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Journal of Environmental Entomology
Physiological Ecology

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Suspension of egg hatching caused by high humidity and submergence in spider mites

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ABSTRACT

We tested the effects of high humidity and submergence on egg hatching of spider mites. In both the high humidity and submergence treatments, many *Tetranychus* and *Panonychus* eggs did not hatch until after the hatching peak of the lower humidity/unsubmerged controls. However, after humidity decreased or water was drained, many eggs hatched within 1–3 h. This was observed regardless of when high humidity or submergence treatments were implemented: either immediately after oviposition or immediately before hatching was due. Normal eyespot formation was observed in most eggs in the high humidity and submergence treatments, which indicates that spider mite embryos develop even when eggs are underwater. Therefore, delays in hatching are not caused by delayed embryonic development. A delay in hatching was always observed in *Panonychus citri* but was more variable in *Tetranychus urticae* and *Tetranychus kanzawai*. The high humidity and submergence treatments affected but did not suppress larval development in these species. In contrast, many *Oligonychus* eggs died following the high humidity treatments. In *Tetranychus* and *Panonychus* spider mites, suspension of egg hatching may mitigate the adverse effects of rainfall.

KEY WARDS Delayed hatching, Embryonic development, Environmental adaptation, Tetranychidae, Acari

Introduction

The time required to develop and reproduce affects the fitness of organisms living under variable environments. For example, egg hatch timing affects the survival and development of larvae, and thus the population dynamics of insects (Pickford 1960; Moriyama and Numata 2006).

Spider mites (Acari, Tetranychidae) are small herbivorous arthropods and include economically important agricultural pests. Many live on the leaf surfaces of their host plants, where they are exposed to various environmental stresses such as desiccation (McEnroe 1961; Ferro and Chapman 1979), heat stress (Perring et al. 1984; Lu et al. 2014), and solar ultraviolet radiation (Sakai et al. 2012; Fukaya et al. 2013). Heavy rains such as during hurricanes and typhoons may also be important to spider mite population dynamics (Osakabe 1965; Boyne and Hain 1983; Ho 2000; Rêgo et al. 2013). However, Klubertanz et al. (1990) found that rainfall did not affect spider mite dynamics.

Females of the citrus red mite, *Panonychus citri* (McGregor), an upper leaf surface user, move from the upper to lower leaf surfaces during rainfall (Kato 1972). Additionally, the Kanzawa spider mite, *Tetranychus kanzawai* Kishida, suspend molting as quiescent deutonymphs if the air humidity is high [relative humidity (RH) ≈ 100%] and resume molting after the humidity decreases (Ikegami et al. 2000). Since quiescent deutonymph females can tolerate more water than adult females (cf. Osakabe 1967), this may be an adaptation to rain (Ikegami et al. 2000).

Studies have reported that under conditions of high humidity, spider mite eggs did not hatch (Kuenen 1946; Osakabe 1959; Bonato et al. 1995), the hatch rate decreased (Mori 1957; Boudreaux 1958; Ferro and Chapman 1979), or egg stages were prolonged (Mori 1957; Boyne and Hain 1983). In these previous studies, however, the authors sought to clarify the effects of

continuous high humidity on egg development and hatching; the adaptive significance of these phenomena remains unclear. If hatch timing can be regulated to avoid rain damage, larval survival rates would increase, which would contribute to the population dynamics of spider mites.

Many plant-dwelling mites generally remain on lower leaf surfaces (Sudo and Osakabe 2011), but some species such as *Panonychus* spp. inhabit both lower and upper leaf surfaces (Foot 1963; Kato 1972; Osakabe et al. 2006; Fukaya et al. 2013). Rainfall likely more directly affects the survival and dynamics of mites living on the upper leaf surfaces. Therefore, species of upper surface users might be adapted to rain, while lower leaf surface users are not.

In this study, we tested the effects of temporal high humidity and submergence in water on egg hatching using spider mites inhabiting both the upper and lower leaf surfaces. We also examined whether the high humidity and submergence treatments in egg stage decrease survival and development in subsequent juvenile stages, and the reproduction of females.

Materials and Methods

***Tetranychus* Species.** A green form laboratory population of the two-spotted spider mite, *Tetranychus urticae* Koch, was established from several different localities in Japan and cultured on potted kidney bean plants for at least 7 years. The red form of *T. urticae* was collected from carnations, *Dianthus caryophyllus* L. (Caryophyllaceae), in a greenhouse at the Nagano Prefecture Vegetable and Ornamental Crops Experiment Station, Nagano, Nagano Prefecture, Japan (36°35′ N–138°13′ E) in July, 2006. *Tetranychus kanzawai* and the bean spider mite, *Tetranychus ludeni* Zacher, were collected from melons, *Cucumis melo* L. (Cucurbitaceae), in a

92 greenhouse at the Shizuoka Prefectural Research Institute of Agriculture and Forestry, Iwata,
93 Shizuoka Prefecture, Japan (34°43′ N–137°50′ E) in November 2010.

94 ***Panonychus* Species.** *Panonychus citri* was collected from satsuma mandarins, *Citrus unshiu*
95 Marc. (Rutaceae), in a citrus grove at the Citrus Research Division, Kuchinotsu, National
96 Agriculture and Food Organization Institute of Fruit Tree Science, Minamishimabara, Nagasaki
97 Prefecture, Japan (32°36′ N–130°11′ E) in June, 2007. *Panonychus osmanthi* Ehara and Gotoh
98 was collected from fragrant olives, *Osmanthus fragrans* Lour. var. *aurantiacus* Makino
99 (Oleaceae), at Kyoto University, Kyoto, Kyoto Prefecture, Japan (35°03′ N–135°79′ E) in March,
100 2011. *Panonychus mori* Yokoyama was collected from Japanese pears, *Pyrus pyrifolia* var. *culta*
101 Nakai (Rosaceae), in an orchard at the Horticultural Experiment Center, Tottori Prefectural
102 Agriculture and Forest Research Institute, Hokuei, Tottori Prefecture, Japan (35°28′ N–133°44′ E)
103 in July, 2012. *Panonychus ulmi* (Koch) was collected from apple trees, *Malus domestica* Borkh
104 (Rosaceae), in an orchard at the Akita Fruit-Tree Experiment Station, Yokote, Akita Prefecture,
105 Japan (39°14′ N–140°31′ E) in July, 2012.

106 ***Oligonychus* Species.** *Oligonychus amiensis* Ehara and Gotoh and *Oligonychus castaneae*
107 Ehara and Gotoh were collected from Japanese stone oaks, *Lithocarpus edulis* (Makino) Nakai
108 (Fagaceae), and Japanese chestnuts, *Castanea crenata* Sieb. et Zucc. (Fagaceae), respectively, at
109 Kyoto University in July 2012. No males appeared in the *O. amiensis* population in reared
110 cultures. Many individuals of *O. amiensis* and *O. castaneae* inhabit upper leaf surfaces.

111 **Rearing Conditions for Stock Cultures.** Spider mites were reared on leaf disks of host
112 plants placed with the adaxial surface up on water-soaked cotton in Petri dishes (90 mm diameter
113 and 20 mm depth). The dishes were placed in a transparent plastic container (350 × 250 × 50 mm)
114 whose lids (200 mm in diameter) were covered with fine polyester fiber mesh and kept in a

115 laboratory at 25 ± 2°C and 50–70% RH, with a 16:8 h L:D light cycle (fluorescent lights were
116 turned on at 07:00 h and off at 23:00 h). All experiments were performed in the same laboratory.

117 All *Tetranychus* spp. and *P. citri* were kept on kidney bean leaf disks. Although kidney beans
118 are not the main hosts of *P. citri*, these mites can develop and reproduce normally on leguminous
119 plants (Ashihara, 1987; Fukaya et al., 2013). *Panonychus osmanthi*, *P. mori* and *P. ulmi* were
120 reared on leaves of Japanese pears, which are a suitable host plants for *P. osmanthi* (Kitashima
121 and Gotoh, 1995). *Oligonychus amiensis* and *O. castaneae* were reared on the leaves of Japanese
122 stone oak and Japanese chestnut, respectively.

123 **Effects of High Humidity on Egg Hatch Timing.** We used three types of treatments to
124 evaluate the effects of high humidity on egg hatch timing: high humidity after oviposition (within
125 24 h) to hatching [Experiment (Exp)-1], high humidity immediately before hatching was due
126 (Exp-2), and high humidity immediately after oviposition and continuing after hatching was due
127 (Exp-3). We used all species of spider mites for Exp-1; *T. urticae* green form, *T. urticae* red form,
128 *T. kanzawai*, *T. ludeni*, and *P. citri* for Exp-2; and *T. urticae* green form, *T. kanzawai*, and *P. citri*
129 for Exp-3.

130 Two leaf squares (20 × 20 mm) of the same plants as those mites were reared on were placed
131 on water-soaked cotton in Petri dishes. We introduced 5 *Tetranychus*, 15 *Panonychus*, or 20
132 *Oligonychus* adult females (3–5 days old) to each leaf square. After 24 h, females were removed
133 (day 0) and eggs were counted. We used two Petri dishes per batch and performed three times in
134 Exp-1 and Exp-2. Whereas, we used five Petri dishes and performed once in Exp-3.

135 We covered one of the two Petri dishes in Exp-1 and four of the five Petri dishes in Exp-3
136 with a transparent plastic lid (high humidity treatments) and left the others open (control) at day 0.
137 In Exp-2, Petri dishes in the high humidity treatments were covered with lids on days 2 and 4 for

138 *Tetranychus* spp. and *P. citri*, respectively. These were the days before expected hatch in the
139 controls. In the high humidity treatment, the RH immediately increased and reached >95% within
140 10 min of the dishes being covered. The temperature was slightly higher in the high humidity than
141 in the control treatment as a result of closing the lid (Table 1).

142 To avoid attachment of water droplets on eggs in the high humidity treatment, water droplets
143 on the leaves and lid were removed using fine point brushes and tissue paper. This was carried out
144 within 30 s once daily.

145 We observed the status of the eggs every day and recorded the number of hatched eggs. On
146 the day after >90% of the eggs hatched in the control treatments (days 5, 7, and 8 for Tetranychus,
147 *Panonychus*, and *Oligonychus* spp., respectively), we checked the development of eyespots in the
148 remaining eggs in the high humidity treatments. In Exp-1 and Exp-2, we removed the lids of Petri
149 dishes to decrease moisture. We recorded the number of eggs hatched just after lid removal and 1,
150 3, 24, and 48 h later. No eggs hatched later than 48 h. In Exp-3, we never opened the lids in the
151 high humidity treatment and observed hatching once a day until day 10.

152 **Effects of Submergence on Egg Hatch Timing.** We used two types of treatments to
153 evaluate the effects of submergence in water on egg hatch timing: submergence from immediately
154 after oviposition to hatching (Exp-4) and submergence immediately before hatching (Exp-5) was
155 due. We used *T. urticae* green form and *P. citri* for this experiment.

156 To set up submergence treatments, we placed water-soaked cotton (10 × 30 × 120 mm) in a
157 transparent plastic case (120 × 120 × 30 mm). One-half (petiole side) of a kidney bean primary
158 leaf was placed on the cotton, and the other half was extended on the bottom and covered with
159 wet paper towels having a square hole (20 × 20 mm; Fig. 1). We prepared eight plastic cases (four
160 for submergence treatments and four for controls) per batch in Ex-4 and Ex-5, and the

161 experiments were performed three times.

162 We introduced 5 *T. urticae* or 15 *P. citri* adult females to the inside of the square hole of
163 paper towel on each leaf. We then removed the mites after 24 h and counted eggs (day 0). To
164 submerge the eggs, we poured distilled water into the two cases (Fig.1) on day 0 for Exp-4 and on
165 day 2 and day 4 (the days of expected hatch for control individuals) for *T. urticae* and *P. citri*,
166 respectively, for Exp-5. By only submerging half of the leaf, leaves were able to be kept fresh
167 during the experiment. The remaining two cases were used as controls.

168 On the day after 90% of the eggs hatched in the control treatments, we checked the
169 development of eyespots in the remaining eggs and drained water (on days 5 and 7 for *T. urticae*
170 and *P. citri*, respectively). We recorded the number of eggs hatched immediately after water was
171 drained and 3, 24, and 48 h later. No eggs hatched later than 48 h.

172 We also observed egg hatching after submergence using stereoscopic microscope with a
173 CCD camera (ARTCAM-274KY-WOM, ARTRAY, Tokyo). We stuck a piece of Parafilm (30 ×
174 40 mm) on the inside bottom of two Petri dishes. The Parafilm was covered with wet paper towel
175 having a square hole (20 × 20 mm). Then, we introduced 5 *T. urticae* (or 15 *P. citri*) adult females
176 to the inside of the square hole of paper towel on each Parafilm. We then removed the mites after
177 24 h and poured distilled water into one of the two Petri dishes, and the remaining one was used
178 as a control. On the day after 90% of the eggs hatched in the control, we drained water and began
179 to photography at 2-min intervals until almost eggs hatched.

180 **Effects of High Humidity and Submergence on Juvenile Development.** We investigated
181 the development of *T. urticae* green form and *P. citri* larvae hatched from eggs exposed to high
182 humidity or submergence from immediately after oviposition to hatching (Exp-6). For this
183 experiment, we prepared two Petri dishes for each of three treatments: high humidity,

submergence, and control (on leaf squares in open Petri dishes). We randomly chose 20 larvae and individually transferred them to kidney bean leaf squares (10 × 10 mm) on water-soaked cotton in Petri dishes (20 leaf squares per dish). We then assessed the time required for mites to emerge as adults. Virgin adult females were individually transferred to new leaf squares (10 × 10 mm), and we recorded the numbers of eggs for the first 5 oviposition days. If a female died during this time, it was excluded from data analysis. Larvae that remained on original leaves were observed for developmental success (to adulthood) and sex ratio. These observations were performed three times.

Statistical Analyses. We used R v. 2.15.2 (R Core Development Team 2012) for statistical analysis except $R \times C$ tests of independence. Hatch rates in high humidity or submergence treatments and controls in Exp-1, 2, 4, and 5 were tested using Fisher's exact test "fisher.test" function in R. Differences in the ratios of eggs that developed eyespots and successfully adjusted hatching (hatched after humidity decreased or water was drained) in Exp-1, 2, 4, and 5; differences in hatch rate in Exp-3; and differences in developmental success and sex ratio in Exp-6 were analyzed with $R \times C$ tests of independence using G-tests (G-values were corrected using Williams's correction: G_{adj}), following unplanned tests of homogeneity (G_H) of treatments for all possible sets of data (Sokal and Rohlf 2000). These analyses were performed using the sum total of eggs or individuals over all Petri dishes used in each experiment.

Effects of the treatments on developmental periods in Exp-6 were evaluated by a one-way analysis of variance (ANOVA; "aov" function in R) followed by a Tukey's HSD post hoc test ("TukeyHSD" function in R) after Bartlett's test for homogeneity of variances ("bartlett.test" function in R). The effects of treatments on egg production in Exp-6 were evaluated using generalized linear models (GLMs with Poisson error). We treated each individual as a sample unit

in these experiments.

Results

Effects of High Humidity on Egg Hatch Timing. In Exp-1, eyespots developed in >89.5% of the eggs of all species in the high humidity treatments (Table 2). Nevertheless, we observed a negative effect of high humidity on the egg hatch rate in all species, especially *P. citri*, for which no eggs hatched (Fig. 2). In contrast, with the exception of *O. castaneae* and *O. amiensis* eggs, many eggs hatched after humidity was decreased. In *Tetranychus* spp. and *P. citri*, many eggs hatched within 1 h of humidity decreasing (Fig. 2). Weaker but similar trends were observed in the remaining three *Panonychus* spp.

The hatch rate in the high humidity treatments was >84.4% for four *Tetranychus* spp., *P. citri*, and *P. mori*. However, except in *T. kanzawai* (Fisher's exact test, $P = 1$), the hatch rate was still lower than for the control (Table 2). The hatch rate decreased to 69.2 and 58.4% for *P. osmanthi* and *P. ulmi*, respectively, and greater negative effects were observed in *Oligonychus* spp. (Table 2, Fig. 2). Hatch rate after humidity decreasing was greatest for *T. kanzawai* and *P. citri*, followed by *T. urticae* green and red forms, *T. ludeni*, and *P. mori* (Table 2).

Negative effects and suspension of egg hatch were also observed in the high humidity treatment in Exp-2 (Fig. 3). *Panonychus citri* eggs did not hatch before the humidity was decreased. A decrease in the hatch rate was observed in three species in the high humidity treatment but not in *T. urticae* red form and *T. kanzawai* (Fisher's exact test, $P = 0.419$ and 0.6626,

228 respectively; Table 3). The highest hatch rate after humidity decreasing was observed in *P. citri*,
229 and the most unsuccessful in *T. kanzawai*, in contrast to the results of Exp-1 (Table 3).

230 The hatch rate in Exp-3 was greatest in *T. kanzawai* (96.0%), followed by *T. urticae* green
231 form (73.2%), and was lowest in *P. citri* (7.3%; *G*-test, d.f. = 2, G_{adj} = 687.5413, $P < 0.001$; Fig. 4).
232 *Tetranychus kanzawai* tended to complete hatching earlier than *T. urticae* in Ex-1 and 2. In Exp-3,
233 the hatch rate was significantly greater in *T. kanzawai* than in *T. urticae* (G_H, d.f. = 2, G_{adj} =
234 66.99835, $P < 0.001$). Hatching was completed by day 7 in *T. kanzawai* and day 8 in *T. urticae*. In
235 contrast, the hatch rate of *P. citri* was significantly lower than that of *T. urticae* (G_H, d.f. = 2, G_{adj}
236 = 425.9087, $P < 0.001$).

237 **Effects of Submergence on Egg Hatch Timing.** Although *T. urticae* green form and *P. citri*
238 eggs were submerged in water from immediately after oviposition to hatching in Exp-4, eyespots
239 developed in most eggs (Table 4a). The hatch rate was lower than in the control treatment but still
240 high; 94.2% of *T. urticae* eggs hatched, but a substantial number hatched in water and drowned
241 (Fig. 5a). In contrast, although the hatch rate of *P. citri* eggs (78.8%) was lower than that of *T.*
242 *urticae* eggs, most hatched after water was drained (Fig. 5a). Consequently, the hatch rate after
243 humidity decreasing was not significantly different between these species (*G*-test, d.f. = 1, G_{adj} =
244 0.7445, $P > 0.05$; Table 4a).

245 Egg hatching was also suspended by the submergence treatments implemented immediately
246 before hatching day was due (Exp-5; Fig. 5b). Submergence affected the hatch rates of eggs of
247 both *T. urticae* and *P. citri* (Table 4b). Although the hatch rate was higher in *T. urticae* (94.1%)
248 than in *P. citri* (84.0%), the hatch rate after humidity decreasing was not significantly different
249 between these species (*G*-test, d.f. = 1, G_{adj} = 2.841845, $P > 0.05$; Table 4b).

250 Embryos of *T. urticae* (Supp. Video_S1) and *P. citri* (Supp. Video_S2) rotated ~360°

immediately before hatching as shown in the animation, after which the shells opened and the eggs hatched. Dorsal setae appeared along the line of rotation, suggesting that egg shells were cut during the rotation.

Effects of High Humidity and Submergence on Juvenile Development. Developmental success after submergence treatment (86–88%) was lower than in the controls (94–96%), but not significantly different from development after high humidity (89–94%; Exp-6; Table 5). No statistical significance was detected between development in the high humidity treatment and the control.

No significant differences were observed between treatments and controls in sex ratio, developmental duration, and egg production, except in the developmental duration of *T. urticae* females. The development of *T. urticae* females was slightly but significantly delayed in comparison to that of control individuals. Similar trends were observed in both sexes of *P. citri*. Consequently, treatments mainly affected developmental success and duration.

Discussion

Submergence in water is frequently a fatal event for terrestrial animals, at least partially due to oxygen deficits. For small spider mites, submergence can be caused even by a drop of rain, especially in upper leaf surface user such as *P. citri*. Spider mites are also intolerant of anoxia conditions (died out within 2 h), although eggs survive slightly longer than other developmental stages (Putman 1968). Nevertheless, the high rate of eyespot formation in *T. urticae* and *P. citri* eggs indicates successful underwater embryonic development. Hatching of eggs in submergence treatments was low, even when the majority of eggs hatched in the control treatments. Then, many

274 eggs hatched immediately after water was drained. This response was reproduced when eggs of
275 *Tetranychus* and *Panonychus* spp. were under high humidity conditions, though the hatch rate
276 decreased in *P. osmanthi* and *P. ulmi*.

277 On the other hand, these responses were not observed in *Oligonychus* spp. eggs placed under
278 high humidity conditions. Why the responses of *O. castaneae* and *O. amiensis* eggs to decreased
279 humidity after high humidity differed from those of other species, and why many eggs died, are
280 unclear. Eggs of other *Oligonychus* spp., the spruce spider mite *O. ununguis* (Jacobi) and the
281 cotton red mite *O. gossypii* (Zacher), were also affected by high humidity (Boyne and Hain 1983;
282 Bonato et al. 1995), indicating that *Oligonychus* eggs may be generally susceptible to high
283 humidity.

284 Herne (1968) reported no effects of submersion in water for up to 48 h on the hatch rate of *P.*
285 *ulmi* eggs. While, submerged eggs did not hatch under water even when submerged immediately
286 prior to normal hatching, while previously submerged eggs hatched later (Herne 1968). Our results
287 largely correspond with these observations. Many eggs hatched only after humidity was decreased
288 and water was drained, even if they had developed to the point of hatching before treatment were
289 implemented, indicating that delayed hatching in high humidity and water for *Tetranychus* and
290 *Panonychus* spp. is not caused by low embryonic developmental rates, but by the suspension of
291 hatching. This regulation is likely a result of the rotation behavior as the shell is cut just before
292 hatching. Eggs might monitor the surrounding environment through a respiratory system
293 consisting of shell perforation organs (the perforation cone) and a centripetal cone directly
294 connected to embryonic tissue (Dittrich 1971).

295 Boudreaux (1958) showed that although high humidity did not cause larval death directly, it
296 affected the juvenile development time in *Tetranychus* mites. In our experiments, the

297 developmental success of individuals that experienced submergence during their egg stages
298 decreased and developmental time was slightly prolonged by submergence. However, these
299 negative effects are minor in comparison to those on larvae wetted by mist or reared under high
300 humidity conditions (Boudreaux 1958). Putman (1970) showed that *P. ulmi* larvae misted for 6–48
301 h had ~80% mortality, development was significantly delayed by a high humidity treatment, and
302 larvae were unable to survive at continuous high humidity. Therefore, the suspension of hatching
303 and passing damp and rainy conditions likely increases larval survival.

304 When eggs remained under high humidity, those of *T. urticae* and *T. kanzawai* gradually
305 hatched. In contrast, most *P. citri* eggs never hatched and eventually died. Leaves of citrus trees,
306 one of the major host plants of *P. citri* in a temperate zone (Osakabe 1987; Kitashima and Gotoh
307 1995), are water-repellent, which means that drops of water remain on leaves, especially on the
308 upper leaf surfaces, during rain. Since *P. citri* oviposits on both upper and lower leaf surfaces
309 (Jones and Parrella 1984; Fukaya et al. 2013), its eggs may be frequently submerged during rain.
310 However, its water repellency also means that citrus leaves dry quickly after rain. Cessation of
311 hatching may be adaptive for *P. citri* to survive rainfall events on the upper leaf surfaces of such
312 hosts.

313 In contrast, *T. urticae* and *T. kanzawai* prefer hairy leaves as habitats (Peters and Berry 1980;
314 Oku et al. 2006). Hairy leaves are soaked during rain, and water droplets are rarely formed.
315 Moreover, many eggs are laid on complicated webs produced by mothers among leaf hairs on the
316 lower leaf surfaces, which might function as shelter from the rain (Gerson 1985). Such habitats
317 may retain moisture longer compared to the habitat of *P. citri*. The high mortality of *P. citri* eggs
318 when they continuously experienced high humidity suggests that limits exist to elongate hatching,
319 after which hatching is unsuccessful. Generally, leaf stomata are concentrated on the lower leaf

surfaces, resulting in higher humidity (Boulard et al. 2002). Thus, *T. kanzawai* and *T. urticae* are likely to experience relatively high humidity more frequently than *P. citri*. If humidity remains high, hatching might lessen the negative effects of rainfall. Duso et al. (2004) found that misty water sprayed using fogging system suppressed *T. urticae* population on cucumber plants. They suggested importance of contact with misty water for the reduction of mite population (Duso et al. 2004). In that, hatched larvae might be damaged from water rather than eggs because eggs do not suspend hatching at lower than 90% RH.

We found suspension of egg hatching caused by high humidity and submergence, in *Panonychus* and *Tetranychus* species, and not *Oligonychus* species. However, mechanisms of the suspension and also resumption are largely unknown. We expect that spider mites can mitigate the adverse effects of rainfall on juvenile stages on account of the suspension of egg hatching. To elucidate this idea, further studies are required.

Acknowledgments

We thank Dr. S. Masui (Shizuoka Prefectural Research Institute of Agriculture and Forestry), Mr. K. Seki (Nagano Prefecture Vegetable and Ornamental Crops Experiment Station), Mr. K. Nakata (Tottori Prefectural Agriculture and Forest Research Institute), Mr. K. Funayama (Akita Fruit-Tree Experiment Station) and Dr. H. Kishimoto (National Agriculture and Food Organization Institute of Fruit Tree Science) for their kind help in collecting spider mites.

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438 **Figure Captions**

439

440 **Fig. 1**. Experimental design for testing the effects of submergence in water on egg hatch
441 timing.

442

443 **Fig. 2.** Effects of high humidity treatments from immediately after oviposition to hatching on
444 egg hatch timing (Exp-1). Solid circles and open triangles represent high humidity and
445 control treatments, respectively. Open circles show the hatch rates after lids of Petri
446 dishes were opened and humidity began decreasing. Vertical lines on plots represent
447 95% fiducial limits.

448

449 **Fig. 3.** Effects of high humidity immediately before hatching was due on egg hatch timing
450 (Exp-2). Solid circles and open triangles represent high humidity and control
451 treatments, respectively. Open circles show the hatch rate after lids of Petri dishes
452 were opened and humidity began decreasing. Vertical lines on plots represent 95%
453 fiducial limits.

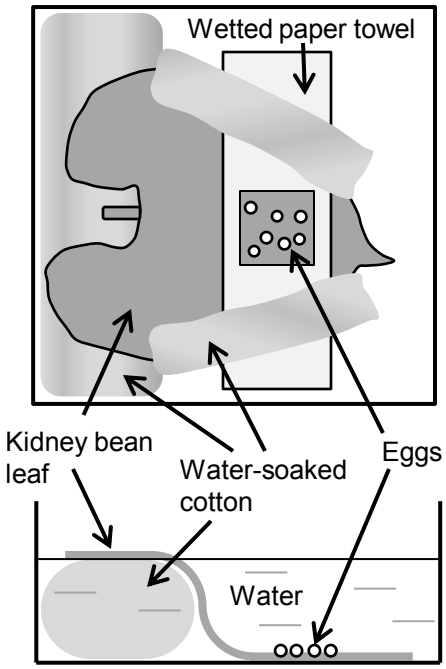
454

455 **Fig. 4**. Effects of high humidity implemented immediately after oviposition and continuously
456 after hatching was due on egg hatch timing (Exp-3). Solid circles and open triangles
457 represent high humidity and control treatments, respectively. Vertical lines on plots
458 represent 95% fiducial limits.

459

460 **Fig. 5**. Effects of submergence in water from (a) immediately after oviposition to hatching
461 (Exp-4) and (b) immediately before hatching was due (Exp-5). Solid circles and open

462 triangles represent submergence and control treatments, respectively. Open circles
463 show the hatch rates after water was drained. Vertical lines on plots represent 95%
464 fiducial limits.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Kidney bean leaf
Water-soaked cotton
Eggs
Water

Fig. 1

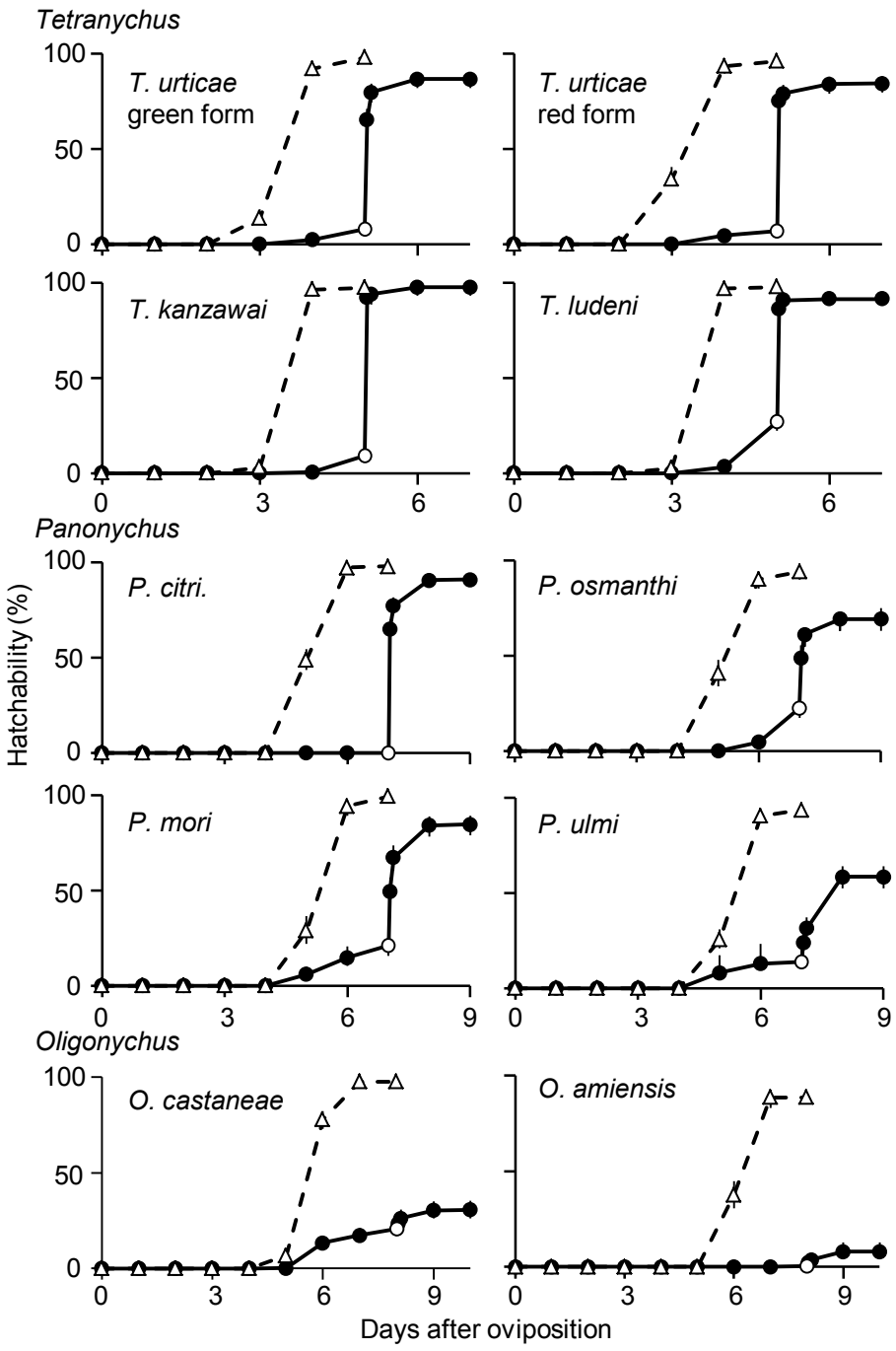
Tetranychus
T. urticae
green form
T. urticae
red form
T. kanzawai
T. ludeni
Panonychus
P. citri.
P. osmanthi
P. mori
P. ulmi
Oligonychus
O. castaneae
O. amiensis
Hatchability (%)
Days after oviposition
100
50
0
0
3
6
9

Fig. 2

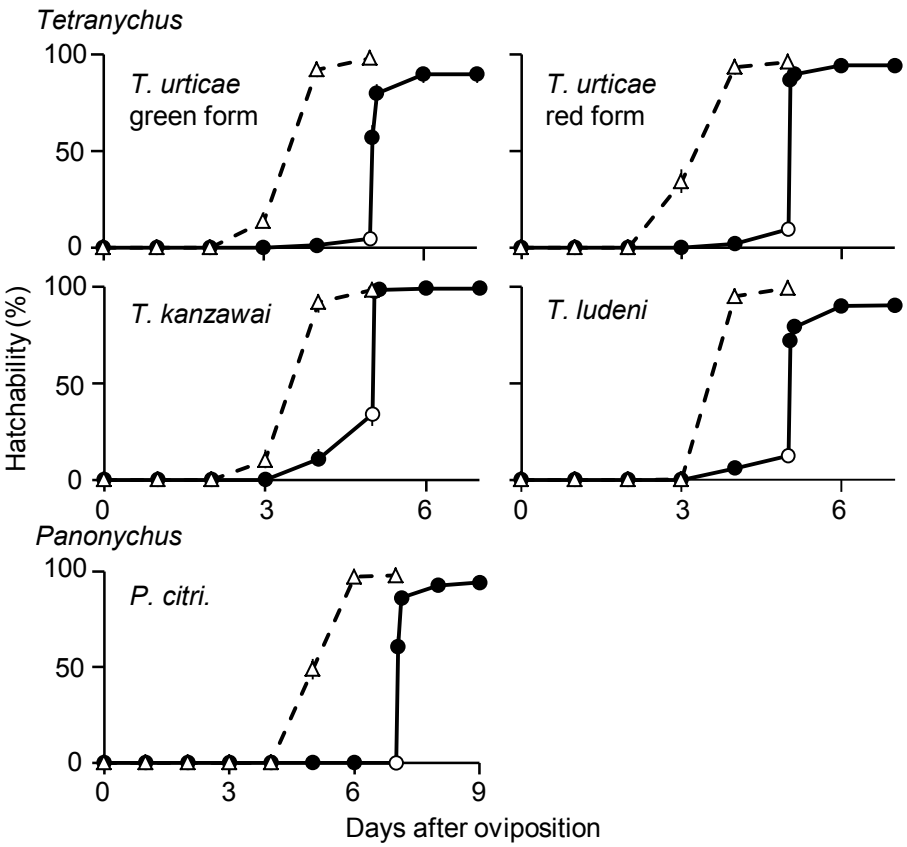
Tetranychus
T. urticae
green form
T. urticae
red form
T. kanzawai
T. ludeni
Panonychus
P. citri.
Hatchability (%)
100
50
0
0
3
6
9
Days after oviposition

Fig. 3

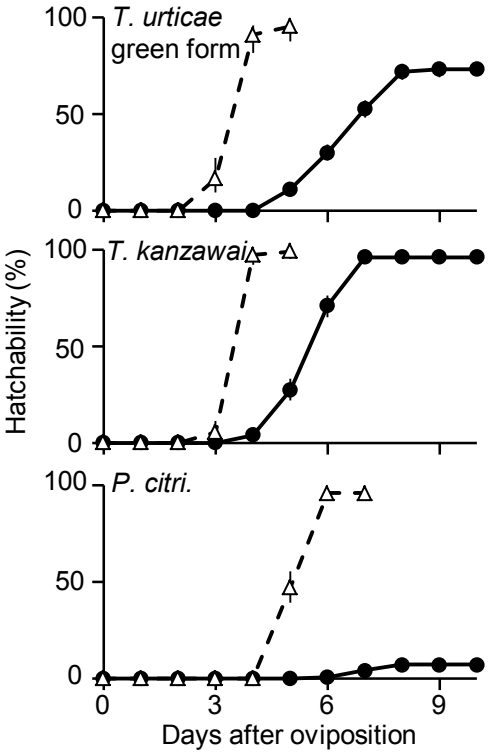
T. urticae
green form
T. kanzawai
P. citri.
100
50
0
Hatchability (%)
0
3
6
9
Days after oviposition

Fig. 4

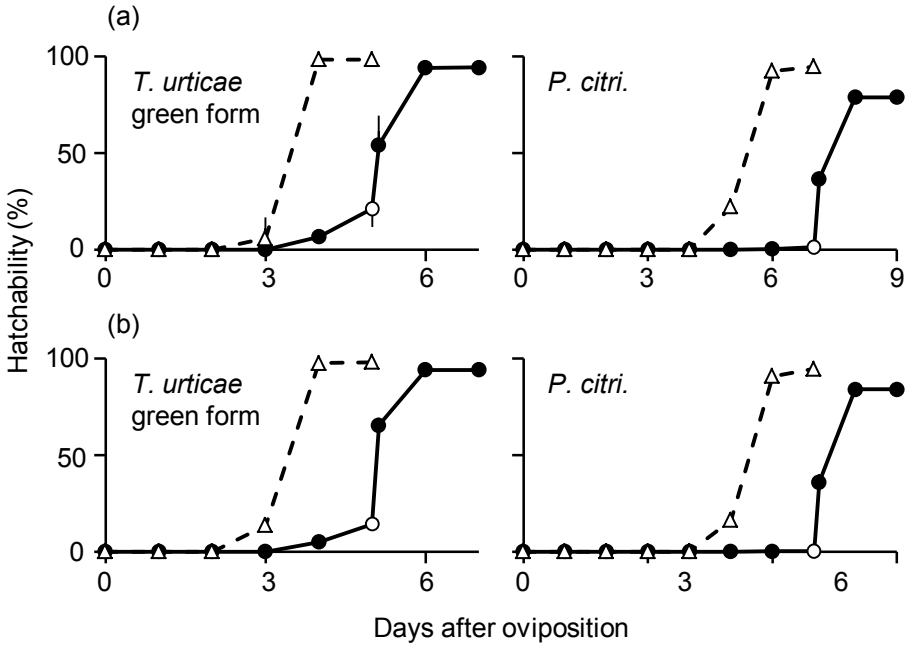
(a)
100
50
0
T. urticae
green form
P. citri.
0
3
6
9
(b)
100
50
0
T. urticae
green form
P. citri.
0
3
6
Hatchability (%)
Days after oviposition

Fig. 5

Table 1 Ranges in temperature and humidity in three types of experiments

	Temperature ± SD (°C)		Humidity ± SD (%)	
	HH[a]	Cont[b]	HH[a]	Cont[b]
Exp-1				
Minimum	24.7 ± 0.5	23.9 ± 0.3	94.4 ± 0.5	81.3 ± 2.7
Maximum	26.1 ± 0.5	25.7 ± 0.5	98.5 ± 0.7	92.4 ± 2.7
Exp-2				
Minimum	24.8 ± 0.5	23.8 ± 0.4	94.4 ± 0.7	77.8 ± 5.6
Maximum	25.7 ± 0.6	24.4 ± 0.5	97.8 ± 0.5	90.9 ± 1.3
Exp-3				
	26.1 ± 0.8	24.9 ± 0.4	96.7 ± 0.5	85.1 ± 5.4

[a] Averaged temperature during the dishes were covered with lids in the high humidity treatment in an experiment. For Exp-1 and Exp-2 we show only the cases of minimum and maximum averages over replications in the same experiment.
[b] Average in the control treatment in an experiment.

Table 2 Success for adjusting the hatch timing in high humidity treatments from immediately after oviposition to hatching (Exp-1)

	Treatments	No. eggs [a]	Eye spots (%) [b]	Hatch rate (%) [c]	Hatch rate after humidity decreasing (%) [d]
T. urticae	High humidity	255	96.5	86.7 ***	78.8 b
(green form)	Control	264	—	98.1	—
T. urticae	High humidity	262	93.9	84.4 ***	77.5 bc
(red form)	Control	228	—	96.1	—
T. kanzawai	High humidity	164	98.2	97.6 ns	88.4 a
	Control	195	—	97.4	—
T. ludeni	High humidity	303	96.4	91.4 **	64.4 c
	Control	224	—	97.8	—
P. citri	High humidity	329	95.1	90.9 ***	90.9 a
	Control	329	—	97.9	—
P. osmanthi	High humidity	237	92.0	69.2 ***	46.8 de
	Control	201	—	94.0	—
P. mori	High humidity	202	96.5	84.6 ***	63.4 cd
	Control	156	—	99.4	—
P. ulmi	High humidity	279	94.6	58.4 ***	44.4 e
	Control	293	—	93.5	—
O. castaneae	High humidity	428	93.5	30.6 ***	9.8 f
	Control	320	—	97.5	—
O. amiensis	High humidity	191	89.5	7.9 ***	7.3 f
	Control	184	—	88.6	—

[a] The sum total of eggs used in experiments three times.

[b] Percentages of eggs in which red eye spots were developed.

[c] Hatch rate in total over experimental periods. Triple, double, and single asterisks at high humidity indicate *P*-values against control to be < 0.001, < 0.01, and < 0.05, respectively, by Fisher's exact test.

[d] Percentage of eggs which hatched after lids of Petri dishes were opened and humidity decreased. The same letter in the column represent that no significant differences were detected among species by an $R \times C$ test of independence using a *G*-test ($P > 0.05$).

Table 3 Success for adjusting the hatch timing in high humidity treatments from immediately before hatching was due (Exp-2)

	Treatments	No. eggs [a]	Hatch rate (%) [c]	Hatch rate after humidity decreasing (%) [d]
T. urticae	High humidity	243	89.7 ***	85.1 b
(green form)	Control	264	98.1	—
T. urticae	High humidity	294	94.2 ns	84.7 b
(red form)	Control	228	96.1	—
T. kanzawai	High humidity	193	99.0 ns	64.7 c
	Control	161	98.1	—
T. ludeni	High humidity	494	90.3 ***	77.7 b
	Control	352	99.1	—
P. citri	High humidity	326	94.2 *	94.2 a
	Control	329	97.9	—

[a] The sum total of eggs used in experiments three times.

[b] Percentages of eggs in which red eye spots were developed.

[c] Hatchability in total over experimental periods. About asterisks see Table 1.

[d] Percentage of eggs which hatched after lids were opened and humidity decreased. The same letters in the column represent that no significant difference was detected in unplanned tests of homogeneity ($P > 0.05$) after an $R \times C$ test of independence using a G-test.

Table 4 Success for adjusting the timing of hatching in submergence treatments from (a) immediately after oviposition to hatching (Exp-4) and (b) immediately before hatching was due (Exp-5) (b)

	Treatments	No. eggs [a]	Eye spots (%) [b]	Hatchability (%) [c]	Hatch rate after drained (%) [d]
(a)					
T. urticae	Submergence	642	97.4	94.2 ***	73.1 ns
(green form)	Control	554	—	98.4	—
P. citri	Submergence	585	97.1	78.8 ***	77.6
	Control	475	—	94.7	—
(b)					
T. urticae	Submergence	578	—	94.1 ***	79.8 ns
(green form)	Control	563	—	98.0	—
P. citri	Submergence	556	—	84.0 ***	83.6
	Control	463	—	94.6	—

[a] The sum total of eggs used in experiments three times.

[b] Percentages of eggs in which red eye spots were developed. No significant differences were detected among species by an $R \times C$ test of independence using a G-test ($P > 0.05$).

[c] Hatchability in total over experimental periods. About asterisks see Table 1.

[d] Percentage of eggs which hatched after the humidity declined (lids were opened). No significant differences were detected among species by an $R \times C$ test of independence using a G-test ($P > 0.05$).

Table 5 Effects of high humidity and submergence treatments on subsequent development and egg production (Exp-6)

			High humidity	Submergence	Control
T. urticae	Development (%) [a]		89.4 (170) ab	86.2 (159) b	94.1 (222) a
green form	Sex ratio ♀/(♀+♂) [b]		0.82 (152)	0.82 (137)	0.86 (209) ns
	Developmental	♀	6.5 ± 0.1 (48) a	6.4 ± 0.1 (38) a	6.1 ± 0.1 (44) b
	duration [c]	♂	6.1 ± 0.1 (9)	6.1 ± 0.1 (10)	6.1 ± 0.1 (14) ns
	Egg production [d]		47.2 ± 0.7 (43)	47.8 ± 1.0 (34)	49.2 ± 1.2 (38) ns
P. citri	Development (%) [a]		94.4 (180) ab	87.9 (149) b	95.5 (179) a
	Sex ratio ♀/(♀+♂) [b]		0.81 (170)	0.88 (149)	0.83 (171) ns
	Developmental	♀	6.7 ± 0.1 (43)	6.8 ± 0.1 (41)	6.5 ± 0.1 (45) ns
	duration [c]	♂	6.9 ± 0.1 (9)	6.9 ± 0.1 (8)	6.5 ± 0.2 (10) ns
	Egg production [d]		19.6 ± 0.7 (36)	19.2 ± 0.6 (34)	19.8 ± 0.6 (38) ns

Figures of parentheses represent the numbers of individuals tested.

[a] Developmental success from larva to adult. The same letters in the column represent that no significant difference was detected in unplanned tests of homogeneity ($P > 0.05$) after an $R \times C$ test of independence using a G-test.

[b] Sex ratio in individuals which developed to adulthood. No significant differences were detected among species by an $R \times C$ test of independence using a G-test ($P > 0.05$).

[c] Developmental times from larva to adult. The same letters in the line for *T. urticae* females were not significantly different at a 5% level in multiple comparisons using Tukey HSD. No significant differences were detected by a one-way ANOVA in *T. urticae* male and *P. citri*.

[d] Number of eggs laid for the first five oviposition days. No significant differences were detected among treatments in both species by Wald tests using GLM.