

Article (refereed) - postprint

This is the peer reviewed version of the following article:

Greenop, Arran; Cecelja, Andreas; Woodcock, Ben A.; Wilby, Andrew; Cook, Samantha M.; Pywell, Richard F. 2019. **Two common invertebrate predators show varying predation responses to different types of sentinel prey.** *Journal of Applied Entomology*, 143 (4). 380-386, which has been published in final form at <https://doi.org/10.1111/jen.12612>

This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

© 2019 Blackwell Verlag GmbH

This version available <http://nora.nerc.ac.uk/522413/>

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at

<http://nora.nerc.ac.uk/policies.html#access>

This document is the authors' final manuscript version of the journal article, incorporating any revisions agreed during the peer review process. There may be differences between this and the publisher's version. You are advised to consult the publisher's version if you wish to cite from this article.

The definitive version is available at <http://onlinelibrary.wiley.com/>

Contact CEH NORA team at
noraceh@ceh.ac.uk

The NERC and CEH trademarks and logos ('the Trademarks') are registered trademarks of NERC in the UK and other countries, and may not be used without the prior written consent of the Trademark owner.

1 **Title:** Two common invertebrate predators show varying responses to different types of
2 sentinel prey in a mesocosm study

3

4 **Authors**

5 Arran Greenop.^{1,2}, Andreas Cecelja., Ben A Woodcock¹, Andrew Wilby², Samantha M Cook³
6 & Richard F Pywell¹.

7 ¹. NERC Centre for Ecology & Hydrology, Maclean Building, Crowmarsh Gifford, Wallingford,
8 Oxfordshire OX10 8BB, UK.

9 ². Lancaster Environment Centre, Library Avenue, Lancaster University, Lancaster LA1 4YQ

10 ³. Biointeractions and Crop Protection Department, Rothamsted Research, Harpenden, Herts,
11 AL5 2JQ, UK.

12

13 **Contact Author:** Arran Greenop, e-mail:arrgre@ceh.ac.uk

14

15 **Acknowledgements**

16 This study was supported by the Centre for Ecology & Hydrology, Rothamsted Research
17 and Lancaster University. The research was also supported by the Natural Environment
18 Research Council (NERC) and the Biotechnology and Biological Sciences Research Council
19 (BBSRC) under research programme NE/N018125/1 LTS-M ASSIST – Achieving
20 Sustainable Agricultural Systems www.assist.ceh.ac.uk.

21

22

23

24

25

26

27 Abstract

28 Sentinel prey (an artificially manipulated patch of prey) are widely used to assess the level of
29 predation provided by natural enemies in agricultural systems. While a number of different
30 methodologies are currently in use, little is known about how arthropod predators respond to
31 artificially-manipulated sentinel prey in comparison to predation on free-living prey
32 populations. We assessed how attack rates on immobilised (aphids stuck to cards) and
33 artificial (plasticine lepidopteran larvae mimics) sentinel prey differed to predation on free
34 moving live prey (aphids). Predation was assessed in response to density of the common
35 invertebrate predators, a foliar active ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae),
36 and a ground active beetle *Pterostichus madidus* (Coleoptera: Carabidae). Significant
37 increases in attack rates were found for the immobilised and artificial prey between the low
38 and high predator density treatments. However, an increased predator density did not
39 significantly reduce numbers of free living live aphids included in the mesocosms in addition
40 to the alternate prey. We also found no signs of predation on the artificial prey by the
41 predator *H. axyridis*. These findings suggest that if our assessment of predation had been
42 based solely on the foliar artificial prey then no increase in predation would have been found
43 in response to increased predator density. Our results demonstrate that predators
44 differentially respond to sentinel prey items which could affect the level of predation recorded
45 where target pest species are not being used.

46

47 **Key words:** ecosystem services, biological control, artificial caterpillars, aphids, ladybirds,
48 ground beetle

49

50

51

52 **Introduction**

53 Biodiversity and ecosystem functioning studies are central to understanding how humans
54 can manage the natural environment to maximise ecosystem services including pollination
55 and pest control (Bianchi et al., 2006; Cardinale et al., 2006; Foster et al., 2011). Of these
56 ecosystem services, pest regulation has received considerable attention, much of it relating
57 to the potential of natural enemies to reduce crop pest populations (Snyder et al., 2008;
58 Gardiner et al., 2009; Rusch et al., 2016; Begg et al, 2017; Greenop et al., 2018).

59

60 Fundamental to understanding the value of natural pest control in agricultural ecosystems is
61 an accurate measure of service delivery (Macfadyen et al., 2015). Several methods exist to
62 assess the suitability and function of pest control provided by natural enemies, ranging from
63 carefully-selected species assemblages in mesocosm studies conducted under laboratory
64 conditions (Straub & Snyder, 2006; Northfield et al., 2010) to the exclusion of entire
65 functional groups under real-world agricultural conditions (Gardiner et al., 2009; Holland et
66 al., 2012; Woodcock et al., 2016; Mansion-Vaquié et al 2017). The current body of literature
67 is dominated by studies that either use natural enemy abundances as a proxy for pest
68 control (Elliott et al. 1999; Schmidt et al. 2005; Bianchi, Booij & Tscharntke 2006) or infer
69 predation rates based on pest abundances (Chaplin-Kramer et al. 2011). However, both
70 approaches have associated problems that may result in the misrepresentation of the true
71 levels of pest control. For example, inferring predation based on natural enemy abundances
72 provides no direct measure of prey suppression (Macfadyen et al., 2015). Additionally pest
73 abundances are often patchy in distribution (Ferguson & Stiling 1996; Winder, Perry &
74 Holland 1999; Wan et al. 2018) and are influenced by bottom up as well as top-down factors
75 (Chaplin-Kramer et al., 2011). Ultimately, over- or under-estimating the efficacy of natural
76 pest control limits our capacity to manage and enhance this service to support sustainable
77 intensification of agricultural systems (Macfadyen et al., 2015; Zalucki et al., 2015).

78

79 To address this issue the use of sentinel prey has become a widely used methodology to
80 infer rates of natural pest control, particularly for agro-ecosystems (Lövei & Ferrante 2017).
81 These approaches use an artificially manipulated patch of prey that can be directly
82 monitored to assess rates of predation under field conditions (Howe et al., 2009; Winqvist et
83 al., 2011; Roslin et al., 2017). As such they provide a quantitative measure of the number of
84 prey consumed or parasitized, which is comparable between experimental treatments
85 (Birkhofer et al., 2017; Lövei & Ferrante, 2017). Two of the most common types of sentinel
86 prey currently used are: 1) live prey that have been immobilised, either by attaching them to
87 sticky labels (Winqvist et al., 2011), cards (Bianchi et al., 2005) or tethering the prey item
88 (Mathews et al., 2004); and 2) artificial prey items that act as lures and elicit a bite response
89 by predators that can then be observed as marks on the lure surfaces. These are
90 constructed out of materials such as modelling clay (Howe et al., 2009; Roslin et al., 2017;
91 Mansion-Vaquié et al., 2017). Both approaches have the practical advantage of allowing
92 studies to control prey densities and as such produce standardised assessments of
93 predation that can be replicated a large number of times at a relatively low cost.

94

95 Several concerns have been raised about the different sentinel prey approaches. One of the
96 most important is that immobilised or artificial prey no longer exhibit ecological mechanisms
97 that play important roles in predation rates. For example, certain aphids show a dropping
98 escape response to foliar-active natural enemies that can reduce predation rates (Losey &
99 Denno, 1998a; Dixon, 1958). Additionally, the state (live, wounded, artificial or dead) of prey
100 items has also been found to influence their attractiveness to predators (Zou et al., 2017;
101 Ferrante et al., 2017). Such ecological mechanisms therefore have potential to impact on the
102 level of predation recorded and consequently our capacity to infer pest control ecosystem
103 services.

104

105 While different sentinel prey methods are currently widely used to infer predation rates, little
106 is known about how many common predators respond to sentinel prey items and the manner
107 in which they are presented. In this study we aim to address this issue by comparing the
108 attack rates by two common predators: the Harlequin ladybird *Harmonia axyridis*
109 (Coleoptera: Coccinellidae), and a carabid beetle *Pterostichus madidus* (Coleoptera:
110 Carabidae) on immobilised prey aphids (*Sitobion avenae* (Hemiptera: Aphididae)) glued to
111 card, and artificial prey (plasticine caterpillars). We compare attack rates on the sentinel
112 methods and also assess how this differs to predation on live pest populations (free moving
113 *S. avenae*) at two different predator densities in a mesocosm study system. We predicted:
114 1) that an increase in attack rates on both sentinel prey (artificial caterpillars and immobilised
115 aphids stuck to cards) and a reduction in live free moving aphid numbers (live pest
116 population) in response to increasing predator density, under assumption that predator
117 attack rates are a linear function of predator density (Abrams & Ginzburg 2000); 2) attack
118 rates on immobilised aphids will be higher than on artificial prey, as the artificial prey do not
119 possess any chemical cues used by both predator species to locate prey and do not
120 represent a valid food item (Lövei & Sunderland, 1996; Kielty et al., 1996; Abassi et al.,
121 2000); and, 3) the ground beetle will be more likely than the ladybird to attack artificial prey
122 as they have been shown to be highly opportunistic and generalist visual hunters (Lang &
123 Gsödl, 2008; Ferrante et al., 2017). In contrast *H. axyridis* is highly dependent on olfactory
124 as well as visual cues to locate prey (Koch, 2003).

125

126 **Methods**

127 **Experimental system**

128 We used an experimental mesocosm design to control predator density and composition
129 between treatments. Each mesocosm comprised a 10L plant pot (28.5cm diameter / 22.5cm

130 deep), filled with peat-free compost and three wheat plants (*Triticum aestivum* L. Em. Fiori &
131 Paol. Variety: KWS Dacanto), enclosed within a porous plastic mesh (height 36.5cm /
132 diameter 28.5cm, pore size 0.05mm, held under standardised environmental conditions of
133 $19.5 \pm 1^\circ\text{C}$ and LD 16:8 h). The combination of a ground-foraging predator, *P. madidus* and
134 a foliar-foraging predator, *H. axyridis* was used as model predator community. Both species
135 are predators of aphids, though have spatially segregated hunting niches (ground vs.
136 canopy, respectively) (Schmitz, 2007; Woodcock & Heard, 2011). Adult *P. madidus* were
137 collected through dry pitfall trapping and then stored in a controlled temperature facility (19.5
138 $\pm 1^\circ\text{C}$ and LD 16:8 h) in plastic cups containing moist soil, and were fed with dog food *ad*
139 *libitum*. Within the same environment, adult *H. axyridis* were collected by hand from the field
140 and stored in plastic 10L pots (28.5cm diameter / 22.5cm deep) covered with a porous
141 plastic mesh (pore size 0.05mm) and were fed *ad libitum* with live aphids. Predators were
142 kept for a maximum of four weeks in the laboratory. The pest species on which predation
143 was assessed was *S. avenae*, an important aphid pest of wheat frequently used as a model
144 prey item for measuring pest control (Mansion-Vaquié et al. 2017; Bosem Baillod et al.
145 2017). This aphid species shows a dropping behaviour in response to predator attacks
146 (Winder 1990).

147

148 We tested two forms of sentinel prey commonly used to assess the delivery of natural pest
149 control ecosystem services under field conditions. Immobilised prey represented by 10
150 aphids glued using superglue (Loctite Super Glue, Henkel, Düsseldorf, Germany) to 4 x 6cm
151 pieces of green card; aphids were placed approx., 0.5 cm apart. This reflects methodologies
152 established by Winqvist et al. (2011). Within each mesocosm we suspended one card in the
153 canopy of the wheat using a pin, and placed another on the soil surface of the plant pot
154 (adapted from Winqvist et al. (2011)). We also used artificial prey designed to mimic
155 lepidopteran caterpillars. Whilst the focus of our experiment was aphid prey, the use of
156 artificial caterpillars has been widely used to infer predation rates in agricultural settings
157 where the target pest species is not necessarily lepidopteran (Mansion-Vaquié et al. 2017).

158 Following approaches described in Howe, Lövei & Nachman (2009), caterpillars were made
159 of non-toxic green plasticine (Newplast, Newclay, Devon, UK) and were 2cm x 0.5cm in
160 diameter (Supplementary material: Appendix S1; Figure S1 & S2). Caterpillars were glued
161 using superglue (Loctite) in pairs to 3x3cm pieces of green card. This ensured once
162 constructed, no further handling of individual caterpillars occurred, avoiding the risk of
163 accidental marks (important as marks were used as a measure of predation). A total of 10
164 artificial prey items were suspended in the canopy by pinning the card with the caterpillars
165 attached to the wheat foliage and 10 caterpillars placed on the soil surface, so the method
166 could be quantitatively compared to the immobilised prey. In each mesocosm we also
167 included live prey so that attack rates on the sentinel prey could be compared to live prey
168 populations. Live prey populations were established as 20 free-moving adult *S. avenae*
169 aphids evenly distributed on the leaves of each wheat plant. Aphids were allowed to settle
170 for 4 hours, after which the two predator species were introduced. In addition to the two
171 sentinel prey treatments, we also included a control treatment for each sentinel prey type
172 that contained no predators. The control treatments were established following the same
173 experimental protocol as above.

174

175 Using this model system, we assessed whether an increased density of predators resulted in
176 higher attack rates on the sentinel prey and lower numbers of live aphids. We prepared a
177 low-density treatment comprising two *H. axyrdis* and two *P. madidus*, and a high-predator
178 density treatment with four individuals each of *H. axyrdis* and *P. madidus*. Each treatment
179 was replicated seven times. All treatments were run at the same time with predators that
180 were starved 24h prior to the experiment (predators were used only once i.e. a total of 84
181 individuals of each species were used over the whole experiment). The proportion of
182 immobilised aphids and the proportion of plasticine caterpillars showing evidence of attack
183 were recorded out of 20 and the number of live aphids were counted after 24h from the point
184 where predators were added.

185

186 **Statistical analysis**

187 We wanted to determine whether prey location (ground vs. foliar) affected predation rates at
188 the two predator densities (low vs. high) and whether these attack rates differed between the
189 sentinel prey methods (immobilised vs. artificial). However, we found no signs of predation
190 by the ladybird on the artificial prey. This resulted in zero variation for this parameter which
191 can lead to unreliable results in generalized linear models (Kuhn & Johnson 2013).
192 Therefore, we first analysed the immobilised prey separately to determine whether attack
193 rates differed between the ground and foliar predators at the different predator densities.
194 Prey items were not analysed individually as statistically independent units, but rather a
195 proportional attack rate across all 10 prey items at either the ground or foliage. We used a
196 binomial distribution reflecting the bounding (0-1) of data. The response variable was attack
197 rate (proportion of prey attacked out of 10) and the explanatory variables were predator
198 density (low and high) and predator feeding location (ground or foliar) and the interaction
199 between these two factors. As there were no predators in the controls for the artificial and
200 immobilised prey treatments we found no signs of attack on the plasticine caterpillars or the
201 aphids glued to cards (except one missing aphid from a card). This again meant that there
202 was near zero variation for the controls and they were excluded from analysis. We then
203 analysed the ground sentinel prey separately to determine whether *P. madidus* had higher
204 attack rates on the plasticine caterpillars in comparison to the immobilised live prey, as it
205 actively attacked both prey types. We used a binomial GLM with attack rate as the response
206 variable and the explanatory variables predator density and prey type and the interaction
207 between these two factors. Significance was assessed against a chi distribution.

208

209 To determine how predator density affected predation on live aphids we used a negative
210 binomial GLM implemented in the MASS package (Venables & Ripley, 2002). A negative
211 binomial GLM was used to account for overdispersion in the count data and for the fact that
212 pest populations have the capacity to reproduce, even over a 24h time frame. The response

213 variable was the number of aphids counted in the mesocosm at the end of the experiment
214 and the explanatory variables were predator density (control (no predators), low and high)
215 and alternative prey type (artificial and immobilised) and the interaction between these two
216 factors. Significance was assessed against a chi distribution. Where the interaction was
217 significant, orthogonal post-hoc contrasts were carried out. All analyses were carried out in R
218 (R Core Team, 2017).

219

220 Results

221 For both sentinel prey methods, evidence of attack was recorded after the 24h foraging
222 period, suggesting that immobilised prey stuck to cards and artificial caterpillars elicited a
223 predation response in the predators. However, we found no signs of predation on the
224 artificial caterpillars in the canopy and found no jaw marks from the predator *H. axyridis* on
225 any of the artificial caterpillars placed on the soil surface; these showed predation only by *P.*
226 *madidus*. Of the immobilised aphid prey, we found no significant interaction between
227 predator feeding location and predator density on predator attack rates ($\chi^2 = 0.210$, df = 1, p
228 = 0.647). Predator feeding location was also not found to have a significant effect on attack
229 rates on the immobilised prey ($\chi^2 = 1.981$, df = 1, p = 0.159), however there was a significant
230 effect of predator density ($\chi^2 = 10.407$, df = 1, p = 0.002). Attack rates were significantly
231 higher at the high predator density compared to the low predator density (proportion of prey
232 attacked out of 10 on immobilised prey: low predator density = $0.207 \pm 1SE 0.046$; high
233 predator density = $0.779 \pm 1SE 0.094$). Where predation was compared between sentinel
234 prey types for *P. madidus* we found there was no significant interaction between prey type
235 and predator density ($\chi^2 = 0.269$, df = 1, p = 0.604). Prey type was also not significant ($\chi^2 =$
236 0.020, df = 1, p = 0.887), however there was a significant increase in attack rates by *P.*
237 *madidus* between predator densities ($\chi^2 = 10.080$, df = 1, p = 0.001) (low predator density
238 mean = $0.114 \pm 1SE 0.038$; high predator density mean = $0.679 \pm 1SE 0.070$).

239

240 There was no significant interaction between predator density and alternative prey type on
241 the number of live prey in each treatment ($\chi^2 = 1.110$, df = 1, p = 0.574), however both main
242 effects predator density and alternative prey type were significant (Alternative prey type $\chi^2 =$
243 6.066, df = 1, p = 0.014; Predator density $\chi^2 = 21.813$, df = 2, p = <0.001). Post hoc
244 comparisons showed that there was a significant difference between the number of live
245 aphids in the control treatments and the predation treatments (z = -4.521, p = <0.001) (Table
246 1). However, there was no significant difference between the low predator density treatment
247 and high predator density treatment (z = 1.100, p = 0.271). The number of live aphids in the
248 artificial prey treatment was significantly lower than the immobilised prey treatment (Table 1).

249 **Discussion**

250 **Effect of predator density on attack rates**

251 In accordance with our first prediction, both the immobilised and artificial prey detected
252 increased attack rates in response to a higher predator density. However, in the case of the
253 live aphids there was no evidence of increased consumption at the higher predator densities.
254 This contrasts with the higher attack rates seen for the sentinel prey under the same
255 conditions. The sentinel prey represented both aggregated and highly conspicuous prey that,
256 in contrast to the live aphids, were unable to escape from predators. In this situation, once
257 the predators located the prey the two predominant limiting effects on attack rates would be
258 predator satiation or negative intraguild interactions (Gagnon, Heimpel & Brodeur 2011).
259 Immobilised prey were viable food items, so would contribute to predator satiation, which
260 could have reduced predation on the free moving prey (the number of free moving aphids
261 was still significantly lower in the predation treatments compared to the control, indicating
262 that predation did occur on the live pest populations). In contrast, the artificial prey is unlikely
263 to contribute to predator satiation as it offers no nutrition, which could lead to an inflation of
264 attack rates on artificial prey (where predators continually attack the prey due to a lack of
265 satiation) or cause them to search for alternative prey (Lövei & Ferrante 2017). We found
266 that significantly more free-moving aphids were consumed in the artificial prey treatment

267 compared to the immobilised prey treatment, suggesting the predators were attacking the
268 live prey to gain food (although the number of aphids consumed did not change as a
269 function of predator density). However, there was strong evidence that at the higher predator
270 densities artificial caterpillars were often attacked multiple times (i.e >40% of caterpillars
271 were attacked). This relatively high attack rate for the artificial caterpillars on the ground may
272 have reduced predation by the ground foraging beetles on the live aphids. A final point is
273 that in comparison to the sentinel prey, the free-moving aphids would be able to avoid
274 predators through either escape responses such as dropping from the plant when attacked,
275 or persisting in refuges where they are less vulnerable to predation; both mechanisms have
276 been found to reduce predation rates (Losey & Denno, 1998a; b; Berryman et al., 2006;
277 Bommarco et al., 2007). This could also explain the lower levels of predation on the free-
278 moving aphid populations also included in the mesocosm.

279

280 **Predation responses to the different sentinel prey methods**

281 We found equivocal evidence in support of our second prediction that attack rates were
282 lower on the artificial prey compared to the immobilised prey, with no significant difference in
283 attack rates by *P. madidus* being identified between the sentinel prey. However, if predation
284 assessments were based only on the artificial caterpillars located in the plant canopy, then
285 no difference in predation would have been detected as *H. axyridis* was not seen to bite
286 these artificial prey items. This agrees with the findings of Lövei and Ferrante, (2017) who
287 demonstrated lower predation on artificial sentinel prey compared to real sentinel prey. Our
288 results suggest this is due to individual predator feeding preferences. The lack of predation
289 by *H. axyridis* supported our third prediction that ground beetles would be more likely to
290 attack artificial prey. Both ground beetles and ladybirds have been found to use visual cues
291 when selecting feeding patches (e.g. attracted to high prey densities) (Lövei & Sunderland,
292 1996; Osawa, 2000; Lee & Kang, 2004) and both groups have also been found to respond
293 to and locate prey based on aphid volatiles (Lövei & Sunderland, 1996; Kielty et al., 1996;

294 Koch, 2003). However, our results suggest that either *H. axyridis* does not view plasticine
295 caterpillars as a prey item, or demonstrates preferences for live aphids over lepidopteran
296 prey. It is worth noting here that *H. axyridis* is polyphagous and will feed on juvenile stages
297 of Lepidoptera (Koch et al., 2003). For this reason other factors may also contribute to the
298 effective avoidance of the artificial caterpillars by *H. axyridis*. For example, *H. axyridis* relies
299 more on olfactory cues and has been shown to be highly attracted to the chemical (*E*)- β -
300 farnesene a key component of the alarm pheromone for most aphid species including *S.*
301 *avenae* (Verheggen et al., 2007). In contrast, ground beetles are more opportunistic
302 predators and may base feeding choices on prey vulnerability (Lang & Gsödl, 2008), which
303 could increase the likelihood of ground beetles attacking artificial prey items. The use of
304 plasticine caterpillars may therefore be a poor measure of predation where the dominant
305 predators in the ecosystem are Coccinellidae or other taxa that show similar hunting
306 strategies.

307

308 Sentinel methods are rarely used to calculate absolute predation and are more frequently
309 used to compare the relative amount of predation between experimental units (Lövei &
310 Ferrante, 2017). When combined with information on crop yield, direct measures of pest
311 damage and conventional quantification of both pest and predator densities, sentinel prey
312 approaches have the potential to provide valuable insights into pest control dynamics in
313 agro-ecosystems. Whilst understanding relative changes in predation between experimental
314 units is useful in elucidating ecosystems dynamics, being able to use sentinel prey items to
315 provide a surrogate measure of pest control for target pest species could be developed into
316 a standardised measure of pest control that can be applied to a range of farming systems.
317 Our study provides a basic demonstration that live and sentinel prey items detect varying
318 levels of predation in response to different predator species and predator densities, which
319 highlights potential limitations of using sentinel prey as proxies for pest suppression.
320 However, as live prey populations are able to reproduce and move, dynamics which cannot

321 be replicated in sentinel prey, the measure of success for real prey is often based on pest
322 threshold densities. As such it is very difficult to draw parallels between predation on live
323 and sentinel prey items.

324

325 There are limitations in this study to the sentinel prey approaches used to evaluate natural
326 pest control that merit consideration if the findings of this research are to inform future work.

327 Firstly, the number of artificial caterpillars may have been unrealistically high as this prey
328 item was included in mesocosms at the same density as the immobilised aphids. This was
329 done in part for practical reasons; if the number of prey were too small then detectable
330 differences between experimental units would be hard to observe, particular where all the
331 prey were consistently attacked or consumed (a problem akin to the “ceiling effect” in
332 statistics) (Austin & Brunner 2003). However, comparable densities to the immobilised prey

333 used in this study are not uncommon for pest populations under field conditions. For

334 example, caterpillars such as *Artogeia rapae* (small cabbage white) can reach similar
335 densities (Hooks, Pandey & Johnson 2003), while aggregations of aphids will normally

336 exceed those used in this study (Sunderland & Vickerman 1980; Sopp, Sunderland &

337 Coombes 1987). Secondly, the sentinel prey types in our study could have been assessed in
338 isolation without alternative real prey. This would have enabled the relative differences in

339 predation between methods to be directly compared more easily. However, to understand
340 how these methods perform in the real-world, where predators are exposed to both naturally
341 occurring free-moving and experimental sentinel prey, using more than one prey species

342 provides a more realistic comparison. In mesocosms a predator may attack the sentinel prey
343 (where it is the only prey) out of necessity (starvation), which directly contrasts to an

344 agroecosystem where alternative prey are likely to be available. Accordingly, this could
345 falsely represent predation by that species on sentinel prey. A similar criticism could also be

346 made where studies use a single real prey species to assess natural pest control. However,

347 typically these studies focus on a model prey species deliberately selected as it represents a

348 pest of economic importance to that crop. In this situation avoidance of that key pest species
349 in preference to alternative prey still provides key biologically relevant information in terms of
350 assessing pest control, at least for that key pest. Finally, it is possible that due to the close
351 spacing of the prey, that the free-moving aphids could walk on the caterpillars and
352 potentially leave prey-related chemical cues on them increasing their level of attraction to the
353 predators. However, we found that more aphids were consumed where the alternate prey
354 were the plasticine caterpillars as opposed to the immobilised prey. This would suggest that
355 the predators were distinguishing between the artificial prey and real prey in the mesocosm
356 without being affected by such chemical cues.

357

358 Conclusion

359 Sentinel prey methods offer a simple way to measure predation that have significant
360 advances over surrogate measures that rely on variation in prey or predator abundances
361 (Lövei & Sunderland, 1996; Chaplin-Kramer et al., 2011; Lövei & Ferrante, 2017). However,
362 when using sentinel prey our results highlight the importance of considering the effects of
363 predator and pest species life-history traits and the influence these have on observed
364 predation. A sensible approach would be to consider more than one measure of prey
365 suppression and tailor it to the desired measure of the study (Macfadyen et al., 2015). For
366 example, using plasticine caterpillars in conjunction with live, free moving prey (of a known
367 focal pest species) would allow a practitioner to record potential key predators within an
368 ecosystem based on the detection of bite marks in the plasticine, whilst also giving an
369 indication of actual pest suppression on the live prey. Correlation in predation rates between
370 the two methods could be used to determine whether the predators revealed by the artificial
371 prey method are the dominant predators responsible for pest control within that particular
372 agroecosystem. Accounting for variation in the attractiveness of different prey items to
373 predators, the effects of inhibiting important ecological escape mechanisms and the effects

374 of different sentinel prey densities will improve estimates of prey suppression. Ultimately,
375 this will improve our understanding of how natural pest control is delivered under field
376 conditions.

377

378 **Author contribution**

- 379 • All authors conceived the research
380 • Author 1 and author 2 designed and conducted experiments
381 • Author 1 conducted statistical analyses
382 • Author 1 wrote the draft manuscript
383 • All authors contributed to writing the final and revised manuscripts
384 • All authors approved the final version of the manuscript

385

386

387 **References**

- 388 Abassi SA, Birkett MA, Pettersson J, Pickett JA, Wadhams LJ & Woodcock CM. (2000).
389 Response of the Seven-spot Ladybird to an Aphid Alarm Pheromone and an Alarm
390 Pheromone Inhibitor is Mediated by Paired Olfactory Cells. *Journal of Chemical Ecology*,
391 26:1765-1771
- 392 Abrams, P.A. & Ginzburg, L.R. (2000) The nature of predation: prey dependent, ratio
393 dependent or neither? *Trends in Ecology & Evolution*, 15, 337–341.
- 394 Austin, P.C. & Brunner, L.J. (2003) Type I error inflation in the presence of a ceiling effect.
395 *The American Statistician*, 57, 97–104.
- 396 Begg, G.S., Cook, S.M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G. Mansion-
397 Vaquie, A., Pell, J., K., Petit, S., Quesada, N., Ricci, B. & Birch, A.N.E (2017) A functional
398 overview of conservation biological control. *Crop Protection* 97: 145-158
- 399 Berryman AA, Hawkins BA & Hawkins BA. (2006). The refuge as an integrating concept in
400 ecology and evolution. *Oikos*, 115:192–196.
- 401 Bianchi FJJA, Booij CJH & Tscharntke T. (2006). Sustainable pest regulation in agricultural
402 landscapes: a review on landscape composition, biodiversity and natural pest control.
403 *Proceedings. Biological Sciences / the Royal Society*, 273:1715–1727.
- 404 Bianchi FJJA, van Wingerden WKRE, Griffioen AJ, van der Veen M, van der Straten MJJ,
405 Wegman RMA & Meeuwsen HAM. (2005). Landscape factors affecting the control of

- 406 *Mamestra brassicae* by natural enemies in Brussels sprout. *Agriculture, Ecosystems &*
- 407 *Environment*, 107:145–150.
- 408 Birkhofer K, Bylund H, Dalin P, Ferlian O, Gagic V, Hambäck PA, Klapwijk M, Mestre L,
- 409 Roubinet E, Schroeder M, Stenberg JA, Porcel M, Björkman C & Jonsson M. (2017).
- 410 Methods to identify the prey of invertebrate predators in terrestrial field studies. *Ecology and*
- 411 *Evolution*, 7:1942–1953.
- 412 Bommarco R, Firle SO & Ekbom B. (2007). Outbreak suppression by predators depends on
- 413 spatial distribution of prey. *Ecological Modelling*, 201:163–170.
- 414 Bosem Baillod, A., Tscharntke, T., Clough, Y. and Batáry, P. (2017). Landscape-scale
- 415 interactions of spatial and temporal cropland heterogeneity drive biological control of cereal
- 416 aphids. *Journal of Applied Ecology*, 54:1804–1813.
- 417 Cardinale BJ, Srivastava DS, Duffy JE, Wright JP, Downing AL, Sankaran M & Jouseau C.
- 418 (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*,
- 419 443:989–992.
- 420 Chaplin-Kramer R, O'Rourke ME, Blitzer EJ & Kremen C. (2011). A meta-analysis of crop
- 421 pest and natural enemy response to landscape complexity. *Ecology Letters*, 14:922–932.
- 422 Dixon AFG. (1958). The escape responses shown by certain aphids to the presence of the
- 423 coccinellid *Adalia decempunctata* (L.). *Transactions of the Royal Entomological Society of*
- 424 *London*, 110:319–334.
- 425 Elliott NC, Kieckhefer RW, Lee J-H & French BW. (1999). Influence of within-field and
- 426 landscape factors on aphid predator populations in wheat. *Landscape Ecology* 14:239–252.
- 427 Ferguson KI & Stiling P. (1996). Non-additive effects of multiple natural enemies on aphid
- 428 populations. *Oecologia*, 108:375–379.
- 429 Ferrante M, Barone G & Lövei GL. (2017). The carabid *Pterostichus melanarius* uses
- 430 chemical cues for opportunistic predation and saprophagy but not for finding healthy prey.
- 431 *Biocontrol*, 62:741–747.
- 432 Foster WA, Snaddon JL, Turner EC, Fayle TM, Cockerill TD, Ellwood MDF, Broad GR,
- 433 Chung AYC, Eggleton P, Khen CV & Yusah KM. (2011). Establishing the evidence base for
- 434 maintaining biodiversity and ecosystem function in the oil palm landscapes of South East
- 435 Asia. *Philosophical Transactions of the Royal Society of London. Series B, Biological*
- 436 *Sciences*, 366:3277–3291.
- 437 Gagnon, A.-È., Heimpel, G.E. & Brodeur, J. (2011) The ubiquity of intraguild predation
- 438 among predatory arthropods. *Plos One*, 6, e28061.
- 439 Gardiner MM, Landis DA, Gratton C, DiFonzo CD, O'Neal M, Chacon JM, Wayo MT,
- 440 Schmidt NP, Mueller EE & Heimpel GE. (2009). Landscape diversity enhances biological
- 441 control of an introduced crop pest in the north-central USA. *Ecological Applications*, 19:143–
- 442 154.
- 443 Greenop A, Woodcock BA, Wilby A, Cook SM & Pywell RF. (2018). Functional diversity
- 444 positively affects prey suppression by invertebrate predators: a meta-analysis. *Ecology*,
- 445 doi.org/10.1002/ecy.2378

- 446 Holland JM, Oaten H, Moreby S, Birkett T, Simper J, Southway S & Smith BM. (2012). Agri-
447 environment scheme enhancing ecosystem services: A demonstration of improved biological
448 control in cereal crops. *Agriculture, Ecosystems & Environment*, 155:147–152.
- 449 Hooks, C.R.R., Pandey, R.R. & Johnson, M.W. (2003) Impact of avian and arthropod
450 predation on lepidopteran caterpillar densities and plant productivity in an ephemeral
451 agroecosystem. *Ecological Entomology*, 28, 522–532.
- 452 Howe A, Lövei GL & Nachman G. (2009). Dummy caterpillars as a simple method to assess
453 predation rates on invertebrates in a tropical agroecosystem. *Entomologia experimentalis et
454 applicata*, 131:325–329.
- 455 Kiely JP, Allen-Williams LJ, Underwood N & Eastwood EA. (1996). Behavioral responses of
456 three species of ground beetle (Coleoptera: Carabidae) to olfactory cues associated with
457 prey and habitat. *Journal of Insect Behavior*, 9:237–250.
- 458 Koch RL, Hutchison WD, Venette RC & Heimpel GE. (2003). Susceptibility of immature
459 monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by
460 *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Control*, 28:265–270.
- 461 Koch RL. (2003). The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its
462 biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, 3:1–16.
- 463 Kuhn, M. & Johnson, K. (2013) *Applied Predictive Modeling*. Springer New York, New York,
464 NY.
- 465 Lang A & Gsödl S. (2008). Prey vulnerability and active predator choice as determinants of
466 prey selection: a carabid beetle and its aphid prey. *Journal of Applied Entomology*, 125:53–
467 61.
- 468 Lee J-H & Kang T-J. (2004). Functional response of *Harmonia axyridis* (Pallas) (Coleoptera:
469 Coccinellidae) to *Aphis gossypii* Glover (Homoptera: Aphididae) in the Laboratory. *Biological
470 Control*, 31:306–310.
- 471 Losey JE & Denno RF. (1998a). The escape response of pea aphids to foliar-foraging
472 predators: factors affecting dropping behaviour. *Ecological Entomology* 23:53–61.
- 473 Losey JE & Denno RF. (1998b). Interspecific variation in the escape responses of aphids:
474 effect on risk of predation from foliar-foraging and ground-foraging predators. *Oecologia*,
475 115:245–252.
- 476 Lövei GL & Ferrante M. (2017). A review of the sentinel prey method as a way of quantifying
477 invertebrate predation under field conditions. *Insect Science*, 24:528–542.
- 478 Lövei GL & Sunderland KD. (1996). Ecology and behavior of ground beetles (Coleoptera:
479 Carabidae). *Annual Review of Entomology*, 41:231–256.
- 480 Macfadyen S, Davies AP & Zalucki MP. (2015). Assessing the impact of arthropod natural
481 enemies on crop pests at the field scale. *Insect Science*, 22:20–34.
- 482 Mansion-Vaquié A, Ferrante M, Cook SM, Pell JK & Lövei GL. (2017). Manipulating field
483 margins to increase predation intensity in fields of winter wheat (*Triticum aestivum*). *Journal
484 of Applied Entomology*, 141:600–611.
- 485 Mathews CR, Bottrell DG & Brown MW. (2004). Habitat manipulation of the apple orchard
486 floor to increase ground-dwelling predators and predation of *Cydia pomonella* (L.)
487 (Lepidoptera: Tortricidae). *Biological Control*, 30:265–273.

- 488 Northfield TD, Snyder GB, Ives AR & Snyder WE. (2010). Niche saturation reveals resource
489 partitioning among consumers. *Ecology Letters*, 13:338–348.
- 490 Osawa N. (2000). Population field studies on the aphidophagous ladybird beetle *Harmonia*
491 *axyridis* (Coleoptera: Coccinellidae): resource tracking and population characteristics.
492 *Population Ecology*, 42:115–127.
- 493 R Core Team (2017). R: A language and environment for statistical computing. R
494 Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- 495 Roslin T, Hardwick B, Novotny V, Petry WK, Andrew NR, Asmus A, Barrio IC, Basset Y,
496 Boesing AL, Bonebrake TC, Cameron EK, Dátilo W, Donoso DA, Drozd P, Gray CL, Hik
497 DS, Hill SJ, Hopkins T, Huang S, Koane B, Laird-Hopkins B, Laukkanen L, Lewis OT, Milne
498 S, Mwesige I, Nakamura A, Nell CS, Nichols E, Prokurat A, Sam K, Schmidt NM, Slade A,
499 Slade V, Suchanková A, Teder T, van Nouhuys S, Vandvik V, Weissflog A, Zhukovich V &
500 Slade EM. (2017). Higher predation risk for insect prey at low latitudes and elevations.
501 *Science*. 356:742–744.
- 502 Rusch A, Chaplin-Kramer R, Gardiner MM, Hawro V, Holland J, Landis D, Thies C,
503 Tscharntke T, Weisser WW, Winqvist C, Woltz M & Bommarco R. (2016). Agricultural
504 landscape simplification reduces natural pest control: A quantitative synthesis. *Agriculture,*
505 *Ecosystems & Environment*, 221:198–204.
- 506 Schmidt MH, Roschewitz I, Thies C & Tscharntke T. (2005). Differential effects of landscape
507 and management on diversity and density of ground-dwelling farmland spiders. *Journal of*
508 *Applied Ecology*, 42:281–287.
- 509 Schmitz OJ. (2007). Predator diversity and trophic interactions. *Ecology*, 88:2415–2426.
- 510 Snyder GB, Finke DL & Snyder WE. (2008). Predator biodiversity strengthens aphid
511 suppression across single- and multiple-species prey communities. *Biological Control*,
512 44:52–60.
- 513 Sopp, P.I., Sunderland, K.D. & Coombes, D.S. (1987) Observations on the number of cereal
514 aphids on the soil in relation to aphid density in winter wheat. *Annals of Applied Biology*, 111,
515 53–57.
- 516 Straub CS & Snyder WE. (2006). Species identity dominates the relationship between
517 predator biodiversity and herbivore suppression. *Ecology*, 87:277–282.
- 518 Sunderland, K.D. & Vickerman, G.P. (1980) Aphid feeding by some polyphagous predators
519 in relation to aphid density in cereal fields. *The Journal of Applied Ecology*, 17, 389.
- 520 Venables WN & Ripley BD. (2002). Modern Applied Statistics with S. Statistics and
521 Computing. (ed by J Chambers, W Eddy, W Härdle, S Sheather & L Tierney) 4th edn.
522 Springer New York, New York, NY.,
- 523 Verheggen FJ, Fagel Q, Heuskin S, Lognay G, Francis F & Haubruge E. (2007).
524 Electrophysiological and behavioral responses of the multicolored Asian lady beetle,
525 *Harmonia axyridis* pallas, to sesquiterpene semiochemicals. *Journal of Chemical Ecology*,
526 33:2148–2155.
- 527 Wan, N.-F., Ji, X.-Y., Kiær, L.P., Liu, S.-S., Deng, J.-Y., Jiang, J.-X. & Li, B. (2018) Ground
528 cover increases spatial aggregation and association of insect herbivores and their predators
529 in an agricultural landscape. *Landscape Ecology*, 33, 799–809.

- 530 Winder, L. (1990). Predation of the cereal aphid *Sitobion avenae* by polyphagous predators
531 on the ground. *Ecological Entomology*, 15:105-110.
- 532 Winder, L., Perry, J.N. & Holland, J.M. (1999) The spatial and temporal distribution of the
533 grain aphid *Sitobion avenae* in winter wheat. *Entomologia experimentalis et applicata*, 93,
534 275–288.
- 535 Winqvist C, Bengtsson J, Aavik T, Berendse F, Clement LW, Eggers S, Fischer C, Flohre A,
536 Geiger F, Liira J, Pärt T, Thies C, Tscharntke T, Weisser WW & Bommarco R. (2011). Mixed
537 effects of organic farming and landscape complexity on farmland biodiversity and biological
538 control potential across Europe. *Journal of Applied Ecology*, 48:570–579.
- 539 Woodcock B., Bullock J., McCracken M, Chapman R., Ball S., Edwards M., Nowakowski M
540 & Pywell R. (2016). Spill-over of pest control and pollination services into arable crops.
541 *Agriculture, Ecosystems & Environment*, 231:15–23.
- 542 Woodcock BA & Heard MS. (2011). Disentangling the effects of predator hunting mode and
543 habitat domain on the top-down control of insect herbivores. *The Journal of Animal Ecology*,
544 80:495–503.
- 545 Zalucki MP, Furlong MJ, Schellhorn NA, Macfadyen S & Davies AP. (2015). Assessing the
546 impact of natural enemies in agroecosystems: toward “real” IPM or in quest of the Holy
547 Grail? *Insect Science*, 22:1–5.
- 548 Zou Y, de Kraker J, Bianchi FJJA, van Telgen MD, Xiao H & van der Werf W. (2017). Video
549 monitoring of brown planthopper predation in rice shows flaws of sentinel methods. *Scientific
550 Reports*, 7:42210.
- 551
- 552
- 553
- 554
- 555
- 556
- 557
- 558
- 559
- 560
- 561
- 562
- 563
- 564
- 565
- 566

567 **Table 1.** Number of live aphids (*Sitobion avenae*) recorded after 24h exposure to the
568 predators *Pterostichus madidus* and *Harmonia axyridis* in a mesocosm where either
569 immobilised prey (20 *S. avenae* aphids glued to card) or artificial prey (20 plasticine
570 caterpillars) were also available. Predator densities control (no predators), low (two *H.*
571 *axyridis* and two *P. madidus*) and high (four *H. axyridis* and four *P. madidus*) are the mean
572 across both alternate prey types. Artificial prey treatment and immobilised prey treatment are
573 the mean across all predator densities.

Treatment	Number of live aphids (mean \pm 1SE)
Control	28.786 \pm 2.823
Low predatory density	18.429 \pm 2.336
High predator density	15.643 \pm 1.561
Artificial prey treatment	18.190 \pm 2.102
Immobilised prey treatment	23.714 \pm 2.212

574

575