentration had no effect
on the mortality of *A. bipunctata* ($Z_{1,75} = -0.92, P = 0.36$), nor was there an interaction
between fertiliser type and concentration ($Z_{1,75} = 0.76, P = 0.44$), or between the species of
aphid being eaten and the effect of fertiliser type ($Z_{1,75} = 0.56, P = 0.58$).
Relative growth rate (RGR) data for *A. bipunctata* feeding on *B. brassicae* was limited as mortality was so high (100% for one treatment combination), so RGR results for *A. bipunctata* feeding on this aphid prey species should be interpreted with caution. Nonetheless, RGR of *A. bipunctata* was considerably greater when fed on *M. persicae* compared with *B. brassicae* \((F_{1,42} = 12.73, P < 0.001)\) but was not affected by fertiliser type \((F_{1,42} = 0.055, P = 0.82)\) or concentration \((F_{1,42} = 1.13, P = 0.29; A. bipunctata\) RGR (mean ± SE) on *M. persicae*: 2.74 ± 0.172, *B. brassicae*: 1.10 ± 0.27). The average first instar duration was 5 days when *Adalia bipunctata* was feeding on *B. brassicae*, and 3.4 days when feeding on *M. persicae*.

### 3.2 Foliar glucosinolate concentration

Two aliphatic (glucoiberin (3-methylsulfinylpropyl glucosinolate) and sinigrin (2-propenyl glucosinolate)) and three indole glucosinolates (4-hydroxy-indol-3-ylmethyl, glucobrassicin (indolyl-3-ylmethyl glucosinolate) and neoglucobrassicin \((N\text{-methoxy-indol-3-ylmethyl})\)) were identified and quantified. Fertiliser type strongly affected foliar aliphatic glucosinolates, with significantly higher concentrations in plants grown in the synthetic fertiliser compared with the organic fertilisers (ANOVA: \(F_{1,32} = 11.98, P = 0.0015\)). In addition, fertiliser type, fertiliser concentration and whether the plant was infested interacted to affect the concentration of foliar aliphatic glucosinolates \((F_{1,32} = 4.76, P = 0.04)\). Neither infestation by aphids nor fertiliser concentration had an effect on the concentration of aliphatic glucosinolates in plants grown in synthetic fertiliser. Uninfested foliage from plants in organic fertiliser had more aliphatic glucosinolates if grown in a high concentration, compared with a low concentration. This relationship was reversed for infested organically fertilised plants, for which aliphatic glucosinolate concentration was greater under the low
fertiliser concentration (Tukey HSD tests, \( P > 0.05 \), Figure 2A and 2B). The concentration of
indole glucosinolates followed the same pattern (fertiliser type: \( F_{1,32} = 25.53, \ P < 0.001; \)
fertiliser type x fertiliser dose x infestation interaction: \( F_{1,32} = 4.90, \ P = 0.03 \)).

3.3 Aphid weight and glucosinolate concentration

Neither the type or amount of fertiliser supplied to host plants affected the concentration of
aliphatic (fertiliser type: \( F_{1,17} = 0.46, \ P = 0.51; \) fertiliser concentration: \( F_{1,17} = 0.11, \ P = 0.75 \))
or indole (fertiliser type: \( F_{1,17} = 0.18, \ P = 0.67; \) fertiliser concentration: \( F_{1,17} = 1.14, \ P = 0.30 \))
glucosinolates in *B. brassicae* (Figure 2C). The concentration of individual glucosinolate
compounds was also unaffected by fertiliser type and concentration (electronic
supplementary material Tables 3 and 4). The concentration of aliphatic glucosinolates was
much higher in *B. brassicae* than in the *B. oleracea* foliage (Figure 2 and electronic
supplementary material Tables 1 and 3).

Aphids that fed on plants fertilised with the high concentrations were larger than those on the
low fertiliser concentrations (fertiliser dose: \( F_{1,17} = 6.12, \ P = 0.02 \)) and there was a trend
towards larger aphids on synthetically fertilised plants (fertiliser type: \( F_{1,17} = 4.01, \ P = 0.06; \)
aphid weight (mean mg ± SE) synthetic high 1.20 ± 0.071, synthetic low 1.02 ± 0.086,
organic high 1.06 ± 0.121, organic low 0.82 ± 0.058).
4. Discussion

*Adalia bipunctata* had a higher relative growth rate and lower mortality when feeding on *Myzus persicae* compared with *Brevicoryne brassicae*, as found previously (Francis et al., 2001b; Kazana et al., 2007; Pratt et al., 2008). This differential mortality has been attributed to *B. brassicae*’s ability to sequester glucosinolates from its host plant, which are released as toxic isothiocyanates when under attack by predators (Kazana et al., 2007). (Francis et al., 2001b) also showed that *A. bipunctata* mortality can be affected by the plant species on which its prey is feeding, due to differences in glucosinolate concentrations. Here, we demonstrate that the type of fertiliser in which Brassica plants are grown can alter predator mortality, with approximately a 10% higher death rate among *A. bipunctata* feeding on aphids from plants growing in synthetic compared with organic fertilisers. This could potentially affect the efficacy of predators as biological control agents in conventional and sustainable farming systems.

*Adalia bipunctata* mortality was increased when they consumed either aphid species that had fed on plants grown in synthetic fertilisers, on which foliar glucosinolate concentrations were higher than plants in organic fertilisers. Previous work suggests that *M. persicae* does not sequester glucosinolates (Francis et al., 2001b) and we found no difference in the glucosinolate content of *B. brassicae* fed on plants growing in the different fertilisers; thus sequestered glucosinolate concentrations do not appear to explain the differential mortality of *A. bipunctata* larvae. Previous studies have also shown that mortality of first instar *A. bipunctata* was higher (Francis et al., 2001b) and egg production lower (Francis et al., 2001a) when feeding on *M. persicae* from plant species with high glucosinolate concentrations compared to species with low foliar glucosinolate concentrations. Although
M. persicae may not actively sequester specific glucosinolate compounds, higher concentrations of glucosinolates may be present passing through the gut of M. persicae feeding on higher glucosinolate host plants. In addition, indole glucosinolates can break down to toxic metabolites without the presence of myrosinase, and thus may have toxic effects on predators feeding on aphid species that do not synthesise the enzyme. Other aphid quality parameters such as nitrogen content, alternative secondary metabolites or behavioural differences may have also have contributed to the differences in coccinellid mortality. For example, nitrogen content of plants and aphids has been shown to alter coccinellid consumption rate, with higher numbers of small aphids eaten on plants growing in low nitrogen concentrations compared with larger aphids on plants growing in high nitrogen concentrations (Aqueel and Leather, 2012). However, in the current study Brevicoryne brassicae were larger on plants grown in the synthetic fertiliser treatment, so aphid size also does not explain the differential A. bipunctata mortality.

The tritrophic interactions hypothesis predicts that generalist herbivores should be more sensitive to variation in host plant quality than specialist herbivores, and thus natural enemy effects will be increased more for generalists feeding on low quality plants (Mooney et al., 2012). In the current study however, we found that coccinellid larval mortality was equally increased when feeding on aphids from plants grown in synthetic fertilizer, regardless of whether they fed on the generalist aphid M. persicae or the more specialist B. brevicoryne.

The type and concentration of fertiliser supplied to Brassica oleracea plants affected constitutive foliar glucosinolate concentrations, though not as we had hypothesised. Constitutive and induced foliar glucosinolates concentrations were greater in synthetically fertilised plants (under high or low treatments) than in plants under one or other organically
fertilised treatment (depending on whether the plant was subjected to aphid feeding or not).

In contrast, in a field trial we found higher glucosinolate concentrations in plants grown in organic fertiliser than those in synthetic fertiliser, regardless of the level of fertiliser (Staley et al., 2010). The plants in the field trial were subjected to feeding by whatever herbivores naturally colonised them, including chewing Lepidoptera larvae as well as aphids. Glucosinolates in these field plants may therefore have been induced differently to those in the current study, as glucosinolate induction can vary with feeding guild as well as species (Poelman et al., 2008).

The induction of a change in glucosinolate concentration by *Brevicoryne brassicae* feeding was also affected by fertiliser type and concentration. Concentrations of constitutive glucosinolates were highest in synthetically fertilised plants, in which neither aliphatic nor indole glucosinolate concentrations were significantly altered by *Brevicoryne brassicae* feeding. Oak trees showed a similar response to feeding by Lepidoptera larvae, as induced changes in astringency and proanthocyanidins did not occur in synthetically fertilised trees, but did in unfertilised ones (Hunter and Schultz, 1995). Optimal defence theory predicts that plants growing in environments with limited resources may be less likely to invest in constitutive defence, and more likely to rely on induced defences (Rhoades, 1979; Siemens and Mitchell-Olids, 1998). In support of this, the current study shows that constitutive foliar glucosinolate concentrations were lower on plants supplied with a low concentration of organic fertiliser compared to those fertilised with synthetic fertiliser, and were intermediate in those grown in high concentrations of organic fertiliser. Feeding by *Brevicoryne brassicae* induced higher glucosinolate concentrations in plants growing in limited resources (those supplied with low levels of organic fertiliser).
The glucosinolate profile of *B. brassicae* differed from that of its host plant, as concentrations of the two aliphatic glucosinolates (sinigrin and glucoiberin) were much higher in the aphid, as found in previous studies (Francis et al., 2001b; Kazana et al., 2007). *Brevicoryne brassicae* may be actively sequestering sinigrin (Kazana et al., 2007) or glucosinolates may be compartmentalised within specific plant cell types (Fahey et al., 2001). *Brassica oleracea* infested with aphids had lower concentrations of both aliphatic and indole glucosinolates if grown in high concentrations of the organic fertiliser compared with the other three treatments, but these differences were not found in the aphids. This supports an active sequestration of glucosinolates by *B. brassicae*. There was no significant effect of fertiliser treatment on the concentration of either grouped or individual glucosinolates within *B. brassicae* or on aphid size, so the differential mortality of *A. bipunctata* was not explained by our aphid quality parameters.

Previous work comparing predators in organic and conventional farming systems has focussed largely on predatory community abundance and diversity (Macfadyen et al., 2009). Here, we show for the first time that tritrophic interactions between herbivores and predators can be mediated by the type of fertiliser supplied to a host plant. The higher mortality of *A. bipunctata* larvae on two different aphid species feeding on plants in synthetic fertiliser demonstrates that the agricultural practices being used on a crop need to be considered when making pest management decisions. In addition, the efficacy of measures that aim to increase biological control through enhancing on-farm predator abundance (e.g. the introduction of beetle banks and field margins encouraged under environmental stewardship schemes; (Natural England, 2010) may vary depending on the type of nutrition being supplied to a crop.
Acknowledgements

Thanks to Tom Oliver for advice on culturing coccinellids, Numrah Nisar for assistance with glucosinolate analyses and to Mark Ramsden for comments on an earlier draft. Thanks also to Gilles San Martin for the coccinellid larval photograph in the graphical abstract. Funding was provided by BBSRC (grant BB/D01154x/1).

References


Francis, F., Haubruege, E., Hastir, P., Gaspar, C., 2001a. Effect of aphid host plant on development and reproduction of the third trophic level, the predator Adalia bipunctata (Coleoptera : Coccinellidae). Environmental Entomology 30, 947-952.

Francis, F., Lognay, G., Wathelet, J.P., Haubruege, E., 2001b. Effects of allelochemicals from first (Brassicaceae) and second (Myzus persicae and Brevicoryne brassicae) trophic levels on Adalia bipunctata. Journal of Chemical Ecology 27, 243-256.


differentially affects plant defence responses to subsequently colonizing herbivores
and their abundance in the field. Molecular Ecology 17, 3352-3365.

Pope, T.W., Girling, R.D., Staley, J.T., Trigodet, B., Wright, D.J., Leather, S.R., van Emden,
host selection by the aphid parasitoid Diaeretiella rapae. Journal of Applied
Entomology, online September 2011, doi: 10.1111/j.1439-0418.2011.01667.x

Ponti, L., Altieri, M.A., Gutierrez, A.P., 2007. Effects of crop diversification levels and
fertilization regimes on abundance of Brevicoryne brassicae (L.) and its parasitization
by Diaeretiella rapae (M'Intosh) in broccoli. Agricultural and Forest Entomology 9,
209-214.

Pratt, C., Pope, T.W., Powell, G., Rossiter, J.T., 2008. Accumulation of glucosinolates by the
cabbage aphid Brevicoryne brassicae as a defense against two coccinellid species.
Journal of Chemical Ecology 34, 323-329.


Interactions among three trophic levels: Influence of plants on interactions between
insect herbivores and natural enemies. Annual Review of Ecology and Systematics
11, 41-65.

R Core Development Team, 2010. R: A Language and Environment for Statistical

the mustard oil bomb. Proceedings of the National Academy of Sciences of the United
States of America 99, 11223-11228.


Figure 1
Mean (± SE) percentage mortality of first instar *Adalia bipunctata* larvae fed aphids (*Brevicoryne brassicae* or *Myzus persicae*) feeding on *Brassica oleracea* growing in an organic animal manure or a synthetic fertiliser at a high or low concentration. Mortality was 100% in all replicates for *A. bipunctata* feeding on *B. brassicae* in the synthetic low fertiliser treatment, so SE = 0 for this treatment.

Figure 2
Mean (± SE) glucosinolate concentration of A) *Brassica oleracea* foliage with no insects feeding on it; B) *B. oleracea* foliage with *Brevicoryne brassicae* feeding on it and C) *B. brassicae*. The *B. oleracea* were grown in an organic animal manure or a synthetic ammonium nitrate fertiliser at a high or low concentration. Within each group of glucosinolates (aliphatic, indole or total) different letters denote significant differences between fertiliser treatments at $P < 0.05$. 
Figure 1

Adalia bipunctata mortality

Mortality (%)

0 20 40 60 80 100 120

Brevicoryne brassicae  Myzus persicae

Mortality: synthetic, high; synthetic, low; organic, high; organic, low
Glucosinolate content of *Brassica oleracea* foliage with no insects feeding on it

Glucosinolate content of *Brassica oleracea* foliage infested with *Brevicoryne brassicae*

*Brevicoryne brassicae* glucosinolate content

Fertiliser type and concentration

**Figure 2**