

## Pest Management Science

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## TITLE

**Predator- and killed prey-induced fears bear significant cost to an invasive spider mite: implications in pest management**

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## SHORT RUNNING TITLE

Predator- and killed prey-induced fears suppress *Tetranychus ludeni* population

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## Abstract

**BACKGROUND:** The success of biological control using predators is normally assumed to be achieved through direct predation. Yet, it is largely unknown how the predator- and killed prey-induced stress to prey may contribute to biological control effectiveness. Here, we investigate variations in life history traits and offspring fitness of the spider mite *Tetranychus ludeni* in response to cues from the predatory mite *Phytoseiulus persimilis* and killed *T. ludeni*, providing knowledge for evaluation of the non-consumptive contribution to the biological control of *T. ludeni* and for future development of novel spider mite control measures using these cues.

**RESULTS:** Cues from predators and killed prey shortened the longevity by 23–25% and oviposition period by 35–40% and reduced the fecundity by 31–37% in *T. ludeni* females. These cues significantly reduced intrinsic rate of increase ( $r_m$ ) and net population growth rate ( $R_0$ ), and extended time to double the population size ( $D_t$ ). Predator cues significantly delayed lifetime production of daughters. Mothers exposed to predator cues laid significantly smaller eggs and their offspring developed significantly more slowly but these eggs had significantly higher hatch rate.

**CONCLUSION:** Predator- and killed prey-induced fears significantly lower the fitness of *T. ludeni*, suggesting that these non-consumptive effects can contribute to the effectiveness of biological control to a great extent. Our study provides critical information for evaluation of biological control effectiveness using predators and paves the way for identification of chemical odors from the predator and killed prey and development of new materials and methods for the control of spider mite pests.

**Keywords:** predation risk; biological control; Phytoseiidae; Tetranychidae

## 1 INTRODUCTION

Predator-prey interactions affect life history traits and behavioral strategies of species involved,<sup>1-4</sup> resulting in demographic changes in these species.<sup>5</sup> Plant feeding mites can detect predation risk through chemical cues from predators and killed conspecifics and respond accordingly.<sup>6-18</sup> Buchanan *et al.*<sup>19</sup> suggested that the risk cues affect both behavioral and physiological traits in prey but have stronger impacts on behavioral traits. For example, spider mites tend to stay away from the areas with predator cues,<sup>20,21</sup> aggregate more tightly in such areas,<sup>22</sup> change locomotion activity,<sup>9</sup> or shift their oviposition site from leaf surface to their webs.<sup>23,24</sup>

Predation risk incurs trade-offs between risk avoidance and other life functions in prey,<sup>25,26</sup> altering their life history traits<sup>13,16,23,27-32</sup> and reducing their fitness and population size.<sup>19,33</sup> These non-consumptive impacts on prey by predation risk may be transgenerational,<sup>32,34,35</sup> and can affect more individuals compared to the consumptive impacts<sup>36</sup> and have influence at least as strong as direct predation on prey population dynamics.<sup>19,37-40</sup> For example, predation risk lowers spider mites' fecundity,<sup>34,41</sup> shortens their adult lifespan<sup>34</sup> and prolongs their offspring development.<sup>34,42</sup> Furthermore, shift of oviposition from leaf surface to webs in response to predation risk results in higher egg mortality due to wind and rain.<sup>43</sup>

Application of predatory mites for biological control is a well-established method against spider mite pests in the world, with *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) being the most used predator for the control of the serious invasive pest, *Tetranychus urticae* Koch (Acari: Tetranychidae).<sup>44-48</sup> These reports mainly evaluated the effectiveness of biological control based on the relationship between the release of *P. persimilis* and population size changes of *T. urticae*, assuming that the spider mite population decline is caused by direct predation. However, non-consumptive effects of predators on prey fitness and forage may also

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contribute to pest biological control but have been rarely evaluated.<sup>49,50</sup> Two recent reports shed some light on this matter using an insect<sup>51</sup> and a mammal<sup>52</sup> predator-prey systems, respectively. The former indicated that predator cues can significantly reduce pest fitness and suppress pest population and the latter shows that these cues can repel the pest. To date, little is known about whether the predator- and killed prey-induced fears could enhance the effectiveness of spider mite biological control and help develop novel pest control measures for this important group of plant pests.

*Tetranychus ludeni* Zacher (Acari: Tetranychidae) is also an important invasive spider mite pest which is native to Europe and now occurs in all continents except Antarctica.<sup>53,54</sup> It attacks over 300 hosts, including many economically important crops such as bean *Phaseolus vulgaris* L. (Fabales: Fabaceae), eggplant *Solanum melongena* L. (Solanales: Solanaceae), cotton rose *Hibiscus mutabilis* L. (Malvales: Malvaceae), China rose *H. rosa-sinensis* L., apple *Malus domestica* Borkh (Rosales: Rosaceae), pumpkin *Cucurbita pepo* L. (Cucurbitales: Cucurbitaceae), and many other cucurbitaceous plants, causing significant economic losses.<sup>53,55</sup> Gotoh *et al.*<sup>56</sup> suggested that *T. ludeni* could replace *T. urticae* to become a major crop pest in the world. Yet, biological control of *T. ludeni* using predatory mites is still poorly understood and knowledge about the effect of predator- and killed prey-induced stress on its fitness is lacking. The limited studies on *T. ludeni* biological control so far mainly focus on the predators from the genus *Neoseiulus* (Acari: Phytoseiidae).<sup>57-59</sup> However, Escudero and Ferragut<sup>60</sup> and Zhang<sup>61</sup> showed that *P. persimilis* performs similarly well on both *T. ludeni* and *T. urticae*, suggesting that this predatory mite can also control *T. ludeni* effectively.

In the present study, we aimed to evaluate how cues from *P. persimilis* and killed *T. ludeni* affected the life history traits and offspring performance of *T. ludeni* females. We exposed

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mated adult females of *T. ludeni* to these cues throughout their life and recorded their longevity, and offspring production and fitness. We then analyzed the changes in their life history traits and calculated life table parameters. This study generated critical information about non-consumptive effects by predation risk on prey population regulations, offering the first knowledge for evaluation of non-consumptive contribution to the biological control of *T. ludeni* and for development of novel spider mite control measures using predator cues.

## 2 MATERIALS AND METHODS

### 2.1 Environmental conditions

Mite colonies were maintained, and all experiments conducted at  $25 \pm 1^\circ\text{C}$  and 50–70% RH with a photoperiod of 16L:8D hours.

### 2.2 *Tetranychus ludeni* colony and experimental mites

*Tetranychus ludeni* were reared on approximately 20 potted kidney bean plants (*Phaseolus vulgaris* L.) in an environmental room of the Massey University Entomology and IPM Laboratory in Palmerston North, New Zealand. Every fortnight we replaced 10 of the oldest plants with new ones. We cut the leaves of the old plants and placed them on the top of the new plants, allowing mites to move to the latter.

To obtain 1-day-old mated adult females for experiment, we randomly took deutonymph females from the colony and introduced them onto a bean leaf disc (3 cm  $\times$  3 cm, 30 individuals per leaf disc) placed on a water saturated cotton pad in a Petri dish (5.5 cm diameter  $\times$  1.0 cm height) until they developed to adult stage. Immediately after adult emergence we transferred 20

of those females and 5 males randomly taken from the colony onto a new leaf disc in a Petri dish and allowed them to stay together for 24 hours before used for experiment.

### **2.3 *Phytoseiulus persimilis* colony and experimental mites**

We obtained the predatory mite *P. persimilis* from Bioforce Ltd, New Zealand, and reared them on four potted kidney bean plants heavily infested with *T. ludeni* in the laboratory. We replaced two oldest plants with new ones every three days and allowed mites to migrate as above. The predatory mite colony was maintained in a metal framed cage (120 cm length  $\times$  60 cm height  $\times$  60 cm width) with transparent mica plastic and fine woven wire mesh walls (0.25  $\times$  0.25 mm aperture) in a separate environmental room.

### **2.4 Effect of predatory and killed conspecific cues on life history traits of *Tetranychus ludeni***

To assess how maternal stress induced by predators and injured conspecifics affected the life history traits of *T. ludeni*, we exposed 1-day-old mated adult females to three different types of cues: (1) bean leaf disc with trace of predators, (2) bean leaf disc with killed conspecifics, and (3) clean bean leaf disc (control with neither predator nor killed conspecific cues). Each treatment had 20 replicates. Predator trace consists of metabolic waste products, eggs and footprints left by the predators on the leaf surface.<sup>30</sup> For each replicate in treatment (1), we randomly selected five adult female predators from the colony and transferred them onto a bean leaf disc (2 cm  $\times$  2 cm) placed on a water saturated cotton pad in a Petri dish (14 cm diameter  $\times$  1.5 cm height). Twenty-four hours later,<sup>30</sup> we removed the predator adults and redundant eggs, ensuring two predator eggs remained on the leaf disc. If the number of eggs on the disc was fewer than two, we transferred predator egg(s) onto the disc from a separate leaf disc (3 cm  $\times$  3

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cm). We maintained two eggs on each disc to keep predator cues consistent in all replicates. For treatment (2), we randomly collected four adult females of *T. ludeni* from the colony and transferred them to a leaf disc. We then killed them with an insect pin and left their bodies on the leaf disc.<sup>9</sup> We used an insect pin instead of a predator to obtain killed *T. ludeni* for two reasons: (1) we aimed to test predator cue and killed prey cue separately and examine relative impact of these two cues on the spider mite's life history traits (see Results), and (2) *T. ludeni* killed by predators would bear cues from both predators and injured prey, making it impossible to evaluate the relative effect of each cue.

In each replicate, we released a 1-day-old mated adult female of *T. ludeni* to the middle of a leaf disc, bearing either predator cues, killed conspecifics, or none of these, placed on a water saturated cotton pad in a Petri dish, and allowed it to stay on the leaf disc for 24 hours. We then transferred the mite to a new leaf disc with the same cues daily until death. We recorded oviposition period (from the first to last eggs laid), daily fecundity (the number of eggs produced per day), lifetime fecundity (total number of eggs produced), and adult lifespan (from emergence to death). We randomly selected two eggs laid by each female daily and measured their diameter under a stereomicroscope (Leica MZ12, Germany) connected to a digital camera (Olympus SC30, Japan), using an imaging software (CellSens® GS-ST-V1.7, Olympus, Japan). We calculated the egg size as:  $\text{volume} = 4/3\pi r^3$ , where  $r$  is radius (= diameter/2). We reared all eggs laid by *T. ludeni* each day on their original leaf disc [predator eggs in treatment (1) were removed]. We allowed eggs laid on each leaf disc to hatch and then transferred mites to a fresh and clean leaf disc without any predators' cues and replaced the leaf disc once every five days until they developed to adults. We checked all leaf discs daily and recorded the number of eggs hatched, number and sex of emerged adults, and developmental time from egg to adult stage. We

calculated the life table parameters<sup>62</sup> for each treatment using the above data (see Statistical analysis below).

## 2.5 Statistical analysis

All data were analyzed using SAS 9.4 with a rejection level set at  $\alpha < 0.05$ . Data on adult survival were compared using a Wilcoxon test (LIFETEST procedure). Data on the  $\ln(x)$ -transformed oviposition period, fecundity and adult emergence rate were normally distributed (Shapiro-Wilk test, UNIVARIATE procedure) and thus analyzed using ANOVA (GLM procedure) followed by a Tukey test for multiple comparisons. A generalized linear model (GLIMMIX procedure) was applied to compare the difference in egg size, egg hatch rate and offspring developmental period, with a *log* function and *Gamma* distribution for egg size and egg hatch rate and *Poisson* distribution for developmental time after the model. Multiple comparisons between treatments were performed using Tukey test.

We modified an exponential functional model<sup>63</sup> to fit the data on the cumulative proportion of daughters produced over female age (NLIN Procedure), i.e., cumulative proportion of daughters =  $a \times \exp(b \times \text{age})$ , where  $a$  is a constant, and  $b$  is the increase rate of cumulation. The difference in  $b$  was compared between socio-environmental cues according to Julious<sup>64</sup>: if the 95% CLs overlap, then there is no significant difference.

We calculated the intrinsic rate of increase ( $r_m$ , daughters/female/day) by solving the Lotka-Euler equation,  $\sum e^{-r_m x} l_x m_x = 1$ , where  $x$  is the female pivotal age,  $l_x$  is the proportion of females surviving to age  $x$ , and  $m_x$  is the number of daughters produced per female at age  $x$ . We also calculated other life table parameters, including the net reproductive rate ( $R_0 = \sum l_x m_x$ ,



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daughters/female/generation), mean generation time [ $T = \log_e(R_0)/r_m$ , days], and doubling time [ $Dt = \log_e(2)/r_m$ , days]. We used the bootstrap method<sup>65,66</sup> with 50,000 bootstrap samples to calculate the pseudo-values of a given parameter and employed the paired-bootstrap test<sup>67-69</sup> for multiple comparisons between any two cues (TTEST Procedure). The significance was determined according to the 95% t-based confidence limits (95% CLs), i.e., if 0 is not within the 95% CLs, the mean difference between the two cue treatments is significantly different.

### 3 RESULTS

#### 3.1 Effect of predation risks on adult survival, fecundity and daughter production

Females exposed to killed conspecific or predator cues died significantly earlier than the control ( $\chi^2_2 = 11.06$ ,  $P = 0.0040$ ) (Fig. 1). Exposure to injured conspecific or predator cues significantly shortened the oviposition period ( $F_{2,55} = 5.60$ ,  $P = 0.0061$ ) (Fig. 2a) and marginally reduced lifetime fecundity ( $F_{2,55} = 3.03$ ,  $P = 0.0566$ ) (Fig. 2b). Predator cues resulted in significantly slower increase of cumulative daughter production compared to control (Non-overlapping 95% CLs) (Fig. 3, Table S1). Killed conspecific cues also caused slow-down of cumulative daughter production to some extent but such effect was not significantly different from that of control (Overlapping 95% CLs) (Fig. 3, Table S1).

#### 3.2 Effect of predation risks on offspring fitness

Eggs laid by mothers exposed to predator cues were significantly smaller than those laid by mothers exposed to either killed conspecific or no cues ( $F_{2,451} = 3.68$ ,  $P = 0.0260$ ) (Fig. 4a). After mothers were exposed to predator cues, the hatch rate of their eggs was significantly higher than that of other treatments ( $F_{2,53} = 4.25$ ,  $P = 0.0194$ ) (Fig. 4b). Furthermore, the developmental time

of immatures produced by mothers exposed to predator cues was significantly longer than that of other treatments ( $F_{2,280} = 3.08$ ,  $P = 0.0475$ ) (Fig. 4c).

### 3.3 Effect of predation risks on life table parameters

As shown in Table 1, females exposed to killed conspecific and predator cues had significantly lower intrinsic rate of increase ( $r_m$ ) and net population growth rate ( $R_0$ ) and required significantly longer time to double the population size ( $D_t$ ). Females had significantly longer generation time ( $T$ ) after exposed to predator cues and significantly shorter generation time after exposed to conspecific cues (Table 1).

## 4 DISCUSSION

In the present study, we demonstrate that cues from predatory mite *P. persimilis* and killed spider mite *T. ludeni* shortened the longevity of *T. ludeni* females by 25 and 23%, respectively (Fig. 1). These findings suggest that in addition to direct predation, the non-consumptive mortality caused by cues from both predators and killed prey can substantially reduce the spider mites' feeding time and thus damage to crops. The earlier prey death induced by these cues could be attributed to various factors, such as the energetic costs of natural enemy avoidance,<sup>26</sup> decrease of foraging rate or food intake<sup>12,35,70</sup> and increase of oxidative damage.<sup>71</sup> Furthermore, predator cues may affect prey through physiological pathways by inducing stress hormones to divert its resource allocation to other physiological process that may translate into lower survivorship.<sup>72,73</sup>

Clinchy *et al.*<sup>74</sup> suggested that the presence of predators could induce sustained stress in prey and compromise their reproductive fitness. We show that fears induced by cues from predators and killed conspecifics lowered reproductive outputs and population growth in *T. ludeni*. For example, these cues shortened oviposition period by 35–40% (Fig. 2a) and reduced fecundity by 31–37% (Fig. 2b). As shown in Fig. 3, cues from predators but not killed *T. ludeni* slowed down lifetime daughter production. Furthermore, predation risk reduced intrinsic rate of increase ( $r_m$ ) and net population growth rate ( $R_0$ ), and extended time to double the population size ( $D_t$ ) (Table 1). These results suggest that predator- and killed conspecifics-induced stress can help suppress pest population growth by reducing their reproductive outputs and delaying production of daughters. Our results support notions that the non-consumptive impacts on prey may be as strong as direct consumption.<sup>37</sup> Similarly, in response to predator cues, both oviposition period<sup>34,75</sup> and fecundity<sup>75-78</sup> significantly decline in *T. urticae* and several other prey species.

The present study partially supports previous reports that non-consumptive impacts of predation risk on prey are transgenerational.<sup>32,34,35</sup> We found that *T. ludeni* mothers exposed to predator cues laid significantly smaller eggs (Fig. 4a) and their offspring had significantly longer developmental period (Fig. 4c) as compared to other treatments. Smaller eggs may result in smaller adults which may have lower reproductive fitness<sup>79,80</sup> but prolonged developmental time may help gain more body mass.<sup>81,82</sup> Moreover, eggs laid by mothers exposed to predator cues had significantly higher hatch rate than those in other treatments (Fig. 4b). These results suggest that *T. ludeni* juveniles can somewhat compensate egg mass loss caused by their mothers' experience in predation risk. The present study did not find any evidence that mothers' experience of killed prey cues could influence their offspring fitness (Fig. 4). Taken together, the

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impact of predation risk on offspring fitness appears to be weaker than on their mothers' and its contribution to pest population suppression could be less significant. However, in the presence of predation risk, *T. kanzawai* females shift their oviposition from leaf surface to webs, resulting in higher egg mortality due to wind and rain.<sup>43</sup> This could increase offspring mortality further in *T. ludeni*, which is worth testing in the future.

We suggest that the non-consumptive effects reported in this study may play a critical role in biological control using predators and should be considered for the evaluation of total *T. ludeni* population suppression by *P. persimilis*. After the release of predators in the field, cues from both predators and killed prey should co-exist. However, it is not yet known whether they have synergistic or additive impacts on prey mortality and reproductive fitness because these two cues were tested separately in the present study. Further investigations into their combined effects on prey are thus warranted. In addition, recent reports show that chemical cues from predators can be used for insect<sup>51</sup> and rodent<sup>52</sup> pest management. These studies have laid new foundations for future research on novel pest control materials and methods for spider mite and other plant pests. For example, future studies can involve extraction of odors released by *P. persimilis* and killed prey, identification of key compounds in these odors, and tests of the effects of individual and combined compounds on prey behavior and fitness and plant damage.

## 5 CONCLUSION

*Tetranychus ludeni* females exposed to cues from predatory mite *P. persimilis* and killed *T. ludeni* have significantly higher mortality, lower reproductive fitness, and slower population growth. In addition to predation, these non-consumptive effects on the spider mite can have

major contribution to the effectiveness of its biological control using predators. The transgenerational impact of the predation risk appears to be weaker and its contribution to pest population suppression could be less significant. Identification and tests of chemical odors from the predator and killed prey may have high potential for the development of novel materials and methods for the control of spider mite and other plant pests.

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#### **AUTHORS' CONTRIBUTION**

D.R., X.Z.H. and Q.W. conceived and designed the study; D.R. collected the data. All authors contributed to data analysis and manuscript preparation.

#### **CONFLICT OF INTEREST DECLARATION**

The authors declare they have no conflict of interest.

#### **SUPPLEMENTARY INFORMATION**

The supplementary material may be found at online version.

## AVAILABILITY OF DATA AND MATERIAL

The datasets from the current study are available from the corresponding author on request.

## REFERENCES

- 1 Abrams PA, The evolution of predator-prey interactions: theory and evidence. *Annu Rev Ecol Syst* **31**:79–105 (2000). <https://doi.org/10.1146/annurev.ecolsys.31.1.79>.
- 2 Ferrari MCO, Manek AK and Chivers DP, Temporal learning of predation risk by embryonic amphibians. *Biol Lett* **6**:308–310 (2010). <https://doi.org/10.1098/rsbl.2009.0798>.
- 3 Moore TY and Biewener AA, Outrun or outmaneuver: predator-prey interactions as a model system for integrating biomechanical studies in a broader ecological and evolutionary context. *Integr Comp Biol* **55**:1188–1197 (2015). <https://doi.org/10.1093/icb/icv074>.
- 4 Otsuki H and Yano S, The stealthiness of predatory mites as spider mite biological control agents. *Biol Control* **136**:104010 (2019). <https://doi.org/10.1016/j.biocontrol.2019.104010>.
- 5 Delić T and Fišer C. Species interactions, in Encyclopedia of Caves, ed. by White WB, Culver DC and Pipan T, Academic Press, pp. 967–973 (2019).
- 6 Grostal P and Dicke M, Recognising one's enemies: a functional approach to risk assessment by prey. *Behav Ecol Sociobiol* **47**:258–264 (2000). <https://doi.org/10.1007/s002650050663>.
- 7 Azandémé Hounmalon G, Torto B, Fiaboe KKM, Subramanian S, Kreiter S and Martin T, Visual, vibratory, and olfactory cues affect interactions between the red spider mite *Tetranychus evansi* and its predator *Phytoseiulus longipes*. *J Pest Sci* **89**:137–152 (2016). <https://doi.org/10.1007/s10340-015-0682-y>.

- 8 Jacobsen SK, Alexakis I and Sigsgaard L, Antipredator responses in *Tetranychus urticae* differ with predator specialization. *J Appl Entomol* **140**:228–231 (2016).  
<https://doi.org/10.1111/jen.12275>.
- 9 Gyuris E, Szép E, Kontschán J, Hettyey A and Tóth Z, Behavioural responses of two-spotted spider mites induced by predator-borne and prey-borne cues. *Behav Processes* **144**:100–106 (2017). <https://doi.org/10.1016/j.beproc.2017.09.002>.
- 10 Calvet É, Lima D, Melo JW and Gondim M, Chemosensory cues of predators and competitors influence search for refuge in fruit by the coconut mite *Aceria guerreronis*. *Exp Appl Acarol* **74**:249–259 (2018). <https://doi.org/10.1007/s10493-018-0233-3>.
- 11 Schoeppner NM and Relyea RA, Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. *Funct Ecol* **23**:1114–1121 (2009).  
<https://doi.org/10.1111/j.1365-2435.2009.01578.x>.
- 12 Hermann SL and Thaler JS, Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. *Oecologia* **176**:669–676 (2014). <https://doi.org/10.1007/s00442-014-3069-5>.
- 13 Eklöv P, Chemical cues from multiple predator-prey interactions induce changes in behavior and growth of anuran larvae. *Oecologia* **123**:192–199 (2000).  
<https://doi.org/10.1007/s004420051005>.
- 14 Oku K, Yano S, Osakabe M and Takafuji A, Spider mites assess predation risk by using the odor of injured conspecific. *J Chem Ecol* **29**:2609–2613 (2003).  
<https://doi.org/10.1023/a:1026395311664>.

- Accepted Article
- 15 Fievet V, Lhomme P and Outreman Y, Predation risk cues associated with killed conspecifics affect the behavior and reproduction of prey animals. *Oikos* **117**:1380–1385 (2008). <https://doi.org/10.1111/j.0030-1299.2008.16629.x>.
  - 16 Lucon-Xiccato T, Chivers DP, Mitchell MD and Ferrari MCO, Making the dead talk: alarm cue-mediated antipredator behaviour and learning are enhanced when injured conspecifics experience high predation risk. *Biol Lett* **12**:20160560 (2016). <https://doi.org/10.1098/rsbl.2016.0560>.
  - 17 Gancedo BJ and Ituarte RB, Responses to chemical cues indicative of predation risk by the freshwater shrimp *Palaemon argentinus* (Nobili, 1901) (Caridea: Palaemonidae). *J Crust Biol* **38**:8–12 (2018). <https://doi.org/10.1093/jcbiol/rux106>.
  - 18 Ameri M, Kemp DJ, Barry KL and Herberstein ME, Predator chemical cues decrease attack time and increase metabolic rate in an orb-web spider. *J Exp Biol* **222**:jeb212738 (2019). <https://doi.org/10.1242/jeb.212738>.
  - 19 Buchanan AL, Hermann SL, Lund M and Szendrei Z, A meta-analysis of non-consumptive predator effects in arthropods: the influence of organismal and environmental characteristics. *Oikos* **126**:1233–1240 (2017). <https://doi.org/10.1111/oik.04384>.
  - 20 Grostal P and Dicke M, Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behav Ecol* **10**:422–427 (1999). <https://doi.org/10.1093/beheco/10.4.422>.
  - 21 Bowler D, Yano S, Amano H and Knell R, The non-consumptive effects of a predator on spider mites depend on predator density. *J Zool* **289**:52–59 (2013). <https://doi.org/10.1111/j.1469-7998.2012.00961.x>.



- 22 Dittmann L and Schausberger P, Adaptive aggregation by spider mites under predation risk. *Sci Rep* **7**:1–9 (2017). <https://doi.org/10.1038/s41598-017-10819-8>.
- 23 Lemos F, Sarmiento RA, Pallini A, Dias CR, Sabelis MW and Janssen A, Spider mite web mediates anti-predator behaviour. *Exp Appl Acarol* **52**:1–10 (2010). <https://doi.org/10.1007/s10493-010-9344-1>.
- 24 Otsuki H and Yano S, Within-patch oviposition site shifts by spider mites in response to prior predation risks decrease predator patch exploitation. *Ethology* **123**:453–459 (2017). <https://doi.org/10.1111/eth.12615>.
- 25 Ferrari MCO, Wisenden BD and Chivers DP, Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can J Zool* **88**:698–724 (2010). <https://doi.org/10.1139/Z10-029>.
- 26 Luong LT, Horn CJ and Brophy T, Mitey costly: energetic costs of parasite avoidance and infection. *Physiol Biochem Zool* **90**:471–477 (2017). <https://doi.org/10.1086/691704>.
- 27 Kats LB and Dill LM, The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* **5**:361–394 (1998). <https://doi.org/10.1080/11956860.1998.11682468>.
- 28 Persons MH, Walker SE and Rypstra AL, Fitness costs and benefits of antipredator behavior mediated by chemotactile cues in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). *Behav Ecol* **13**:386–392 (2002). <https://doi.org/10.1093/beheco/13.3.386>.
- 29 Nelson EH, Predator avoidance behavior in the pea aphid: costs, frequency, and population consequences. *Oecologia* **151**:22–32 (2007). <https://doi.org/10.1007/s00442-006-0573-2>.

- 30 Walzer A and Schausberger P, Threat-sensitive anti-intraguild predation behaviour: maternal strategies to reduce offspring predation risk in mites. *Anim Behav* **81**:177–184 (2011).  
<https://doi.org/10.1016/j.anbehav.2010.09.031>.
- 31 Orrock JL, Preisser EL, Grabowski JH and Trussell GC, The cost of safety: refuges increase the impact of predation risk in aquatic systems. *Ecology* **94**:573–579 (2013).  
<https://doi.org/10.1890/12-0502.1>.
- 32 Tariel J, Plénet S and Luquet É, Transgenerational plasticity in the context of predator-prey interactions. *Front Ecol Evol* **8**:548660 (2020).  
<https://doi.org/10.3389/fevo.2020.548660>.
- 33 Sheriff MJ, Peacor SD, Hawlena D and Thaker M, Non-consumptive predator effects on prey population size: A dearth of evidence. *J Anim Ecol* **89**:1302–1316 (2020).  
<https://doi.org/10.1111/1365-2656.13213>.
- 34 Li G-Y and Zhang Z-Q, Development, lifespan and reproduction of spider mites exposed to predator-induced stress across generations. *Biogerontology* **20**:871–882 (2019).  
<https://doi.org/10.1007/s10522-019-09835-0>.
- 35 Des Roches S, Robinson RR, Kinnison MT and Palkovacs EP, The legacy of predator threat shapes prey foraging behaviour. *Oecologia* **198**:79–89 (2022). <https://doi.org/10.1007/s00442-021-05073-9>.
- 36 Preisser EL, The physiology of predator stress in free-ranging prey. *J Anim Ecol* **78**:1103–1105 (2009). <https://doi.org/10.1111/j.1365-2656.2009.01602.x>.
- 37 Preisser EL, Bolnick DI and Benard MF, Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* **86**:501–509 (2005).  
<https://doi.org/10.1890/04-0719>.

- 38 Zanette LY, White AF, Allen MC and Clinchy M, Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* **334**:1398–1401 (2011). <https://doi.org/10.1126/science.1210908>.
- 39 Macleod CD, Macleod R, Learmonth JA, Cresswell W and Pierce GJ, Predicting population-level risk effects of predation from the responses of individuals. *Ecology* **95**:2006–2015 (2014). <https://doi.org/10.1890/13-1795.1>.
- 40 Pessarrodona A, Boada J, Pagès J, Arthur R and Alcoverro T, Consumptive and non-consumptive effects of predators vary with the ontogeny of their prey. *Ecology* **100**:1–13 (2019). <https://doi.org/10.1002/ecy.2649>.
- 41 Dias CR, Bernardo AMG, Mencalha J, Freitas CWC, Sarmiento RA, Pallini A and Janssen A, Antipredator behaviours of a spider mite in response to cues of dangerous and harmless predators. *Exp Appl Acarol* **69**:263–276 (2016). <https://doi.org/10.1007/s10493-016-0042-5>.
- 42 Freinschlag J and Schausberger P, Predation risk-mediated maternal effects in the two-spotted spider mite, *Tetranychus urticae*. *Exp Appl Acarol* **69**:35–47 (2016). <https://doi.org/10.1007/s10493-016-0014-9>.
- 43 Okada S and Yano S, Oviposition-site shift in phytophagous mites reflects a trade-off between predator avoidance and rainstorm resistance. *Biol Lett* **17**:20200669 (2021). <https://doi.org/10.1098/rsbl.2020.0669>.
- 44 Opit GP, Nechols JR and Margolies DC, Biological control of twospotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), using *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseidae) on ivy geranium: assessment of predator release ratios. *Biol Control* **29**:445–452 (2004). <https://doi.org/10.1016/j.biocontrol.2003.08.007>.

- 45 Yanar D, Geboloğlu N, Cakar T and Engür M, The use of predatory mite *Phytoseiulus persimilis* (Acari: phytoseiidae) in the control of two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) at greenhouse cucumber production in Tokat Province, Turkey. *Appl Ecol Environ Res* **17**:2033–2041 (2019).  
[https://doi.org/10.15666/aeer/1702\\_20332041](https://doi.org/10.15666/aeer/1702_20332041).
- 46 Chacón-Hernández JC, Cerna-Chávez E, Vanoye-Eligio V, Ocho-Fuentes YM, Ordaz-Silva S, Soria-Díaz L and Landeros-Flores J, *Phytoseiulus persimilis* (Acari: Phytoseiidae) functional response in the biological control of *Tetranychus urticae* (Acari: Tetranychidae) eggs on rose foliage discs. *J Entomol Sci* **54**:275–287 (2019).  
<https://doi.org/10.18474/JES18-95>.
- 47 Migeon A, Tixier M-S, Navajas M, Litskas VD and Stavrínides MC, A predator-prey system: *Phytoseiulus persimilis* (Acari: Phytoseiidae) and *Tetranychus urticae* (Acari: Tetranychidae): worldwide occurrence datasets. *Acarologia* **59**:301–307 (2019).  
<https://doi.org/10.24349/acarologia/20194322>.
- 48 Tiftikçi P, Kök Ş and Kasap İ, Biological control of twospotted spider mites [*Tetranychus urticae* Koch (Acari: Tetranychidae)] using *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) at different ratios of release on field-grown tomatos. *Biol Control* **151**:104404 (2020). <https://doi.org/10.1016/j.biocontrol.2020.104404>.
- 49 Hermann SL and Landis DA, Scaling up our understanding of non-consumptive effects in insect systems. *Curr Opin Insect Sci* **20**:54–60 (2017).  
<https://doi.org/10.1016/j.cois.2017.03.010>

- 50 Kahl HM, Leslie AW and Hooks CRR, Consumptive and non-consumptive effects of wolf spiders on cucumber beetles and cucumber plant damage. *Ann Appl Biol* **178**:109–120 (2021). <https://doi.org/10.1111/aab.12643>.
- 51 Wen J and Ueno T, Application of predator-associated cues to control small brown planthoppers: non-consumptive effects of predators suppress the pest population. *BioControl* **66**:813–824 (2021). <https://doi.org/10.1007/s10526-021-10115-5>.
- 52 Villalobos A, Schlyter F, Birgersson G, Koteja P and Löf M, Fear effects on bank voles (Rodentia: Arvicolinae): testing for repellent candidates from predator volatiles. *Pest Manag Sci* (online 07 January 2022). <https://doi.org/10.1002/ps.6787>.
- 53 Bolland HR, Gutierrez J and Flechtmann C, World Catalogue of the Spider Mite Family (Acari: Tetranychidae), Brill, Leiden (1998).
- 54 CABI, *Tetranychus ludeni* [Distribution map]. CABI, Wallingford, pp. Map 758 (2011). <https://www.cabi.org/ISC/abstract/20113409546>.
- 55 Zhang Z-Q, Mites of Greenhouses: Identification, Biology and Control. CABI Pub. (2003). <https://doi.org/10.1079/9780851995908.0000>.
- 56 Gotoh T, Moriya D and Nachman G, Development and reproduction of five *Tetranychus* species (Acari: Tetranychidae): Do they all have the potential to become major pests? *Exp Appl Acarol* **66**:453–479 (2015). <https://doi.org/10.1007/s10493-015-9919-y>.
- 57 Reichert MB, Toldi M and Ferla NJ, Feeding preference and predation rate of *Neoseiulus idaeus* (Acari: Phytoseiidae) feeding on different preys. *Syst Appl Acarol* **21**:1631–1640 (2016). <https://doi.org/10.11158/saa.21.12.4>.
- 58 Reichert M, Toldi M, Rode P, Ferla J and Ferla N, Biological performance of the predatory mite *Neoseiulus idaeus* (Phytoseiidae): a candidate for the control of tetranychid mites in

- brazilian soybean crops. *Braz J Biol* **77**:361–366 (2016). <https://doi.org/10.1590/1519-6984.14915>.
- 59 Ballal C, Gupta S, Gupta T and Varshney R, A simple protocol for rearing a native predatory mite *Neoseiulus indicus*. *Curr Sci* **120**:1923–1926 (2021).
- 60 Escudero LA and Ferragut F, Life-history of predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Acari: Phytoseiidae) on four spider mite species as prey, with special reference to *Tetranychus evansi* (Acari: Tetranychidae). *Biol Control* **32**:378–384 (2005). <https://doi.org/10.1016/j.biocontrol.2004.12.010>.
- 61 Zhang Z-Q, Taxonomy of *Tetranychus ludeni* (Acari: Tetranychidae) in New Zealand and its ecology on *Secchium edule*. *N Z Entomol* **25**:27–34 (2002). <https://doi.org/10.1080/00779962.2002.9722091>.
- 62 Jervis MA, Copland MJW and Harvey JA, The life-cycle, in *Insects as Natural Enemies: A Practical Perspective*, ed. by Jervis MA, Springer Netherlands: Dordrecht, pp. 73–165 (2005). [https://doi.org/10.1007/978-1-4020-2625-6\\_2](https://doi.org/10.1007/978-1-4020-2625-6_2).
- 63 Archontoulis SV and Miguez FE, Nonlinear regression models and applications in agricultural research. *Agron J* **107**:786–798 (2015). <https://doi.org/10.2134/agronj2012.0506>.
- 64 Julious SA, Using confidence intervals around individual means to assess statistical significance between two means. *Pharm Stat* **3**:217–222 (2004). <https://doi.org/10.1002/pst.126>.
- 65 Huang Y-B and Chi H, Assessing the application of the jackknife and bootstrap techniques to the estimation of the variability of the net reproductive rate and gross reproductive rate: A

case study in *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). *J Agri Fore* **61**:37–46 (2012).

66 Yu L-Y, Chen Z-Z, Zheng F-Q, Shi A-J, Guo T-T, Yeh B-H, Chi H and Xu Y-Y, Demographic analysis, a comparison of the jackknife and bootstrap methods, and predation projection: a case study of *Chrysopa pallens* (Neuroptera: Chrysopidae). *J Econ Entomol* **106**:1–9 (2013). <https://doi.org/10.1603/EC12200>.

67 Efron B and Tibshirani RJ, An Introduction to the Bootstrap. Taylor & Francis (1994). <https://doi.org/10.1201/9780429246593>.

68 Mou D-F, Lee C-C, Smith CL and Chi H, Using viable eggs to accurately determine the demographic and predation potential of *Harmonia dimidiata* (Coleoptera: Coccinellidae). *J Appl Entomol* **139**:579–591 (2015). <https://doi.org/10.1111/jen.12202>.

69 Reddy GVP and Chi H, Demographic comparison of sweetpotato weevil reared on a major host, *Ipomoea batatas*, and an alternative host, *I. triloba*. *Sci Rep* **5**:11871 (2015). <https://doi.org/10.1038/srep11871>

70 Wineland SM, Kistner EJ and Joern A, Non-consumptive interactions between grasshoppers (Orthoptera: Acrididae) and wolf spiders (Lycosidae) produce trophic cascades in an old-field ecosystem. *J Orthoptera Res* **24**:41–46 (2015). <https://doi.org/10.1665/034.024.0101>.

71 Janssens L and Stoks R, Predation risk causes oxidative damage in prey. *Biol Lett* **9**:1–4 (2013). <https://doi.org/10.1098/rsbl.2013.0350>.

72 Hawlena D and Schmitz OJ, Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *Am Nat* **176**:537–556 (2010). <https://doi.org/10.1086/656495>.

- 73 Sitvarin M, Breen K and Rypstra A, Predator cues have contrasting effects on lifespan of *Pardosa milvina* (Araneae: Lycosidae). *J Arachnol* **43**:107–110 (2015).  
<https://doi.org/10.1636/J14-48.1>.
- 74 Clinchy M, Sheriff MJ and Zanette LY, Predator-induced stress and the ecology of fear. *Funct Ecol* **27**:56–65 (2013). <https://doi.org/10.1111/1365-2435.12007>.
- 75 Moghadasi M, Golpayegani A, Saboori A, Allahyari H and Dehghani H, *Tetranychus urticae* changes its oviposition pattern in the presence of the predatory mites, *Phytoseiulus persimilis* and *Typhlodromus bagdasarjani*. *Acta Biologica* **26**:65–81 (2019).  
<https://doi.org/10.18276/ab.2019.26-07>.
- 76 Škaloudová B, Zemek R and Krivan V, The effect of predation risk on an acarine system. *Anim Behav* **74**:813–821 (2007). <http://doi.org/10.1016/j.anbehav.2007.02.005>.
- 77 Fernández Ferrari MC and Schausberger P, From repulsion to attraction: species- and spatial context-dependent threat sensitive response of the spider mite *Tetranychus urticae* to predatory mite cues. *Naturwissenschaften* **100**:541–549 (2013).  
<https://doi.org/10.1007/s00114-013-1050-5>.
- 78 Venkanna Y, Suroshe SS and Dahuja A, Non-consumptive effects of the zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fab.) on its prey, the cotton aphid, *Aphis gossypii* Glover. *Biocontrol Sci Technol* **31**:1204–1219 (2021).  
<https://doi.org/10.1080/09583157.2021.1937521>.
- 79 Fox C, The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus*. *Oikos* **71**:321–325 (1994). <https://doi.org/10.2307/3546280>.
- 80 Honěk A, Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**:483–492 (1993). <https://doi.org/10.2307/3544943>.



- 81 Fox CW, The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia* **96**:139–146 (1993). <https://doi.org/10.1007/bf00318042>.
- 82 Fox CW and Dingle H, Dietary mediation of maternal age effects on offspring performance in a seed beetle (Coleoptera: Bruchidae). *Funct Ecol* **8**:600-606 (1994). <https://doi.org/10.2307/2389921>.

**Table 1.** Mean ( $\pm$  SE) intrinsic rate of increase ( $r_m$ ), net reproductive rate ( $R_0$ ), generation time ( $T$ ) and doubling time ( $D_t$ ) of *Tetranychus ludeni* in response to no cues, killed conspecific cues and predator cues.

Cue	$r_m$	$R_0$	$T$	$D_t$
No cues (No)	$0.1002 \pm 0.0004$ a	$5.04 \pm 0.04$ a	$16.15 \pm 0.07$ b	$6.92 \pm 0.03$ b
Killed conspecific (Kil.)	$0.0828 \pm 0.0014$ b	$3.35 \pm 0.06$ c	$14.58 \pm 0.14$ c	$8.42 \pm 0.15$ a
Predator (Pred.)	$0.0852 \pm 0.0010$ b	$4.20 \pm 0.06$ b	$16.84 \pm 0.14$ a	$8.16 \pm 0.10$ a
95% CLs (No vs Kil.)	$0.0144 \sim 0.0204$	$1.56 \sim 1.83$	$1.25 \sim 1.89$	$-1.81 \sim -1.19$
95% CLs (No vs Pred.)	$0.0128 \sim 0.0172$	$0.13 \sim 0.25$	$-0.10 \sim -0.39$	$-1.44 \sim -1.04$
95% CLs (Kil. vs Pred.)	$-0.0059 \sim 0.0011$	$0.19 \sim 0.37$	$-2.65 \sim -1.87$	$-0.09 \sim 0.62$

Estimated values in columns followed by different letters are significantly different. For each parameter, 95% CLs  $> 0$  or  $< 0$  indicates a significant difference between treatments.

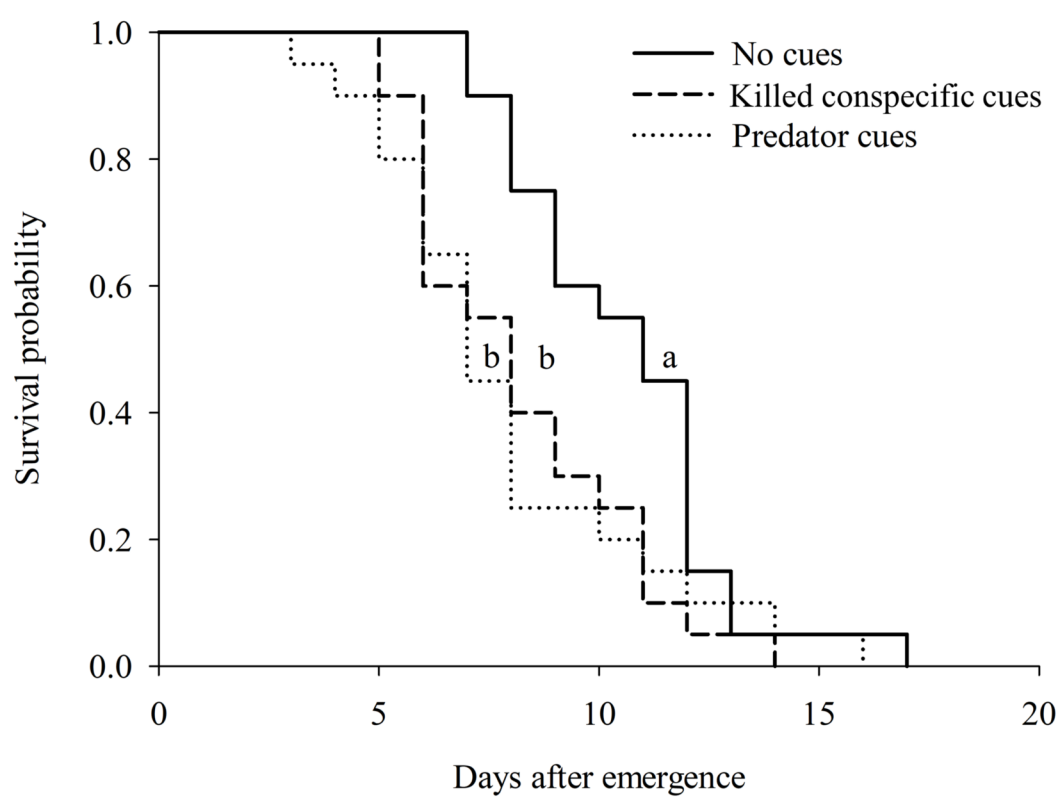
## Figure Legends

**Figure 1.** Survival probability of *Tetranychus ludeni* in response to no cues, killed conspecific cues and predator cues. Lines with the same letters are not significantly different ( $P > 0.05$ ).

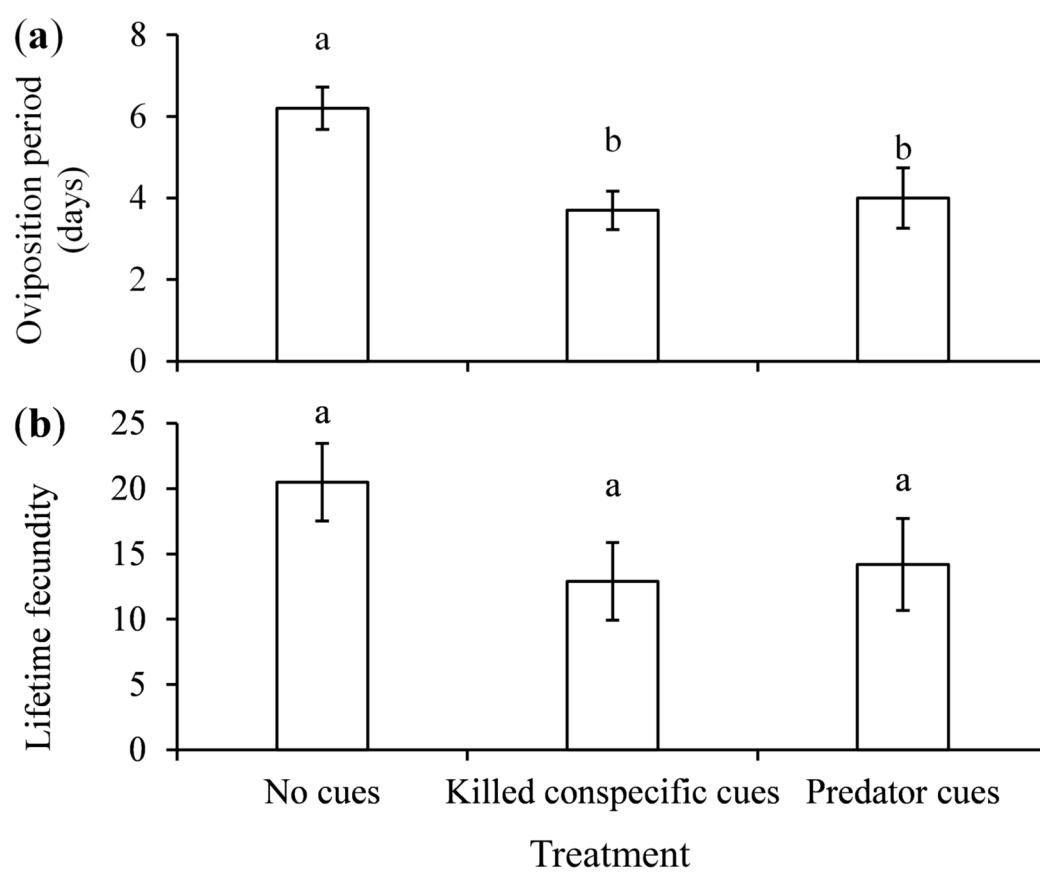
**Figure 2.** Mean ( $\pm$  SE) oviposition period (**a**) and lifetime fecundity (**b**) of *Tetranychus ludeni* females in response to no cues, killed conspecific cues and predator cues. Columns with the same letters are not significantly different ( $P > 0.05$ ).

**Figure 3.** Cumulative proportion of daughters produced by *Tetranychus ludeni* females in response to no cues, killed conspecific cues and predator cues. Lines with the same letters are not significantly different (Overlapping 95% CLs).

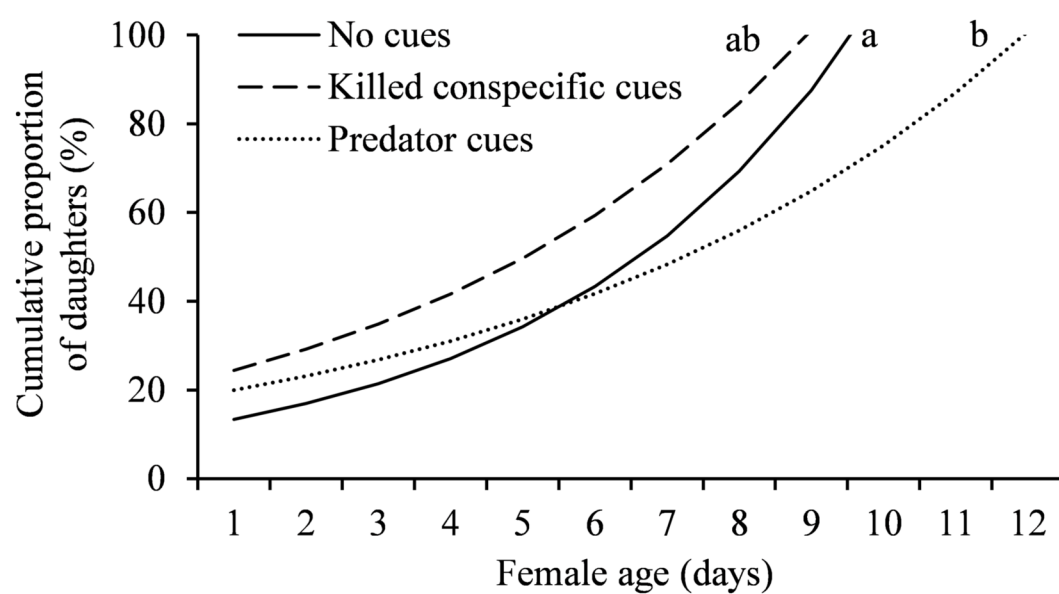
**Figure 4.** Effect of mothers' exposure to no cues, killed conspecific cues and predator cues on mean ( $\pm$  SE) egg size (**a**), egg hatch rate (**b**), and offspring developmental period (**c**) in *Tetranychus ludeni*. Columns with the same letters are not significantly different ( $P > 0.05$ ).



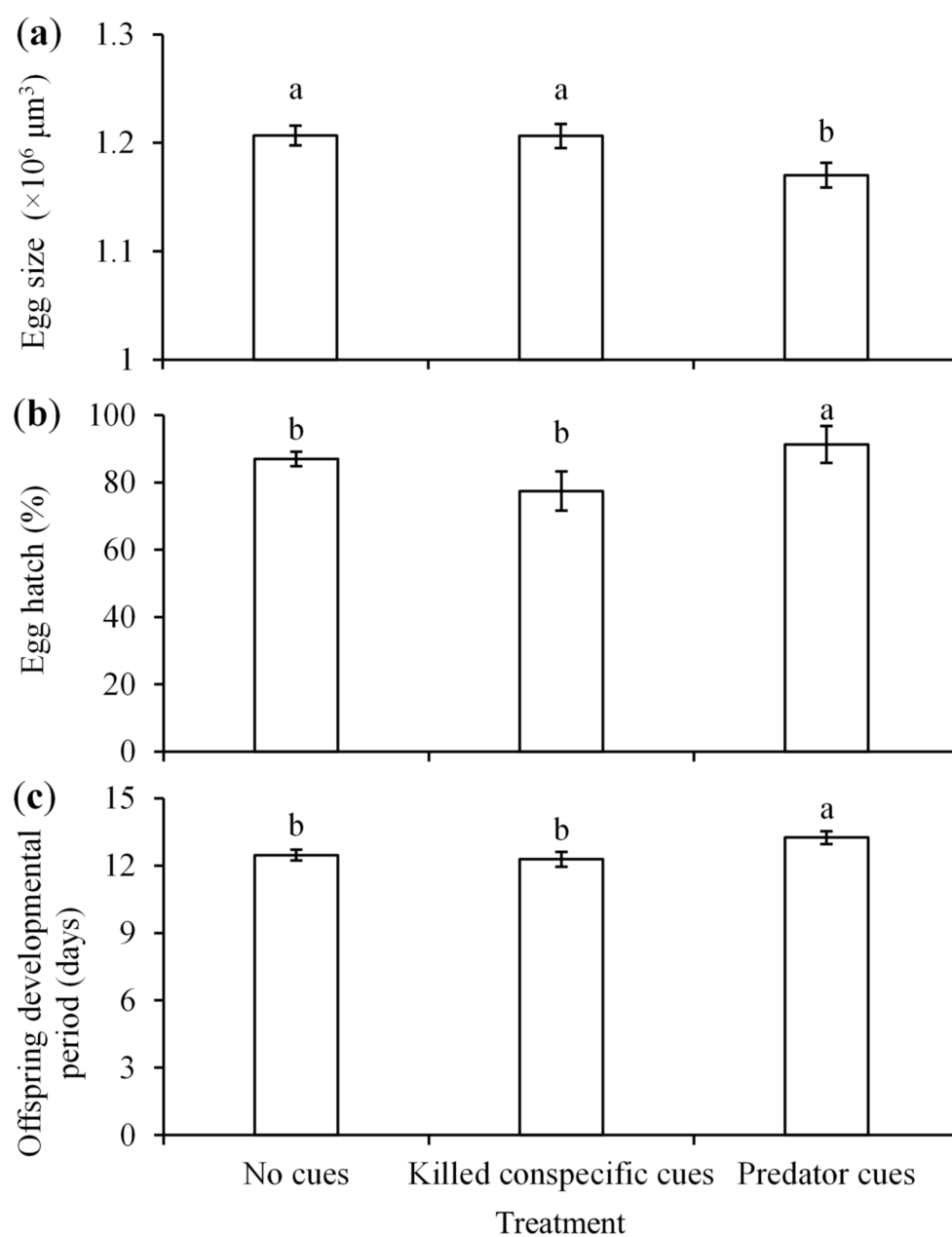
PS\_7168\_Fig. 1 (revised).tif



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PS\_7168\_Fig. 3 (revised).tif



PS\_7168\_Fig. 4 (revised).tif

**Predator- and killed prey-induced fears bear significant cost to an invasive spider mite: implications in pest management**

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**Graphical Abstract Text**

Findings on impact of predator- and killed prey-induced fears on an important invasive pest, *Tetranychus ludeni*, help develop new materials and methods for controlling spider mite pests. Cues from predators and killed prey shorten female longevity by a quarter and reproductive fitness by a third, significantly reducing its population growth.



