Function of quiescence of *Tetranychus kanzawai* (Acari: Tetranychidae),
as a defense mechanism against rain

Yoko Ikegami,* Shuichi Yano, Junji Takabayashi and Akio Takafuji

Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University, Kyoto 606–8502, Japan

(Received 4 February 2000; Accepted 27 March 2000)

**Abstract**

We compared the mortality of quiescent female deutonymphs and adult females of the kanzawa spider mite, *Tetranychus kanzawai*, on leaf-discs that received simulated rain. The mortality of adult females was significantly higher than that of quiescent nymphs under the simulated rain. This may be due to a difference in water tolerance between the two stages, because the mortality rate did not differ in the absence of the simulated rain. The quiescent period was extended under increased moisture caused by the rain, and the quiescent deutonymphs emerged in response to a decrease in moisture. This plasticity in development seemed to ensure their molting safely after rainfall. The presence of both active and quiescent stages in the life cycle may function as a bet-hedging strategy against unpredictable rain.

**Key words:** *Tetranychus kanzawai*, quiescent deutonymph, rain, bet-hedging

**INTRODUCTION**

Spider mites develop through egg, larva, quiescent larva (protochrysalis), protonymph, quiescent protonymph (deutochrysalis), deutonymph, quiescent deutonymph (teleiochrysalis) to the adult stage. Mites in the quiescent stages are more likely to be attacked by predators (e.g., Shimoda et al., 1997). Nevertheless, they spend almost half of their time between egg hatching and adult emergence as quiescent stages [e.g., ca. 46% in *T. kanzawai* females (Osakabe, 1967) and ca. 53% in *T. urticae* (Herbert, 1981)]. This may simply be explained by a physiological constraint related to molting. However, this long quiescent period may have ecological significance that excels the risk of predation. The objective of this study was to demonstrate the ecological function of the quiescent stage.

A major mortality factor in spider mites is rain. Tanaka and Inoue (1962) showed that rain negatively affected the population increase of *Panonychus citri*. Artificial rain decreased the population density of *T. kanzawai* on tea leaves (Osakabe, 1967), and a population of *T. kanzawai* in Japanese pear orchards decreased drastically due to rainfall (T. Shimoda, personal communication). During the quiescent stage, spider mites anchor themselves to the substrate (Crooker, 1985). Males spin their own web over quiescent deutonymphs (Penman and Cone, 1974). Such webs would further protect the quiescent mites from being washed off by rain. We hypothesized that if the quiescent mites showed a lower mortality than those in active stages exposed to rain fall, then the function of the long quiescent stage may be to protect the mites against rain. To test this hypothesis, we compared the effect of rain on the adult female and the quiescent deutonymph of *T. kanzawai*. We also studied whether the mites are capable of regulating the duration of the quiescent stage in response to changing humidity.

**MATERIALS AND METHODS**

Plants. We used kidney bean plants (*Phaseolus vulgaris*) as food for kanzawa spider mites (*T. kanzawai*). The plants were reared in plastic pots (12 cm diameter and 10 cm depth) in a climate room (25±2°C, 50–70% R.H., 16L–8D). Plants were offered to the spider mites two to three weeks after germination.

*To whom correspondence should be addressed at: E-mail: imageki@kais.kyoto-u.ac.jp*
Mites. We collected *T. kanzawai* from a rose garden in Kyoto City in 1998. The spider mites were kept on kidney bean leaf discs placed on water-soaked cotton in petri dishes (9 cm diameter and 2 cm depth). The leaves were placed with the adaxial surface facing upward. We placed the dishes in a transparent plastic container in a climate room (25 ± 2°C, 50–70% R.H., 16L–8D). Herbert (1981) reported that males and females of *T. urticae* showed different durations of both active and quiescent periods. In the following experiments, we used only quiescent female deutonymphs and adult females because in other quiescent stages (quiescent larva and quiescent protonymph), males and females, which may show different durations of active and quiescent periods, were visually indistinguishable.

Experiment 1. Water-tolerance in the quiescent and active stages. We compared the mortality of the quiescent female deutonymphs and the adult females under simulated rain. Forty-two kidney bean leaf pieces (1 × 1 cm) were placed on moist cotton in a petri dish (14 cm diameter and 2 cm depth). The petri dish was then divided into left and right sides, each having 21 leaf pieces. Twenty-one female deutonymphs selected at random from the stock culture were individually introduced onto the leaf pieces on one side. After 24 h, all mites entered the quiescent stage. The same number of adult females, also taken from the culture, were introduced onto the leaf pieces placed on the other side.

To simulate rain, we sprayed 6.5 ml of water over the dish with a sprayer three times each day, beginning at 9:00, 12:00 and 15:00. After two days of simulated rain, we kept the dish in the climate room for two more days. We then recorded the mortality of the mites in both stages on the leaf pieces. We did not count the mites that had died before the simulated rain was initiated. For the control treatment, we recorded the mortality of the mites in both stages on leaf pieces that had not been subjected to simulated rainfall. The experiment was done twice.

Experiment 2. Effects of water droplets on the duration of the quiescent deutonymph period. To detect the effects of rain on the duration of the quiescent deutonymph period, female deutonymphs randomly selected from the stock culture were video-recorded with respect to the simulated rain. A kidney bean leaf piece (0.5 × 0.5 cm) was placed on moist cotton in a plastic dish (3.5 cm diameter and 1 cm depth). The area of the leaf piece was the largest piece that could fit in the viewing area of the video camera, but was enough to feed a female deutonymph for two days (Y. Ikegami, unpublished data).

A single female deutonymph was individually introduced onto each leaf piece. We then video-recorded the females using a CCD camera (WAT-202, Watec) and a time-lapse video cassette recorder (EVT-820, Sony). The videotapes were re-played later to determine the start and finish time of the quiescent stage. Since the quiescent mites assume a characteristic posture in which they arrange their legs in parallel (Fig. 1), they were easily distinguished from those in active stages in the re-played display. To record a large number of quiescent deutonymphs at the same time, 36 dishes with mites were arranged along the edge of a round table that rotated once every 10 min in a climate room (25 ± 2°C, 50–70% R.H., 16L–8D) (Fig. 2).

After 24 h of video recording, all the deutonymphs on the table had entered the quiescent stage. Then, water (0.5 ml each leaf) was dropped with a syringe to cover each leaf in half of the dishes on the table. The leaf pieces in the

![Fig. 1. *T. kanzawai* in (a) quiescent stage and (b) active stage.](image-url)
rest of the dishes were not watered as controls. Each leaf square was recorded when it passed right under the camera, which was fixed perpendicularly against the table edge. The recording was started at 15:30, and finished when all the deutonymphs became adults. We used red light to illuminate the mites during the night time.

Experiment 3. Effects of moisture on the duration of the quiescent deutonymph. Forty kidney bean leaf pieces (1 × 1 cm) were placed on water-soaked cotton in a petri dish (14 cm diameter and 2 cm depth). Forty quiescent female deutonymphs from the stock culture were individually introduced onto the leaf squares. We prepared two plastic dishes with 40 leaf pieces and kept them in a climate room (25 ± 2°C, 50–70% R.H., 16L–8D). Then, we sprayed water (6.5 ml) over the dish with a sprayer to simulate rain. To obtain two different humidity conditions, one dish was covered with a transparent plastic lid, while the other one was kept open. We did not count the dead mites. The number of mites that reached the adult stage on each dish was recorded daily at 10:30 in the climate room. The proportion of adults, i.e., (the number of mites that became adults/the number of surviving mites at the end of the experiment)×100, was defined as “emergence rate (%).” On the fourth day, the lid was taken off and the number of adults was recorded at 10-min intervals. After 120 min of observation, both dishes were placed in the climate room for another day to record the emergence rate on the fifth day, at which time the experiment was terminated.

RESULTS AND DISCUSSION

Experiment 1. Water tolerance of the mites in quiescent and active stages

Under the simulated rain condition, the mortality of adult females was significantly higher than that of quiescent deutonymphs (32.5% vs. 8.1%, p = 0.0110, Fisher’s exact probability test) (Fig. 3). In contrast, the mortality between the adults and quiescent deutonymphs did not differ in the absence of the stimulated rain (0% vs. 2.4%, p = 1.0000, Fisher’s exact probability test). The mortality of the quiescent deutonymphs with rain and that without the rain did not differ significantly (8.1% vs. 2.4%, p = 0.34, Fisher’s exact probability test). These data indicate that the quiescent deutonymph is more water-tolerant than the adult. The quiescent larvae and protonymphs may also be more water-tolerant than the respective active larvae and protonymphs.

Our results are not consistent with those of Osakabe (1967), who reported that the quiescent stage was less water-tolerant than

![Fig. 2. Equipment used to record the quiescent period in the climate room (25 ± 2°C, 30–50% R.H., 16L–8D). Plastic dishes (max. 36 dishes) were arranged along the edge of a round table (ca. 48 cm in diameter) which was rotated once every 10 min. The CCD camera was connected to a video cassette recorder set for time-lapse recording.](image)

![Fig. 3. Mortality of quiescent female deutonymphs and adult females treated with simulated rain and that of the controls (without rain).](image)
the active stage. He dipped the mites for 48 h in water, while we sprayed water directly on the mites. The different results may be attributed to this difference.

**Experiment 2. Effects of water droplets on the duration of quiescent deutoymph period**

The duration of the quiescent deutoymph period with water droplets was significantly longer than that without water (38.2 ± 1.2 h vs. 31.6 ± 0.2 h, p < 0.0001, t-test) (Fig. 4). Based on the results of Experiment 1, we conclude that rain is a mortality factor for adult female *T. kanzawai* but not for the quiescent deutoynymphs and the elongation of the quiescent period in response to water droplets functioned as an avoidance mechanism of the mites against rain.

**Experiment 3. Effects of moisture on the duration of the quiescent deutoymph**

In the uncovered dish, the emergence rate was more than 90% on day 2, whereas the rate in the covered dish was only 3% even on day 4 (Fig. 5). The body color of the quiescent females is known to turn white just before molting. All individuals in the covered dish had turned white by day 4. Interestingly, 80% of the quiescent females in the covered dish emerged within 30 min after the dish was uncovered, and all of the mites molted within 120 min after the lid was taken off (Fig. 5).

As shown in Experiment 2, the quiescent period was extended only ca. 6.6 h by the water droplets. The moisture around the mites in the dish covered with a lid was apparently higher than that in the uncovered dish. Thus, the extension of the quiescent period would be attributed to the high moisture content in the closed dish.

In addition, the data of experiment 3 suggest that the female adults responded to the opening of the lid allowing them to emerge. When the lid was opened, the moisture decreased immediately and the droplets on the leaf pieces evaporated quickly. This change would be similar to the change after natural rain. Thus, the simultaneous molting after the opening of the lid suggests that the mites, which are ready to emerge, could wait until the rain stopped. It is hypothesized that a plastic phenotype is more advantageous than a fixed phenotype for organisms living in an environment that fluctuates spatiotemporally within a local habitat and/or the organism’s generation time (Bradshaw, 1965). Plasticity in the duration of the quiescent deutoymph period would be an effective defense against unpredictable rain. It is known that astigmatid mites have a quiescent stage called "hypopus" in which they are more tolerant against adverse ecological conditions (Ehara and Shinkaji, 1996).

We showed here that female quiescent deutoynymphs were more tolerant against water than adult females. Further, their quiescence period was extended in response to rain and moisture, and the mites could emerge in response to a decrease in moisture. These data indicate that one of the ecological functions of the quiescent deutoymph stage (and probably of other quiescent stages as well) is to protect against

![Fig. 4. Quiescent period (in hours) of the mites treated with water and that of the control.](image)

![Fig. 5. Emergence rate (%) of quiescent deutoynymphs. Broken line indicates the rate in the control experiment (without a lid), and the solid line indicates the rate in the treated experiment (with a lid). Arrow indicates the time when the lid was removed from the covered dish in the treated experiment.](image)
rain. As for spider mites living under outdoor conditions such as T. kanzawai, rain would be one of the unpredictable environmental changes that decrease the mean fitness of individual organisms. Bet-hedging is thought to be an important strategy to survive under unpredictable environments (Schaffer, 1974). This strategy decreases the fitness variance of an organism caused by a fluctuating environment, thereby increasing the mean fitness of individual organisms. As the duration of oviposition is long in spider mites, offspring from one female consist of both active and quiescent phenotypes at any time. So we hypothesize that having relatively long quiescent stages in a life cycle functions as a bet-hedging strategy against rain.

ACKNOWLEDGEMENTS

We thank Dr. K. Takakura for valuable technical advice. We wish to express our gratitude to Dr. Y. Saito for critical comments. We thank Dr. T. Shimoda for his useful comments. This study was supported by the Research for the Future program (JSPS-RFTF96L006703) and by the Program for the Promotion of Basic Research Activities for Innovative Biosciences (Bio-oriented Technology Research Advancement Institution).

REFERENCES


