Live and inanimate predator-associated cues suppress the population of sap-feeding prey and induce polyphenism

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Declarations

Conflict of interests

None declared.

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Author contributions

All authors contributed critically to the drafts and gave final approval for publication.

MSK designed the experiment, co-executed the experimental work with TD, co-shaped and co-optimised the analysis with OYB, co-built the illustrations with TD, and expanded the manuscript and improved the visualisation.
TD executed the experimental work with MSK, did the data entry in collaboration with MSK, provided the first drafts of this manuscript with illustrations, produced the bibliography. TD co-wrote the manuscript with MSK.
OYB contributed to the enhancement of the experimental design, accomplished the analysis with MSK, provided insights on the refinement and polishing of the visualisation and contributed to the writing up and enhancement of the manuscript.
SW hosted the work, provided suggestions for the optimisation of the experimental design and contributed to the enhancement of the writing up of the manuscript.

Abstract

Non-consumptive effect of predation is a well-researched subject of which certain temporal, non-consumptive and predator-mimetic facets are yet to be investigated in plant-parasite systems. Here we focus on the consequences of the exposure of a pest population to predator-associated risk.

One clone of the green peach aphid *Myzus persicae* (Sulzer), raised on a model crop *Brassica oleracea* (L.), was exposed to different regimes of risks associated with the aphidophagous predator ladybird *Coccinella septempunctata* (L.). This encompassed consumption, consumption alternated by non-competitive predation risk, presence of isolated predators with and without aphid feed, dead predator, predator dummy, scented (mimed) dummy, treated plants (or soil) with predator-borne cues, and predator removal.

Over time, the respective risk regimes led to variable yet conspicuous suppression of the prey population. For example, unlike predator removal, ladybird corpses and dummies had considerable persistent negative effects on aphid reproductive success. Also, by the end of the experiment, polyphenism (winged morph production) varied across the risk regimes and was animated under the presence of a starved isolated predator, but faded when a predator corpse was present, and vanished in the presence of a predator dummy.

Using a model aphid-crop system, we provide timely and novel insights on differential impacts of a variety of predator-associated cues on the population growth and phenotypic plasticity of the parthenogenetic pest in question. This work has implications for the rapidly developing area of the ecology of fear and thus merits applications across different eco-agricultural contexts.

Keywords: Aphid, ladybird, non-consumptive risk, temporality.

Introduction

Across natural and agricultural ecosystems, the impact of predation on the population dynamics and survival of prey is multifaceted. It takes place in the form of direct consumption and also occurs non-consumptively (*i.e.*, via fear of predation or *intimidation*) (Lima, 1998; Preisser *et al.*, 2005; McCauley *et al.*, 2011; Zöttl *et al.*, 2013; Kersch-Becker and Thaler, 2015). This can be in effect through different types of stimulation, *e.g.*, the presence of impaired predators (*i.e.*, altered experimentally to no longer consume the prey) (Nelson *et al.*, 2004; Nelson and Rosenheim, 2006), the mere presence of a predator (McCauley *et al.*, 2011; Kersch-Becker and Thaler, 2015), and predation threat including induction by predator-borne cues (Preisser and Bolnick, 2008; Ferrari *et al.*, 2010; Ninkovic *et al.*, 2013; Khudr *et al.*, 2017).

Information cues in action

The prey assess info-chemicals from their surroundings, including cues associated with natural enemies (Lima and Dill, 1990; Kats, and Dill, 1998; Lima and Steury, 2005; Ferrari *et al.*, 2010). Inducible prey defences after perception of environmental risk have been reported to be changeable relevant to the risk and hence may be adjustable and adaptive, via transgenerational effects (Keiser and Mondor, 2013), when they lead to fitness gains (Evans and Schmidt, 1990; Coslovsky and Richner, 2011; Zöttl *et al.*, 2013). However, the lingering question remains on what sensory modalities of the prey are most important in shaping the perception of and the response to predation risk.

Prey responses to predation risk may span feeding cessation, escape and avoidance of predators (Nelson, 2007; Keiser and Mondor, 2013), dispersion (Roitberg *et al.*, 1979, Nelson and Rosenheim, 2006, Hatano *et al.*, 2010), and habituation (Shalter, 1984; Holomuzki and Hatchett, 1994). Also, prey reproductive success may undergo inhibition, or reproduction may be altered under intimidation by predators (Preisser *et al.*, 2005; McCauley *et al.*, 2011), alarm pheromone effects (de Vos *et al.*, 2010), and behavioural changes (Nelson *et al.*, 2004; Hoki *et al.*, 2014). Whichever choice the prey make, the decision of developing anti-predator responses and reacting to environmental risks incur an ecological cost (Agabiti *et al.*, 2016; Ingerslew and Finke, 2017; Hermann and Landis, 2017), leading in general to compromised prey fitness and temporarily altered dynamics and behaviours (Lima and Dill, 1990; Sih, 1994; Sih, 1997; Nelson *et al.*, 2004; Preisser *et al.*, 2005) to the advantage of survival (Francke *et al.*, 2008; Keiser, 2012).

The effects triggered by predator cues (Norin, 2009) diffuse through prey population and intensify in effect due to aphid pheromone communication (de Vos *et al.*, 2010; Keiser, 2012, Ingerslew and Finke, 2017), thereby inducing varied responses such as polyphenism (Weisser *et al.*, 1999), and/or changes in reproductive success (Khudr *et al.*, 2017). In this regard, using isolated

predators or inanimate objects cued by predator info-chemicals as a form of risk to affect parthenogenetic phloem-feeding insects has not received enough attention compared to the effects of the deployment of impaired predators (Nelson *et al.*, 2004; Nelson and Rosenheim, 2006).

Polyphenism and inducible defences

In the case of parthenogenetic phloem-feeding insects, the population, under temperate conditions, comprises female congeners of the same genetic identity with very strong maternal/transgenerational effects (Mousseau and Fox, 1998; Weisser *et al.*, 1999; Hu *et al.*, 2018). The responses to stimuli become heightened by maternal preconditioning of daughters and granddaughters via telescoping generations (Kindlmann and Dixon, 1989), as consecutive generations of offspring may develop and become preconditioned simultaneously (Mousseau and Fox, 1998; Weisser *et al.*, 1999; Hu *et al.*, 2018), leading for instance to the production of winged aphids (dispersive morphs) (Weisser *et al.*, 1999; Kunert *et al.*, 2005).

Non-consumptive effects of predation can be associated with the emergence of anti-predator distinct phenotypic plasticity, *e.g.*, production of winged morphs (alates) as a polyphenic response (Dixon and Agarwala, 1999; Mondor *et al.*, 2005). Alates are associated with dispersal and settlement aside from adverse circumstances (Dixon and Agarwala, 1999; Kunert and Weisser, 2003; Mondor *et al.*, 2005). Roitberg *et al.* (1979) reported that coccinellid predation risk induced aphids to scatter on plant and in between plants, which was followed by reduced reproductive success, indicating energy costs, allocated for the preconditioning of progeny (escapees), at the expense of reproduction (Mackay and Wellington, 1975; Roitberg *et al.*, 1979; Keiser and Mondor, 2013). Moreover, Fievet *et al.* (2008) demonstrated that exposure to indirect predation cues in the form of dead conspecifics induced behavioural changes that were accompanied by a decline in the aphid population. Furthermore, a relation between reduced feeding time and dwindling reproductive success (Sih, 1992) has been described by Nelson (2007). The reduction of feeding time was related, in the cited work, to predator disturbance frequency, where the presence of non-consumptive (impaired) predators led to reduced aphid fitness (Nelson *et al.*, 2004). Further, the phenotypically plastic phenotypes of aphids may, in turn, have variable extended effects on other trophic levels, highlighting the importance of consequences of the effects of fear of predation over time (Preisser *et al.*, 2005; Creel and Christianson, 2008; Steffan and Snyder, 2010). As such, optimal habitats for prey may change with time, as do anti-predator behaviours, thereby forcing the prey to resort to safer micro-habitats, but avoidance of ecological risk can become increasingly costly on poor or scarce resources (Turner, 1997), where optimal feeding sites are rare. Interestingly, recognition of predator-associated cues (*e.g.*, dead predators and/or predator models [mimics]) and inducible anti-

predator defences by insects with extreme phenotypic plasticity, such as aphids, have not been sufficiently investigated and understood as yet.

Brevity versus longevity of non-consumptive effects

The evidence for the wide-spread impact of fear of predation is mounting across a vast array of taxa (Preisser *et al.*, 2005; Hermann and Landis, 2017). However, the current understanding of the role of the duration of risk exposure is meagre, particularly in respect to the influence of non-consumptive effects on phloem-feeding insect biology, but see (Van Dievel *et al.*, 2016). Prolonged or more frequent exposure to high frequencies/intensities of predation risk imposed by natural enemies can cause a state of environmental uncertainty (Sih, 1992; Koops, 2004; Trussell *et al.*, 2011) that may dilute the strength of the non-consumptive effects. This can alter the assessment and response of prey against risk cues and thus may induce prey habituation to risk by time. This, in turn, may be contextual and contingent on prey physiology and energy dynamics (Trussell *et al.*, 2011; Matassa and Trussell, 2014). Moreover, unlike older predator-borne cues, fresh ones might be associated with higher localised risk and hence may provide more certainty/reliability. Faster and more adaptive prey responses, based on a perception of reliable and localised signals (Dixon and Agarwala 1999; Podjasek *et al.*, 2005; Keiser and Mondor, 2013), could reduce ecological costs (Koops, 2004; Ninkovic *et al.*, 2013; Zöttl *et al.*, 2013). Nevertheless, temporal aspects of anti-predator responses remain obscure, including whether and how constant versus fluctuating risks may affect prey population dynamics in the short and the long-term (Lima and Bednekoff, 1999; Hamilton and Heithaus, 2001).

The intensity and consistency of the effects of predator-borne cues, when consumption and/or non-consumptive effects change through time, may result in trait-mediated prey responses; the effect of which may scale up through ecosystems (Lima and Bednekoff, 1999, Steffan and Snyder, 2010; Matassa and Trussell, 2014). To date, comprehensive studies on profiling reproductive success and phenotypic plasticity of parthenogenetic prey under respective exposure to an array of non-consumptive risk types over time are scarce. Although prey intimidation by the constant presence of an isolated predator (non-consumptive effects) has been demonstrated before in insects, *e.g.*, dragonfly (McCauley *et al.*, 2011), this, however, has never been sufficiently investigated over a generation time and/or with variation in predator isolations. This also still needs to be investigated in contrast with other types of inanimate predator-associated cues, using agroecological model systems of plants and plant parasites.

Non-consumptive effects via biomimicry

Ladybirds are effective in the biocontrol of aphid (Hoki *et al.*, 2014) and in greenhouse settings (Riddick, 2017), and their footprints are known to elicit avoidance behaviour in aphids

175 (Ninkovic *et al.*, 2013). Phloem-feeding plant parasites, such as aphids, mainly chemically detect
176 the presence of ladybird, *e.g.*, *Coccinella septempunctata*, through direct contact and exposure to
177 the tracks and excretions left by the predator (Ninkovic *et al.*, 2013), with evidence for
178 concentration and sex-dependency effects of ladybird olfactory cues (Youren, 2012).

179 On the one hand, it has been well-established that aphids respond to non-consumptive cues
180 associated with their predators. For example, impaired predators have been shown to negatively
181 impact on aphid reproduction (Nelson and Rosenheim, 2006). Moreover, encounters between green
182 peach aphid *Myzus persicae* and corpses of its lacewing predator *Chrysoperla carnea* (Stephens) or
183 plants pre-treated with lacewing cues have been reported to induce anti-predator avoidance
184 behaviour and to lead to a differential reduction in aphid fitness as a consequence of fear of
185 predation (Khudr *et al.*, 2017). However, defining the effects of different non-consumptive cues
186 (*e.g.*, cued predator dummies and cued host plants), to test whether predator-borne info-chemicals
187 may repress aphid reproduction, has been largely overlooked.

188 On the other hand, the literature provides examples of research on the employment of
189 predator replicas (false predators) (*e.g.*, Mitrovich and Cotroneo, 2006) or artificial prey (*e.g.*,
190 Morrell and Turner, 1970; Roslin *et al.*, 2017) to study contextual dynamics of predator-prey and
191 risk-prey interactions, but these studies usually focus on higher taxa rather than insects. To the best
192 of our knowledge, mimicking natural enemies by exposing aphids to synthetic predators that highly
193 resemble coccinellids or any other aphid natural enemy has not been done before. Moreover, studies
194 on the application of inanimate mimetic objects, *e.g.*, cued predator dummies, to control plant insect
195 pests are thus far lacking. Indeed, treating aphid/pest environment, including host plants, predator
196 decoys and soil, with suspensions bearing predator-borne info-chemicals may break new ground in
197 pest control. Moreover, while most research on the ecology of fear focuses on prey performance
198 changes, following behaviour modifications, there is an emergent need to decipher previously
199 uncharted non-consumptive effects on prey fitness and pest control where innovative ecological
200 means are employed (Ingerslew and Finke, 2017).

201 In the present work, we test the effects of exposure to various types of consumptive and non-
202 consumptive risks, associated with and/or mimicking an aphidophagous insect *C. septempunctata*
203 (L.), on prey reproductive success (total aphid numbers per census) and phenotypic plasticity
204 (polyphenism in terms of winged morph count at the end of the experiment). We use one genotype
205 of a significant crop pest that is green peach aphid *M. persicae* (Sulzer). See (Figure 1) for an
206 overview of the conceptual design of the work.

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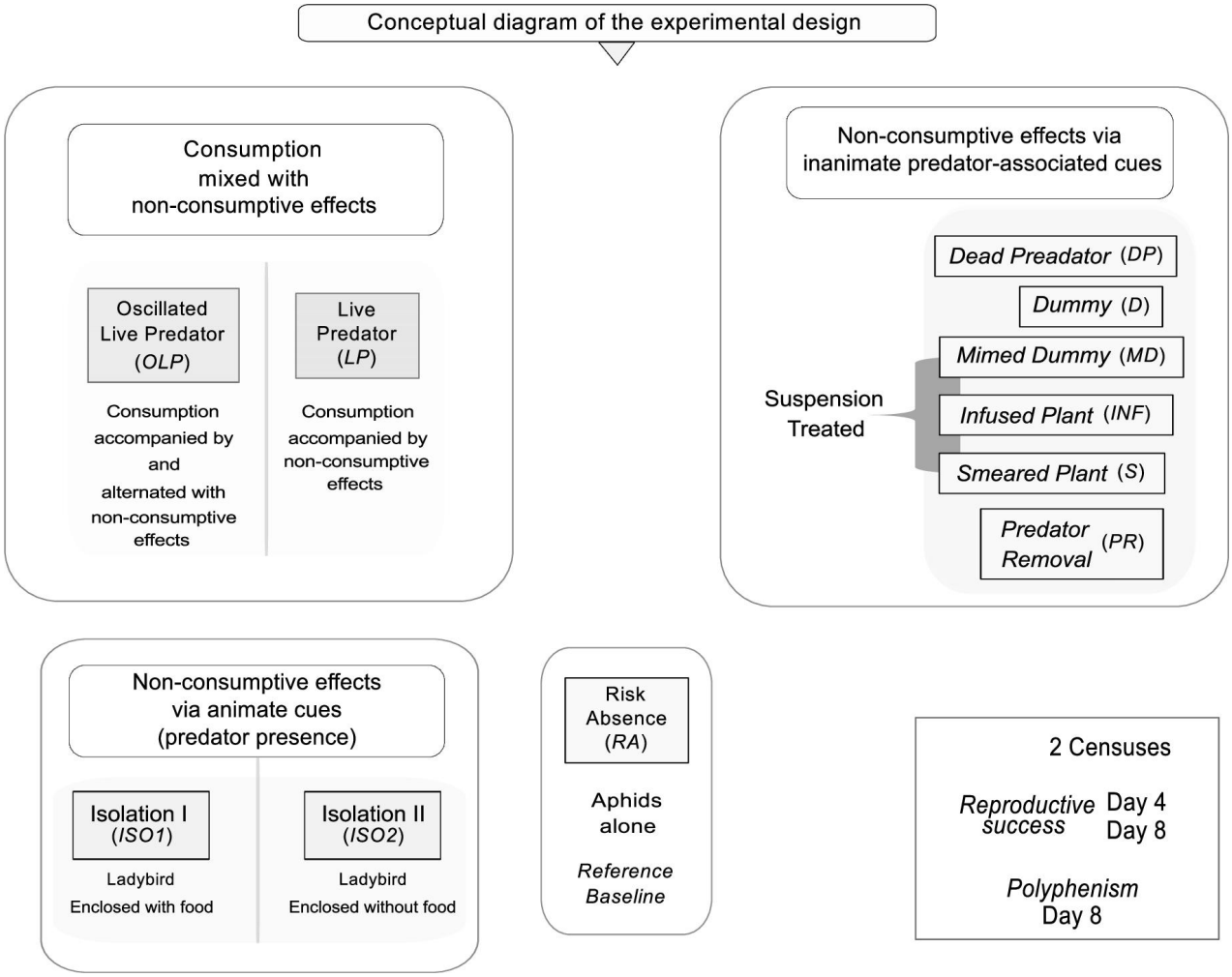

Figure 1. Schematic diagram of the conceptual set-up of the experimental work. The diagram illustrates the characteristics of the applied predator-associated risk treatments and the structure of the analyses that examined aphid raw total numbers (as a proxy for reproductive success) on Day 4 and Day 8, and winged morphs (alata production denoting distinct phenotypic plasticity [polyphenism]) on Day 8, as functions of treatments.

We test the following questions:

- Do aphid reproductive success and phenotypic plasticity vary over time in response to consumption, an alternation between consumption and non-consumptive effects, and by non-consumptive risks caused by animate or inanimate stimuli?
- How do the different types of predator-associated risks rank in their repressive capacity of aphid population growth?

Materials and Methods

Model organisms

The experiment was performed at the Freie Universität Berlin, Germany, greenhouse conditions (22-24°C and 16:8 L:D). Savoy cabbage *Brassica oleracea var. sabauda*, cultivar Vertus 2, purchased form (Sperli©, Germany), was the treatment host plant. Two seeds were sown on opposite sides of plastic potware (11cm height & diameter) filled with steam-sterilised soil. All the treatment plants were 30 days old and watered as needed.

To control for aphid preconditioning, one clonal lineage of *M. persicae* was reared on kale *Brassica oleracea* var. Sabellica (L.), cultivar 'Lerchenzunge', purchased from (Quedlinburger Saatgut© via a local supplier in Germany), and maintained under the aforementioned conditions prior to the commencement of the experiment. Culture and treatment plants are both attractive for *M. persicae*; an important polyphagous pest (Blackman and Eastop, 2007). A population adult (females) *C. septempunctata* (seven-spotted ladybird) was obtained from a commercial supplier (Katz Biotech AG, Baruth, Germany). After receipt, ladybirds were kept in the fridge (9°C), for one night, in a plastic container that included paper strips. Before trials, the refrigerated ladybirds were fed individually with 5 nymphs of *M. persicae* from the stock culture. Additionally, thirty ladybirds (~1,067g in total) were frozen for 48h and utilised afterwards to prepare predator suspension (described below). The freezing of ladybirds occurred once received from the supplier, with no food provided before freezing.

Set-up and experimental design

The microcosm of the experiment was made by installing a grid to hold up an ultra-fine mesh fitted with a zip used to enclose the plants in their pots. Twenty apterous 3rd aphid instars were placed per microcosm, midway of the two plants. The aphids were carefully transmitted, into each microcosm, on a rectangle of waxed paper (2x1cm), using a fine wet brush. The treatments were run synchronised. We had 11 treatments × 7 repeats (microcosms) × 20 instars per microcosm totalling up to 1540 genetically identical aphids. Each treatment lasted eight days, starting with Day 0 as the set-up day (see details below). Aphids were counted halfway (day 4) and on the last day of the experiment (Day 8) to determine treatment effects on the total raw numbers of *M. persicae.* On the final count on Day 8, alata production (*i.e.*, the number of alates [winged morphs indicating phenotypic plasticity]) was also surveyed per microcosm.

For the treatments involving the influence of prey intimidation induced by predator-associated non-consumptive effects, cues were attached to one plant only of the two available in the microcosm, referred to as the 'cue-treated plant'. See (Figure 1) for a schematic diagram of the

design, and (Table S1, Supplementary Materials) for further details. Microcosm preparation and aphid transmission were as described above. There were eleven treatment lines:

1- Risk Absence (*RA*): Aphids were alone in the microcosm, risk-free.

2- Live Predator (*LP*): After 24h, ladybirds were taken from the fridge 1h before treatment. Once aphids were transferred into respective microcosms as described above, one ladybird was introduced per microcosm and remained enclosed for the full experiment time where it was left free to forage the microcosm. For aphid counting on Day 4, ladybirds were captured momentarily in separate plastic containers. After aphid census, each ladybird was re-located into its relevant microcosm. The risk effect here was mainly consumption, but also the predator roaming the microcosm could generate a non-consumptive effect by their bio-signature (semiochemicals, tracks and excretion).

3- Oscillated Live Predator (*OLP*): After 24h, ladybirds were singly introduced into microcosms following 1h tune-up off the fridge. With this treatment, the predator was left in the microcosm for 2 days followed by one day 'out of microcosm' and so forth periodically until the end of the experiment. We kept the ladybirds in the fridge (9°C) during the 'time-out', in separate glass containers covered with mesh and one provision of 5 stock individuals of *M. persicae* nymphs as a feed. Containers were numbered and every ladybird was returned to its original corresponding microcosm. For aphid counting on Day 4, ladybirds were captured momentarily in separate plastic containers. The risk effect here entailed phases of (ladybird in microcosm) including periodic consumption accompanied by possible non-consumptive effect due to microcosm roaming by the predator, which was alternated with phases (intervals of ladybird out of microcosm) including intimidation by inanimate ladybird-borne cues left within the aphids' environment. This presence/absence rhythm of predation/predation risk served the purpose of creating fluctuation/oscillation in risk exposure.

The following treatments (4-11) all exclude direct prey consumption, and examine prey intimidation resulting from the influence of different *animate* or *inanimate* cues associated with predation risk:

4- Isolation I (*ISO1*): Each ladybird was enveloped with 20 aphids to feed on, in a fine-mesh sachet (3x3cm). The mesh was firmly sealed with a rubber band and tied to the stem of one plant in the microcosm using a string (3cm above soil surface). Instantaneously, aphids were transferred into the microcosm. Ladybird individuals were left enveloped continuously throughout the treatment. The risk effect here was prey intimidation (non-consumptive) via the constant presence of isolated animate predator accompanied by the emission of alarm pheromone by the consumable conspecific aphids confined in the predator sachet.

5- *Isolation II* (*ISO2*): Ladybirds were individually enveloped, as mentioned above in *ISO1*, but without aphid feed. On Day 4, during aphid census, the ladybirds were captured briefly and fed with 5 aphids from the stock culture in separated plastic containers before they were re-enveloped in the corresponding sachets. The risk effect here was non-consumptive via the *mere* presence of an animate predator (without consumption of prey conspecifics in the predator sachet).

6- *Dead Predator* (*DP*): Fourteen ladybirds were frozen. Then, two randomly selected ones were transferred into each microcosm; one ladybird was placed on the ground close to the stem, while the other was carefully tied to the plant stem (3cm above soil surface), using a sewing kit. The risk effect here was non-consumptive by inanimate predator-borne cues. This treatment draws on our recent work on the effects of predator cadavers on aphid population growth (Khudr *et al.,* 2017).

7- *Dummy* (*D*): Lookalike mock ladybirds were prepared using shaped masses of odourless synthetic paste and colour markers; mimicking *C. septempunctata*. The dummies were waterproofed beforehand, using a transparent polish. The first dummy was placed on the soil surface, close to the stem. The second was tied to the plant stem (as in the treatment *Dead Predator*). A string was attached to the dummy's end to tether it to the stem. See (Figure 2) for a picture of the dummy. The risk effect here was non-consumptive by inanimate cues (visual mimicry).

Figure 2. Predator dummy. The composite photograph displays the ladybird replica (termed as 'dummy'). The same dummy was cued with the ladybird suspension, conveying predator-borne chemical cues, as described in the main text to create the decoy termed 'mimed dummy'. The left part represents the set-up of the dummy or the mimed dummy, while the part on the right represents an exemplary dummy with reference in cm.

For the treatments 8-10 below, the aforementioned suspension was prepared by fine-crushing thirty frozen ladybirds, using a grinding glass kit. Water was added up to a volume of 150ml. The suspension was vigorously shaken before each application.

8- *Mimed Dummy* (*MD*): Dummies were bound to one plant in the microcosm as described in the *Dummy* treatment above. Then, they were carefully smudged several times with the dead-ladybird suspension prior to aphid introduction into microcosms. The risk effect here was non-consumptive by inanimate cues (visual and olfactory mimicry).

9- *Smeared Plant* (*S*): The leaves and stem of one plant in each corresponding microcosm were generously daubed with the dead-ladybird suspension prior to aphid introduction. The risk effect here was non-consumptive by inanimate predator-borne cues (*e.g.*, tracks, excretion, and semiochemicals including olfactory cues).

10- *Soil Infused* (*INF*): A syringe with a modified elongated nozzle was inserted into the soil to gradually inject the dead-ladybird suspension (described above) at varying depths in contact with the roots of one plant of the two available per microcosm. The infusion process of 10 mini-shots (1.9 ml per shot) covered the root system up to the disc where the stem came forth; 19 ml suspension was used in total per microcosm. This created a fine surrounding 'prey intimidation zone' in and on the soil surrounding the root system and the stem base of the treated plant. The risk effect here was non-consumptive due via predator-borne cues (*e.g.*, olfactory cues). This treatment draws on our recent work on the applied infusion method for soil manipulation to directly or indirectly impede pest population growth (Khudr *et al.,* 2017).

11- *Predator Removal* (*PR*): Ladybirds were taken off the fridge for 1h to tune-up and fed with 5 nymphs of *M. persicae* from the stock population. For 48 h, one ladybird was left with one plant only per microcosm for each repeat of this treatment in order to have the plants marked by the bio-signature of the ladybird. Subsequently, after the removal of the predator, a fresh plant (untreated) was carefully transferred to each microcosm, with a mass of soil surrounding the roots. This was followed by the aphid introduction. The risk effect here was non-consumptive by inanimate predator-borne cues (*e.g.*, olfactory cues).

Statistical analysis

We used R version R 3.3.1 (R Core Team, 2016) for the statistical analyses. First, we investigated aphid raw total numbers per plant in the microcosm as a proxy for reproductive success, with two respective counts on Day 4 and Day 8 of the experiment. To examine the repressive effect of each predator-associated treatment on the reproductive success from two standpoints, we applied two generalised linear mixed effect models (GLMM), with the function 'glmer', 'bobyqa' optimisation, and Gamma family (due to the non-normal shape of distribution, confirmed by Shapiro test, and

362 high positive skewness), using R package '*lme4*' (Bates *et al.*, 2015). The microcosm was nested
363 within the count day times and randomised in the model; the main effects of the models were
364 respectively revealed using an Anova command, R packages 'car' (Fox and Weisberg, 2011).
365 *Model I (Risk type effect*): The explanatory variable is of five levels: Risk-free [Absence of risk
366 (*RA*)], mixed consumptive and possible non-consumptive risks (*LP*), mixed consumptive and
367 possible non-consumptive risks alternated with non-consumptive effect (prey intimidation) [*OLP*],
368 prey intimidation by animate cues [*ISO1* and *ISO2*], and prey intimidation by inanimate cues [*DP*,
369 *D*, *MD*, *S*, *INF*, *PR*]. The control (*RA*) was the model baseline. This was followed by a posthoc pair-
370 wise multiple comparison test (Tukey's HSD) using the R package 'lsmeans' (Lenth, 2016).
371
372 *Model II (Treatment effect*): The explanatory variable was of 11 levels; see experimental design
373 above and (Table S1, Supplementary Materials); the treatment "Risk Absence (*RA*)" made the
374 baseline in this model. This was followed by a posthoc pair-wise multiple comparison test (Tukey's
375 HSD), R package 'lsmeans'.
376
377 Second, we investigated alata production. Alates were counted on Day 8 at the end of the
378 experiment. This was investigated using two generalised linear models (GLM) with a quasipoisson
379 family (due to over-dispersion and non-normality [confirmed by Shapiro test]), using the R package
380 'multcomp' (Hothorn *et al.*, 2008). The explanatory variables in *Model I (Risk type effect*) were as
381 described in the above-mentioned *Model I, plus a covariate* that was total aphid numbers in the
382 microcosm representing numerical population pressure that might contribute to the production of
383 alates. The other model (II) had the 11-level treatment effect as an explanatory variable (see *Model*
384 *II* above); the treatment "Risk Absence (*RA*)" was the model baseline. The main effects of the
385 models were respectively revealed using an Anova command, R packages 'car' (Fox and Weisberg,
386 2011).
387 See (Figure 1) for a conceptual schematic diagram of the design, and (Table S1,
388 Supplementary Materials) for further structural details. Data for this study are available at the
389 figshare repository via the following URL: https://figshare.com/s/b99e89b8a3c6160ab74f

390 **Results**

391 *Aphid reproductive success*

392 Aphid numbers were lower than in the control for all treatments. As shown in (Figure 3),
393 aphids suffered a clear differential loss in reproductive success that was contextual and contingent
394 upon the type of the imposed predator-associated risk. The latter highly influenced aphid

reproductive success in the microcosm ($\chi^2_{4,268}$= 50.90, P < 0.0001), where, according to *Model I* summary, all risk types had differential significant effects: consumption (via *LP* [Live Predator]) (P = 0.023), mixed consumptive and non-consumptive effects alternated with non-consumptive effects (via *OLP* [Oscillated Live Predator]) (P<0.0001), non-consumptive animate cues (via *ISO1* [Isolation I] and *ISO2* [Isolation II]) (P = 0.008), non-consumptive inanimate cues (via *DP* [Dead Predator], *D* [Dummy], *MD* [Mimed Dummy], *S* [Smeared Plant], *INF* [Soil Infused], and *PR* [Predator Removal]) (P = 0.002), (Tables S2, Supplementary Materials) for details of the model, and (Table S3, Supplementary Materials) for posthoc multiple pairwise comparisons.

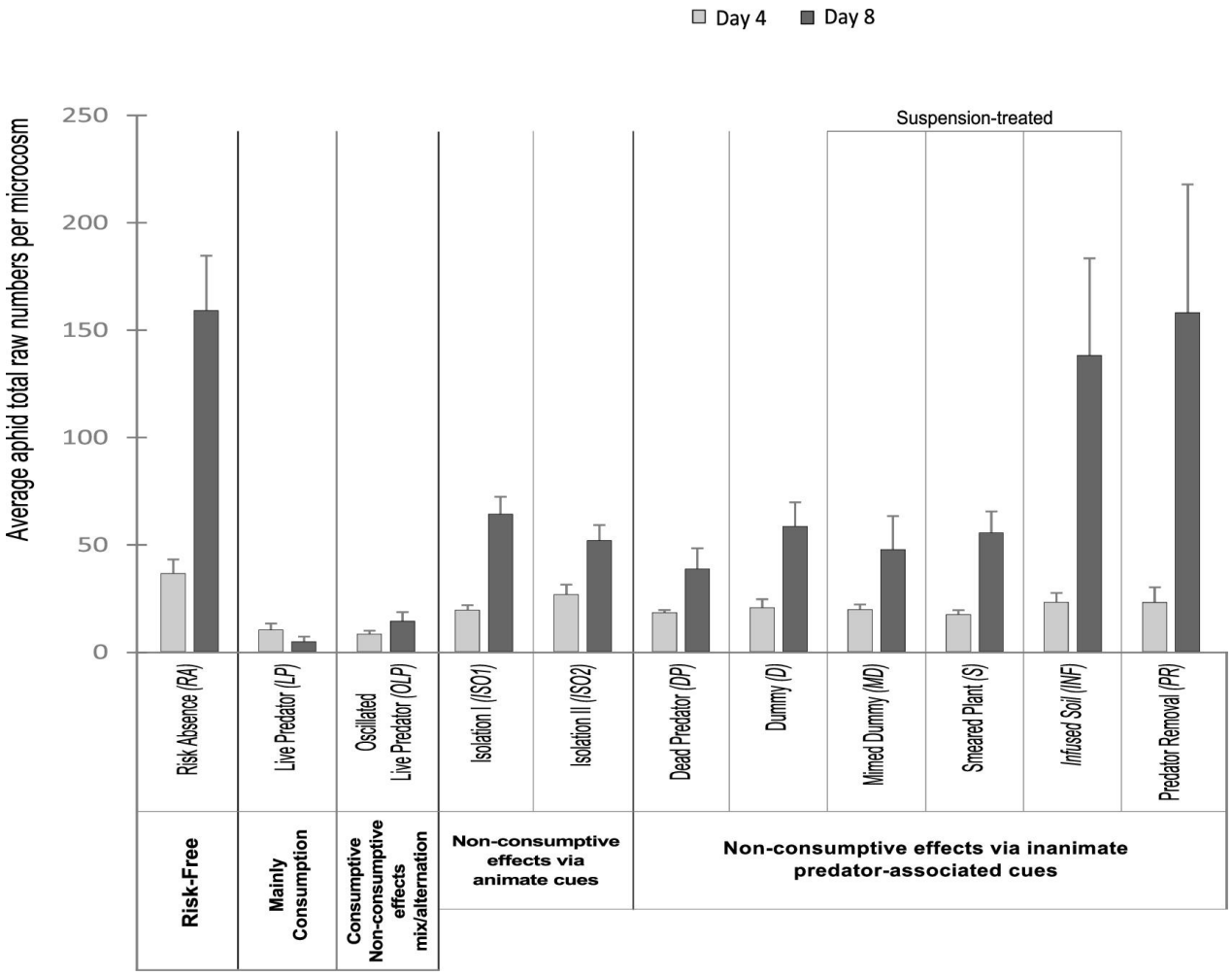

Figure 3. Aphid reproductive success under the effect predator-associated risks. The average aphid total raw numbers (±SEM) in the microcosm (as a proxy for reproductive success) are illustrated. The light grey bar shows the mean reproductive success on Day 4 (1st census); the darker bar describes mean reproductive success on the final day of the experiments (2nd census).

410 According to *Model II*, there was a strong treatment effect on aphid reproductive success in
411 the microcosm ($\chi^2_{10,262}$= 69.07, P < 0.0001). Specifically, the influence of risk distinctly varied
412 across risk treatments: *LP* (P<0.0001), *OLP* (P<0.0001), *ISO1* (P = 0.019), *ISO2* (P = 0.011), *DP* (P
413 = 0.0003), *D* (P = 0.013), *MD* (P = 0.003), *S* (P = 0.004), but *INF* and *PR* were insignificant. See
414 (Tables S4, Supplementary Materials) for details of the model, and (Table S5, Supplementary
415 Materials) for posthoc multiple pairwise comparisons.

416 Ranking first and second alternatively in impeding aphid reproductive success, the risks
417 imposed by (*LP*, constant consumption with possible non-consumptive risk) *and (OLP*, periodic
418 consumption, accompanied by non-consumptive effects, with intervals of exposure to predator-
419 borne cues) had together the strongest negative impacts on aphid numbers throughout the
420 experiment. By the first aphid census, (*LP*) (x-bar = 10 ±3SEM aphids) was only marginally bested,
421 in impeding the reproductive success, by (*OLP*) (x-bar = 8 ±3SEM aphids). Whereas, the second
422 census portrayed a slightly different reversed pattern to the advantage of (*LP*) (x-bar = 5 ±2SEM
423 aphids) compared to (*OLP*) (x-bar = 14 ±4SEM aphids), (Table 1, Figure 3).

424 Overall, compared to the absence of risk, the non-consumptive risk cues had a clear negative
425 impact on the aphid population that differed by context. The least effective treatment on Day 4,
426 ranking 10th in impeding aphid reproductive success, was (*ISO2*) (x-bar = 26.57 ±4.59SEM aphids)
427 compared to the control (RA) (x-bar = 36 ±6.65SEM aphids). The least impeding one on Day 8 was
428 (*PR*) with a very poor decrement of the aphid reproductive success (x-bar =158 ±60SEM aphids).
429 The animate cues (*ISO1* at Day 4, and *ISO2* at Day 8) ranked 5th on the scale of impeding aphid
430 reproductive success; whereas, the 3rd and 4th ranks were always held by the inanimate-cue
431 treatments *Smeared Plant* (*S*) and *Dead Predator* (*DP*) on Day 4, and (*DP*) and (*MD*) at Day 8, as
432 they were more efficient in their impeding effects than *ISO1* and *ISO2*, (Table 1, Figure 3).

433 The inanimate cues of the (*S*) had the most negative impact on aphid reproductive success
434 (x-bar = 17 ±2SEM aphids) on Day 4, with 52% decrement of reproductive success compared to
435 what was recorded in the control (*Risk Absence,* x-bar = 36 ±7SEM aphids). Nevertheless, (*S*) was
436 only 23% and 20% less effective than (*OLP*) and (*LP*), respectively, while at the bottom of the chart
437 was the non-consumptive risk of the isolated starved predator (*ISO2*) with 25% reduction of
438 reproductive success in comparison with the control, (Table 1, Figure 3).

439 Noticeably, aphid numbers at the second census indicated a nuanced differential effect of
440 impeding aphid reproductive success by inanimate cues, with *Dead Predator* (*DP*), being the most
441 effective in impeding aphid reproductive success, resulting in ~ 76% lower reproductive success (x-
442 bar = 38 ±10SEM aphids) than the control's reading, (x-bar = 159 ±26SEM aphids). Further, the
443 effects of (*DP*) was only 21% and 15% less effective than the cases of (*LP*) and (*OLP*), respectively.
444 By contrast, *M. persicae* population thrived after the first census and reached a high score at Day 8

445 after the soil was cued (*INF*) (x-bar = 138 ±45SEM aphids) *and also* under *Predator Removal* (*PR*)
446 (x-bar = 158 ±60SEM aphids) entailing a short-lived effect for the suspension treatment and the
447 predator bio-signature (traces/trails) therein.

448 In all cases at Day 8, albeit (*INF*) and (*PR*) being the poorest suppressors of the aphid
449 population, they both led to densities lower than the risk-free control did (x-bar = 159 ±25.6SEM
450 aphids), (Table 1, Figure 3). Further, what is also interesting is that (*DP*) maintained the same rank
451 (7th) of the impediment of the reproductive success at both censuses. The same was shown for (*INF*)
452 keeping the 9th rank at both day counts. Additionally, the inanimate-cue treatments (*DP*, *S*, and *MD*)
453 were always within the rank range 3rd-6th, and the dummy treatment (*D*) maintained always the 7th
454 rank on Day 4 and Day 8.

455 *Aphid polyphenism*

456 The proportions of alates differentially varied under the influence of risk; as extracted from *Model I*,
457 the type of predator-associated risk highly influenced alata production ($LR\chi^2_{(4,608)}$= 27.07,
458 P<0.0001), so did the pressure exerted by the pressure exerted by the entire population size in the
459 microcosm ($LR\chi^2_{(1,60)}$= 8.63, P = 0.003); the interaction between these two predictors was
460 marginally significant ($LR\chi^2_{(4,60)}$= 9.5, P = 0.0497), (Figure 4).

461 There were no alates to report under the consumptive risk (*LP*) contrary to the unexpected
462 percentage of (x-bar = 2.8% ±2.78SEM) under (*OLP*). More surprisingly, we found the highest
463 morph proportion (x-bar = 16.7% ±5.3SEM) under (*ISO2)* (fed ladybirds that then were left with no
464 nourishment in the predator sachet), but (*ISO1*) (ladybirds enveloped with additional aphid feed)
465 resulted in a considerably small percentage of alates (x-bar = 0.7% ±0.7SEM), (Figure 4). Note that
466 the proportion of alates was (x-bar = 1.5% ±0.4SEM) under the control (*RA*), attributable to
467 crowding.

468 Interestingly, amongst the inanimate risk-cue treatments, alates proportions were the highest
469 in the presence of the *Mimed Dummies (MD*) (x-bar = 8.4% ±1.3SEM), followed by the case of
470 *Smeared Plant* (*S*) (x-bar = 5.8% ±1.3SEM), both of the suspension-treated group. Conversely, the
471 microcosms with notable aphid reproductive success under (*INF)* led to the second smallest
472 proportion of alates (x-bar = 0.04% ±0.04SEM) across all risk treatments. Moreover, aphid
473 reproductive success, at Day 8, was 75% lower under (*DP*) compared to the reading under (*PR*), yet
474 counterintuitively, these risk treatments by inanimate cues resulted in almost identical proportions
475 of alates (x-bar = 1% ±1.02SEM) and (x-bar = 0.9% ±0.76SEM), respectively. Lastly, the risk
476 imposed by the dummy (*D*) did not elicit any production of alates, (Figure 4).
477

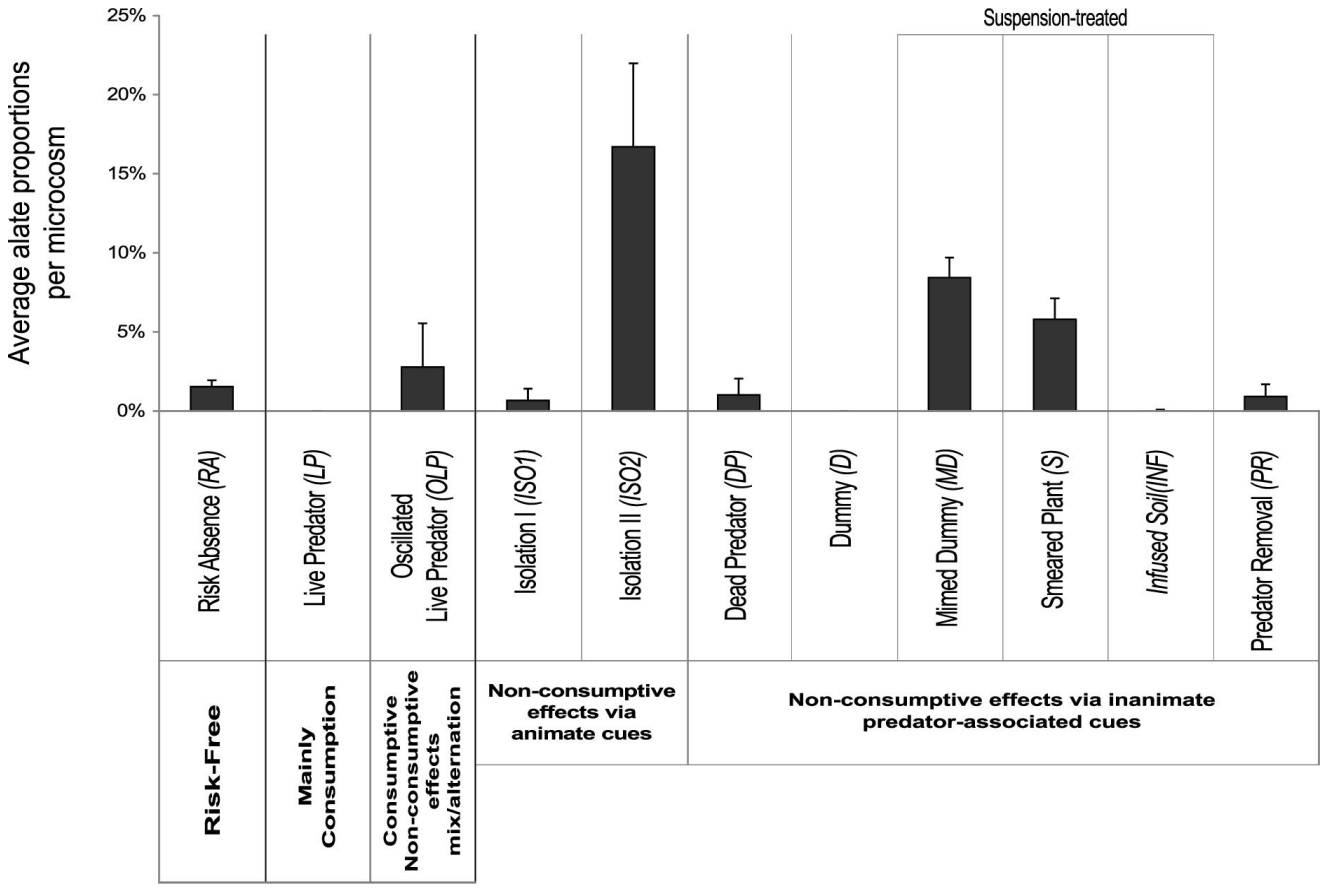

Figure 4. Aphid polyphenism under the effect of predator-associated risks. Aphid polyphenism (distinct phenotypic plasticity) is described as the mean percentage of alates (±SEM) produced by the end of the experiment on Day 8.

Further, *Model II* indicated that the numerical pressure of the population in the microcosm significantly influenced alata production ($LR\chi^2_{(1,48)}$= 6.49, P = 0.011); both the risk treatment and and the interaction between the risk treatment and the said numerical population pressure had highly highly significant effects on alata production ($LR\chi^2_{(10,48)}$= 180.34, P<0.0001) and ($LR\chi^2_{(10,48)}$= 42.32, P<0.0001), respectively. See (Tables S6 and S7, Supplementary Materials) for model details, and (*Notes 1* and *2*, Supplementary Materials).

Discussion

In this work, we tested differential and time-limited responses of one clone of *M. persicae to* continuous and discontinuous risk stimuli, in terms of reproductive success and phenotypic plasticity (polyphenism), to a range of different consumptive and/or non-consumptive risk cues. *Myzus persicae* reacted to the presence of a potential predator as well as to chemical or visual cues

associated with its coccinellid predator. Prey intimidation via non-consumptive effects led to the remarkably variable impedance of aphid reproductive success and also resulted in varied production of winged morphs. Using a model agroecosystem of an important parthenogenetic plant pest raised on a significant crop, this timely and novel work addresses repressive effects of fear of predation that have not been previously sufficiently investigated. Our findings bear significant theoretical, empirical, and applied facets and implications.

Reproductive success and polyphenism under risk

Compared to the risk-free treatment (control), *Live Predator* (*LP*) and *Oscillated Live Predator* (*OLP*) strongly decreased the densities of *M. persicae*. In (*LP*) the diminished aphid population was the result of constant consumption mixed with possible non-consumptive effect due to the live predator making forages for food within the microcosm. Whereas, (*OLP*) was an alternation between two states: (1) consumptive + non-consumptive effects, and (2) non-consumptive effects only. Thus, (*OLP*) presented a complex periodic mixed-effect challenge for the pest as episodes of consumption accompanied by non-consumptive effects were interposed by predator time-outs (prey intimidation by predator-borne cues only) where the marks of the predator, bearing its semiochemicals, remained on the plants. The treatment (*OLP*) was scoped to be significant in controlling aphid numbers; the specific difference of temporal variation in consumption and prey intimidation was the decisive factor for (*OLP*) ranking second after the most efficient treatment (*LP*) at the second census. Furthermore, the described idiosyncrasy of (*OLP*) induced more alates; whereas no morphs appeared under (*LP*). It could be argued, therefore, that temporal variation of risk and alternation of risk types (consumptive and non-consumptive effects) can induce differential adaptive prey responses (Keiser, 2012; Keiser and Mondor, 2013). Thus, the frequency of exposure to risky surroundings will shape the outcome of prey escape and survival propensities (Mackay and Wellington, 1975; Lima and Bednekoff, 1999; Keiser and Mondor, 2013).

Moreover, the effects of the animate risk associated with the isolated ladybirds, through their mere presence in the microcosm, had strong negative impacts on aphid reproductive success as well as phenotypic plasticity and thereby population dynamics. Surprisingly, the isolated ladybird without food (*ISO2*) elicited a high percentage of alates, although the average reproductive success under this treatment was lower at the second census when compared to (*ISO1)* (ladybird enveloped with aphid conspecific prey). This was possibly due to induction by chemical signals emanating from the isolated ravenous predator in communication with the aphids (Norin, 2009). These findings shine a novel light on the assumption that aphids perceiving their conspecifics being preyed upon might become triggered to produce dispersive morphs (alates) in response to escalated risk; see (Fievet *et al.*, 2009). The conspecifics in our example in (*ISO1*) were consumed alive in a

529 micro-porous sachet poised in the vicinity of the clone. By Day 4, the ladybird having consumed its
530 aphid feed in isolation, there were presumably plenty of aphid alarm signalling diffusing from the
531 sachet hence (*ISO1*) thus ranking 5th on the scale of impeding the reproductive success of *M.*
532 *persicae*. By contrast, on the same day, the ladybird in (*ISO2*) was presumably emitting
533 semiochemicals but that was not backed up enough by aphid alarm pheromone to trigger a tangible
534 suppression of the population hence (*ISO2*) ranking only 10th. Remarkably, on Day 8, the ladybird
535 in (*ISO2*) became increasingly hungry for aphids and might have been, therefore, emitting more
536 intense or more frequent semiochemicals that helped trigger an alarm in the clone hence (*ISO2*)
537 ranking 5th and inducing many alates to flee the risk. Whereas, on Day 8, (*ISO1*) ranked 8th with
538 clearly poorer induction of alates perhaps because there were no conspecifics left in the population
539 to be preyed upon by then and thus not enough trigger of the alarm state in the clone. It is
540 noteworthy that during the brief feeding period in (*ISO2*), it is also likely that the ladybird became
541 smeared with cornicle secretions produced by the stock aphids (the same clone as the experimental
542 one). Alarm signals/volatiles might have emanated from the secretions after the ladybirds were re-
543 introduced to the experimental units (Mondor and Roitberg, 2004) and hence contributed to the
544 induction of the alata production and the population diminishing capacity in (*ISO2*). In the animate-
545 cue risk treatments, the prey-originated and the predator-originated cues were seemingly
546 intertwined. On the whole, this implies that the non-consumptive effects of the isolated predators
547 can have noticeable negative impact on the aphid population, but their impact may need coupling
548 with aphid alarm pheromones for the effect of the "scent of fear" (Leather, 2015) to heighten
549 (Ingerslew and Finke, 2017); see also (Kats and Dill, 1998).

550 The difference in aphid reproductive success in the animate risk treatments might also be
551 partly explained by the higher percentages of winged offspring, entailing reduced fecundity for the
552 advantage of dispersal in (*ISO2*). More investment in dispersal as an anti-predator defence can be
553 read as a transgenerational adaptive tactics in aphids (Keiser, 2012; Keiser and Mondor, 2013), but
554 the inducible defence can be costly in the sense that it entails a lower reproductive success that may
555 negatively affect population growth due to decreased production of fecund apterae (Mackay and
556 Wellington, 1975; Dixon, 1998; Dixon and Agarwala, 1999; Ingerslew and Finke, 2017); survival
557 of the clone may, however, increase in the long term due to dispersion (Francke *et al.*, 2008; Keiser,
558 2012). Again, note, that although aphid reproductive success was lower under (*ISO1*) than (*ISO2*)
559 by Day 4, the pattern of difference was reversed at Day 8 with more decrement of aphid
560 reproductive success in (*ISO2*) but remarkably accompanied with ~ 25-fold (*i.e.*, 96%) increase in
561 alata production. Could it be that the longer the isolated predator starves the more effective/intense
562 the release of the alert/alarm cues (eliciting flight defence) is in the vicinity of the prey population?

By and large, prey intimidation via animate cues yielded similar impediment of the reproductive success of the clonal prey population in question, when compared with the intimidation via predator-associated inanimate cues. Interestingly, four out of six risk treatments by inanimate cues were generally effective in decreasing aphid reproductive success generally similar in magnitude to the effects demonstrated for animate cues. In turn, the different risk treatments, comprising different signals, led to variable inhibition of aphid population growth as well as differential induction of alates. Our results support earlier findings on the disruptive or suppressive effects of non-consumptive predation risk on aphid population dynamics (Nelson and Rosenheim, 2006, Nelson, 2007) including the effects of dead conspecifics (Fievet *et al.*, 2008), predator bio-signature (Steffan and Snyder, 2010, Ninkovic *et al.*, 2013), and impaired predators (Nelson *et al.*, 2004; Nelson and Rosenheim, 2006).

The population-diminishing effects of the inanimate cues pertaining to (*DP*), (*D*), (*MD*), and (*S*) were largely persistent at the second census of the experiment, suggesting an extended negative impact on aphid reproductive success against time. In stark contrast, the effects of the inanimate cues of soil-infused (*INF*) and predator removal (*PR*) were short-lived and weakened after Day 4. It should be noted that by the second census on Day 8, the effect of dummies in the *Dummy* treatment (*D*), representing predator mimicry, became augmented in impeding aphid reproductive success after the daubing of the dummy with predator suspension bearing visual and olfactory cues in the *Mimed Dummy* treatment (*MD*). Still, the effect of (*MD*) could not surpass that of the dead predator in (*DP*) at both censuses. Also, the considerably strong negative effect of the smeared plant (*S*) on Day 4 lost 3 ranks on the scale of the impediment of aphid reproductive success by Day 8, while (*DP*) climbed up a notch to become the third most efficient risk treatment after (*LP*) and (*OLP*).

Overall, (*DP*) had eventually a stronger negative influence on *M. persicae* reproductive success than the predator-mimicking treatments (*Dummy, Mimed Dummy, Smeared Plant,* and *Infused Soil*) and the animate-risk treatments (*ISO1*) and (*ISO2*). The predator cadavers in (*DP*), and the dummies (especially the mimed [scented]) bore visual and/or olfactory plus tactile cues, in direct contact with aphids, unlike the rest of the risk treatments which mostly entailed either visual or olfactory signals. This receives credence from the findings documented by Ingerslew and Finke (2017) on the palpation of aphids by a parasitoid wasp, suggesting an under-studied non-consumptive negative tactile effect on aphid fitness that might be applicable for other natural enemies of aphids. The combination of different perceptions by the aphids exposed to predation risk could be the decisive factor behind the superlative repressive effect of (*DP*) in the long run, especially in light of the evidence that aphids rely on combined modalities to perceive and assess their embedding contexts (Ben-Ari and Inbar, 2014). As such, our findings comparatively showcase that *biomimetic* designs along with manipulating non-consumptive effects of isolated or dead

predators can lead to suppression of pest population growth in a novel fashion. Alata production can be triggered mainly by aphid density (Dixon, 1998), but also by a variety of factors spanning exhausted resources and the presence of natural enemies (Dixon, 1998; Kunert *et al.*, 2005; Creel and Christianson, 2008). Moreover, previous studies showed that ladybird traces might, as well, trigger the production of alates (Dixon and Agarwala, 1999). Our results indicate that signals of the intimidation by animate (*ISO2*) and inanimate (*M*) and (*S*) cues might have played a critical role in the induction of winged morphs as phenotypically plastic response, irrespective of the smaller corresponding populations suffering from considerable suppression pressure of the ranks 4th, 5th, and 6th, respectively, at Day 8. At any rate, it is important to comparatively highlight that a study on transgenerational polyphenism of pea aphid, by Purandare *et al.* (2014), demonstrated that the tracks of a coccinellid predator induced the least number of dispersive morphs, if compared with the control or the effect of crowding. The cited authors suggested that the polyphenic responses might have depended on the intensity and sufficiency of predation risk.

Nevertheless, the proportions of winged offspring continued to differ under the influence of non-consumptive effects in our study. For example, the treatments (*MD*) and (*S*) triggered the production of a greater number of alates, whilst they decreased aphid reproductive success, compared to the treatments (*INF*) and (*PR*) where *M. persicae* populations thrived (yet produced negligible to relatively small numbers of alates). This implies that alarm signals (pheromones) and risk cues (semiochemicals), not crowdedness in these cases, were important for triggering aphid polyphenism as an inducible defence against risk. The treatment including mimetic dummies or host plants prompted (sprayed) with predator-borne cues unveiled novel aspects of extreme phenotypic plasticity in aphids as means of indirect avoidance of novel threats (Grostal and Dicke, 1999). That means that alongside the alteration of population size, perception of the predator-borne cues might influence the production of alates as a plastic anti-predator flight defence in aphids (Mondor *et al.*, 2005). Again, the winged aphids have relatively lower fitness in comparison to the wingless (Dixon, 1998). Thus, alata production can also account for the reduced aphid densities close to environmental risk in our work under the treatments (*MD*) and (*S*) of prey intimidation by inanimate cues, in particular, due to increased investment in the generation of less fecund, but more dispersive phenotypes (Roitberg *et al.*, 1979, Dixon, 1998, Kunert and Weisser, 2003).

On the one hand, based on the risk allocation hypothesis (Lima and Bednekoff, 1999), we suggest that the variation in aphid response to the range of constant or periodic consumption, and the animate, as well as inanimate cues of predation risk, applied in our study, can be largely attributed to *M. persicae*'s differential ability to perceive, identify and respond to different kinds, intensities and frequencies of predation risk. It can be inferred, therefore, that the influence of fear of predation on green peach aphid fitness might be pronounced in environments with frequent

633 disturbance intervals and/or disproportionate intensities of risk. This connotes an ability of the
634 phloem-feeding prey to apply different strategies in reaction to a range of olfactory and visual cues
635 that are associated with fitness deprivation as a result of exposure to environmental risk; see (Keiser,
636 2012).

637 On the other hand, it can be argued that the risk treatments presented unreliable cues for
638 aphids and hence led to perceiving the embedding contexts as rather inhospitable; this would have
639 created, in turn, a varying state of sensory ambiguity that altered prey's decisions and reproductive
640 success. The difference shown in aphid response to those treatments was due to the differential
641 perception of such ambiguity of predator-borne or predator-associated cues that confused aphid
642 mothers and hampered their fitness (Koops, 2004). This argument receives support from Ben-Ari
643 and Inbar (2014), who suggested that aphid assessment of the environment and hence aphid
644 responses, including transgenerational adaptations (Keiser, 2012), to environmental cues would be
645 based on a multi-modal perception of cues and cue reliability. For example, the short-lived effects
646 regarding the cues of the (*INF*) and (*PR*) treatments conform to the work provided by Koops (2004),
647 suggesting that the acquisition of ambiguous information may have a shorter temporal influence on
648 prey decision making than the information acquired from reliable cues. In other words, accurate or
649 more reliable info-chemicals acquired by prey are important to make the right decisions (Keiser,
650 2012; Keiser and Mondor, 2013) and thus the lack of certainty would lead to less accurate risk
651 assessment and consequently maladaptive decisions. Notwithstanding, it has been shown that prey
652 responsiveness to predation risk may decrease due to habituation after continuous exposure to non-
653 lethal cues (Shalter, 1984; Holomuzki and Hatchett, 1994). The impact of predator-lookalike
654 mimics (dummies) on aphid traits, in our study, was evident and suggests that aphids utilise
655 chemical as well as visual cues in assessing their surroundings (Ben-Ari and Inbar, 2014). However,
656 the mimics (dummy and mimed dummies) can trick aphids as they seemed to be perceived as a real
657 threat. Other possible explanations for the impeded reproductive success of *M. persicae* in our work
658 might be assignable to mortality as a result of predator-induced stress, as suggested by McCauley *et*
659 *al.* (2011), and/or reproductive costs due to abrupt changes in diet and disruption of feeding time
660 (Nelson, 2007).

661 It has been demonstrated that temporal variation in exposure to predation risk can also
662 influence prey decisions (Lima and Bednekoff, 1999). However, the extent of the influence of
663 predation risk on prey fitness and population dynamics has also been proposed to be dependent on
664 prey physiology and on temporal variability (Trussell *et al.*, 2011, Matassa and Trussell, 2014); as
665 prey may reduce investment/allocation and display of anti-predator behaviours under episodes of
666 high or prolonged risk (McNamara and Houston, 1987, Lima and Bednekoff, 1999; Hamilton and
667 Heithaus, 2001; Kotler *et al.*, 2004). In this regard, Creel and Christianson (2008) suggested that

668 anti-predator responses might involve energetic and physiological tolls with tangible impacts on the
669 reproduction and survival of prey population (Dixon and Agarwala, 1999; Nelson *et al.*, 2004;
670 Mondor *et al.*, 2005; Nelson, 2007). It is worth to note that we did not observe any clear avoidance
671 of the cue-treated plants in the microcosm by aphids, but increased phenotypic plasticity, denoting
672 dispersal, was notable as described above; see (*Note 1*, Supplementary Materials). Further, *M.*
673 *persicae* also varied its inducible defences under (*ISO2*) of prey intimidation by animate cues, as
674 there was less investment in the production of apterae, increased maternal conditioning of offspring
675 into alates, and relatively more aphids abandoned and/or dropped off the host plants. See also (*Note*
676 *2* including Table S8 and Figure S1, Supplementary Materials) for a spotlight on an interesting
677 complementary off-plant behaviour.

678 In summary, our findings stress the relevance of intimidation in predator–prey systems
679 (Preisser *et al.*, 2005; Creel and Christianson, 2008). Aphids can assess environmental risk in their
680 surroundings, where they adjust their progenies phenotypes to procreate and adapt to change (Dixon,
681 1998; Dixon and Agarwala, 1999; Keiser, 2012; Ben-Ari and Inbar, 2014). The variation in aphid
682 densities and polyphenism across the risk treatments can be incurred as a result of a subtle
683 differential perception of predator-borne cues by aphids; concomitant modified reproduction and
684 alata production (Nelson *et al.*, 2004, Nelson, 2007; Khudr *et al.*, 2017), as an anti-predator defence,
685 seem to be contextual tactics that may change relevant to aphid physiological state and
686 environmental stressors (McAllister *et al.*, 1990; Mousseau and Fox, 1998; Weisser *et al.*, 1999;
687 Villagra *et al.*, 2002; Hu *et al.*, 2018) as well as the encounter between aphid and predation risk
688 (Nelson *et al.*, 2004; Nelson and Rosenheim, 2006; Nelson, 2007). Such traits and tactics may be
689 subject to alteration depending on the type and frequency of the predator cues (Sih *et al.*, 2000;
690 Matassa and Trussell, 2014). Examining prey responses to pulsated variable intensities of predation
691 risk (Lima and Bednekoff, 1999; Trussell *et al.*, 2011) should receive further investigation in future
692 studies.

693 *Conclusions*

694 Amid research scarcity on the effects of indirect and imitative stimuli associated with an
695 aphidophagous predator on its phloem-feeding prey, our assay provides empirical evidence of the
696 negative impacts of several animate and inanimate types of predation risk on a clonal aphid
697 population over time. Our findings contribute to improving our understanding of the influence of
698 non-consumptive effects on the reproduction and polyphenism of an important plant pest, using
699 predator corpses as well as mimetic predator replicas and cued inanimate materials.

700 Our results demonstrate that non-consumptive cues related to a natural enemy led to
701 differential impeding of aphid reproductive success and variable induction of dispersive winged

morphs as an inducible polyphenic anti-predator defence. *Myzus persicae*'s concurrent reaction to risk treatments was differential and associated with fitness losses but a relative propensity of flying out the hostile contexts was apparent. We also found that the effects of the exposure to the risk cues may have a limited duration in terms of effect longevity which impacted the prey population in a context-dependent fashion; specific types of intimidation (*e.g.*, dead predators and mimed dummies) were persistent and considerably more efficient than others (*e.g.*, soil infused). This further highlights that the impact of non-consumptive risk effects and their longevity may change by context and by time.

Interestingly, the effects of the inanimate cues led to better or similar negative impacts on aphid reproductive success when compared to those of the animate cues (*i.e.*, the presence of alive, but isolated predator). Clearly, the isolated starved predator led to unparalleled intensified induction of phenotypic plasticity in our study. Our findings have the potential to open the gate for unorthodox ways to contribute to the sphere of research on predation risk as well as provide novel eco-friendly means to protect agroecosystems from pest irruptions *in natura*.

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Table 1. Relative impediment of aphid reproductive success in ranks across two censuses. The different levels of the risk treatments are ordered in terms of the decrement of aphid densities (as a proxy for reproductive success). The encircled number refers to the rank of the impediment of aphid reproductive success. Each risk treatment has a rank number above and a percentage beneath that elucidates the decrement of aphid population density relative to the risk-free control (Risk absence [*RA*]). For example, at Day 8, aphid reproductive success in (*DP*) was 76% lower than the reproductive success in (*RA*), *i.e.*, put differently; the reproductive success in (*DP*) was 24% the reproductive success in the control (*RA*). There were a census on Day 4 and another on Day 8. The treatments are: Risk Absence (*RA*), Live Predator (*LP*), Oscillated Live Predator (*OLP*), Isolation I (*ISO1*), Isolation II (*ISO2*), Dead Predator (*DP*), Mimed Dummy (*MD*), Dummy (*D*), Smeared Plant (S), Soil Infused (*INF*), and Predator Removal (*PR*).

Rank	**Most efficient** ①	②	③	④	⑤	⑥	⑦	⑧	⑨	Least efficient ⑩	Risk Free ⑪
1st Census (Day 4)	***OSC*** 77%	***LP*** 72%	***S*** 52%	***DP*** 50%	***ISO1*** 46%	***MD*** 44%	***D*** 42%	***PR*** 37%	***INF*** 36%	***ISO2*** 25%	*RA* x-bar = 36 ±6.65SEM
2nd Census (Day 8)	***LP*** 97%	***OSC*** 91%	***DP*** 76%	***MD*** 70%	***ISO2*** 67%	***S*** 65%	***D*** 64%	***ISO1*** 60%	***INF*** 13%	***PR*** 1%	*RA* x-bar = 159 ±25.6SEM