Associational resistance or susceptibility: the indirect interaction between chemically-defended and non-defended herbivore prey via a shared predator

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10 Abstract

11 Many organisms possess chemical defences against their natural enemies, which
12 render them unpalatable or toxic when attacked or consumed. These chemically-
13 defended organisms commonly occur in communities with non- or less-defended prey,
14 leading to indirect interactions between prey species, mediated by natural enemies.
15 Although the importance of enemy-mediated indirect interactions have been well
16 documented (e.g., apparent competition), how the presence of prey chemical defences
17 may affect predation of non-defended prey in terrestrial communities remains unclear.
18 Here, an experimental approach was used to study the predator-mediated indirect
19 interaction between a chemically-defended and non-defended pest aphid species.
20 Using laboratory-based mesocosms, aphid community composition was manipulated
21 to include chemically-defended (CD) aphids (*Brevicoryne brassicae*), non-defended
22 (ND) aphids (*Myzus persicae*) or a mixed assemblage of both species, on *Brassica*
23 *oleracea* cabbage plants, in the presence or absence of a shared predator (*Chrysoperla*
24 *carnea* larvae). Aphid population growth rates, aphid distributions on host plants and
25 predator growth rates were measured. In single-species treatments, *C. carnea* reduced
26 *M. persicae* population growth rate, but had no significant impact on *B. brassicae*
27 population growth rate, suggesting *B. brassicae* chemical defences are effective
28 against *C. carnea*. *C. carnea* had no significant impact on either aphid species
29 population growth rate in mixed-species treatments. *M. persicae* (ND) therefore
30 experienced reduced predation in the presence of *B. brassicae* (CD) through a
31 predator-mediated indirect effect. Moreover, predator growth rates were significantly
32 higher in the *M. persicae*-only treatments than in either the *B. brassicae*-only or
33 mixed-species treatments, suggesting predation was impaired in the presence of *B.*
34 *brassicae* (CD). A trait-mediated indirect interaction is proposed, consistent with

35 associational resistance, in which the predator, upon incidental consumption of
36 chemically-defended aphids is deterred from feeding, releasing non-defended aphids
37 from predatory control.

38

Introduction

Many mechanisms that shape ecological communities involve indirect interactions. For example, trophic cascades (where enemies of herbivores indirectly affect plant communities, Pace et al. 1999; Schmitz et al. 2004), exploitation competition (where organisms indirectly compete for shared resources, Holt et al. 1994; Denno et al. 2000) and apparent competition (where organisms 'compete for survival' through sharing natural enemies, Holt 1977, van Veen et al. 2006) have long been known to affect community structures and persistence. Only within the last decade has our knowledge of 'neighbour effects' or 'associational interactions' been synthesized and their contribution to interactions at population and community levels been addressed (Barbosa et al. 2009; Underwood et al. 2014). As studies of associational interactions among higher trophic level terrestrial communities are sparse, here, we investigate the occurrence and strength of associational interaction between chemically-defended and non-defended aphids, sharing a generalist predator in a model terrestrial system.

Associational effects are 'when consumer effects on individuals of one resource organism type, at a given density, are a function of the neighbourhood composition of other resource types at particular spatial scales' (Underwood *et al.* 2014). The strength and nature of these interactions can be influenced by traits of resource organisms (Underwood et al. 2014) such as chemical defences – that may render organisms unpalatable to enemies. Associational resistance (AR) has been defined as 'reduced consumer effects in a community with non-focal neighbours compared to a monoculture of the focal organism' (Underwood et al. 2014), that could result from traits of one resource species (such as chemical defences) deterring consumers from using neighbouring resource species. In contrast, associational

susceptibility (AS) has been defined as 'increased consumer effects in a community with non-focal neighbours compared to a monoculture of the focal organism' (Underwood et al. 2014), that could result from traits of one resource species (such as palatability) encouraging consumers to use neighbouring resource species. At present, AR and AS have mostly been observed between palatable and chemically-defended, non-palatable plant species consumed by herbivores (Hay 1986; Wahl and Hay 1995; Kostenko et al. 2012; Castagneyrol et al. 2013). However, taxa including amphibians (Daly 1995; Kats et al. 1988), reptiles (Williams et al. 2004; Fry et al. 2005) and invertebrates (Opitz and Müller 2009) also possess chemical defences, rendering them unpalatable, toxic or venomous. Thus, associational interactions among terrestrial higher order communities are underexplored while mechanisms that determine whether AR or AS occur remain unclear (Barbosa *et al.* 2009).

The occurrence and strength of associational interactions are likely to depend on whether consumers are selective in their prey choice, and how desirable and non-desirable prey distribute among their shared habitat (Fig. 1; Holt 1984; Holt and Kotler 1987; Schmitz et al. 2004). If predators are selective, they may avoid consuming undesirable prey in favour of better quality prey, irrespective of how prey types are distributed (Figure $1a_1$ & $1b_1$; Eisner et al. 2000; Boivin et al. 2010). However, for unselective predators, the distribution of each prey species may greatly affect relative rates of predation. Where prey occupy distinct spatial niches, an unselective predator encountering a patch of non-defended, good-quality prey, may continue to use that patch until prey are depleted or predation is at a sub-optimal rate (Fig. $1a_2$). If an unselective predator encounters a patch of harmful or undesirable prey, consumption of prey may harm or kill the predator, or encourage it to seek a new patch (Fig. $1a_3$; MacArthur and Pianka 1966; Charnov 1976; Heller 1980). Where

prey types mix among their habitat, an unselective predator would encounter both prey types while foraging increasing the potential for associational resistance (Fig. $1b_2$) or associational susceptibility (Fig. $1b_3$), as the likelihood of incidental prey consumption increases (Prasad and Snyder 2006). The nature of any associational interaction between prey species may therefore be affected by whether predators are selective in choosing their prey and whether prey species mix or segregate in their habitat.

Brassica plants, including cabbage and broccoli, provide an opportunity to test associational interactions among terrestrial invertebrate communities. Two aphid pests, which can occur on the same plants (Kalule and Wright 2002b), possess different adaptations to *Brassica* plants' glucosinolate-based chemical defences (Halkier and Gerschenzon 2006; Hopkins, et al. 2009). Specialist *Brevicoryne brassicae* (Linnaeus) aphids sequester the plants' chemical defences (Francis et al. 2001; Bridges et al. 2002; Kazana et al. 2007) rendering them toxic, or inhibitory to the growth rates of generalist predators including *Adalia bipunctata* (Linnaeus) ladybird larvae, *Episyrphus balteatus* (De Geer) hoverfly larvae and *Chrysoperla carnea* (Stephens) lacewing larvae upon consumption (Francis et al. 2001; Kazana et al. 2007; Kos et al. 2011; 2012). Generalist *Myzus persicae* (Sulzer) aphids, however, possess no chemical defences against enemies (Francis et al. 2001, Bridges et al. 2002). Previously, we observed that *C. carnea* did not innately select, or learn to select *M. persicae* over *B. brassicae* when given a choice (Nesbit et al. 2015). However, these behavioural assays were conducted over a short time scale (5 hours) in Petri dishes, not among host plants, where the spatial distributions of aphids may affect the outcome (as Fig. 1).

113 *B. brassicae* have been observed to aggregate among younger leaves higher up
114 the stem, whereas *M. persicae* aggregate among older, lower leaves (Trumble 1982;
115 Staley et al. 2011). Variation in chemical defences between plant cultivars and organs
116 could feasibly contribute to the difference in aphid distributions. For example,
117 generalist *M. persicae* aphids may aggregate more heavily than specialist *B. brassicae*
118 among low-tier leaves (Trumble 1982; Staley *et al.* 2011) because they are typically
119 less well defended than newer leaves (Fagerstrom *et al.* 1987; McCall & Fordyce
120 2010; van Dam *et al.* 1996), while both species may aggregate more heavily among
121 less defended organs on more defended plants.

122 Here, we assess the nature of associational interaction between neighbouring
123 non-defended prey (*M. persicae*) and chemically-defended prey (*B. brassicae*), via a
124 shared predator (*C. carnea*), in a terrestrial higher trophic level community. The
125 following predictions were tested: (1) suppression of aphid population growth rate by
126 the shared predator will be greater against non-defended *M. persicae* than chemically-
127 defended *B. brassicae* aphids (following Kalule and Wright 2002a; 2002b; Chaplin-
128 Kramer et al. 2011). (2) Predator efficacy against each prey species when presented
129 together will vary depending on how prey species distribute among their shared
130 habitat. As *C. carnea* have previously been shown to be unselective in their prey
131 choice (Nesbit et al. 2015), relative consumption of harmful/non-harmful prey will
132 depend on relative encounter rates (Fig. 1). If prey species show a high degree of
133 spatial heterogeneity then we expect an associational interaction will occur (following
134 Fig. $1b_2$ and $1b_3$). If prey species are spatially segregated, we expect the unselective
135 predator to consume *M. persicae*, as predators may find a good-quality resource patch
136 (Fig. $1a_2$), or relocate from a poor-quality patch (Fig. $1a_3$). (3) Predator efficacy will

vary depending on the variety of cabbage plant hosting the prey species, as aphid distributions will vary between varieties (Kalule and Wright 2002a; 2002b).

Materials and methods

A tri-trophic model system was used with treatments including combinations of aphid composition (*Brevicoryne brassicae* alone; *Myzus persicae* alone; or a mixed treatment including both aphid species), predation (*Chrysoperla carnea* lacewing larvae present or absent) and host plant cabbage cultivar (*Brassica oleracea* cv. Derby Day and cv. f1 Minicole), resulting in a total of 12 treatment combinations. Derby Day has consistently been reported as susceptible to herbivory (Kalule and Wright 2002a; 2002b), while Minicole is reported to possess a degree of resistance against *B. brassicae* and *M. persicae* due to greater antibiosis (Kalule and Wright 2002a; 2002b). Four replicate cages of each treatment combination were included per experimental block. The experiment was conducted in two consecutive temporal blocks, giving a total of eight replicates of each treatment combination. Seeds of both cabbage cultivars (Nicky's Nurseries, Broadstairs, UK) were sown in John Innes n^{o}.2 compost (August and September 2010) in 15 cell seed trays (65 mm width, 65 mm length, 60 mm depth per cell) and grown for five weeks in a glasshouse. At five weeks after sowing, all cabbages were re-potted (10 cm diameter by 9 cm depth pots) and moved to a controlled environment (CE) room (12 h light: 12 h dark, temp. 22^{o}C). *B. brassicae* were originally supplied from lab stocks maintained at HRI Warwick and *M. persicae* were obtained from lab stocks maintained at Rothamsted Institute (both species were sourced close to the respective institutions). They were maintained in cultures at Lancaster University for a year prior to this experiment. Cultures of both

aphid species were maintained on Derby Day cabbage plants in a CE room, with conditions as previously stated. *C. carnea* larvae (2nd instar, Fargro Ltd., Littlehampton, UK) were stored in a refrigerator at 4°C and maintained on a diet of the buckwheat seeds they were supplied with, for approximately 3 days until the experiment began.

Experimental Set-up

One week before the experiment began, plants were transferred to experimental mesh cages (30 cm diameter, approx. 60 cm high) in the CE room (conditions as above), one plant per cage. Plants were watered daily and given a week to acclimatise to the conditions. Measurements of plant height (mm, measured from the base of the stem to tip of the budding leaf) and leaf number were used to assign plants to treatments; the mean height and mean leaf number of plants was equalised between treatments.

At the start of the experiment, twenty mixed-age wingless aphids (ten of each species for mixed-species treatments) were transferred to 3 cm diameter Petri dishes and left in contact with the base of the host plant stem, allowing the aphids to freely distribute on the plants. On day three, an aphid count was conducted by removing the plant carefully from the cage, counting the number of aphids on each leaf and on the plant 'core' (the stem, cotyledons and growing points of the plant). *C. carnea* (2nd instar), stored individually in 3 cm diameter Petri dishes, were then released at the base of the stem, one individual *C. carnea* per cage. *C. carnea* had been weighed prior to starvation overnight, before being assigned to treatments.

184 The experimental duration was eight days including the day *C. carnea* were
185 released. The duration was chosen following a preliminary investigation which
186 showed population growth of *B. brassicae* and *M. persicae* to continue to grow in an
187 exponential phase during this time. *C. carnea* would also remain as predatory larvae
188 during this time (before spinning cocoons and maturing into non-predatory adults).
189 Plants were watered daily until the compost was saturated. The cages were randomly
190 re-distributed around the CE room every day. Plants were destructively sampled on
191 the last day to count aphids, after which cages were searched for *C. carnea*, which
192 were then weighed.

193

194 *Statistical Analysis*

195 The effect of experimental treatments on aphid populations was analysed using
196 linear mixed effects (LME) models. As single species treatments started with twenty
197 aphids and mixed-species treatments started with ten of each species, the final aphid
198 counts were transformed to population growth rates using the formula below to enable
199 comparisons of treatment effects:

200 Population growth rate = ln(final population count +1/initial population count +1)

201 Population growth rates for each aphid species were analysed separately. The maximal
202 model for each aphid species included mixing with the other respective aphid species
203 (monoculture or mixed), predation (the presence/absence of *C. carnea*) and cultivar
204 (Derby Day or Minicole) with all interactions. Experimental block (1 or 2) and the
205 total number of leaves per plant (4 to 9) were included as individual random effects
206 terms. The significance of fixed effects was assessed by sequential deletion from the
207 maximal model using maximum likelihood parameter estimation. Deviance change

between models with and without individual terms was tested using chi-squared (χ^2) tests (hereafter: analysis of deviance, Zuur et al. 2009). The final model including significant fixed effects and the random effects, was re-fitted under REML parameter estimation and checked for mis-specification by inspection of residuals, as outlined in Zuur et al. (2009).

To test the effects of the experimental treatments on *C. carnea* predators, the growth of individual predators was estimated as:

Predator growth rate = ln(recovered fresh mass (mg)/initial fresh mass (mg))

Fixed effects in the maximal LME model included cultivar, aphid species (*B. brassicae*, *M. persicae* or mixed *B. brassicae* and *M. persicae*) and the interaction term. The random effect was experimental block. The significance of fixed effects was assessed by analysis of deviance following the procedures described above (Zuur et al. 2009).

To assess variation in aphid distributions within the plants, the final counts of aphids at four sites within the plant were analysed: core (stem, cotyledons and growing points), low-tier leaves (oldest, lowest position on the stem), middle-tier leaves and top-tier leaves (youngest at the start of the experiment, with highest position on the stem). The number of leaves counted in each tier varied between plants of different total leaf numbers (Four-leaved plant: 2,1,1; five-leaved plant: 2,2,1; six-leaved plant 2,2,2 and seven-leaved plant: 3,2,2 respectively for top-, middle- and low-tier leaf sites, etc.). Data were analysed separately for single and mixed-species treatments due to the different starting population sizes. Data were tested for overdispersion and maximal models were fit to two available parameterisations of the negative binomial distribution using generalised linear mixed effects models

232 (GLMMs) (Zuur et al. 2009) each with and without a mixture-zero-inflation parameter
233 (Zuur et al. 2009), giving four possible maximal models. The most suitable maximal
234 model was chosen based on the lowest AIC score. The fixed effects of each maximal
235 model included aphid species (*B. brassicae* or *M. persicae*), predation (presence or
236 absence of *C. carnea*), cultivar (Derby Day or Minicole) and plant site with all two
237 and three-way interaction terms. Due to the variation in number of leaves counted per
238 tier between plants of different numbers of leaves, total leaf number was included as a
239 random effect, in addition to experimental block and host plant ID, as counts were
240 made from sites of the same plant. The significance of fixed effects was tested by
241 analysis of deviance, as described above (Zuur et al. 2009).

242 All analyses were conducted using the 'R.v.2.15.2' statistical software (R
243 Development Core Team 2012). All LME models were fitted using the 'lme4'
244 package (Bates et al. 2012). All GLMMs were fitted using the 'glmmADMB' package
245 (Fournier et al. 2012). Overdispersion tests were conducted using the 'qcc' package
246 (Scrucca 2004).
247

248 **Data deposition**

249 Data available from the Dryad Digital Repository:
250 <http://dx.doi.org/10.5061/dryad.ks10q> (Nesbit et al. 2016).

251

252 ## Results

253 Neither mixing with *Myzus persicae* aphids, predation from *Chrysoperla*
254 *carnea* larvae or variation in cabbage cultivar had any significant effect on

Brevicoryne brassicae population growth rates (Table 1a; Fig. 2a). In contrast, predators effectively reduced *M. persicae* population growth rates in single-species treatments, but had no significant impact when *M. persicae* were in mixed-species treatments with *B. brassicae* (Table 1b, Fig. 2b).

For single aphid species treatment combinations, in the absence of predators, (Table 2a), aphid counts across plant sites were significantly influenced by the interaction between aphid species and plant site (Table 2a): numbers of *M. persicae* and *B. brassicae* were similar among top-tier and middle-tier leaves, but *M. persicae* counts were much higher than *B. brassicae* on the low-tier leaves and the plant core (Fig. 3a). Numbers of both aphid species were also significantly affected by the interaction between cultivar and plant site (Table 2a), as counts of both aphid species were higher on the core of Derby Day plants than on the core of Minicole plants. For *B. brassicae*, therefore, counts on Derby Day plants were comparatively uniform across plant sites, whereas on Minicole plants, *B. brassicae* counts were low on the plant core and highest on the middle-tier leaves. For *M. persicae*, counts on Derby Day plants were highest on the core, while on Minicole plants, counts were equally high on the core and low-tier leaves (Fig. 3a). Aphid numbers per plant site were also significantly reduced in the presence of *C. carnea* (Table 2a). However, this effect was mediated by the interaction between predation and plant site (Table 2a), as *C. carnea* reduced aphid abundance on the core and low-tier leaves, but not on the middle- and top-tier leaves on both cultivars (Fig. 3a).

Among mixed-species treatments, distributions of both aphid species within the plant, in the absence of predators, were similar to those of single-species treatments. Again, aphid species and the interaction term between aphid species and plant site were significant (Table 2b), as *M. persicae* counts were similar to *B.*

brassicae counts among top- and middle-tier leaves, but *M. persicae* counts were higher on low-tier leaves and the plant core (Fig. 3b). Again, the interaction between plant site and cultivar was significant (Table 2b) as both aphid species counts were higher on the core of Derby Day plants than on the core of Minicole plants. For *B. brassicae*, counts on Derby Day plants were fairly uniform across plant sites, but were again lower on the plant core and highest on middle-tier leaves on Minicole plants. For *M. persicae,* counts were highest on the core on Derby Day, while on Minicole plants counts were higher on the low-tier leaves (Fig. 3b). Predator impacts in mixed-species treatments were more varied than in single-species treatments. Predation was significant as a fixed effect, but the effect was further influenced by two and three-way interaction terms (Table 2b). Firstly, a significant interaction was found between plant site, aphid species and predation, as *C. carnea* reduced *B. brassicae* numbers on the plant core consistently on plants of both cabbage cultivars, but had no effect on *M. persicae* counts (Fig. 3b). Secondly, the interaction between plant site, predation and cultivar was also significant, as *C. carnea* reduced numbers of both aphid species on low-tier leaves of Derby Day plants only (Fig. 3b).

Predator growth rate was significantly affected by aphid species ($\chi^2 = 7.80$, df = 2, $p = 0.020$) irrespective of plant cultivar, with higher growth rates observed for *C. carnea* from *M. persicae* treatments than either *B. brassicae* or mixed-species treatments (Fig. 4).

Discussion

The aim of this investigation was to assess how chemically-defended *Brevicoryne brassicae* and non-defended *Myzus persicae* aphids indirectly interact via

304 a shared generalist predator, *Chrysoperla carnea* lacewing larvae. When both aphid
305 species were present and under predation pressure, a predator-mediated indirect
306 interaction was observed, consistent with associational resistance (Barbosa et al. 2009,
307 Underwood et al. 2014), in which *M. persicae* indirectly benefited from the presence
308 of neighbouring *B. brassicae*, due to reduced efficacy of *C. carnea*. Additionally, the
309 importance of predator selectivity in their prey choice and the spatial distribution of
310 prey species (Fig. 1) in determining whether associational resistance or susceptibility
311 occurred was assessed. Both the inability of the predators used here to avoid
312 consuming harmful prey (Nesbit et al. 2015) and the high degree of mixing of both
313 aphid species on the same host plants (Fig. 3b) are likely to have affected the nature of
314 indirect interaction between aphid species.

315 Our first prediction was that suppression of aphid population growth rate by
316 the shared predator will be greater against non-defended *M. persicae* than chemically-
317 defended *B. brassicae* aphids (following Kalule and Wright 2002a; 2002b; Chaplin-
318 Kramer et al. 2011). In the single-species treatments, as predicted, predation of *M.*
319 *persicae* was greater than predation of *B. brassicae*, but there was also considerable,
320 consistent spatial variation in predation of both aphid species. *C. carnea* reduced
321 counts of both aphid species on the plant core (stem, cotyledons and new growth
322 material) and low-tier leaves (Fig. 3a), which suggests that *C. carnea* maintained a
323 consistent pattern of site use while foraging on plants. From ground level, *C. carnea*
324 would have used the stem to access the cotyledons, then low-, middle- and top-tier
325 leaves respectively, and are likely to have consumed aphids they encountered first
326 while foraging (as previously observed, Nesbit et al. 2015). Use of the plant core and
327 low-tier leaves may also have been promoted if the top- and middle-tier leaves were
328 more difficult to access. It is known that epicuticular waxes, which vary with plant

age, organ and organ surface (Eigenbrode and Espelie 1995) can impede mobility of predators including *C. carnea* (Eigenbrode et al. 1996). The consistent spatial variation, but different strength of predation against each aphid species meets expectations of a predator encountering differentially-defended prey. We previously observed that survival of *C. carnea* fed diets of *B. brassicae* was significantly lower than those fed diets of *M. persicae* (Nesbit et al. 2015). Furthermore, consumption of *B. brassicae* can increase mortality and/or reduce the growth rates of other generalist predators as well as *C. carnea* (Francis et al. 2001; Kazana et al. 2007; Kos et al. 2011; 2012), while other glucosinolate-sequestering herbivores can be unpalatable to enemies upon attack or consumption (Müller et al. 2002; Vlieger et al. 2004). The glucosinolate-based defences of *B. brassicae* may therefore potentially deter predators from further feeding. For example, predatory *Ceraeochrysa cubana* (Hagen) lacewing larvae have been found to abandon egg clusters of the moth *Utetheisa ornatrix* (Linnaeus) if, upon inspection, eggs are identified as chemically-defended (Eisner et al. 2000). Predatory fish can also avoid consuming unpalatable amphibian and invertebrate larvae to the extent that unpalatable prey can achieve competitive dominance in habitats with predators (Kats et al.1988; Lindquist and Hay 1996). In our system, *C. carnea* upon encountering and consuming *B. brassicae* may have been physically impaired or deterred from further feeding, feeding only to avoid starvation (Sherratt et al. 2004), resulting in the observed low predator growth rate (Fig. 4) and no reduction of *B. brassicae* population growth rate (Fig. 2a). In contrast, predation of *M. persicae* was likely only limited by satiation, resulting in a high predator growth rate (Fig. 4) and reduction of *M. persicae* population growth rate (Fig. 2b).

Our second prediction was that predator efficacy will vary depending on how prey species distribute among their shared habitat. When both aphid species were

present together, differences in their distributions on the host plant were observed. Among the leaves, *M. persicae* counts were highest on low-tier leaves while *B. brassicae* were more abundant among middle-tier leaves (Fig. 3b). However, spatial segregation between aphid species was not strong, in contrast to what has been found by other authors in the same system (Trumble 1982; Staley et al. 2011). This suggests that predators were likely to encounter aphids of both species when foraging anywhere on the plant, which may have heavily influenced the resulting predator-mediated indirect interaction (following Fig. 1b).

We predicted that if prey species showed a high degree of spatial heterogeneity then an associational interaction will occur (following Fig. $1b_2$ and $1b_3$). In contrast to the single prey species treatments, *C. carnea* reduced neither *B. brassicae* nor *M. persicae* population growth rates when the aphids were presented together (Fig. 2). Among the plant sites, the number of *B. brassicae* individuals were only consistently reduced on the plant core (Fig. 3b). It appears, therefore, that *C. carnea* encountered and consumed *B. brassicae* on the plant core while foraging, and were impaired or deterred from predation, resulting in lower predator growth rates (Fig. 4) and a release of *M. persicae* from strong predation; associational resistance/apparent commensalism via a trait-mediated indirect interaction. It should also be acknowledged that the necessary confounding of treatments with population size may affect the strength of predation rates and predator performance as well, due to the difference in aphid densities between mixed and monoculture treatments. However, the prevalence of associational resistance is consistent with the results of previous behavioural assays, which showed that when *C. carnea* encountered and consumed *B. brassicae* at a relatively high rate, *M. persicae* were released from predation pressure (Nesbit et al. 2015). Here, the same result is evident between these aphids and their shared predator

379 *in situ* among host plants, over a longer experimental duration of days rather than
380 hours. As well as by the trait-mediated indirect interaction described (sub-lethal
381 effects of *B. brassicae* consumption), associational resistance/apparent commensalism
382 could conceivably have arisen from a density-mediated indirect interaction if
383 consumption of *B. brassicae* killed *C. carnea* (Francis et al. 2001; Kos et al. 2011b;
384 2012a). This seems unlikely to have influenced our results, however, as predator
385 recapture rates were similar between aphid treatments (12/16 predators from *B.*
386 *brassicae* treatments; 11/16 predators from *M. persicae* treatments*;* 11/16 predators
387 from mixed-species treatments), which suggests no treatment effect on *C. carnea*
388 mortality.

389 How prey species distribute among their shared habitat is known to affect the
390 nature and strength of indirect apparent interactions (Holt 1984; Holt and Kotler 1987;
391 Schmitz et al. 2004) and here, seems to have influenced the nature of associational
392 interaction between two aphid species, in accordance with Fig.1b_3. At the whole plant
393 level, the high spatial dispersion of both aphid species suggests *C. carnea* were likely
394 to encounter and consume *B. brassicae* at all plant sites, however, variation in
395 numbers of each species within sites may affect the strength of AR experienced at
396 finer spatial scales. In previous behavioural assays, it was observed that when *C.*
397 *carnea* encounter and consume *B. brassicae* at a low rate, predation of both species
398 may be maintained (Nesbit et al. 2015). A similar trend may be inferred when
399 comparing predation on low-tier Minicole leaves compared to low-tier Derby Day
400 leaves.

401 Our third prediction was that predator efficacy will vary depending on the
402 variety of cabbage plant hosting the prey species, as aphid distributions will vary
403 between varieties. Although this was not evident on a whole-plant scale, there was a

404 significant difference in predation of aphids on lower tier leaves in mixed-species
405 treatments between plants of different cultivars. *C. carnea* had no effect on *B.*
406 *brassicae* or *M. persicae* numbers on the Minicole low-tier leaves, but reduced counts
407 of both species on the Derby Day low-tier leaves, where the ratio of *M. persicae*: *B.*
408 *brassicae* numbers was much greater (Fig. 3b). Thus, differences in prey distributions
409 may affect the strength and nature of associational interactions by affecting the
410 likelihood of predators encountering and consuming harmful prey.

411 Due to the difference in starting populations used between treatments,
412 statistical comparison of aphid distributions in single- and mixed-species treatments is
413 precluded. However, our observations suggest that *M. persicae* may use the plant core
414 and low-tier leaves less in mixed-species treatments than in single-species treatments
415 (Figure 3). The effect of this may be two-fold. Firstly, *M. persicae* may distribute
416 more heavily in areas less visited by *C. carnea* when *B. brassicae* is present (the
417 middle and top-tier leaves) and suffer lower predation as a result. Secondly, as *M.*
418 *persicae* numbers were relatively lower on the core and low-tier leaves, this increases
419 the likelihood of predators encountering and consuming *B. brassicae*; *B. brassicae*
420 were less 'diluted' by *M. persicae* and thus, the likelihood of associational resistance
421 may be promoted.

422 Multiple mechanisms may drive associational interactions between higher
423 order consumers. Using an aphid parasitoid system, van Veen et al (2005)
424 demonstrated that associational resistance can occur between host *Acyrthosiphon*
425 *pisum* (Harris) pea aphids and non-host, chemically-defended *Megoura viciae*
426 (Buckton) vetch aphids via *Aphidius ervi* (Haliday) parasitoids. van Veen et al. (2005)
427 found parasitism of *A. pisum* to be significantly reduced by the presence of *M. viciae*,
428 due to a reduction in parasitoid foraging efficiency (van Veen et al. 2005). Where van

Veen et al. (2005) demonstrate that associational resistance may occur in terrestrial higher trophic level systems through 'reduced prey apparancy' (where a palatable species is less visible due to unpalatable species), here, associational resistance occurred through a predator-mediated indirect interaction in which predation of non-defended prey was impaired through incidental consumption of harmful prey. Thus, associational resistance may occur also in terrestrial higher trophic level systems through 'reduced enemy efficacy'.

Through associational resistance afforded by *B. brassicae* anti-predator chemical defences, *M. persicae* may be released from predation pressure despite possessing no anti-predator defences of their own, though the scale over which these effects may last requires further investigation. We previously observed a pattern of associational resistance between these aphids in Petri dishes over a short time scale (5 hours) (Nesbit et al. 2015) and have now observed associational resistance between these aphids *in situ* among host plants over an eight day duration. Further experiments could usefully assess the strength and prevalence of these effects over a longer timescale, over different spatial scales (following Underwood et al. 2014). Associational interactions however should be considered as important ecological mechanisms in a wider context than merely plants and their associated herbivores (Barbosa et al. 2009). Associational interactions may be prevalent in any system where vulnerable prey distribute in close proximity among more physically, or chemically-defended prey species.

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459 ## References

460 Barbosa, P. et al. 2009. Associational Resistance and Associational Susceptibility:
461 Having Right or Wrong Neighbors. – Ann. Rev. Entomol. 40: 1-20.

462 Bates, B. et al. 2012. lme4: Linear mixed-effects models using S4 classes. - R package
463 version 0.999999-0. http://CRAN.R-project.org/package=lme4

464 Boivin, G. et al. 2010. Learning affects prey selection in larvae of a generalist
465 coccinellid predator. - Entomol. Exp. Appl. 135: 48-55.

466 Bridges, M. et al. 2002. Spatial organization of the glucosinolate-myrosinase system
467 in brassica specialist aphids is similar to that of host plants. - Proc. R. Soc. B. 269:
468 187-191.

469 Castagneyrol, B. et al. 2013. Plant apparency, an overlooked driver of associational
470 resistance to insect herbivory. – J. Ecol. 101: 418-429.

471 Chaplin-Kramer, R. et al. 2011. Chemically mediated tritrophic interactions: opposing
472 effects of glucosinolates on a specialist herbivore and its predators. – J. Appl. Ecol.
473 48: 880-887.

474 Charnov, E.L. 1976. Optimal foraging, the Marginal Value Theorem - Theor. Popul.
475 Biol. 9: 129-136.

476 Daly, J.W. 1995. The chemistry of poisons in amphibian skin. - P. Natl. Acad. Sci.
477 USA 92: 9-13.

478 Denno, R.F. et al. 2000. Feeding-induced changes in plant quality mediate
479 interspecific competition between sap-feeding herbivores. - Ecology 81: 1814-1827.

480 Eigenbrode, S.D. and Espelie, K.E. 1995. Effects of plant epicuticular lipids on insect
481 herbivores. – Ann. Rev. Entomol. 40: 171-194.

482 Eigenbrode, S.D., et al. 1996. Mobility of three generalist predators is greater on
483 cabbage with glossy leaf wax than on cabbage with a wax bloom. - Entomol. Exp.
484 Appl. 81: 335-343.

485 Eisner, T. et al. 2000. Chemical defences against predation in an insect egg. - P. Natl.
486 Acad. Sci. USA 97: 1634-1639.

487 Fagerstrom, T. et al. 1987. On Optimal Defence in Plants. – Funct. Ecol. 1:73-81.

488 Fournier, D.A. et al. 2012. AD Model Builder: using automatic differentiation for
489 statistical inference of highly parameterized complex nonlinear models. – Optim.
490 Method. Softw. 27: 233-249.

491 Francis, F. et al. 2001. Effects of allelochemicals from first (Brassicaceae) and second
492 (*Myzus persicae* and *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. – J.
493 Chem. Ecol. 27: 243-256.

494 Fry, B.G. et al. 2005. Early evolution of the venom system in lizards and snakes. -
495 Nature 439: 584-588.

496 Halkier, B.A., & Gerschenzon, J. 2006. Biology and Biochemistry of Glucosinolates. -
497 Annu. Rev. Plant Biol. 57: 303-333.

498 Hay, M.E. 1986. Associational Plant Defenses and the Maintenance of Species
499 Diversity: Turning Competitors into Accomplices. – Am. Nat. 128: 617-641.

500 Heller, R. 1980. On Optimal Diet in a Patchy Environment. - Theor. Popul. Biol. 17:
501 201-214.

Holt, R.D. 1977. Predation, Apparent Competition, and the Structure of Prey Communities. – Theor. Popul. Biol. 12: 197-229.

Holt, R.D. 1984. Spatial Heterogeneity, Indirect Interactions, and the Coexistence of Prey Species. – Am. Nat. 124: 377-406.

Holt, R.D. and Kotler, B.P. 1987. Short-Term Apparent Competition. – Am. Nat 130: 412-430.

Holt, R.D. et al. 1994. Simple Rules for Interspecific Dominance in Systems with Exploitative and Apparent Competition. – Am. Nat. 144: 741-771.

Hopkins, R.J. et al. 2009. Role of Glucosinolates in Insect-Plant Relationships and Multitrophic Interactions. - Annu. Rev. Entomol. 54: 57-83.

Kalule, T. and Wright, D.J. 2002a. Effect of cabbage cultivars with varying levels of resistance to aphids on the performance of the parasitoid, *Aphidius colemanii* (Hymenoptera: Braconidae). - B. Entomol. Res. 92: 53-59.

Kalule, T. and Wright, D.J. 2002b. Tritrophic interactions between cabbage cultivars with different resistance and fertilizer levels, cruciferous aphids and parasitoids under field conditions. - B. Entomol. Res. 92: 61-69.

Kats, L.B. et al. 1988. Antipredator defences and the persistence of amphibian larvae with fishes. - Ecology 69: 1865-1870.

Kazana, E. et al. 2007. The cabbage aphid: a walking mustard oil bomb. - Proc. R. Soc. B. 274: 2271-2277.

Kos, M., et al. 2011. Prey-mediated effects of glucosinolates on aphid predators. – Ecol. Entomol. 36: 377-388.

524 Kos, M., et al. 2012. Herbivore-Mediated Effects of Glucosinolates on Different
525 Natural Enemies of a Specialist Aphid. – J. Chem. Ecol. 38: 100-115.

526 Kostenko, O. et al. 2012. Effects of diversity and identity on the neighbouring plant
527 community on the abundance of arthropods on individual ragwort (*Jacobaea vulgaris*)
528 plants. – Entomol. Exp. Appl. 144: 27-36.

529 Lindquist, N. and Hay, M.E. 1996. Palatability and Chemical Defense of Marine
530 Invertebrate Larvae. - Ecol. Monogr. 66:431–450.

531 MacArthur, R.H. and Pianka, E.R. 1966. On optimal use of a patchy environment. -
532 Am. Nat. 100: 603-609.

533 McCall, A.C. and Fordyce, J.A. 2010. Can optimal defence theory be used to predict
534 distribution of plant chemical defences? – J. Ecol. 98: 985-992.

535 Müller, C. et al. 2002. Host plant derived feeding deterrence towards ants in the turnip
536 sawfly *Athalia rosae.* - Entomol. Exp. Appl. 104: 153-157.

537 Nesbit, C.M. et al. 2015. Selection of aphid prey by a generalist predator: do prey
538 chemical defences matter? – Ecol. Entomol. 40: 767-775.

539 Nesbit, C. M. et al. 2016. Data from: Associational resistance or susceptibility: the
540 indirect interaction between chemically-defended and non-defended herbivore prey
541 via a shared predator. – Dryad Digital Repository,
542 <http://dx.doi.org/10.5061/dryad.ks10g>.

543 Opitz, S.E.W. and Müller, C. 2009. Plant chemistry and insect sequestration. -
544 Chemoecology 19: 117-154.

545 Pace, M.L., et al. 1999. Trophic cascades revealed in diverse ecosystems. - Trends
546 Ecol. Evol. 14: 483-488.

547 Prasad, R.P. and Snyder, W.E. 2006. Diverse Trait-Mediated Interactions in a Multi-
548 Predator, Multi-Prey Community. - Ecology 87: 1131-1137.

549 R Core Development Team. 2012. R: A language and environment for statistical
550 computing. - R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-
551 900051-07-0, URL http://www.R-project.org/.

552 Schmitz, O.J. et al. 2004. Trophic cascades: the primacy of trait-mediated indirect
553 interactions. – Ecol. Lett. 7: 153-163.

554 Scrucca, L. 2004. qcc: an R package for quality control charting and statistical process
555 control. - R News 4: 11-17.

556 Sherratt, T.N. et al. 2004. Natural selection on unpalatable species imposed by state-
557 dependent foraging behaviour. – J. Theor. Biol. 228: 217-226.

558 Staley, J.T. et al. 2011. Plant nutrient supply determines competition between
559 phytophagous insects. - Proc. R. Soc. B. 278: 718-724.

560 Trumble, J.T. 1982. Within-Plant Distribution and Sampling of Aphids (Homoptera:
561 Aphididae) on Broccoli in Southern California. - J. Econ. Entomol. 75: 587-592.

562 Underwood, N. et al. 2014. A conceptual framework for associational effects: when
563 do neighbors matter and how would we know? - Q. Rev. Biol. 89: 1-19.

564 van Dam, N. M. et al. 1996. Optimal Distribution of Defences: Are Plants Smart
565 Investors? – Funct. Ecol. 10: 128-136.

van Veen, F.J.F. et al. 2005. Stable coexistence in insect communities due to density- and trait-Mediated indirect interactions. - Ecology 86: 1382-1389.

van Veen, F.J.F. et al. 2006. Apparent Competition, Quantitative Food Webs, and the Structure of Phytophagous Insect Communities. - Annu. Rev. Entomol. 51: 187-208.

Vlieger, L. et al. 2004. Effectiveness of the defence mechanism of the turnip sawfly, *Athalia rosae* (Hymenoptera: Tenthredinidae), against predation by lizards. – B. Entomol. Res. 94: 283-289.

Wahl, M. and Hay, M.E. 1995. Associational resistance and shared doom: effects of epibiosis on herbivory - Oecologia 102: 329-340.

Williams, B.L. et al. 2004. A resistant predator and its toxic prey: Persistence of newt toxin leads to poisonous (not venomous) snakes - J. Chem. Ecol. 30: 1901-1919.

Zuur, A.F. et al. 2009. Mixed Effects Models and Extensions in Ecology with R - Springer Science.

579 Tables and Table Legends

580

581 Table 1: Results from deletion tests assessing the impacts of treatments, on the
582 population growth rates of (a) *Brevicoryne brassicae* and (b) *Myzus persicae* aphids in
583 linear mixed effects models. Fixed effects include: aphid treatment (monoculture or
584 mixed with the other respective aphid species), predation (*Chrysoperla carnea* larvae
585 present or absent) and plant cultivar (Derby Day or Minicole cabbage cultivar).
586 Significant effects are highlighted bold. All fixed effects had one degree of freedom.

Aphid Species:	(a) *B. brassicae*		(b) *M. persicae*	587
Response:	Aphid Population Growth Rate			588
Fixed Effects	χ^2	*p*	χ^2	*p* 589
Aphid Treatment	0.46	0.496	3.27	0.071
Predation	2.06	0.151	10.54	**0.001**
Cultivar	0.01	0.908	0.70	0.404
Aphid Treatment:Predation	0.01	0.938	4.54	**0.033**
Predation:Cultivar	0.06	0.813	2.01	0.157
Aphid Treatment:Cultivar	0.09	0.760	1.68	0.195
Aphid Treatment:Predation:Cultivar	0.01	0.923	0.02	0.895

595 Table 2: The significance of fixed effects on aphid count per plant site at the end of
596 the experiment for (a) single-species treatments and (b) mixed-species treatments.
597 Fixed effects include plant site (core/top-tier leaves/mid-tier leaves/low-tier leaves),
598 aphid species (*Brevicoryne brassicae* or *Myzus persicae*), predation from *Chrysoperla*
599 *carnea* larvae and host cabbage cultivar (Derby Day or Minicole). Included are the
600 overdispersion test results to assess the suitability of a Poisson distribution (rejected at
601 $p < 0.05$) and selection of the negative binomial response distribution (highlighted in
602 bold) for generalized linear mixed models based on lowest AIC scores. ZI denotes
603 inclusion of a mixture zero-inflation parameter, using one degree of freedom.
604 Significant effects are highlighted in bold. The negative binomial dispersion
605 parameter (Theta) and zero-inflation parameter of the minimum adequate model
606 (MAM) are also included.

607

Treatments:	(a) Single Species			(b) Mixed Species		
Response:	Aphid count per site					
Overdispersion	D		*p*	D		*p*
	19.9		< 0.001	17.8		< 0.001
Distribution	AIC		Theta (θ)	AIC		Theta (θ)
n.binom	1918		1.43 ± 0.19	1678		0.95 ± 0.10
n.binom (ZI)	1892		2.43 ± 0.38	1658		1.86 ± 0.34
n.binom1	1895		9.62 ± 1.10	1662		10.3 ± 1.32
n.binom1 (ZI)	**1875**		7.42 ± 0.96	**1644**		7.74 ± 0.02
Fixed Effects	χ^2	df	*p*	χ^2	df	*p*
Plant Site	4.13	3	0.247	2.06	3	0.561
Aphid Species	8.05	1	**0.005**	40.80	1	**< 0.001**
Predation	15.92	1	**< 0.001**	4.24	1	**0.045**
Cultivar	0.23	1	0.632	3.21	1	0.073
Plant Site:Aphid Species	39.90	3	**< 0.001**	48.76	3	**< 0.001**
Plant Site:Predation	20.07	3	**< 0.001**	4.91	3	0.179
Aphid Species:Predation	2.00	1	0.157	0.25	1	0.620
Plant Site:Cultivar	12.39	3	**0.006**	19.57	3	**< 0.001**
Aphid Species:Cultivar	0.69	1	0.408	3.08	1	0.079
Predation:Cultivar	0.86	1	0.354	0.97	1	0.325
Plant Site:Aphid Species:Predation	5.27	3	0.153	10.10	3	**0.018**
Plant Site:Aphid Species:Cultivar	1.10	3	0.778	3.71	3	0.294

Plant Site:Predation:Cultivar	6.70	3	0.082	7.95	3	**0.047**
Aphid Species:Predation:Cultivar	< 0.01	1	0.950	0.40	1	0.528
Theta (θ) (MAM)			8.56 ± 1.12			8.41 ± 1.23
Zero-Inflation (MAM)			0.04 ± 0.02			0.07 ± 0.03

608

609 Figure Legends

610 Figure 1: An overview of how the spatial distribution of prey may affect the impacts
611 of predators on good quality and poor quality (unpalatable or harmful) prey.

612

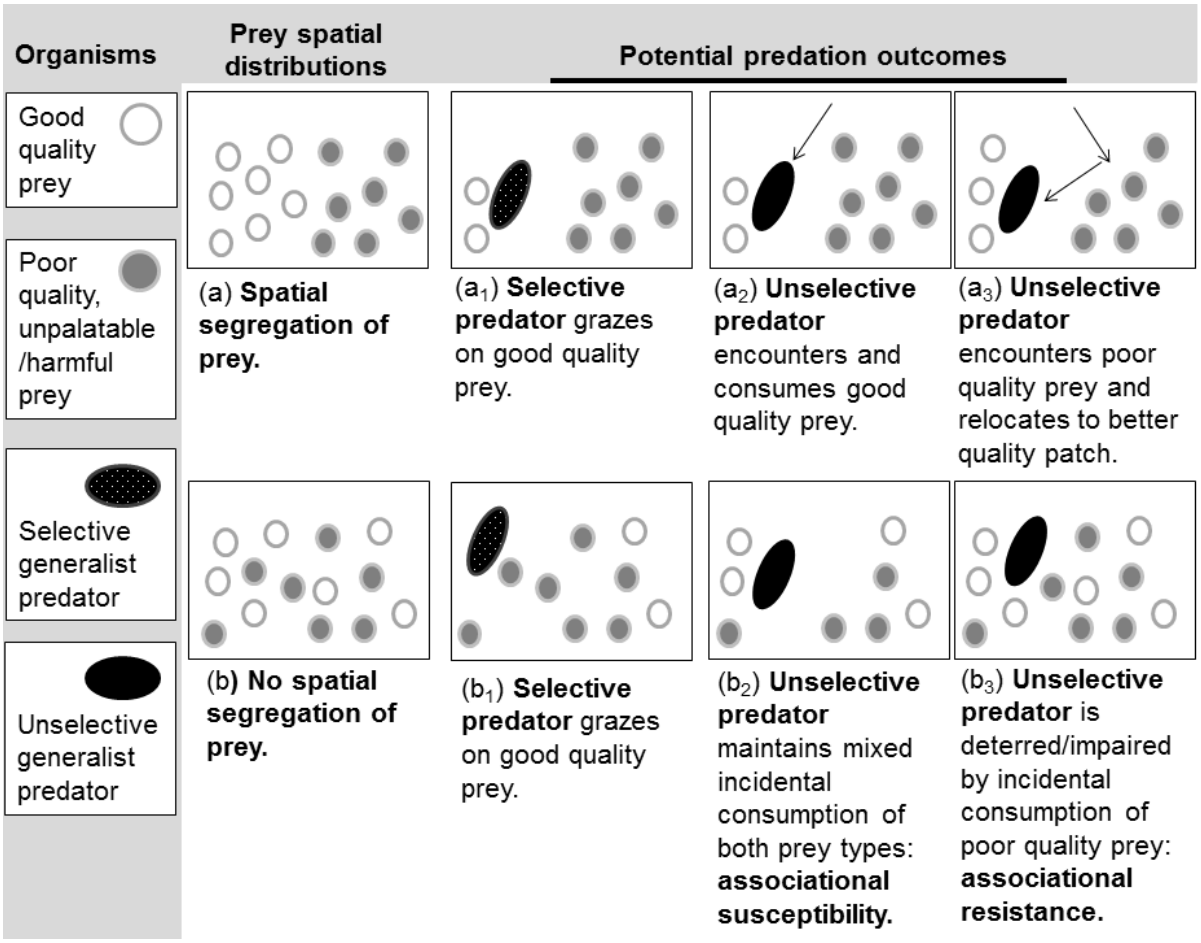
613 Figure 2: Population growth rates (ln(final count+1/initial count +1)) of (a)
614 *Brevicoryne brassicae* (b) *Myzus persicae* for each experimental treatment:
615 Monoculture (aphid species alone), Predation (aphid species in monoculture, but
616 under predation pressure from *Chrysoperla carnea* larvae), Mixed (other aphid
617 species also present, no predator) and Mixed+Pred (both mixed with the other
618 respective aphid species and under predation pressure). The grey dots denote the raw
619 data including random effects. The black dots denote the means and black error bars
620 denote the standard error of the means.

621

622 Figure 3: The counts of aphids at different sites within the host plant for (a) single-
623 species and (b) mixed-species treatments of *Brevicoryne brassicae* (Bb) or *Myzus*
624 *persicae* (Mp) aphids on either Derby Day (DD) or Minicole (Min) cabbage cultivars,
625 in the presence (dark grey) or absence (light grey) of predacious *Chrysoperla carnea*
626 larvae. Sites include the plant 'core' (cotyledons, stem and growing points), low-tier
627 leaves (low), middle-tier leaves (middle) and top-tier leaves with highest relative
628 positioning on the stem (top). Bars denote the parameter estimates, back-transformed
629 from a log-link, from the minimum adequate generalised linear mixed effects model.
630 Error bars denote the back-transformed standard errors. Asterisks denote significant
631 reductions in aphid counts between predator absent and present treatments.

632

633 Figure 4: Growth rates of predatory *Chrysoperla carnea* larvae (ln(final
634 weight(g)/initial weight (g))) recovered from *Brevicoryne brassicae* (Bb; n = 12),
635 *Myzus persicae* (Mp; n = 11) or mixed *M. persicae* and *B. brassicae* (Mp+Bb; n = 11)
636 treatments after 7 days. Grey dots denote the raw data including random effects. Black
637 dots denote the mean and the black error bars denote the standard error of the means.
638

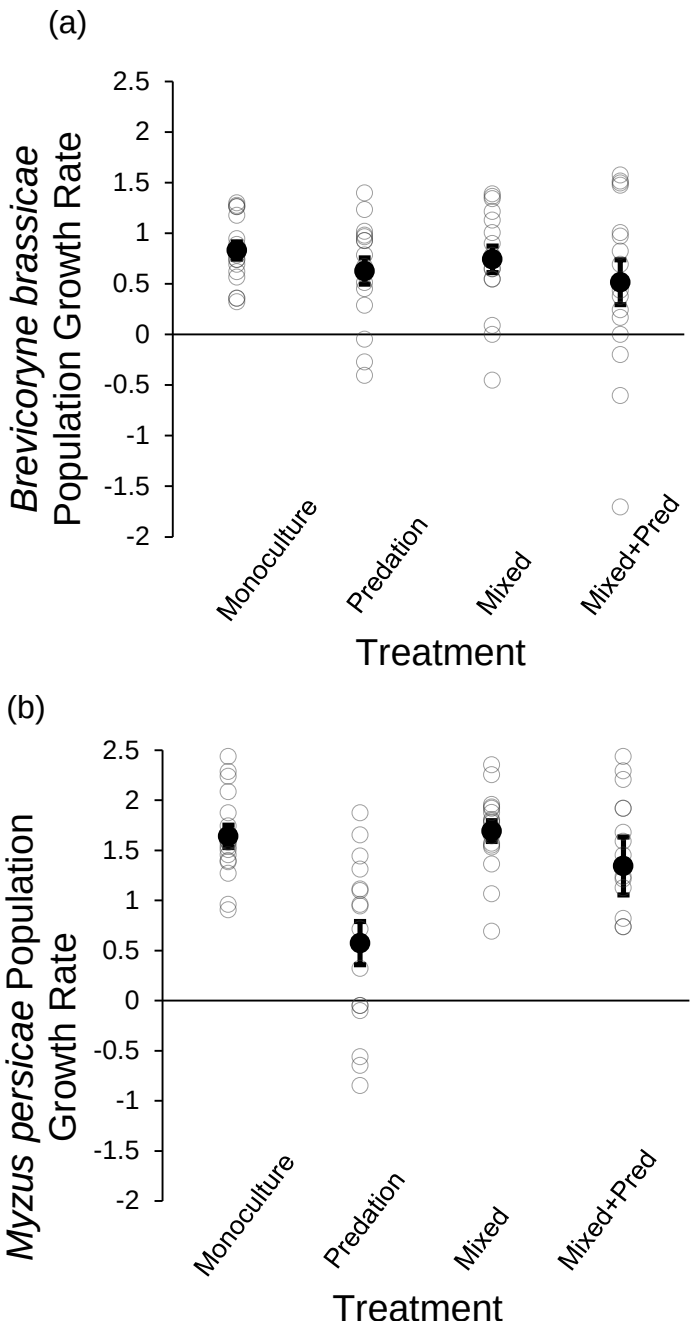


639

640 Figure 1.

641

642



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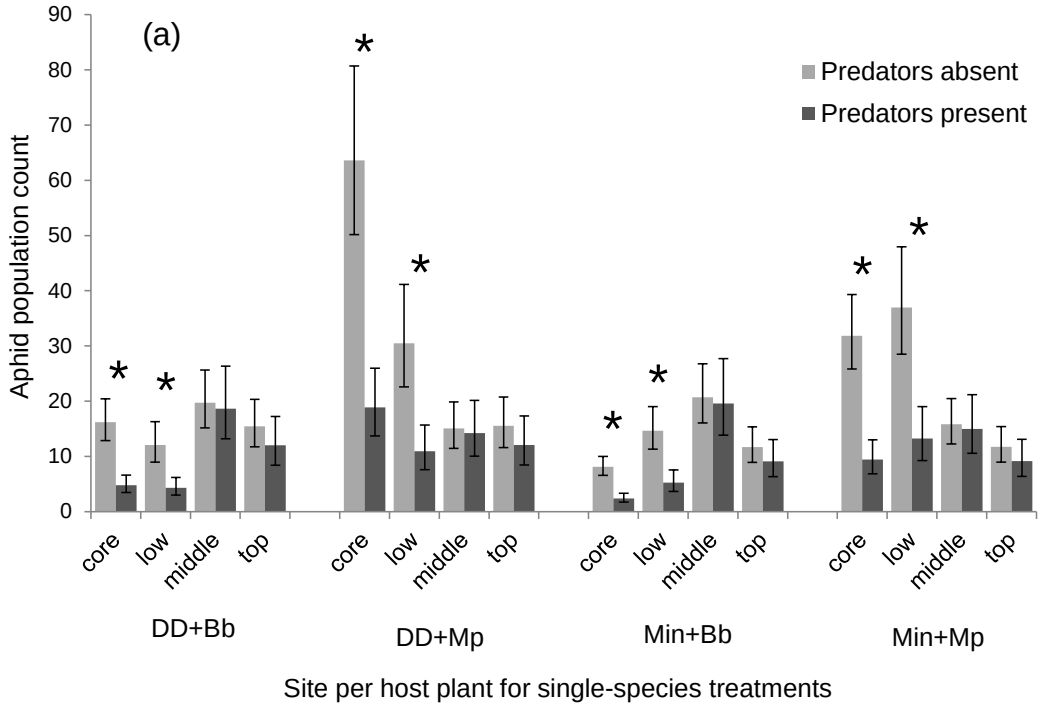
645 Figure 2.


(a)
Predators absent
Predators present
Aphid population count
0
10
20
30
40
50
60
70
80
90
core
low
middle
top
DD+Bb
DD+Mp
Min+Bb
Min+Mp
Site per host plant for single-species treatments

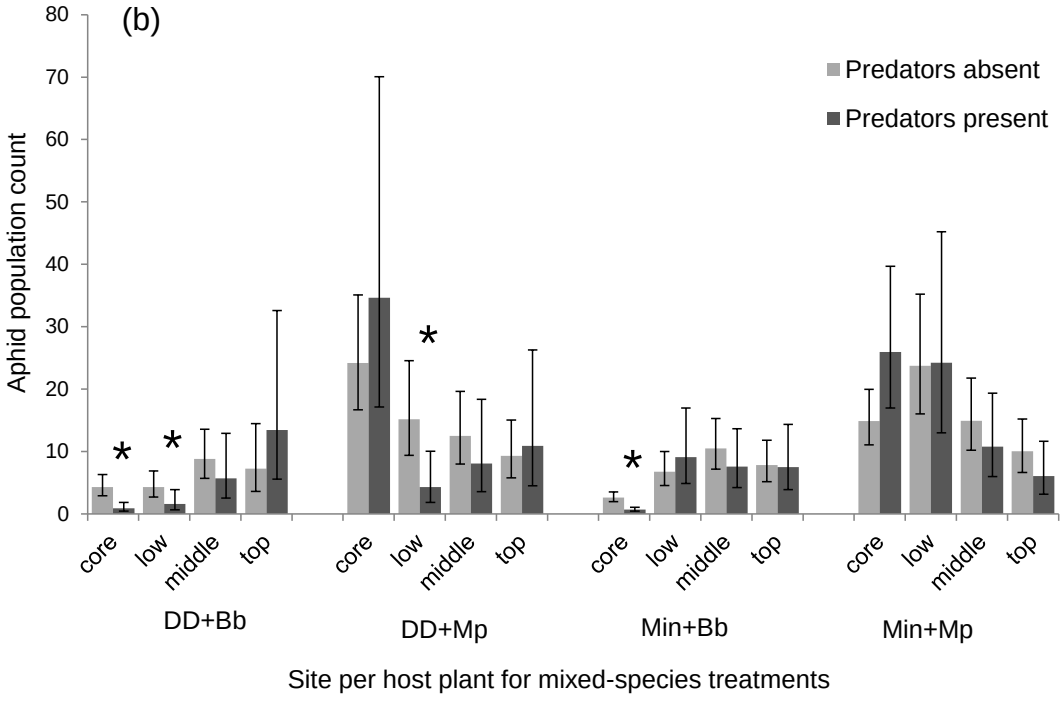
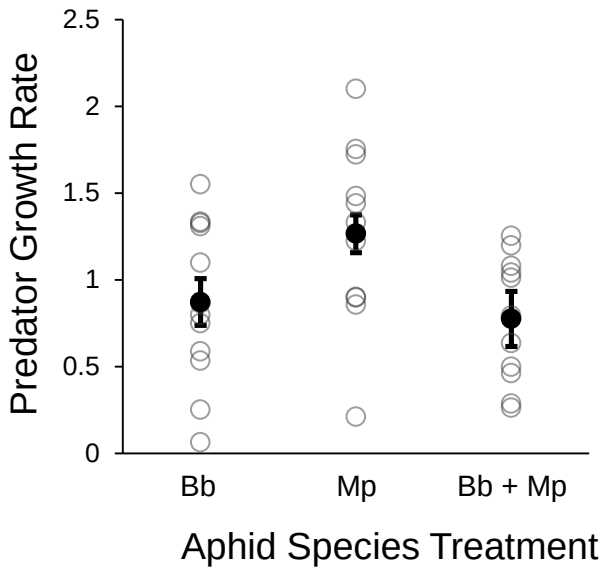

(b)
Predators absent
Predators present
Aphid population count
0
10
20
30
40
50
60
70
80
core
low
middle
top
DD+Bb
DD+Mp
Min+Bb
Min+Mp
Site per host plant for mixed-species treatments

Figure 3.



650

651 Figure 4.