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Role of excreta in predator avoidance by the Kanzawa spider mite, *Tetranychus kanzawai* (Acari: Tetranychidae)

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Abstract. The Kanzawa spider mite, *Tetranychus kanzawai* (Acari: Tetranychidae) constructs webs over leaf surfaces and usually lives under these webs. *T. kanzawai* produces two types of excreta, black and yellow pellets, and uses its webs as a place for excretion. *T. kanzawai* also uses its webs as a refuge when the predatory mite, *Neoseiulus womersleyi* (Acari: Phytoseiidae) is present. To clarify what factors deter *N. womersleyi* from foraging on *T. kanzawai* webs, I experimentally examined the effects of *T. kanzawai* excreta on its own fitness (fecundity) and the foraging behaviour of *N. womersleyi*. When the excreta of *T. kanzawai* was put on leaf surfaces, the fecundity of *T. kanzawai* adult females was reduced by the black but not the yellow faecal pellets. On the other hand, predation by *N. womersleyi* was reduced by the yellow but not the black pellets. Although this effect of the yellow pellets on *N. womersleyi* did not last on leaf surfaces, it deterred *N. womersleyi* from staying on the web regardless of its freshness. These results suggest that *T. kanzawai* deposits black pellets on webs to avoid its negative effect on their own fitness, and yellow pellets are deposited on webs to reduce the risk of predation.

INTRODUCTION

Many herbivorous arthropods use refuges on plants to avoid the risk of predation. Herbivores select parts of host plants that are less likely to be searched by predators/parasitoids (e.g., Damman, 1987; Hopkins & Dixon, 1997). Although absolute enemy free space seldom or never exists (Jeffries & Lawton, 1984), places where physical factors, such as plant structures, deter predators/parasitoids from foraging provide refuges for prey (e.g., Gross & Price, 1988; Venzon et al., 2000; Kasai et al., 2005).

The polyphagous spider mite, *Tetranychus kanzawai* (Acari: Tetranychidae), constructs complicated webs over the leaf surfaces of its host plants and usually lives under the webs (Saito, 1983; Oku et al., 2004). In the presence of the predatory mite, *Neoseiulus womersleyi* (Acari: Phytoseiidae), however, *T. kanzawai* uses its webs as a refuge (Oku et al., 2003, 2004). Although *N. womersleyi* can easily invade and move around on the webs of *T. kanzawai* (Ehara & Amano, 1993), it prefers to forage on the surfaces of leaves (Oku et al., 2006). Although this may explain why *T. kanzawai* webs function as a refuge for *T. kanzawai*, it is unknown what factors deter *N. womersleyi* from foraging on *T. kanzawai* webs.

T. kanzawai also uses its webs as a place for excretion (Saito, 1983). Spider mites excrete two types of excreta, yellow-white viscous pellets (hereafter referred as "yellow pellets") and black solid pellets, separately (Wiesmann, 1968). The former mainly consist of nitrogenous metabolites, such as guanine (McEnroe, 1961), while the latter consist of the residue left after digesting their food (Mothes-Wagner, 1985). Although social spider mites deposit excreta at particular sites (Saito, 1983, 1995), *T. kanzawai* deposits excreta randomly (Saito, 1983; K. Oku, pers. observ.). Excreta of prey are often attractive to their predators (e.g., Wainhouse et al., 1992; Müller & Hilker, 1999), including the black pellets of tetranychid mites [for example the *T. urticae*-*Phytoseiulus persimilis* system (Sabelis et al., 1984)]. However, excreta can also function as an effective defense against predators (e.g., Vencl et al., 1999). Thus, I hypothesized that *T. kanzawai* excreta prevent *N. womersleyi* from foraging on webs and so provide *T. kanzawai* with a refuge. Saito (1983) suggested that spider mites deposit excreta on webs in order to prevent leaf surfaces from becoming polluted because they are not only a habitat but also a source of food for spider mites; however, there are no studies on the negative effects of excreta on spider mites. In this study, the effects of *T. kanzawai* excreta on its own fitness and the foraging behaviour of *N. womersleyi* are examined and the relationships between the excretory behaviour of *T. kanzawai* and predator avoidance discussed.

MATERIAL AND METHODS

Mites

The mite *T. kanzawai* was collected from convolvulus, *Calystegia japonica*, on the campus of Kyoto University, Japan, and maintained on leaf discs of kidney bean (*Phaseolus vulgaris*), a suitable host plant for *T. kanzawai*, pressed onto water-saturated cotton wool in Petri dishes (90 mm diameter, 14 mm depth). *N. womersleyi* was also collected in Kyoto and reared on bean leaf discs heavily infested with the prey species, *T. urticae* (30–50 female adults and individuals of other stages per leaf). All of the dishes were placed together in a transparent plastic container and kept at 25°C, 50% relative humidity and a photoperiod of 16L : 8D.

To obtain *T. kanzawai* eggs, 20 mated *T. kanzawai* adult females were randomly selected from stock cultures and placed on a bean-leaf disc on water-saturated cotton wool in a Petri dish, as described above. They were then allowed to oviposit for 2–3 days under laboratory conditions. All *T. kanzawai* eggs used in this study were obtained in this way. Furthermore, to standardize the age of *N. womersleyi* adult females, their eggs were collected from stock cultures and placed on leaf discs infested with *T. urticae* for 7 days under laboratory conditions. The *N. womersleyi* adult females that developed were used in the following experiments.

The preferred excretory sites of adult females of *T. kanzawai*

To determine the sites where adult females of *T. kanzawai* excrete, 15 mated females (2 days old) were isolated on each leaf square (10 × 10 mm). After 24 h, the number of both black and yellow pellets and their location were recorded (i.e., on leaf surfaces or on webs). I determined whether the sites for excretion of each pellet type by *T. kanzawai* is biased by using a paired-comparison t-test. Furthermore, the ratio of the distribution of each pellet type on webs and leaf surfaces was compared using a paired-comparison t-test of arcsine-transformed data.

Effects of *T. kanzawai* excreta on its own fitness

On average, *T. kanzawai* adult females excreted 1.9 ± 0.15 (mean ± SEM) black and 13.2 ± 0.79 (mean ± SEM) yellow pellets during 24 h (see Fig. 1). Thus, over a period of ten days they excrete 20 black pellets and 120 yellow pellets, respectively. Furthermore, the number of eggs laid by *T. kanzawai* females within a certain period after maturation (hereafter "fecundity") was used as the fitness index (e.g., Gotoh et al., 1999; Oku et al., 2002).

To examine the effects of excreta on the fecundity of adult females of *T. kanzawai*, 54 mated *T. kanzawai* females (2 days old) were randomly selected from stock cultures, transferred onto a 40 × 40-mm sheet of parafilm (Parafilm "M", American National Can) and starved for 1 day under laboratory conditions. 54 leaf squares (10 × 10 mm) were placed in Petri dishes and 20 black pellets spread evenly on each of half of the leaf squares (n = 27), but not on the rest, which were the controls (n = 27; clean leaf). One *T. kanzawai* female was placed on each leaf square. Next day, the number of eggs laid by the *T. kanzawai* females on each leaf square was counted. A similar experiment using 120 yellow pellets instead of 20 black pellets was conducted and the fecundity in the two treatments compared using a t-test.

Effects of *T. kanzawai* excreta on the foraging behaviour of *N. womersleyi*

The predatory mite, *N. womersleyi*, prefers *T. kanzawai* eggs to adult females (Furuichi et al., 2005). To determine whether *T. kanzawai* excreta affect the foraging behaviour of the predatory mite, 92 leaf squares (10 × 20 mm) were put on water-saturated cotton wool (15 × 25 mm) in a Petri dish and each leaf square divided into two equal parts by a water-saturated thread. The water-saturated thread prevented mites crossing from one side of a leaf to the other. Five newly emerged *T. kanzawai* females were put on one half of each leaf square and allowed to construct webs over the leaf surface for 5 h. Since *T. kanzawai* females do not lay eggs within 24 h of emergence under laboratory conditions (Furuichi et al., 2005) the webs remained free of eggs. The method described in Furuichi et al. (2005) was used to obtain newly emerged *T. kanzawai* females. At the same time, 20 black pellets (n = 30) or 120 yellow pellets (n = 30) of excreta of *T. kanzawai* were placed evenly on the other half (the area without *T. kanzawai* females) of each of the leaf squares, and the rest of the leaf squares served as a control (n = 32; clean leaf). Twenty *T. kanzawai* eggs were placed on the halves of each leaf square with excreta (the area without *T. kanzawai* females). Subsequently, the *T. kanzawai* females and the water-saturated thread were removed from each square, and one mated *N. womersleyi* adult female (3–5 days old), which had been starved for two days, was placed on the area of leaf square with *T. kanzawai* webs. The webs prevent *N. womersleyi* from escaping from leaf squares without prey (K. Oku, pers. observ.). After 5 h, the number of eggs that were not eaten by *N. womersleyi* on each leaf square was counted. Trials in which *N. womersleyi* escaped from the leaf squares were excluded from the data set. The results for the control, black and yellow pellets were compared using a Dunnett's test (SAS, 2006).

The effect of the age of yellow excreta of *T. kanzawai* on the foraging behaviour of *N. womersleyi*

The effect of the age of *T. kanzawai* excreta on the foraging behaviour of *N. womersleyi* was determined on 115 leaf squares (10 × 20 mm), each divided into two equal parts by a water-saturated thread in the same manner as described above. 120 yellow pellets excreted by *T. kanzawai* were evenly spread on the surfaces of one half of 38 leaf squares (old pellets) and then kept for 2 days under laboratory conditions. Another 120 yellow pellets were evenly spread on the surfaces of one half of the other 39 leaf squares (fresh pellets) and the remaining leaf squares served as a control (n = 38; clean leaf). At the same time, five newly emerged *T. kanzawai* females were placed onto the half of the leaf squares without pellets and allowed to construct webs on the leaf surface for 5 h. Then 20 *T. kanzawai* eggs were placed onto the halves of each leaf square with excreta (the area without *T. kanzawai* females), after which the *T. kanzawai* females and the water saturated threads removed from all the squares and one mated *N. womersleyi* adult female (3–5 days old), previously starved for two days, was placed onto the areas with *T. kanzawai* webs on each leaf square. After 5 h, the number of eggs that were not eaten by *N. womersleyi* on each leaf square was counted. Trials in which *N. womersleyi* escaped from the leaf squares were excluded from the data set. The results for the control, old pellet and fresh pellet treatments were compared using a Dunnett's test (SAS, 2006).

Furthermore, in order to determine the effect of *T. kanzawai* excreta on the percentage of visits made by *N. womersleyi* to the webs, each of 60 leaf squares (20 × 20 mm) were placed on water-saturated cotton wool (20 × 20 mm) in Petri dishes. These were divided into three groups, each of 20 leaf squares, designated "5 day old", "2 day old" and "fresh", depending on the age of the excreta. Ten newly emerged *T. kanzawai* females were placed onto each of the 20 leaf squares for 1 day under laboratory conditions (5 day old). In this way leaf squares with excreta on the webs was obtained, since *T. kanzawai* excretes on webs rather than leaf surfaces (see Fig. 1). The *T. kanzawai* females were then removed from each leaf square with minimal damage to the webs. After 2 days, ten newly emerged *T. kanzawai* females were placed on each of another 20 leaf squares for 1 day under laboratory conditions, which resulted in excreta on webs as described above (2 day old). Next day, ten newly emerged *T. kanzawai* females were placed on each of the remaining 20 leaf squares, and the same process repeated (fresh excreta). Then one *N. womersleyi* (3–5 days old), which had been starved for one day, was placed on each leaf square. After allowing the predators one hour to acclimatize to the laboratory conditions, the location of the *N. womersleyi* (i.e., on leaf surfaces or on webs) at 10-min intervals for 2 h. Trials in which *N. womersleyi* escaped from the leaf were excluded from the data

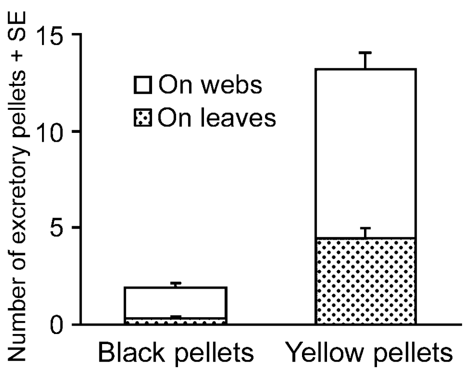

Fig. 1. The number of black and yellow excretory pellets deposited by adult females of *Tetranychus kanzawai* on webs and leaves (n = 15).

set. The percentage of individuals that remained on the webs in the three treatments were compared using a Tukey-Kramer HSD test (SAS, 2006) after the results were arcsine-transformed.

RESULTS

The preferred excretory sites of adult females of *T. kanzawai*

Adult females of *T. kanzawai* excreted more pellets on webs than on leaf surfaces (black pellet, $t = -3.676$, df = 14, $P = 0.0025$; yellow pellet, $t = -3.600$, df = 14, $P = 0.0029$; Fig. 1), with 83.3 ± 0.08 (mean ± SEM) % of the black pellets and 65.4 ± 0.04 (mean ± SEM) % of yellow pellets excreted on webs. The percentage of black pellets excreted on webs was higher than of yellow pellets ($t = -3.524$, df = 14, $P = 0.0034$; Fig. 1).

Effect of *T. kanzawai* excreta on its own fitness

In the presence of black pellets *T. kanzawai* adult females had a lower fecundity than when on clean leaves (clean, 8.3 ± 0.28 (mean ± SEM); black pellets, 7.4 ± 0.18 (mean ± SEM); $t = 2.597$, df = 49, $P = 0.012$; Fig. 2A), but the presence of yellow pellets did not affect their fecundity [clean, 6.7 ± 0.25 (mean ± SEM); yellow pellets, 6.9 ± 0.25 (mean ± SEM); $t = 0.585$, df = 46, $P = 0.561$; Fig. 2B].

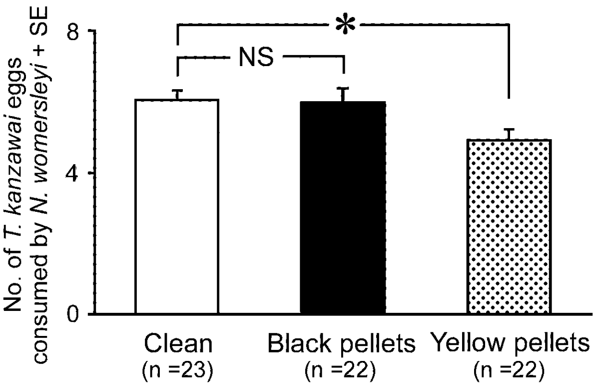

Fig. 3. Effect of *T. kanzawai* excreta on the foraging behaviour of *Neoseiulus womersleyi*. The results for the control (clean), black and yellow pellets were compared using Dunnett's test. * $P < 0.05$

Effects of *T. kanzawai* excreta on the foraging behaviour of *N. womersleyi*

The number of eggs consumed by *N. womersleyi* was negatively affected when yellow pellets were present ($d = 2.263$, $\alpha = 0.05$, $P = 0.035$; Fig. 3), but not black pellets ($P = 0.994$).

The effect of the age of yellow excreta of *T. kanzawai* on the foraging behaviour of *N. womersleyi*

Fresh yellow pellets of *T. kanzawai* negatively affected the number of *T. kanzawai* eggs consumed by *N. womersleyi* compared with clean leaves ($d = 2.253$, $\alpha = 0.05$, $P = 0.043$; Fig. 4), but the presence of old yellow pellets on leaf surfaces did not affect the consumption of eggs by *N. womersleyi* ($P = 0.861$). These results indicate that the effect of yellow pellets on the egg consumption of *N. womersleyi* declined with time. However, the percentage of individuals of *N. womersleyi* that remained on the webs did not differ between treatments at the $P < 0.05$ confidence level [$q = 2.454$, $\alpha = 0.05$; fresh excreta, 25.8 ± 3.15 (mean ± SEM) %; 2 days old, 17.5 ± 2.55 (mean ± SEM) %; 5 days old, 22.2 ± 3.22 (mean ± SEM) %].

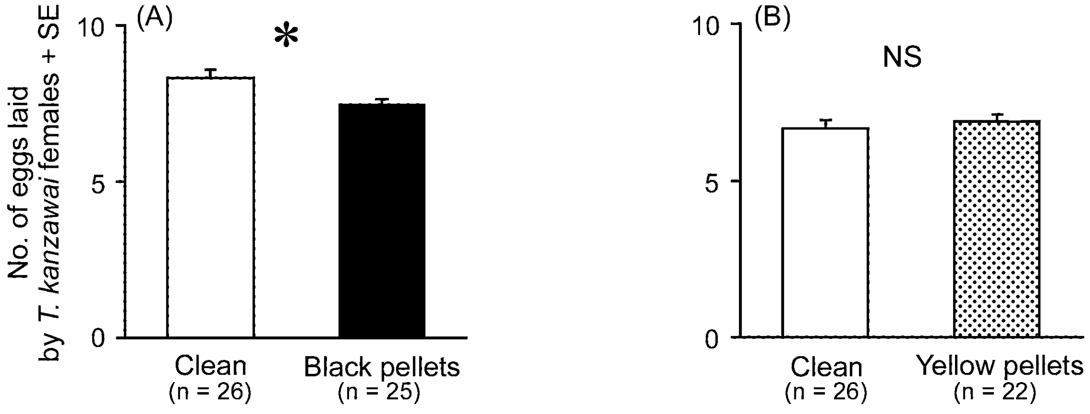

Fig. 2. Effect of black (A) and yellow pellets (B) on *T. kanzawai* fitness. * $P < 0.05$ (t-test).

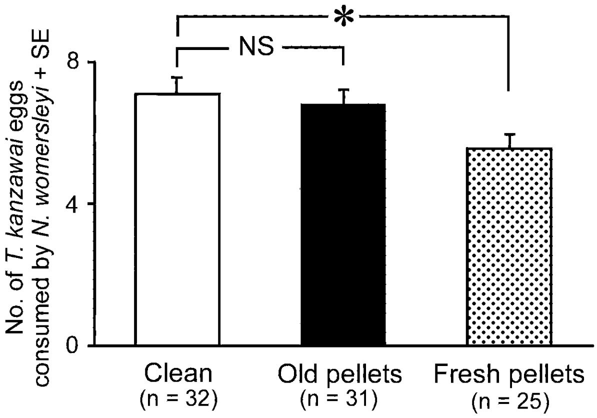

Fig. 4. Effects of the age of *T. kanzawai* yellow excreta on the foraging behaviour of *N. womersleyi* on leaf surfaces. The results for the control (clean), old and fresh pellets were compared using Dunnett's test. * $P < 0.05$

DISCUSSION

T. kanzawai excreted both black and yellow pellets on webs rather than leaf surfaces. Black pellets reduced the fecundity of *T. kanzawai* adult females, but not the yellow pellets. This seems to explain why the black pellets were preferentially excreted on webs compared to yellow pellets. The results presented in Fig. 2 indicate there was a difference between the controls, which might be attributed to different levels of crowding the females experienced during their development (Oku et al., 2002). Female spider mites produce eggs using resources eaten within a day (e.g., Yano et al., 1998). Black pellets pollute leaf surfaces and reduce the amount of food available for *T. kanzawai* females. As suggested by Saito (1983), *T. kanzawai* possibly deposits black pellets on webs in order to avoid their negative effect on their own fitness. However, there seem to be other reasons why *T. kanzawai* deposits yellow pellets on webs.

The presence of *T. kanzawai* yellow pellets reduced the consumption of eggs by *N. womersleyi*. Yellow pellets are viscous (Wiesmann, 1968), which would prevent *N. womersleyi* from foraging. Although the yellow pellets on webs remain viscous for at least for 5 days, those on leaf surfaces soon dry out (K. Oku, pers. observ.). This accords with the observation repeated here that old yellow pellets did not have a negative effect on predation by *N. womersleyi*. On the other hand, the percentage of *N. womersleyi* remaining on *T. kanzawai* webs was low and did not change regardless of the freshness of the excreta. This result suggests that the negative effect of yellow pellets on webs lasts for longer than on leaf surfaces. Following the scenario, *T. kanzawai* is also expected to deposit proportionally more yellow pellets on webs than on leaf surfaces, which was observed. As a result, *N. womersleyi* forages mainly on leaves and *T. kanzawai* escapes predation by seeking a refuge on the webs. That is, although *T. kanzawai* black pellets might be attractive to predatory mites (see Sabelis et al., 1984), their yellow pellets act as a defense against predation. *T. kanzawai* webs are so fragile that it was not possible to remove excreta from the webs without damaging them. Thus, the percentage of records of *N. womersleyi* on the webs contained with different ages of excreta was compared here.

Some arthropods produce two types of excreta; one is useful for them and the other is deposited in latrine sites (see Weiss, 2006). Furthermore, some arthropods defend themselves against predators/parasitoids using secretions (e.g., Dani et al., 1996; Eisner et al., 1997; Machado et al., 2005). Since *T. kanzawai* sometimes produces a mixture of black and yellow pellets (K. Oku, pers. observ.), it is unlikely that *T. kanzawai* excretes two different types of excreta separately due to a constraint. Therefore, in *T. kanzawai* the black pellets are excreta and yellow pellets act to deter predators. Although the black and yellow pellets are classed as excreta, spider mites may "secrete" the yellow pellets in order to actively deter predators.

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