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Diapause incidence in the two-spotted spider mite increases due to predator presence, not due to selective predation

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Abstract. We recently reported evidence for increased diapause incidence in the spider mite *Tetranychus urticae* in presence of the predatory mite *Typhlodromus pyri*. This effect may arise from (1) selective predation on non-diapause spider mites, (2) predator-induced diapause in spider mites, or (3) both. Using a different strain of *T. urticae*, we first recovered increased diapause incidence in association with predators. Then, we tested for selective feeding in two-choice experiments with equal numbers of non-diapause and diapause spider mites. We found that the predatory mite had a significant preference for the latter. This indicates that increased diapause incidence in association with predatory mites is not due to selective predation. Therefore, predator-mediated physiological induction of diapause seems a more likely explanation. The cues leading to induction appear to relate to the predators, not their effects, since predation simulated by spider-mite removal or puncturing did not significantly affect diapause incidence. Why spider mites benefit from this response, remains an open question.

Introduction

In a previous study we demonstrated that the physical presence of the predatory mite *Typhlodromus pyri* Scheuten significantly enhanced diapause induction in a Greek strain of the spider mite *Tetranychus urticae* Koch (Kroon et al. 2004). The proportion of diapause spider mites at long-night photoperiods and 18 °C increased by more than 50% in the presence of predatory mites. This higher incidence of diapause could be explained in two ways: either the predators physiologically induce diapause among the surviving female spider mites, or the predators selectively feed on spider mites that do not enter diapause.

Using a Dutch strain of spider mites, we test the effect of predators on diapause incidence again, and to what extent this effect results from selective predation. In addition, we investigate whether the effect arises from the actual presence of the predatory mite or its impact on the prey, by simulating predatory activity through prey removal or prey puncturing.

Materials and methods

Experimental animals

Using a Dutch strain of *T. urticae*, we tested whether induction of diapause could be enhanced by predators, like in a Greek strain tested in an earlier series of experiments (Kroon et al. 2004). In this Dutch strain diapause incidence as a function of long-night photoperiods in the laboratory (at 18 °C) rises very steeply from 0 to 100% around LD 14.5:9.5 (D. Kroon and A. Kroon, unpublished results). To detect any effect of predators, spider mites were exposed to predatory mites at an intermediate nightlength, where only part of the spider mite population enters diapause.

The Dutch strain of *T. urticae* was collected in 1999 in the dunes near Santpoort-Noord, The Netherlands, from spindle tree (*Euonymus europaeus* L.), overgrown with hop (*Humulus lupulus* L.). The spider mites were reared in the laboratory on detached bean leaves (*Phaseolus vulgaris* L.) under long-day illumination (17 h of light and 7 h of darkness, or LD 17:7).

The predatory mite *T. pyri* was collected in 1978 in an apple orchard near Goes, the Netherlands. Ever since, the predators were reared in the laboratory on plastic arenas as described by Overmeer (1981), and fed pollen of broad bean, *Vicia faba* L. Prior to our experiments, predatory mites were reared for several weeks on spider mites.

Diapause induction

Four treatments were compared for their effect on diapause induction. In the ‘predation’ treatment, spider mites developed from larvae to adults in presence of predatory mites; in the ‘control’ treatment they developed without disturbance; in the ‘removal’ and ‘puncture’ treatments different aspects of the predators’ feeding activity were simulated throughout the development of juvenile spider mites. In the removal treatment, a proportion of the juvenile spider mites was taken away to mimic a decrease in spider mite density. In the puncture treatment, juvenile spider mites were punctured and left in place to mimic predator attacks.

For all treatments, adult spider mites were allowed to lay eggs for 24 h at LD 16:8 and 25 °C on detached bean leaves. Five leaves with 200–250 spider mite eggs per leaf (laid by 40 females/leaf) were used in each of the treatments, and five leaves with 150–200 spider mite eggs (from 30 females/leaf) in the control treatment. The control treatment started with a lower number of eggs, because in this treatment no eggs were removed later on. On day 2 of the experiment, leaves with spider mite eggs were transferred to three temperature and photoperiod-controlled incubators, where the spider mites completed their development at LD 14.25:9.75 and 18 °C. This photoperiod and temperature induce a low percentage diapause in this spider mite strain (roughly 20%; D. Kroon

and A. Kroon, unpublished results). All four treatments were carried out simultaneously within each of the three incubators.

When most of the spider mite eggs had hatched – after about 10 days of incubation – three adult predator females were introduced on each of the leaves of the predation treatment. Three times a week, all leaves were checked to verify that predators were alive and that predator eggs were present. Dead predatory mites were replaced and – in the rare case where no eggs had been laid since the previous inspection – all three predators were replaced. Ten days after the introduction of the predators, most of the spider mites had reached adulthood, and all predator stages were removed.

Predatory mites feed on eggs and immature stages, thus reducing spider mite density and potentially disturbing the survivors. In a pilot experiment at 18 °C the predation rate of *T. pyri* on the various spider mites stages was assessed. This information was used in the removal and puncture treatment, which lasted 10 days, similar to the experiments with the predation treatment. In the removal treatment, spider mites were taken away daily using a fine brush to cause minimum disturbance (over the 10 days 75 spider mites per leaf were removed, initially 12 larvae per day and ultimately three deutonymphs per day). In the puncture treatment, spider mites were pierced daily using a dissecting needle and the corpses remained on the leaf surface, as a potential source of spider mite disturbance (over the 10 days 75 spider mites per leaf were punctured, initially 12 larvae per day, ultimately three deutonymphs per day).

After another week of incubation diapause proportions were determined. Diapause females are orange, in contrast to the greenish summer form, and they do not produce eggs (Veerman 1985). The colour of females was used to determine the diapause state.

Feeding preference

Typhlodromus pyri females were offered a mixture of diapause and summer form *T. urticae* females on bean leaf discs. To obtain spider mites in diapause of roughly the same age, females were allowed to lay eggs on detached bean leaves for 24 h at LD 16:8 and 25 °C. The next day, leaves with eggs were transferred to an incubator where the mites completed development at LD 10:14 and 18 °C (a diapause-inducing photoperiod for mites of this strain). After diapause expression, mites were stored at 4 °C and continuous darkness (DD), until use in the experiment. To obtain summer-form spider mites of similar age, females in their last quiescent stage (teleiochrysalids) were placed on bean leaves. Males were added to inseminate newly emerged females. Three days after the collection of the teleiochrysalids, the experiment was started.

A single predatory female was allowed to forage on a bean leaf disc (Ø 35 mm) with 10 adult summer-form and 10 diapause spider mite females ($n = 10$). The relative death rates of the diapause and summer-form preys were

taken as a measure for the predator's food preference. As a control, death rates of summer-form and diapause spider mites were assessed on 10 bean leaf discs with the same numbers of spider mites present, but with no predators.

The leaf discs were placed on moist cotton wool and incubated at LD 17:7 and 18 °C. Three times a week, the dead summer-form and diapause spider mites were counted and replaced, and prey and predator eggs were removed, as well as (much of) the spider mite web (*T. pyri* are hindered by the dense *T. urticae* webbing; McMurtry et al. 1970; Sabelis and Bakker 1992). Ten days after the start of the experiment, predators were transferred to fresh leaf discs. After 18 days the experiment was ended. At the end of the experiment 8 of the 10 predatory mites were still alive, one had died on day 9, one on day 16. The impact of actual predation was determined as follows: for each leaf disc the daily death rate of diapause and summer-form spider mites with a live predator present were lowered with the average daily death rate of the respective control group.

Statistical analysis

Diapause proportions were analyzed with a factorial ANOVA for a randomized block design, treating data from each incubator as a block. If the null hypothesis (H0: diapause induction does not differ among predation treatments) was rejected, the four treatments were compared in all pairwise combinations using Bonferroni's *post-hoc* test. The analysis was carried out using the software programme Statistica 6. To meet the assumption of normally distributed data, diapause proportions were arcsine square root transformed.

The feeding preference experiment was analyzed with the (two-tailed) paired *t*-test (Zar 1999), because death rates of diapause and summer-form spider mites are dependent. The null hypothesis (death rates of diapause and summer-form spider mites are similar) was tested against the alternative hypothesis: the death rates differ.

Results

Diapause induction

Spider mites exposed to predators throughout juvenile development (predation treatment) showed a higher diapause incidence than spider mites that had not been exposed to predators – including not only the control treatment, but also the removal and puncture treatments (Figure 1). This difference ranged from 11 to 27% and was significant for all three pairwise treatment comparisons (see Table 1; Bonferroni *post hoc* test: predation vs. control $p = 0.001$, predation vs. removal $p = 0.004$, predation vs. puncturing $p = 0.01$). Thus, presence of *T. pyri* enhanced diapause also in this Dutch strain of *T. urticae*.

Table 1. Analysis of variance on diapause incidence (see Figure 1)

	SS	df	MS	F	p
Predation treatment	0.111005	3	0.037002	25.103	0.000854
Incubator (block)	0.201418	2	0.100709	68.323	0.000074
Error	0.008844	6	0.001474		

The predation treatments were blocked by incubator; hence, no interaction term was calculated between the two factors (Zar 1999).

Figure 1. Effect of presence of predatory mites (*T. pyri*) and of mimicked predator activity on diapause induction in the spider mite *T. urticae* at LD 14.25:9.75 and 18 °C. Mites numbers tested are shown on top of the bars. Diapause proportions were significantly different between the predation treatment and all three other treatments (see Table 1 and text for more details). Predation treatment: predators were present during development of spider mites; control treatment: undisturbed development of spider mites; removal treatment: removing a proportion of the juvenile spider mites; puncture treatment: puncturing and leaving in place a proportion of the juvenile spider mites.

Pairwise comparisons among control, removal and puncture treatments did not yield significant differences for any of the incubators, suggesting that simply removing or puncturing of juvenile mites was insufficient to trigger the diapause response of the surviving mites in a way similar to those exposed to actual predator presence. Diapause proportion did show a fairly consistent relation with the degree of disturbance of spider mites: in all three incubators diapause was lowest in the control treatment, intermediate in the disturbance treatments and highest in the predation treatment.

Predators' feeding preference

The control experiment revealed that in absence of predators the average daily death rate per leaf disc was 0.05 ± 0.02 (SE) diapause females vs. 0.13 ± 0.03

Figure 2. Death rates (10 replicates) and average death rate of adult diapause and summer-form spider mite females, *T. urticae*, caused by foraging predatory mites, *T. pyri*. The death rates have been adjusted for mortality caused by other factors than predation (see Material and methods). Each replicate bar represents the death rate of adult female spider mites on one leaf disc due to one predatory mite (reproducing adult female).

summer-form females. In presence of predators, the average daily death rate per leaf disc was 0.68 ± 0.10 diapause females vs. 0.24 ± 0.05 summer-form females; all predators laid eggs, implying that they had all been foraging on the spider mites. Hence, average daily spider mite mortality due to actual predation was $0.63 (\pm 0.10)$ diapause females/leaf disc vs. $0.13 (\pm 0.04)$ summer-form females/leaf disc (Figure 2). These adjusted death rates differed significantly ($t = 4.04$; d.f. = 9, $p < 0.005$). Thus, *T. pyri* killed significantly more diapause than summer-form spider mite females.

Discussion

In this article, we reconfirm that the proportion spider mites entering diapause is significantly higher in the presence of predators than in their absence, using a Dutch strain of *T. urticae*. In principle, this enhanced spider-mite diapause could have been the consequence of disproportional mortality caused by the predators, rather than the consequence of a physiological response to the predators' presence. Selective predation on summer-form mites could explain an increase in diapause proportion in the surviving spider mites. To test whether predators impose such disproportional mortality, they were given a choice between adult diapause and non-diapause prey.

Adult diapause and non-diapause spider mites are known to differ; diapause spider mites have a higher content of fat, sugar (Boudreaux 1963), and carotenoids (Veerman 1974), they are somewhat smaller since they do not contain developing eggs, and they move around less. It appeared that on average the mortality of summer-form mites was *lower* than that of diapause mites. In other words, there is no evidence that selective predation underlies the difference in diapause response found. It should be realised, however, that a preference of the predators might still influence the net response seen in the spider mites. In our selective predation assay only adult mites were offered, whereas in the diapause-induction experiments predators were present when the prey were developing from larvae to young adults. It seems unlikely, however, that predators would display an opposite preference when offered a choice between the two types of juvenile mites (for which differences between the two are unknown).

The diapause characteristics of the Dutch strain used in this study and the Greek strain used in the previous study differ considerably. For example, under long-night photoperiods and 18 °C only part of the Greek strain enters diapause, which is shallow and easily interrupted (Koveos et al. 1993a, b; Kroon et al. 2004); under the same conditions all mites of the Dutch strain enter a deep diapause, which cannot be broken readily (D. Kroon and A. Kroon, unpublished results). Most importantly, under the climatic conditions in our experiments, diapause induction in the Dutch strain is much more sensitive to night-length than in the Greek strain. Nevertheless, in the small window of night-length where diapause induction is not complete, predation risk still has a significant effect on diapause incidence. Hence, predation risk seems to be able to affect diapause induction irrespective of the strength of other diapause-inducing factors such as night-length.

Given that diapause induction is stimulated by the presence of natural enemies, the response must be associated with predator cues. We mimicked two aspects of predation: decrease in prey density by simply removing juvenile spider mites and decrease in prey density by puncturing (and leaving behind) the prey. Neither of the two treatments affected diapause induction significantly. Thus, the results do not provide compelling evidence for the idea that removal and/or injury of the prey act as a cue. Weisser et al. (1999), who found that presence of predators had a stimulatory effect on winged offspring production in pea aphids, also did not find an effect of simulated disturbances. Of course, simulated disturbances differ in many ways from disturbances caused by actual predators. For example, removal or puncturing of mites occurred once per day, whereas predatory mites were present 24 h per day. This temporal aspect of disturbance was obviously not present in our experiments.

When can diapause induction lead to escape from predation?

Prey responses to predation risk usually involve short, instantaneous behavioural reactions. Ontogenetical development into diapause, however, is a

slow process. In the two-spotted spider mite this process starts in the juvenile phase and is only fully expressed in the adult female (males do not enter diapause). This begs the question under which conditions diapause induction can result in escape from predation. Clearly, this process cannot work if predators can quickly eradicate prey populations. The nature of predation needs to be such that predation rates are limited by satiation (plateau of the functional response) and high predation risk only occurs after a time delay due to food conversion and development (an inherent property of the predator's numerical response). In our study system, this is the case: the adult female predatory mite is somewhat smaller than the adult female spider mite and easily satiated (Kroon, pers. obs.).

Interestingly, the only other terrestrial predator-prey system known where predation risk induces a physiological change in prey development, also satisfies this condition. Predatory ladybirds (*Coccinella septempunctata* and *Adalia bipunctata*) induce a shift from unwinged to winged morphs in the pea aphid, *Acyrtosiphon pisum* (Dixon and Agarwala 1999; Weisser et al. 1999). The winged morph is produced in response to adverse environmental conditions (Dixon 1998), but may also facilitate escape from predation, because ladybirds do not eradicate aphid populations in one aphid generation, and neither in one ladybird generation.

Given the slow response of diapause induction to predation risk, the question arises how this can result in escape from predation. We have shown here that diapause induction does not yield *direct* protection against predation: predators do kill diapause females. Hence, the protection has to be achieved *indirectly*, through behaviour associated with diapause. In the spider mite *T. urticae*, diapause females search for hibernation sites (Popov and Veerman 1996), whereas ovipositing summer-form spider mites remain on their host plant (Kennedy and Smitley 1985). We hypothesize that it is this behavioural response that indirectly leads to escape from predation in times of deteriorating climatic conditions. Predation risk gives females the last push in their decision making whether or not to enter the hibernation state. Whether this results in a higher chance to survive until the next spring and produce more offspring, remains to be assessed in future experiments.

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