Article (refereed) - postprint

Banfield-Zanin, J.A.; Rossiter, J.T.; Wright, D.J.; Leather, S.R.; **Staley, J.T.**. 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

Accepted in *Biological Control*, 30 May 2012

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

70 populations of a generalist aphid were reduced (Staley et al., 2010). These changes to plant
71 defensive chemistry in response to fertiliser type have been shown to alter competition
72 between two herbivore species (Staley et al., 2011) and also have the potential to alter
73 interactions between natural enemies and their prey.
74
75 Predators and parasitoids use volatile organic compounds (VOCs) emitted by the plant to
76 locate their prey. These VOCs are breakdown products of secondary metabolites emitted by
77 plants in response to herbivore feeding damage (Hopkins et al., 2009; Vet and Dicke, 1992).
78 Some herbivore species have the ability to sequester these defensive chemicals, to use in their
79 own defence against predators (Ratzka et al., 2002; Winde and Wittstock, 2011). The
80 specialist Brassica aphid, *Brevicoryne brassicae*, has a defence system which mimics that of
81 its host plants. *Brevicoryne brassicae* produces an enzyme (myrosinase), which catalyses the
82 hydrolysis of glucosinolates to potentially toxic isothiocyanates (Kazana et al., 2007). The
83 aphid accumulates sinigrin and other glucosinolate compounds, which are present in higher
84 concentrations in the aphid than its host plant. If *B. brassicae* is attacked by a predator the
85 myrosinase comes into contact with glucosinolates and volatile isothiocyanates are released
86 (Francis et al., 2001b) resulting in high mortality of the first instar of the coccinellid
87 *Adalia bipunctata* (Kazana et al., 2007; Pratt et al., 2008). By contrast, a more generalist
88 aphid species (*Myzus persicae*) does not accumulate high concentrations of glucosinolates,
89 and mortality of *A. bipunctata* larvae was lower when fed *M. persicae* compared with
90 *B. brassicae* (Francis et al., 2001b). Some predators of *B. brassicae* also have reduced
91 performance if feeding on hosts that have developed on plant species or cultivars with high
92 concentrations of glucosinolates (Chaplin-Kramer et al., 2011; Kos et al., 2011), suggesting a
93 direct link between plant glucosinolate concentration and herbivore defence against their
94 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 1cm^2 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/m^2. 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.*

164 *persicae*, and five left uninfested. Seven days later adult aphids were removed leaving only
165 deposited nymphs, and after a further 7 days aphids from the colonies were used for predator
166 bioassays or glucosinolate analyses.
167
168 *2.4 Predator performance*
169
170 Two-spot coccinellid beetles (*Adalia bipunctata*) were reared on a diet of *Acyrthosiphon*
171 *pisum* aphids (cultured on *Vicia faba* var. Aquadulce Claudia), and maintained in a CE room
172 (as above). Larvae hatching within 24 h of each other were collected from multiple egg
173 clusters, weighed on a microbalance (Sartorius MP3, Sartorius AG, Germany) and placed
174 individually into a Petri dish (11cm diameter). For each fertiliser-treated aphid-infested plant,
175 three coccinellid larvae were fed an excess of adult apterous aphids collected from that plant.
176 Each experimental replicate thus consisted of the response of three coccinelid larvae to
177 feeding on aphids from a colony reared for a generation on a single plant under one of four
178 fertiliser treatments. Coccinellid larvae were maintained in a CE room (conditions as above)
179 and survival was recorded daily throughout the first instar. The weight of larvae surviving
180 and moulting into the second instar was recorded. First instar *A. bipunctata* larvae are more
181 susceptible to sequestered defences in their prey than later instars (Francis *et al.*, 2001a), and
182 ongoing development to adulthood is dependent on survival through this initial
183 developmental stage.
184
185 *2.5 Foliar glucosinolate concentration*
186
187 Five *B. brassicae*-infested plants were selected at random from each of the four fertiliser
188 treatments. The fourth oldest leaf was excised from each of these plants and from five

189 uninfested plants from each fertiliser treatment. Each leaf was wrapped in aluminium foil and
190 frozen immediately in liquid nitrogen. The samples were transferred into an -80˚C freezer,
191 prior to being placed in a freeze-drier (ThermoSavant, Micro Modulyo) for 48 h before
192 glucosinolate analysis. The dried leaves were crushed and 20 mg from each placed into an
193 Eppendorf tube for glucosinolate extraction and analysis.
194
195 Following the addition of 1ml methanol, 20 µl of 2 mg/ml benzyglucosinolate as a standard,
196 and a methanol-conditioned steel bead to the Eppendorfs tubes, the samples were run through
197 a tissue-lyser for 2 min and then vortexed. Desulphoglucosinolates were then extracted as
198 detailed by (Kazana et al., 2007). Samples were analysed by high-performance liquid
199 chromatography (HPLC; Agilent 1200 series with a Synergy (150 x 2 mm) column, in a 229
200 nm variable wavelength detector), where desulphoglucosinolates were separated on a water-
201 acetonitrile gradient. Glucosinolate concentrations were then calculated.
202
203 *2.6 Aphid glucosinolate concentration and weight*
204
205 Ten apterous adult *B. brassicae* aphids were removed and weighed (Sartorius MP3, Sartorius
206 AG, Germany) from the five selected infested plants for each fertiliser treatment. They were
207 frozen in liquid nitrogen within one hour of removal from the plant, and then transferred to an
208 -80˚C freezer. The samples were then freeze-dried for 48 hours, before being re-weighed to
209 establish dry weight and transferred to an Eppendorf tube for glucosinolate extraction and
210 analysis. Sample preparation and analysis process was the same as used for foliar
211 glucosinolates (described above).
212
213 *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 ($Z_{1,75} = 0.56$, $P = 0.58$).

239 Relative growth rate (RGR) data for *A. bipunctata* feeding on *B. brassicae* was limited as
240 mortality was so high (100% for one treatment combination), so RGR results for
241 *A. bipunctata* feeding on this aphid prey species should be interpreted with caution.
242 Nonetheless, RGR of *A. bipunctata* was considerably greater when fed on *M. persicae*
243 compared with *B. brassicae* ($F_{1,42}$ = 12.73, P < 0.001) but was not affected by fertiliser type
244 ($F_{1,42}$ = 0.055, P = 0.82) or concentration ($F_{1,42}$ = 1.13, P = 0.29; *A. bipunctata* RGR (mean ±
245 SE) on *M. persicae*: 2.74 ± 0.172, *B. brassicae*: 1.10 ± 0.27). The average first instar
246 duration was 5 days when *Adalia bipunctata* was feeding on *B. brassicae*, and 3.4 days when
247 feeding on *M. persicae*.
248
249 *3.2 Foliar glucosinolate concentration*
250
251 Two aliphatic (glucoiberin (3-methylsulfinylpropyl glucosinolate) and sinigrin (2-propenyl
252 glucosinolate)) and three indole glucosinolates (4-hydroxy-indol-3ylmethyl, glucobrassicin
253 (indolyl-3-ylmethyl glucosinolate) and neoglucobrassicin (*N*-methoxy-indol-3-ylmethyl))
254 were identified and quantified. Fertiliser type strongly affected foliar aliphatic
255 glucosinolates, with significantly higher concentrations in plants grown in the synthetic
256 fertiliser compared with the organic fertilisers (ANOVA: $F_{1,32}$ = 11.98, P = 0.0015). In
257 addition, fertiliser type, fertiliser concentration and whether the plant was infested interacted
258 to affect the concentration of foliar aliphatic glucosinolates ($F_{1,32}$ = 4.76, P = 0.04). Neither
259 infestation by aphids nor fertiliser concentration had an effect on the concentration of
260 aliphatic glucosinolates in plants grown in synthetic fertiliser. Uninfested foliage from plants
261 in organic fertiliser had more aliphatic glucosinolates if grown in a high concentration,
262 compared with a low concentration. This relationship was reversed for infested organically
263 fertilised plants, for which aliphatic glucosinolate concentration was greater under the low

264 fertiliser concentration (Tukey HSD tests, $P > 0.05$, Figure 2A and 2B). The concentration of
265 indole glucosinolates followed the same pattern (fertiliser type: $F_{1,32} = 25.53$, $P < 0.001$;
266 fertiliser type x fertiliser dose x infestation interaction: $F_{1,32} = 4.90$, $P = 0.03$).
267
268 *3.3 Aphid weight and glucosinolate concentration*
269
270 Neither the type or amount of fertiliser supplied to host plants affected the concentration of
271 aliphatic (fertiliser type: $F_{1,17} = 0.46$, $P = 0.51$, fertiliser concentration: $F_{1,17} = 0.11$, $P = 0.75$)
272 or indole (fertiliser type: $F_{1,17} = 0.18$, $P = 0.67$, fertiliser concentration: $F_{1,17} = 1.14$, $P = 0.30$)
273 glucosinolates in *B. brassicae* (Figure 2C). The concentration of individual glucosinolate
274 compounds was also unaffected by fertiliser type and concentration (electronic
275 supplementary material Tables 3 and 4). The concentration of aliphatic glucosinolates was
276 much higher in *B. brassicae* than in the *B. oleracea* foliage (Figure 2 and electronic
277 supplementary material Tables 1 and 3).
278
279 Aphids that fed on plants fertilised with the high concentrations were larger than those on the
280 low fertiliser concentrations (fertiliser dose: $F_{1,17} = 6.12$, $P = 0.02$) and there was a trend
281 towards larger aphids on synthetically fertilised plants (fertiliser type: $F_{1,17} = 4.01$, $P = 0.06$;
282 aphid weight (mean mg ± SE) synthetic high 1.20 ± 0.071, synthetic low 1.02 ± 0.086,
283 organic high 1.06 ± 0.121, organic low 0.82 ± 0.058).
284

285 **4. Discussion**

286

287 *Adalia bipunctata* had a higher relative growth rate and lower mortality when feeding on
288 *Myzus persicae* compared with *Brevicoryne brassicae*, as found previously (Francis et al.,
289 2001b; Kazana et al., 2007; Pratt et al., 2008). This differential mortality has been attributed
290 to *B. brassicae*'s ability to sequester glucosinolates from its host plant, which are released as
291 toxic isothiocyanates when under attack by predators (Kazana et al., 2007). (Francis et al.,
292 2001b) also showed that *A. bipunctata* mortality can be affected by the plant species on
293 which its prey is feeding, due to differences in glucosinolate concentrations. Here, we
294 demonstrate that the type of fertiliser in which Brassica plants are grown can alter predator
295 mortality, with approximately a 10 % higher death rate among *A. bipunctata* feeding on
296 aphids from plants growing in synthetic compared with organic fertilisers. This could
297 potentially affect the efficacy of predators as biological control agents in conventional and
298 sustainable farming systems.

299

300 *Adalia bipunctata* mortality was increased when they consumed either aphid species that had
301 fed on plants grown in synthetic fertilisers, on which foliar glucosinolate concentrations were
302 higher than plants in organic fertilisers. Previous work suggests that *M. persicae* does not
303 sequester glucosinolates (Francis et al., 2001b) and we found no difference in the
304 glucosinolate content of *B. brassicae* fed on plants growing in the different fertilisers; thus
305 sequestered glucosinolate concentrations do not appear to explain the differential mortality of
306 *A. bipunctata* larvae. Previous studies have also shown that mortality of first instar
307 *A. bipunctata* was higher (Francis et al., 2001b) and egg production lower (Francis et al.,
308 2001a) when feeding on *M. persicae* from plant species with high glucosinolate
309 concentrations compared to species with low foliar glucosinolate concentrations. Although

310 *M. persicae* may not actively sequester specific glucosinolate compounds, higher
311 concentrations of glucosinolates may be present passing through the gut of *M. persicae*
312 feeding on higher glucosinolate host plants. In addition, indole glucosinolates can break
313 down to toxic metabolites without the presence of myrosinase, and thus may have toxic
314 effects on predators feeding on aphid species that do not synthesise the enzyme. Other aphid
315 quality parameters such as nitrogen content, alternative secondary metabolites or behavioural
316 differences may have also have contributed to the differences in coccinellid mortality. For
317 example, nitrogen content of plants and aphids has been shown to alter coccinellid
318 consumption rate, with higher numbers of small aphids eaten on plants growing in low
319 nitrogen concentrations compared with larger aphids on plants growing in high nitrogen
320 concentrations (Aqueel and Leather, 2012). However, in the current study
321 *Brevicoryne brassicae* were larger on plants grown in the synthetic fertiliser treatment, so
322 aphid size also does not explain the differential *A. bipunctata* mortality.
323
324 The tritrophic interactions hypothesis predicts that generalist herbivores should be more
325 sensitive to variation in host plant quality than specialist herbivores, and thus natural enemy
326 effects will be increased more for generalists feeding on low quality plants (Mooney et al.,
327 2012). In the current study however, we found that coccinellid larval mortality was equally
328 increased when feeding on aphids from plants grown in synthetic fertilizer, regardless of
329 whether they fed on the generalist aphid *M. persicae* or the more specialist *B. brevicoryne*.
330
331 The type and concentration of fertiliser supplied to *Brassica oleracea* plants affected
332 constitutive foliar glucosinolate concentrations, though not as we had hypothesised.
333 Constitutive and induced foliar glucosinolates concentrations were greater in synthetically
334 fertilised plants (under high or low treatments) than in plants under one or other organically

335 fertilised treatment (depending on whether the plant was subjected to aphid feeding or not).
336 In contrast, in a field trial we found higher glucosinolate concentrations in plants grown in
337 organic fertiliser than those in synthetic fertiliser, regardless of the level of fertiliser (Staley et
338 al., 2010). The plants in the field trial were subjected to feeding by whatever herbivores
339 naturally colonised them, including chewing Lepidoptera larvae as well as aphids.
340 Glucosinolates in these field plants may therefore have been induced differently to those in
341 the current study, as glucosinolate induction can vary with feeding guild as well as species
342 (Poelman et al., 2008).
343
344 The induction of a change in glucosinolate concentration by *Brevicoryne brassicae* feeding
345 was also affected by fertiliser type and concentration. Concentrations of constitutive
346 glucosinolates were highest in synthetically fertilised plants, in which neither aliphatic nor
347 indole glucosinolate concentrations were significantly altered by *Brevicoryne brassicae*
348 feeding. Oak trees showed a similar response to feeding by Lepidoptera larvae, as induced
349 changes in astringency and proanthocyanidins did not occur in synthetically fertilised trees,
350 but did in unfertilised ones (Hunter and Schultz, 1995). Optimal defence theory predicts that
351 plants growing in environments with limited resources may be less likely to invest in
352 constitutive defence, and more likely to rely on induced defences (Rhoades, 1979; Siemens
353 and Mitchell-Olds, 1998). In support of this, the current study shows that constitutive foliar
354 glucosinolate concentrations were lower on plants supplied with a low concentration of
355 organic fertiliser compared to those fertilised with synthetic fertiliser, and were intermediate
356 in those grown in high concentrations of organic fertiliser. Feeding by *Brevicoryne brassicae*
357 induced higher glucosinolate concentrations in plants growing in limited resources (those
358 supplied with low levels of organic fertiliser).
359

360 The glucosinolate profile of *B. brassicae* differed from that of its host plant, as concentrations
361 of the two aliphatic glucosinolates (sinigrin and glucoiberin) were much higher in the aphid,
362 as found in previous studies (Francis et al., 2001b; Kazana et al., 2007).
363 *Brevicoryne brassicae* may be actively sequestering sinigrin (Kazana et al., 2007) or
364 glucosinolates may be compartmentalised within specific plant cell types (Fahey et al., 2001).
365 *Brassica oleracea* infested with aphids had lower concentrations of both aliphatic and indole
366 glucosinolates if grown in high concentrations of the organic fertiliser compared with the
367 other three treatments, but these differences were not found in the aphids. This supports an
368 active sequestration of glucosinolates by *B. brassicae*. There was no significant effect of
369 fertiliser treatment on the concentration of either grouped or individual glucosinolates within
370 *B. brassicae* or on aphid size, so the differential mortality of *A. bipunctata* was not explained
371 by our aphid quality parameters.
372
373 Previous work comparing predators in organic and conventional farming systems has
374 focussed largely on predatory community abundance and diversity (Macfadyen et al., 2009).
375 Here, we show for the first time that tritrophic interactions between herbivores and predators
376 can be mediated by the type of fertiliser supplied to a host plant. The higher mortality of
377 *A. bipunctata* larvae on two different aphid species feeding on plants in synthetic fertiliser
378 demonstrates that the agricultural practices being used on a crop need to be considered when
379 making pest management decisions. In addition, the efficacy of measures that aim to increase
380 biological control through enhancing on-farm predator abundance (e.g. the introduction of
381 beetle banks and field margins encouraged under environmental stewardship schemes;
382 (Natural England, 2010) may vary depending on the type of nutrition being supplied to a
383 crop.
384

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

Alyokhin, A., Porter, G., Groden, E., Drummond, F., 2005. Colorado potato beetle response to soil amendments: A case in support of the mineral balance hypothesis? Agriculture Ecosystems & Environment 109, 234-244.

Aqueel, M.A., Leather, S.R., 2012. Nitrogen fertiliser affects the functional response and prey consumption of *Harmonia axyridis* (Coleoptera: Coccinellidae) feeding on cereal aphids. Annals of Applied Biology 160**,** 6-15.

Bengtsson, J., Ahnstrom, J., Weibull, A.C., 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. Journal of Applied Ecology 42, 261-269.

Chaplin-Kramer, R., Kliebenstein, D.J., Chiem, A., Morrill, E., Mills, N.J., Kremen, C., 2011. Chemically mediated tritrophic interactions: opposing effects of glucosinolates on a specialist herbivore and its predators. Journal of Applied Ecology 48, 880-887.

Costello, M.J., Altieri, M.A., 1995. Abundance, growth rate and parasitism of *Brevicoryne brassicae* and *Myzus persicae* (Homoptera, Aphididae) on broccoli grown in living mulches. Agriculture Ecosystems & Environment 52, 187-196.

Crawley, M.J., 2007. The R Book. John Wiley & Sons, Chichester, UK.

Culliney, T.W., Pimentel, D., 1986. Ecological effects of organic agricultural practices on insect populations. Agriculture Ecosystems & Environment 15, 253-266.

Fahey, J.W., Zalcmann, A.T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56, 5-51.

Francis, F., Haubruge, 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., Haubruge, 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.

Garratt, M.P.D., Leather, S.R., Wright, D.J., 2010a. Tritrophic effects of organic and conventional fertilisers on a cereal-aphid-parasitoid system. Entomologia Experimentalis et Applicata 134, 211-219.

Garratt, M.P.D., Wright, D.J., Leather, S.R., 2010b. The effects of organic and conventional fertilizers on cereal aphids and their natural enemies. Agricultural and Forest Entomology 12, 307-318.

Garratt, M.P.D., Wright, D.J., Leather, S.R., 2011. The effects of farming system and fertilisers on pests and natural enemies: A synthesis of current research. Agriculture Ecosystems & Environment 141, 261-270.

Hairston, N.G., Smith, F.E., Slobodkin, L.B., 1960. Community structure, population control and competition. The American Naturalist 94, 421 - 425.

Hopkins, R.J., van Dam, N.M., van Loon, J.J.A., 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annual Review of Entomology 54, 57-83.

Hunter, M.D., Schultz, J.C., 1995. Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. Ecology 76, 1226-1232.

433 Kazana, E., Pope, T.W., Tibbles, L., Bridges, M., Pickett, J.A., Bones, A.M., Powell, G.,
434 Rossiter, J.T., 2007. The cabbage aphid: a walking mustard oil bomb. Proceedings of
435 the Royal Society B-Biological Sciences 274, 2271-2277.
436 Kos, M., Kabouw, P., Noordam, R., Hendriks, K., Vet, L.E.M., van Loon, J.J.A., Dicke, M.,
437 2011. Prey-mediated effects of glucosinolates on aphid predators. Ecological
438 Entomology 36, 377-388.
439 Letourneau, D.K., Bothwell, S.G., 2008. Comparison of organic and conventional farms:
440 challenging ecologists to make biodiversity functional. Frontiers in Ecology and the
441 Environment 6, 430-438.
442 Letourneau, D.K., Drinkwater, L.E., Shennan, C., 1996. Effects of soil management on crop
443 nitrogen and insect damage in organic vs conventional tomato fields. Agriculture
444 Ecosystems & Environment 57, 179-187.
445 Natural England, 2010. Entry Level Stewardship - Environmental Stewardship Handbook.
446 Third edition.
447 Macfadyen, S., Gibson, R., Polaszek, A., Morris, R.J., Craze, P.G., Planque, R., Symondson,
448 W.O.C., Memmott, J., 2009. Do differences in food web structure between organic
449 and conventional farms affect the ecosystem service of pest control? Ecology Letters
450 12, 229-238.
451 Mooney, K.A., Pratt, R.T., Singer, M.S., 2012. The tri-trophic interactions hypothesis:
452 interactive effects of host plant quality, diet breadth and natural enemies on
453 herbivores. PloS one 7**,** e34403.
454 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, S., Team, R.D.C., 2011. nlme: Linear and
455 Nonlinear Mixed Effects Models. R package version 3.1-98.

456 Poelman, E.H., Broekgaarden, C., Van Loon, J.J.A., Dicke, M., 2008. Early season herbivore
457 differentially affects plant defence responses to subsequently colonizing herbivores
458 and their abundance in the field. Molecular Ecology 17**,** 3352-3365.
459 Pope,T.W., Girling, R.D., Staley, J.T., Trigodet, B., Wright, D.J., Leather, S.R., van Emden,
460 H.F., Poppy, G.M. (2012) Effects of organic and conventional fertilizer treatments on
461 host selection by the aphid parasitoid *Diaeretiella rapae*. Journal of Applied
462 Entomology, online September 2011, doi: 10.1111/j.1439-0418.2011.01667.x
463 Ponti, L., Altieri, M.A., Gutierrez, A.P., 2007. Effects of crop diversification levels and
464 fertilization regimes on abundance of *Brevicoryne brassicae* (L.) and its parasitization
465 by *Diaeretiella rapae* (M'Intosh) in brocolli. Agricultural and Forest Entomology 9,
466 209-214.
467 Pratt, C., Pope, T.W., Powell, G., Rossiter, J.T., 2008. Accumulation of glucosinolates by the
468 cabbage aphid *Brevicoryne brassicae* as a defense against two coccinellid species.
469 Journal of Chemical Ecology 34, 323-329.
470 Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N., Weis, A.E., 1980.
471 Interactions among three trophic levels: Influence of plants on interactions between
472 insect herbivores and natural enemies. Annual Review of Ecology and Systematics
473 11, 41-65.
474 R Core Development Team, 2010. R: A Language and Environment for Statistical
475 Computing. R Foundation for Statistical Computing, Vienna, Austria.
476 Ratzka, A., Vogel, H., Kliebenstein, D.J., Mitchell-Olds, T., Kroymann, J., 2002. Disarming
477 the mustard oil bomb. Proceedings of the National Academy of Sciences of the United
478 States of America 99, 11223-11228.

Rhoades, D.F., 1979. Evolution of plant chemical defence against herbivores. In: Rosenthal, G.A., Janzen, D.H., Eds.), Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press Inc, New York, USA, pp. 4 - 54.

Siemens, D.H., Mitchell-Olds, T., 1998. Evolution of pest-induced defenses in Brassica plants: tests of theory. Ecology 79, 632-646.

Staley, J.T., Stafford, D.B., Green, E.R., Leather, S.R., Rossiter, J.T., Poppy, G.M., Wright, D.J., 2011. Plant nutrient supply determines competition between phytophagous insects. Proceedings of the Royal Society B-Biological Sciences 278, 718-724.

Staley, J.T., Stewart-Jones, A., Pope, T.W., Wright, D.J., Leather, S.R., Hadley, P., Rossiter, J.T., Van Emden, H., Poppy, G.M., 2010. Varying responses of insect herbivores to altered plant chemistry under organic and conventional treatments. Proceedings of the Royal Society B-Biological Sciences 277, 779-786.

Vet, L.E.M., Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37, 141-172.

Winde, I., Wittstock, U., 2011. Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. Phytochemistry 72, 1566-1575.

497 **Figure legends**

498

499 Figure 1

500 Mean (± SE) percentage mortality of first instar *Adalia bipunctata* larvae fed aphids
501 (*Brevicoryne brassicae* or *Myzus persicae*) feeding on *Brassica oleracea* growing in an
502 organic animal manure or a synthetic fertiliser at a high or low concentration. Mortality was
503 100% in all replicates for *A. bipunctata* feeding on *B. brassicae* in the synthetic low fertiliser
504 treatment, so SE = 0 for this treatment.

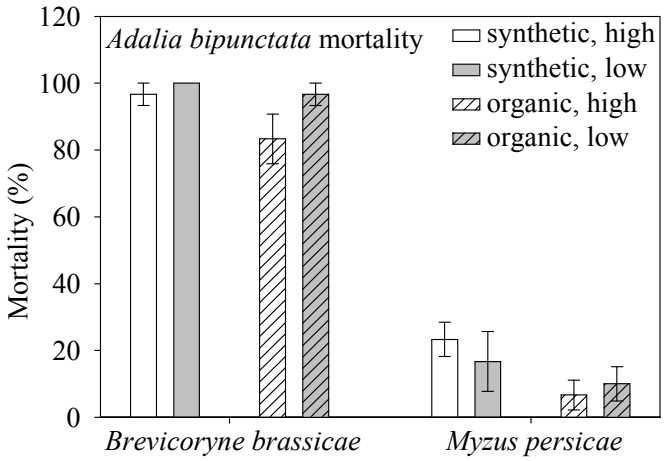
505

506 Figure 2

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

513

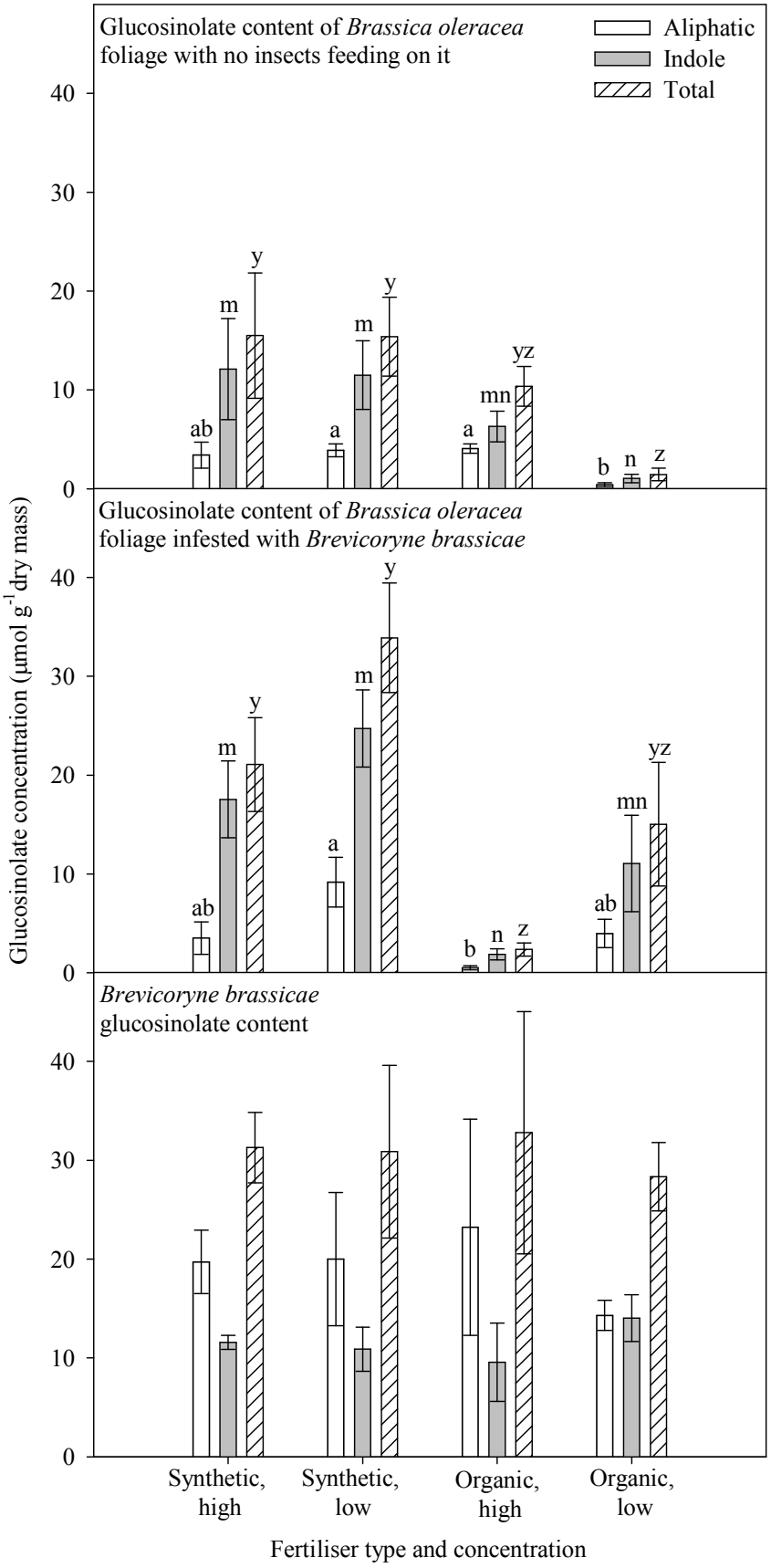
514 Figure 1



515

516

517 Figure 2



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