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

Authors

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Abstract

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

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

Introduction

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

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

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

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

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

Methods

Experimental system

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

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

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

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

Statistical analysis

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

To determine how predator density affected predation on live aphids we used a negative biniomial GLM implemented in the MASS package (Venables & Ripley, 2002). A negative binomial GLM was used to account for overdispersion in the count data and for the fact that pest populations have the capacity to reproduce, even over a 24h time frame. The response variable was the number of aphids counted in the mesocosm at the end of the experiment and the explanatory variables were predator density (control (no predators), low and high) and alternative prey type (artificial and immobilised) and the interaction between these two factors. Significance was assessed against a chi distribution. Where the interaction was significant, orthogonal post-hoc contrasts were carried out. All analyses were carried out in R (R Core Team, 2017).

Results

For both sentinel prey methods, evidence of attack was recorded after the 24h foraging period, suggesting that immobilised prey stuck to cards and artificial caterpillars elicited a predation response in the predators. However, we found no signs of predation on the artificial caterpillars in the canopy and found no jaw marks from the predator *H. axyridis* on any of the artificial caterpillars placed on the soil surface; these showed predation only by *P. madidus*. Of the immobilised aphid prey, we found no significant interaction between predator feeding location and predator density on predator attack rates ($\chi_2 = 0.210$, df = 1, p = 0.647). Predator feeding location was also not found to have a significant effect on attack rates on the immobilised prey ($\chi_2 = 1.981$, df = 1, p = 0.159), however there was a significant effect of predator density ($\chi_2 = 10.407$, df = 1, p = 0.002). Attack rates were significantly higher at the high predator density compared to the low predator density (proportion of prey attacked out of 10 on immobilised prey: low predator density = 0.207 ±1SE 0.046; high predator density = 0.779 ±1SE 0.094). Where predation was compared between sentinel prey types for *P. madidus* we found there was no significant interaction between prey type and predator density ($\chi_2 = 0.269$, df = 1, p = 0.604). Prey type was also not significant ($\chi_2 = 0.020$, df = 1, p = 0.887), however there was a significant increase in attack rates by *P. madidus* between predator densities ($\chi_2 = 10.080$, df = 1, p = 0.001) (low predator density mean = 0.114 ±1SE 0.038; high predator density mean = 0.679 ±1SE 0.070).

There was no significant interaction between predator density and alternative prey type on the number of live prey in each treatment (χ_2 = 1.110, df = 1, p = 0.574), however both main effects predator density and alternative prey type were significant (Alternative prey type χ_2 = 6.066, df = 1, p = 0.014; Predator density χ_2 = 21.813, df = 2, p = <0.001). Post hoc comparisons showed that there was a significant difference between the number of live aphids in the control treatments and the predation treatments (z = -4.521, p = <0.001) (Table 1). However, there was no significant difference between the low predator density treatment and high predator density treatment (z = 1.100, p = 0.271). The number of live aphids in the artificial prey treatment was significantly lower than the immobilised prey treatment (Table 1).

Discussion

Effect of predator density on attack rates

In accordance with our first prediction, both the immobilised and artificial prey detected increased attack rates in response to a higher predator density. However, in the case of the live aphids there was no evidence of increased consumption at the higher predator densities. This contrasts with the higher attack rates seen for the sentinel prey under the same conditions. The sentinel prey represented both aggregated and highly conspicuous prey that, in contrast to the live aphids, were unable to escape from predators. In this situation, once the predators located the prey the two predominant limiting effects on attack rates would be predator satiation or negative intraguild interactions (Gagnon, Heimpel & Brodeur 2011). Immobilised prey were viable food items, so would contribute to predator satiation, which could have reduced predation on the free moving prey (the number of free moving aphids was still significantly lower in the predation treatments compared to the control, indicating that predation did occur on the live pest populations). In contrast, the artificial prey is unlikely to contribute to predator satiation as it offers no nutrition, which could lead to an inflation of attack rates on artificial prey (where predators continually attack the prey due to a lack of satiation) or cause them to search for alternative prey (Lövei & Ferrante 2017). We found that significantly more free-moving aphids were consumed in the artificial prey treatment compared to the immobilised prey treatment, suggesting the predators were attacking the live prey to gain food (although the number of aphids consumed did not change as a function of predator density). However, there was strong evidence that at the higher predator densities artificial caterpillars were often attacked multiple times (i.e >40% of caterpillars were attacked). This relatively high attack rate for the artificial caterpillars on the ground may have reduced predation by the ground foraging beetles on the live aphids. A final point is that in comparison to the sentinel prey, the free-moving aphids would be able to avoid predators through either escape responses such as dropping from the plant when attacked, or persisting in refuges where they are less vulnerable to predation; both mechanisms have been found to reduce predation rates (Losey & Denno, 1998a; b; Berryman et al., 2006; Bommarco et al., 2007). This could also explain the lower levels of predation on the free-moving aphid populations also included in the mesocosm.

Predation responses to the different sentinel prey methods

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

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

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

There are limitations in this study to the sentinel prey approaches used to evaluate natural pest control that merit consideration if the findings of this research are to inform future work. Firstly, the number of artificial caterpillars may have been unrealistically high as this prey item was included in mesocosms at the same density as the immobilised aphids. This was done in part for practical reasons; if the number of prey were too small then detectable differences between experimental units would be hard to observe, particular where all the prey were consistently attacked or consumed (a problem akin to the “ceiling effect” in statistics) (Austin & Brunner 2003). However, comparable densities to the immobilised prey used in this study are not uncommon for pest populations under field conditions. For example, caterpillars such as *Artogeia rapae* (small cabbage white) can reach similar densities (Hooks, Pandey & Johnson 2003), while aggregations of aphids will normally exceed those used in this study (Sunderland & Vickerman 1980; Sopp, Sunderland & Coombes 1987). Secondly, the sentinel prey types in our study could have been assessed in isolation without alternative real prey. This would have enabled the relative differences in predation between methods to be directly compared more easily. However, to understand how these methods perform in the real-world, where predators are exposed to both naturally occurring free-moving and experimental sentinel prey, using more than one prey species provides a more realistic comparison. In mesocosms a predator may attack the sentinel prey (where it is the only prey) out of necessity (starvation), which directly contrasts to an agroecosystem where alternative prey are likely to be available. Accordingly, this could falsely represent predation by that species on sentinel prey. A similar criticism could also be made where studies use a single real prey species to assess natural pest control. However, typically these studies focus on a model prey species deliberately selected as it represents a pest of economic importance to that crop. In this situation avoidance of that key pest species in preference to alternative prey still provides key biologically relevant information in terms of assessing pest control, at least for that key pest. Finally, it is possible that due to the close spacing of the prey, that that the free-moving aphids could walk on the caterpillars and potentially leave prey-related chemical cues on them increasing their level of attraction to the predators. However, we found that more aphids were consumed where the alternate prey were the plasticine caterpillars as opposed to the immobilised prey. This would suggest that the predators were distinguishing between the artificial prey and real prey in the mesocosm without being affected by such chemical cues.

Conclusion

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

Author contribution

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

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

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